THE MODE OF ACTION OF MUSCLE RELAXANTS AND ALLIED DRUGS

A Thesis submitted to the University of Glasgow

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in the

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by

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LIST OF PUBLICATIONS

Certain aspects of the work described in this thesis have been published. The publications are as follows:-

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   Pages 3 to 13.
   Symposium on the use of modern diuretics in the control of hypertension.
   Edited by Heseltine, W.W., Liverpool; C. Birchall and Sons Ltd.

2. B. J. Mir and J. J. Lewis (1960).
   Some observations on the pharmacology of 10-methoxydeserpidine.
   J. Pharm., 12, 677-684.

PAPERS IN PREPARATION

3. Observations on the effects of ether and other volatile anaesthetics on ion fluxes.

4. The effects of 10-methoxydeserpidine on in vitro adenosine triphosphatase activity, anaerobic glycolysis and respiration in certain tissues.

STUDIES ON THE MODE OF ANTIHYPERTENSIVE ACTION OF 10-METHOXYDESERPIDINE AND SOME ALLIED DRUGS

PART I (a)

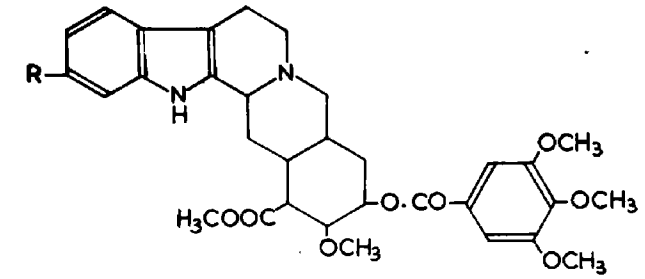
C H A P T E R I.

CHAPTER I

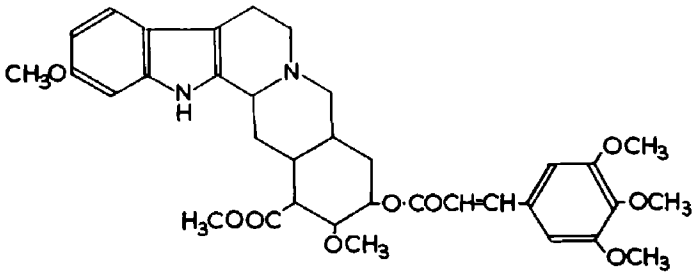
INTRODUCTION

Species of the genus Rauwolfia (family Apocynaceae) are indigenous to the subtropical zones of Asia, Africa and South America, and the natives of these regions have employed the roots of the plant for treating a variety of illnesses. The genus was named in the honour of Leonhard Rauwolf, a German physician and botanist who travelled to India in search of plants of medicinal interest and in 1582 published a book on medicinal plants. The use of Rauwolfia serpentina, so named because of the snake-like appearance of the root, goes back in native Indian medicine to most ancient times. It is only, however, within the past decade that the plant has gained a place in orthodox therapeutics. In accounts of the early use of Rauwolfia in India, its sedative properties were most prominently mentioned as being beneficial in the treatment of excited and maniacal individuals.

In more recent times, Roy (1931) found that large doses of R. serpentina induced sleep, caused a dulling of the sensations and a diminution of the reflexes. In the same year, Sen and Bose (1931) studied the pharmacological effects of R. serpentina on the cat and other animal species. They reported a small drop in the blood pressure level and stimulation of respiration. Fatal doses caused death from respiratory failure; the heart continuing to beat for some time after respiration /



R = H , Deserpidine
R = CH_3O, Reserpine



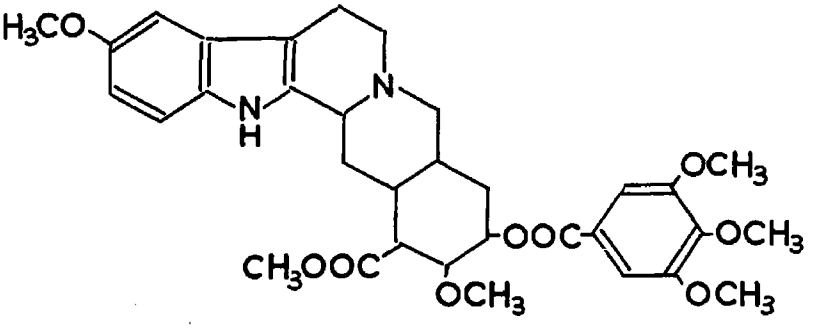
Rescinnamine

Rauwolfia Alkaloids

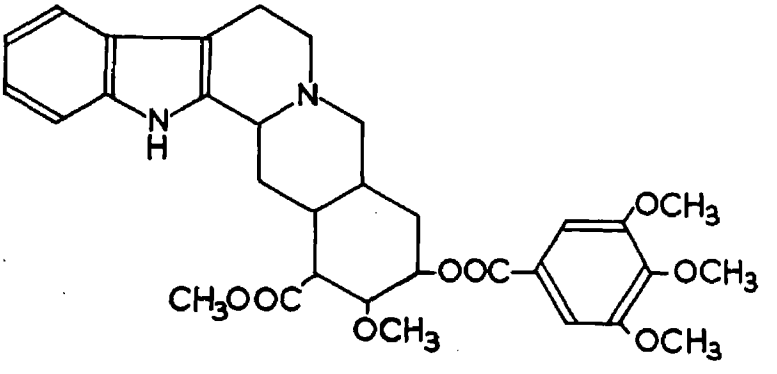
Fig. 1.

respiration had ceased. The characteristic hypotensive effect of R. serpentina was confirmed by Chopra, Bose, Gupta and Chopra (1942) who observed, following the administration of an extract of R. serpentina, a fall in the blood pressure level of animals with experimental hypertension. In 1949, Vakil reported upon the clinical value of R. serpentina in human hypertension. A systematic chemical investigation of the alkaloids of R. serpentina had already been made by Siddiqui and Siddiqui in 1931, but none of the substances isolated, which included ajmaline and serpentine, had shown the characteristic hypotensive and sedative activity of the whole root. The ultimate isolation of the substance responsible for the sedative actions noted by earlier workers was achieved by Müller, Sohlittler and Bein (1952). Subsequent pharmacological investigations by Bein (1953) indicated that this substance, which was given the name of reserpine, exerted typical antihypertensive and tranquillizing actions in experimental animals including dogs, cats, monkeys and mice. These characteristic effects were found to be slow in onset but prolonged in duration.

Two alkaloids subsequently obtained from R. serpentina have also shown typical reserpine-like activity. These are rescinnamine, (Fig. 1, page 2) identified by Klohs, Draper and Keller (1954) and deserpidine (11-desmethoxyreserpine) (Fig. 2, page 4) first isolated by Sohlittler, Ulshafer, Pandow, Hunt and Dorfman (1955). Rescinnamine is the trimethoxycinnamate of methylreserpate, and deserpidine differs from /



10-methoxydeserpidine



11-desmethoxyreserpine (deserpidine)

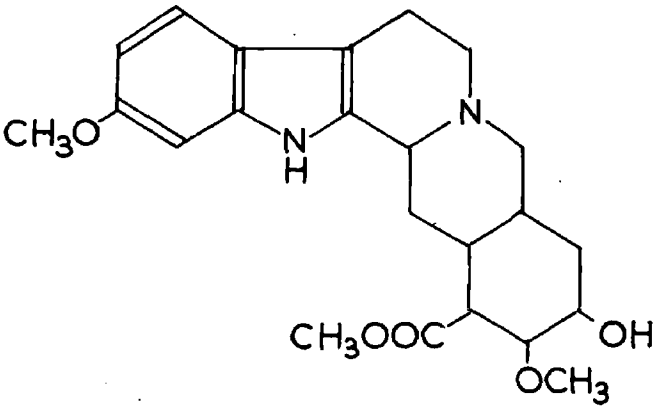
Fig. 2.

from reserpine by the absence of the methoxy group in position 11 of Ring A.

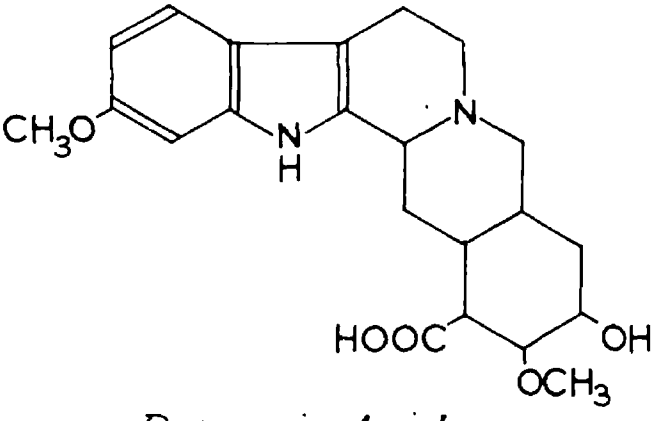
Pharmacological and clinical investigations carried out by a number of workers have indicated that the general pharmacological actions of reserpine are also manifested by deserpidine (Schneider, Plummer, Earl, Barrett, Rinehart and Dibble, 1955 and Achor and Hanson, 1956).

The pharmacological actions of reserpine were studied intensively by Bein (1953); Tripod and Meier (1954); Trapold, Plummer, and Yonkman (1954); Vakil (1955); Lewis (1956); Bein (1956) and Kohli and Mukerji (1961). During the past decade, it has been widely employed in the treatment of hypertension and certain psychiatric disorders. Reserpine has enjoyed a world wide popularity, but its usefulness in the treatment of arterial hypertension has become limited due to its side-effects, the most important of which include mental depression, drowsiness, nasal congestion and diarrhoea. The incidence of these unpleasant reactions in relatively large numbers of patients has led to a search for compounds with reserpine-like antihypertensive actions but with fewer side-effects.

In 1958, Velluz and his associates synthesised an isomer of reserpine known as 10-methoxydeserpidine (methoserpidine) (Fig. 2 , page 4) and reported the separation of the antihypertensive actions from the central nervous system depressant effects of reserpine. The drug was reported to produce none of the side-effects associated with reserpine /



Methyl Reserpate



Reserpic Acid

Fig. 3.

reserpine therapy.

Little information is available upon the actions of 10-methoxydeserpidine on isolated tissues and organs. Investigations were carried out upon the effects of 10-methoxydeserpidine on the cardiovascular system of the normotensive, anaesthetised cat and rat. The central nervous system depressant effects were investigated using intact mice and rats.

In order to investigate the possible mechanism or mechanisms by which 10-methoxydeserpidine exerted its antihypertensive actions, studies upon the effects of this drug on tissue respiration, anaerobic glycolysis and the adenosine-triphosphatase activity of the rat brain, liver and skeletal muscle were performed.

To compare the effects of 10-methoxydeserpidine with a drug which exhibits reserpine-like antihypertensive and central nervous system depressant effects, the pharmacological properties of 10-methoxydeserpidine were compared with those of deserpidine which has consequently also been investigated.

During the isolation of reserpine in 1954, Schlittler and his associates obtained reserpic acid and methylreserpate. These products are obtained on hydrolysing reserpine. Reserpic acid (Fig. 3, page 6) when esterified with diazomethane yielded a methyl ester known as methylreserpate (Fig. 3, page 6). Reserpic acid is reported to possess none of the hypotensive or sedative actions of reserpine whereas methyl-

reserpate /

methylreserpate is known to possess about one third of the potency of reserpine (Rubin and Burke, 1954 and Plummer, Barrett and Rutledge, 1954). Plummer and his associates concluded that esterification was necessary for the production of the characteristic central nervous system depressant effects of reserpine.

To compare the pharmacological actions of reserpio acid and methylreserpate with those of 10-methoxydeserpidine, studies were performed upon isolated tissues and organs and intact animals.

10-Methoxydeserpidine

Chemistry - 10-methoxydeserpidine is a structural isomer of reserpine (Fig. 2, page 4), the methoxy group in Ring A being on carbon atom 10, instead of on carbon atom 11 (Velluz, 1959).

Pharmacological Properties of 10-Methoxydeserpidine -

Effects on the Cardiovascular System. 10-methoxydeserpidine produces a moderate fall in the arterial blood pressure of the normotensive, anaesthetised cat and rabbit (Velluz, Peterfalvi and Jequier, 1958). This antihypertensive response was found to be more significant in rats, made experimentally hypertensive by the administration of saline and implantation of desoxycorticosterone-acetate pellets. 10-methoxydeserpidine, like reserpine, is characterised by the slowness in onset of its pharmacological actions and their prolonged duration. Peterfalvi and Jequier (1960) observed that whatever the dosage used, the route of administration /

administration, or the animal species, there was no sedative action. The carotid sinus pressor reflex in the anaesthetised cat was markedly reduced or abolished following the administration of 10-methoxydeserpidine (Peterfalvi and Jequier, 1960 and La Barre, 1960). The hypertensive response to the intravenous injection of adrenaline in the anaesthetised cat was potentiated, whereas the response of the nictitating membrane to adrenaline was unaltered. As with reserpine, (Bein, 1953 and Plummer, Earl, Schneider, Trapold and Barrett, 1954) the fall in blood pressure level is accompanied by a distinct bradycardia which is more marked in the dog and less so in the cat (Peterfalvi and Jequier, 1960). Clinical investigations of the antihypertensive actions of 10-methoxydeserpidine were first carried out by Meriel, Galinier, Suc and Bounhoure (1959)who reported it to be a safe antihypertensive agent of low toxicity and with no side-effects. In the subsequent clinical reports of Gross, Peterfalvi and Jequier (1959), it was indicated that at adequate therapeutic dose levels, 10-methoxydeserpidine produced a fall in the blood pressure level in all types of hypertension with few or no side-effects. Holt (1961) revealed that two-thirds of her ambulant patients treated with 10-methoxydeserpidine showed a good hypotensive response with an absence of reserpine-like side-effects.

Burn and Rand (1958) demonstrated that reserpine depleted the thoracic aorta of the rabbit of its stores of noradrenaline. 10-Methoxydeserpidine when given to mice at dose levels of 25 mg. per kg. intraperitoneally /

intraperitoneally did not alter the catecholamine content of the heart and brain (Leroy and Schaepdryver, 1961).

Effects on the Central Nervous System. Unlike reserpine, 10-methoxydeserpidine has been reported to possess no sedative or tranquillizing actions (Velluz, Peterfalvi and Jequier, 1958; Gross, Peterfalvi and Jequier, 1959; Peterfalvi and Jequier, 1960; and Holt, 1961). When given to mice, in dose levels of 20 to 500 mg. per kg. intraperitoneally and 2 g. per kg. orally, 10-methoxydeserpidine did not produce reserpine-like sedative effects or toxic actions. The subcutaneous administration to rats of 1 mg. per kg. of 10-methoxydeserpidine daily for a period of two months and in dogs for three months did not cause loss in weight, retardation of growth or mortality (Peterfalvi and Jequier, 1960). On the other hand, La Barre (1960) has demonstrated that intraperitoneal administration of 10-methoxydeserpidine (4 to 6 mg. per kg.) to rats, produced a reduction in motility. This effect lasted for a period of two days after the administration of the drugs and was said to be due to a sedative action.

Although 10-methoxydeserpidine is claimed to be devoid of side-effects, sleepiness and mental depression were demonstrated in a proportion of cases following prolonged treatment with this drug (Holt, 1961; Read, 1961; and Bellis and his associates, 1961). It has also been reported by Holt (1961) that 11 of her patients who had shown symptoms of anxiety were relieved of these following the use of 10-methoxydeserpidine as an antihypertensive /

antihypertensive agent.

Side-effects following 10-methoxydeserpidine have been recorded by other investigators and those most frequently observed clinically were nasal congestion, nausea, lethargy and a gain in weight, (Gross, Peterfalvi and Jequier, 1959; and Read, 1961). La Barre (1960) has demonstrated that 10-methoxydeserpidine has less tendency than reserpine to produce gastric ulceration and vomiting.

The median lethal dose (LD 50) of 10-methoxydeserpidine in mice has been estimated by Peterfalvi and Jequier (1960) to be 2 g. per kg. intraperitoneally whereas that of reserpine is 8.0 mg. per kg.

Deserpidine

Deserpidine has been studied by a number of workers who have reported on its effects upon intact animals. It has been shown to possess reserpine-like hypotensive and sedative actions and in all other respects appears to have the typical pharmacological properties of reserpine, indicating that the removal of the 11-methoxy group causes no qualitatively important effects (Schlittler, Ulshafer, Pandow, Hunt and Dorfman, 1955; Slater, Rathbun, Henderson and Neuss, 1955; Schneider, Plummer, Earl, Barrett, Rinehart and Dibble, 1955; Harrisson, Packman, Smith, Hosansky and Salkin, 1955; and Packman, Abbott and Harrisson, 1956).

Pharmacological Properties of Deserpidine. Deserpidine produces a fall /

fall in the blood pressure of the normotensive, anaesthetised cat and dog. Like reserpine, its antihypertensive and sedative actions are delayed in onset and prolonged in duration. When given intravenously to the anaesthetised cat or dog, a latent period of from one to three hours elapses before a significant reduction in the blood pressure level is noticed. The pressor response elicited by faradization of the central end of the vagus in dogs (Cronheim, Orcutt and Toekes, 1955) and cats (Slater, Rathbun, Henderson and Neuss, 1955) is completely abolished following the administration of deserpidine. When given intravenously to the cat and dog, deserpidine depresses the pressor responses to bilateral occlusion of the common carotid arteries (Schneider, Plummer, Earl, Barrett, Rinehart and Dibble, 1955, and Slater, Rathbun, Henderson and Neuss, 1955). The pressor effect of intravenous administration of adrenaline is enhanced following injection of deserpidine, whereas the depressor responses to injections of histamine and carbachol are not modified. The responses of the nictitating membrane to preganglionic stimulation of cervical sympathetic and to injection of adrenaline are not reduced following the administration of deserpidine (Slater, Rathbun, Henderson and Neuss, 1955).

Cronheim, Orcutt and Toekes (1955) observed bradycardia associated with a fall in the blood pressure after deserpidine was administered to dogs. In the perfused, innervated but otherwise isolated hindquarters of the rat, in which the vasomotor tone was increased by infusion of noradrenaline /

noradrenaline, vasopressin or barium chloride, both deserpidine and reserpine exerted a direct vasodilator effect (McQueen and Blackman, 1955).

A clinical study by Ford, Borreson, Lindley and Moyer, (1957) has indicated that deserpidine has antihypertensive actions qualitatively similar to those of reserpine.

Innes, Krayer and Waud (1958) in their investigations on the heart rate and on the functional refractory period of atrio-ventricular transmission in the heart-lung preparation of the dog, observed that the cardio-accelerator effects of deserpidine resembled those of reserpine. In the early period of their action, reserpine and deserpidine increased the heart rate and decreased the functional refractory period and atrio-ventricular propagation time. After a period of from 90 to 150 minutes, using a dose equivalent in effect to 3 mg. of reserpine, deserpidine caused a decrease in the heart rate while the functional refractory period and the atrio-ventricular propagation time returned towards their original duration. Deserpidine and reserpine lacked this cardio-accelerator action in experiments where the heart-lung preparation was depleted of intrinsic catechol amines by previous treatment with reserpine. The transient cardio-acceleration and facilitation of atrio-ventrioular transmission following the use of reserpine and deserpidine can be explained by the release from the heart of catechol amines, especially noradrenaline which is known to cause these /

these effects. The depressant action is concealed until the release of the amines is no longer sufficient to antagonise it. A number of workers have shown that reserpine causes a reduction in the catechol amine content of brain, peripheral sympathetic nervous tissues, the adrenal medulla, heart and artery walls (Holzbauer and Vogt, 1956; Muscholl and Vogt, 1958; Carlsson and Hillarp, 1956; Krayer and Paasonen, 1957; Paasonen and Krayer, 1958; and Burn and Rand, 1958). Deserpidine was also shown to cause a decrease in the noradrenaline content of the rat heart. The adrenaline content was not markedly affected (Paasonen and Krayer, 1959). It has also been shown to cause depletion of the noradrenaline content of the adrenal medulla of the rat (Greenberg, Toman and Jeffay, 1959) and mouse (de Schaep-dryver and Preziosi, 1959).

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C H A P T E R II.

C H A P T E R II

A. MATERIALS

The drugs used in the investigation which is described in this section of the thesis, together with their shortened names, are as follows:-

1. Acetylcholine chloride is described as acetylcholine.
2. Sodium pentobarbitone (Veterinary Nembutal, Abbott) is described as pentobarbitone.
3. Histamine acid phosphate is described as histamine.
4. 5-Hydroxytryptamine creatinine sulphate is described as 5-hydroxytryptamine.
5. (-)-Adrenaline bitartrate is described as adrenaline.
6. (-)-Noradrenaline bitartrate is described as noradrenaline.
7. Decamethonium iodide is described as decamethonium.
8. Nicotine hydrogen tartrate is described as nicotine.
9. Atropine sulphate is described as atropine.
10. (+)-Tubocurarine chloride is described as tubocurarine.

The following drugs were also used in the investigations presented in this part of the thesis.

10-Methoxydeserpidine (Roussel).

Deserpidine (Ciba).

Reserpic /

Reserpic acid (Riker).

Methyl reserpate (Riker).

The compositions and methods of preparation of all physiological saline solutions used in this investigation are to be found in Appendix I, page 452.

Solutions of 10-methoxydeserpidine, deserpidine, methyl reserpate and reserpic acid were made by dissolving the solid in the minimal amount of glacial acetic acid and adjusting the volume with distilled water. The pH of the final solutions was from 3.4 to 3.6. Control solutions prepared in the same way and at the same pH were used throughout for purposes of comparison. The statistical method employed to calculate the experimental observations is described in Appendix I, page 451.

B. EXPERIMENTAL

10-Methoxydeserpidine is a structural isomer of reserpine, the methoxy group being on carbon atom 10 instead of carbon atom 11 in ring A. In deserpidine the 11-methoxy group present in reserpine is absent.

In order to investigate the effects of this change in chemical structure upon pharmacological activity, five types of experiments were carried out. These were:-

1. Experiments on the blood pressure level and vasomotor reflexes of normotensive anaesthetised cats and rats.
2. Experiments on the responses of isolated tissue preparations taken /

taken from organs containing smooth or skeletal muscle.

(a) Isolated strips of guinea pig ileum.

(b) Isolated strips of rabbit duodenum.

(c) Isolated strips of horse carotid artery.

(d) Blood vessels of the isolated, perfused rat hindquarters in situ.

(e) Isolated frog rectus abdominis muscle.

(f) Experiments were also carried out on isolated cardiac muscle, including isolated guinea pig auricles and the isolated perfused rabbit heart.

3. Experiments on the ganglion blocking activity.

4. Experiments on the tranquillizing and central nervous system depressant effects. For example, the induction of ptosis, miosis and the prolongation of pentobarbitone-induced sleep.

5. The determination of acute toxicity in mice.

1. Experiments on the blood pressure level and vasomotor reflexes of normotensive anaesthetised cats and rats.

(a) Experiments on the blood pressure of the anaesthetised cat.

Method:

Cats of either sex, weighing from 2.0 to 4.0 kg. were used. Anaesthesia was induced by means of the intraperitoneal injection of 40 to 60 mg. per kg. sodium pentobarbitone. The cat was left from 20 to 40 minutes after the injection for surgical anaesthesia to develop. /

develop. When anaesthetised, the cat was laid on its back upon an operating table; the legs were secured to the table by means of strings and the head extended by passing a strong thread through the skin at the apex of the lower jaw. The skin covering the neck was cut away from the sternum to the apex of the mandible. The fascia covering the trachea was divided at the midline and the blunt point of an aneurysm needle passed between the muscles of the neck and thence around the trachea. A strong linen thread was now passed around the trachea and a transverse incision made in the latter by means of a scalpel. The cut edge of the partly severed trachea was held firmly in a pair of blunt ended forceps and a trachea 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 animal alive by means of artificial respiration. The amounts of air entering and leaving the cannula could be controlled by means of an adjustable sleeve or side tube. When experiments involving the leg muscles were performed, the external jugular vein, usually of the left side, was cannulated. In the other experiments the left femoral vein was cannulated and used for intravenous injections. To cannulate the external jugular vein the skin of the left antero-lateral part of the neck was removed and the left external jugular vein exposed. The fascia covering the vein was carefully removed, a thread tied around the cephalad end and a bulldog clip put

on /

on to the vein at the cardiac aspect. A small transverse out 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 in saline was then inserted into the incision with the pointed end towards the heart. Taking care that no air bubbles were left in the system, the cannula was connected by means of rubber tubing to a 50 ml. burette containing normal physiological saline. 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 trachea and vein one of the carotid arteries was then cannulated. The artery was first tied off at a point as near to the head as possible. A bulldog clip was then placed on the artery about 3 cm. below the ligature and a thread 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 inserted into the incision with the pointed end towards the heart. The cannula was connected by means of polythene tubing to a mercury manometer and the tube filled with normal physiological saline containing 1 unit per ml. of heparin 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 flag on one arm of the mercury manometer recorded the blood pressure on a moving, smoked paper /

paper surface.

Drug solutions were injected into the rubber connection between the vein cannula and the burette. Each injection was washed in slowly by the infusion of 3 ml. of saline from the burette. The blood pressure was 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 a cannulated femoral artery.

In a number of experiments upon cats the blood pressure level was raised by the continuous infusion of solutions of adrenaline or noradrenaline, and the drugs were injected when the elevated blood pressure level had become steady. The adrenaline and noradrenaline solutions contained from 0.05 to 0.1 mg. per ml; the rate of infusion was 1 ml. per minute using a Palmer's constant rate slow infusion apparatus. Respiratory movements were recorded by means of the apparatus shown in Fig. 4, page 30. To record the respiratory volume of the anaesthetised cat the trachea cannula was connected to the side limb of a Palmer's respiratory valve (modified from Gaddum, 1941) by means of a small piece of rubber tubing. The expired air was passed into a 1 litre capacity Buchner flask which was connected by means of rubber tubing from its side arm to a tambour. Changes in the volume of gas entering the flask are transmitted to the tambour by the rubber tubing and recorded on a moving, smoked paper surface using a gimbal lever.

When /

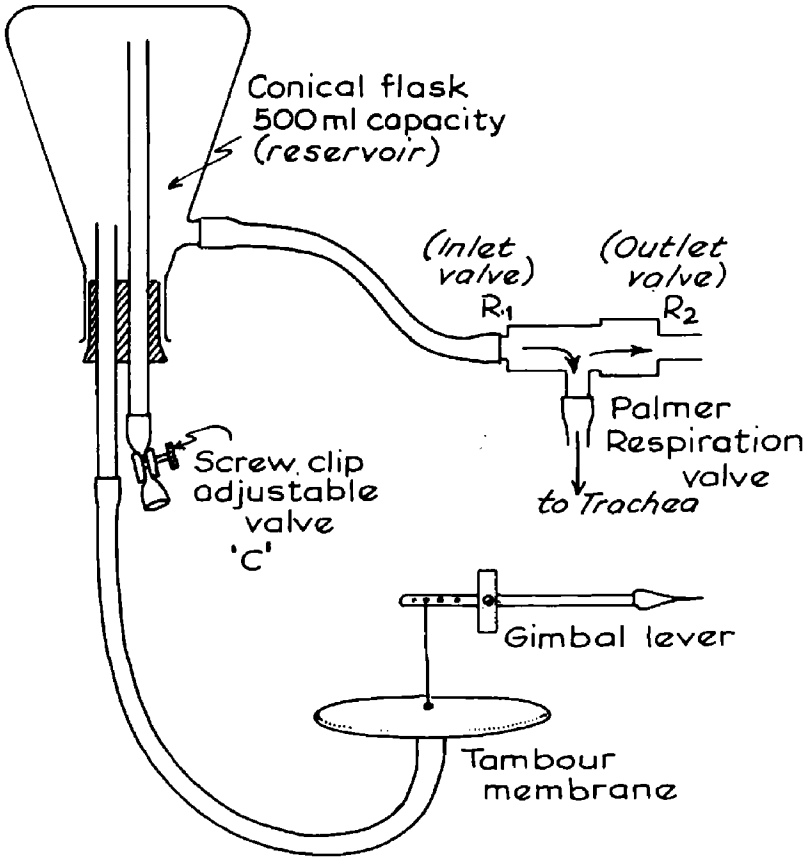

Fig. 4.

Diagram of the apparatus used for recording the respiration of the anaesthetised cat.

When administered intravenously 10-methoxydeserpidine and deserpidine both produced a significant fall in the systolic and diastolic arterial blood pressure levels. In order to analyse as far as possible the cause and nature of the fall in blood pressure, experiments were carried out on certain vasopressor reflexes. The effects of the drugs were studied by comparing the nature and magnitude of the reflex responses observed before and after their administration. At least two or three responses were elicited before the drug injection. Control solvent solutions of the same volume were injected and the effects on the same reflex responses observed.

Vasomotor reflexes

Certain vasopressor reflexes in which the central reflex pathways are in the medulla, hypothalamus and cerebrum have been investigated.

Afferent nerve impulses are constantly impinging upon the vasomotor centre in the medulla from all the regions of the body but by far the most important of these appear to be those from the carotid sinuses, carotid bodies and aortic arch. Efferent impulses are thus initiated in the vasomotor centre and appropriate adjustments then made in the blood pressure according to the needs of the organism. The afferent nerves from the pressor receptor and chemoreceptor endings in the carotid sinuses and carotid bodies are very important in the reflex control of blood pressure, heart rate and respiration. Pressor responses can also apparently be elicited when any afferent nerve is stimulated.

Investigations /

Investigations were carried out on the following pressor responses:-

(b) Carotid sinus pressor reflex

The carotid sinus is a dilatation normally present at the bifurcation of the common carotid artery. In the wall of the carotid sinus are characteristic sensory nerve endings which have the typical histological structure of stretch receptors and resemble those found in the aortic arch. These receptors in the carotid sinus respond to the alterations in the blood pressure. The afferents from the carotid sinus pass mainly in the glossopharyngeal nerve to the vasomotor centre in the medulla. When the pressure in the sinuses is lowered artificially there is a reflex rise in the blood pressure, vasoconstriction, cardiac acceleration and an increase in the rate and depth of respiration. These effects are due to the secretion of adrenaline and noradrenaline by the adrenal medullae and vasoconstriction is due to an increase in the tone of the vasomotor centre. Reserpine has been shown to reduce markedly the pressor responses elicited by bilateral carotid occlusion (Bein, 1953).

Method

The common carotid arteries were carefully separated from the sheath carrying the vago-sympathetic trunk and a loop of thread tied around each of them below the level of the carotid sinus. Pressor responses were elicited by bilateral occlusion of the common carotid arteries /

arteries either by placing bulldog clips on the arteries or by raising the threads looped loosely around them.

(c) Occlusion of the abdominal aorta

The abdominal cavity was opened by a midline incision. The rectus abdominis muscle and the fascia 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 coeliac artery. The abdominal aorta was occluded for a period of from fifteen to twenty seconds by raising the looped threads. The effects of drugs injected intravenously on these reflexes were followed for three to four hours after drug injection.

(d) Stimulation of the greater splanchnic nerve

The splanchnic nerves supply the major part of the abdominal viscera and are visceral sympathetic nerves. The greater splanchnic nerve emerges from the fifth to eighth thoracic segments of the spinal cord and passes through the segmental ganglia of the lateral sympathetic chain to relay synapses in the coeliac, superior mesenteric and renal ganglia. The preganglionic or grey fibres travel in the muscle coats of the larger vessels to the arterioles and thus reach the viscera these blood vessels supply. The afferent nerves from the viscera end in cell bodies lying in the dorsal root ganglion are connected to the /

the cells of efferent nerves with their central axons and to the other segments of the spinal cord by means of internuncial neurons. The sympathetic nerve supply of the viscera is controlled by the vasomotor centre and posterior part of the hypothalamus.

When the central end of the splanchnic nerve is stimulated a reflex rise in blood pressure results due to the increase in tone of the vasomotor centre which causes vasoconstriction of the splanchnic area.

Reserpine does not cause inhibition of the response elicited by electrical stimulation of the cut central end of the splanchnic nerve (Bein, 1953). To investigate and compare the effects of 10-methoxy-deserpidine and deserpidine on this reflex the following experiment was carried out.

Method

The coeliac ganglion in the abdomen was exposed and dissected free, the greater splanchnic nerve traced upwards and carefully cleared of connective and fatty tissue. The nerve was divided close to the ganglion and the cut central end was placed on a pair of shielded platinum electrodes which were left in place and the abdomen closed. The nerve was stimulated with 15 to 20 second bursts of impulses by means of a Dobbie McInnes square wave stimulator at a frequency of 800 to 1,400 per minute, at 5 to 10 volts with a pulse width of 1.0 to 1.5 msec. The experiment was performed for three to four hours and /

and responses elicited at intervals of 10 minutes.

(e) Stimulation of the cut central end of the cervical vagus

Method

The right vago-sympathetic trunk was carefully dissected free from its fascia and separated from adjacent tissues. The vagus was freed from the cervical sympathetic trunk with which it runs. It was divided by means of scissors at as low a point in the neck as possible. The central end was placed on a pair of platinum electrodes which were connected to the output of a Dobbie McInnes 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 15 to 20 seconds at intervals of 5 minutes.

(f) Experiments on the blood pressure of the anaesthetised rat

Experiments were performed on the blood pressure of the anaesthetised rat in order to study the effects of drugs on (a) the blood pressure and (b) on the pressor or depressor responses elicited by intravenous administration of adrenaline and noradrenaline.

Method

The method adopted for recording the blood pressure of the anaesthetised rat was that described by Dekanski (1952) using Condon's (1953) manometer (Fig.5, page 38).

Male albino rats weighing from 275 to 300 g. were used. The rat was injected subcutaneously with urethane, 175 mg. per 100 g. of body weight /

weight and left for one hour to attain surgical anaesthesia. After one hour the rat was laid on its back on the operating table, the hind legs were secured to the table with strings and the head was fixed on the table by passing a cotton thread through the skin of the lower jaw. The skin covering the front of the neck from the sternum to the apex of the mandible was removed and the fascia covering the trachea divided at the mid-line and the trachea exposed. A linen thread was passed around the trachea with the help of a blunt aneurysm needle and a transverse incision made in the latter by means of a fine scalpel. A polythene cannula of 2.5 mm. external diameter was inserted into the trachea and tied into place. The skin over the inguinal region of the thigh was removed and the femoral vein exposed and separated from the femoral artery. Another deep branch reaching the femoral vein was tied off and the superficial pudendal vein retracted to one side. The femoral vein was clamped on the cardiac end and a ligature made at the peripheral end. A small transverse cut was made by means of iris scissors in the dilated piece of vein and a polythene cannula filled with heparin was inserted into the incision with the pointed end towards the heart. Taking care that no air bubbles were left in the system the cannula was connected by means of a short piece of fine rubber tubing to a 1 ml. burette containing normal physiological saline. Heparin, 200 units per 100 g. body weight was injected into the vein cannula using a 1 ml. tuberculin syringe and washed in with 0.2 ml.

of /

of physiological saline.

Having completed the cannulation of the trachea and vein one of the carotid arteries was then cannulated. The artery was first tied off at a point as near to the head as possible. A bulldog clip was then placed on the artery about 1 cm. below the ligature and a thread passed under the vessel midway between the ligature and the bulldog clip. A small transverse cut was made in the artery by means of sharp-pointed iris scissors. A polythene cannula filled with a solution of heparin was inserted into the incision with the pointed end towards the heart and tied into place. The cannula was connected by means of polythene tubing to the Condon's mercury manometer, the tube being filled with normal physiological saline containing 1 unit per ml. of heparin as an anticoagulant. Air having been displaced from the system and the pressure in the manometer set at about 80 mm.Hg., the artery clip was removed. The flag on one arm of the mercury manometer recorded the blood pressure on a moving smoked paper surface.

Drug solutions were injected into the rubber connection between the vein cannula and the burette. Each injection was washed in slowly with 0.1 ml. of saline. Drug solutions were never injected in quantities of more than 0.2 ml.

Responses to the intravenous injection of adrenaline (1 µg.) and noradrenaline (1 µg.) were taken and the effect of the drugs under investigation studied on these responses at intervals for a period of from /

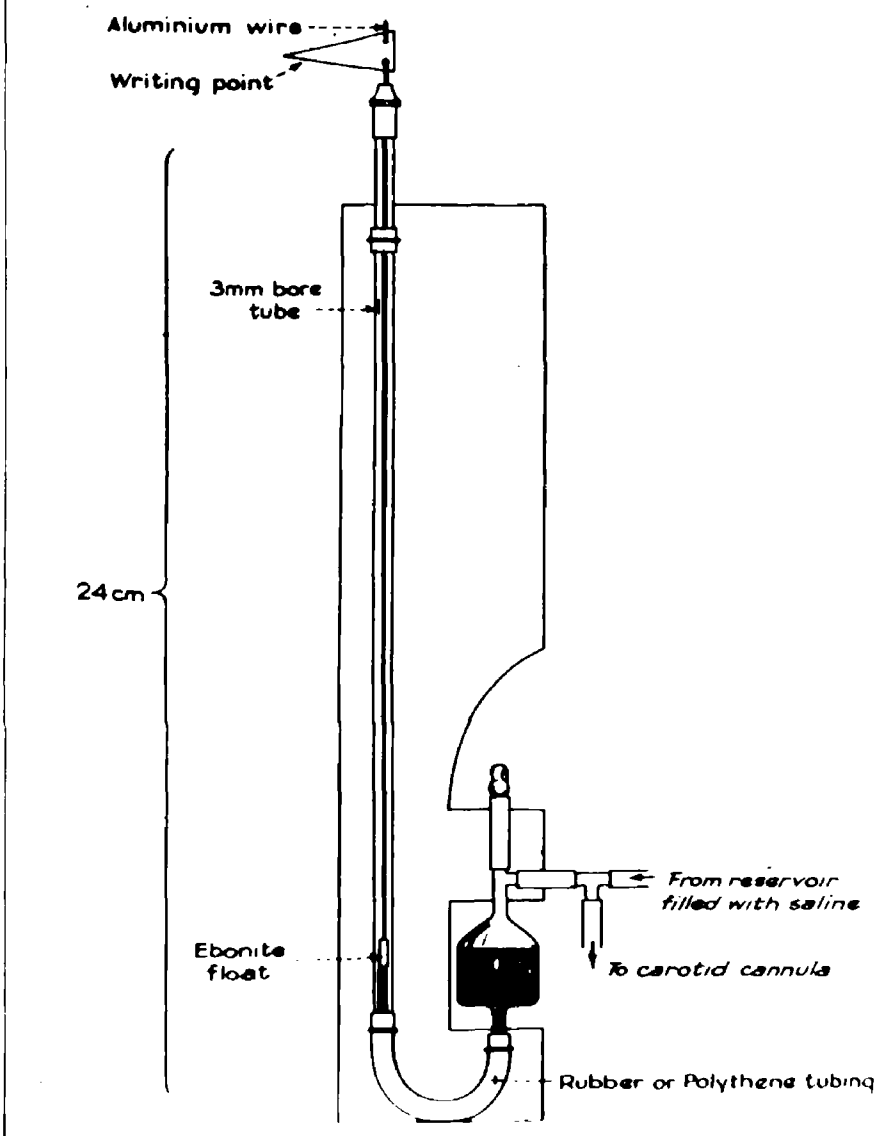

Fig. 5.

Diagram of the manometer used for recording the arterial blood pressure of the anaesthetised rat.

from two to three hours.

Urethane (25 g.) was dissolved in 100 ml. of distilled water. 0.7 ml. per 100 g. body weight was used for subcutaneous injection.

2. Experiments on the responses of isolated tissue preparations taken from organs containing smooth or skeletal muscle.

(a) Experiments on the isolated guinea pig ileum.

The method adopted for recording the contractions of isolated guinea pig ileum was essentially the same as that used by Guggenheim and Löffler (1916). Experiments were performed on the isolated guinea pig ileum to investigate the effects of drugs (a) on the smooth muscle of the gut and (b) on the responses of the gut to certain stimulant drugs.

Method

Guinea pigs of either sex weighing from 300 to 500 g., and which had been fasted overnight, 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 removed from the region about 3 to 5 cm. proximal to the ileocaecal junction. The contents of the ileum were washed out by inserting a pipette into one end of the gut and perfusing the gut with Tyrode's solution (Appendix I, page 452). Threads were tied to both ends of the segment which was then set up in a 2 ml. organ bath (Fig. 6, page 40) containing oxygenated Tyrode's solution. One thread was attached to a modified frontal point writing lever and the other end to a hook fixed

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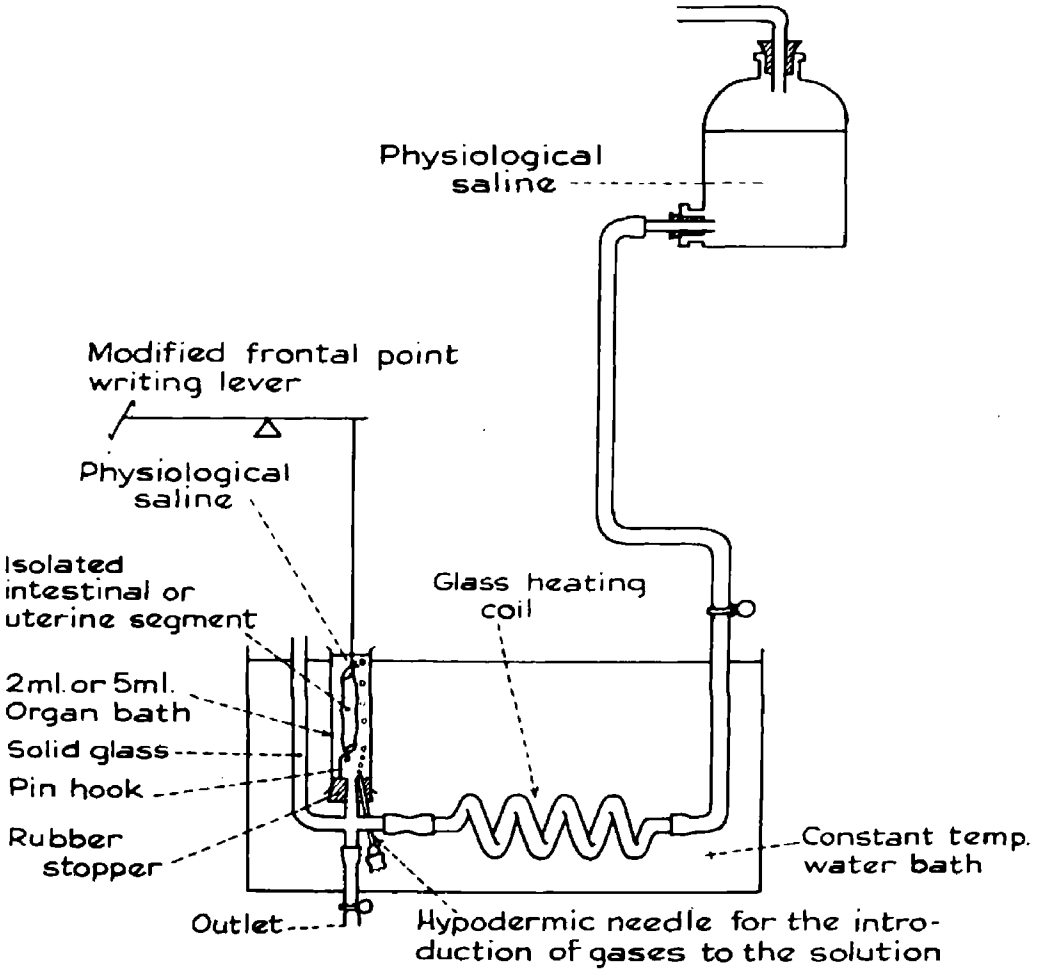

Fig. 6.

Diagram of the organ bath used for experiments upon isolated strips of guinea pig ileum or strips of horse carotid artery

at the base of the bath, the temperature being maintained thermostatically at 36 ± 0.5°C. Solutions containing the stimulant drugs were added and washed out by the overflow method. The inlet tube at the lower end of the bath was connected via heating coils to reservoirs which contained Tyrode's solution. 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 with a 1 ml. tuberculin syringe. Reproducible submaximal contractions were obtained at three minute intervals to an appropriate dose of the spasmogen which was allowed to remain in the bath for from 20 to 30 seconds. The drug solution was added to the bath one minute before the next addition of the spasmogen (acetylcholine, 5-hydroxytryptamine, histamine or barium chloride). The length of the tissue was allowed to return to its resting level before the next addition of the drug.

(b) Experiments on the isolated rabbit duodenum

Method

Rabbits of either sex weighing from 2 to 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 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 this was set up in a 10 ml. organ bath containing oxygenated Locke's solution (Appendix I, page 452) at 36± 0.5°C. The thread at one end of the duodenum was fixed /

fixed to the lower end of the 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 eight times. Adrenaline or acetylcholine was added to the bath by means of a 1 ml. tuberculin syringe and, at 5 minute intervals, the effect was observed for a period of 40 to 60 seconds. At the end of each addition of the drug the fluid in the bath was changed by running in fresh Locke's solution. The solution of the drug under investigation was added to the bath 2 minutes before the next addition of acetylcholine or adrenaline and the effects were observed. The tissue was allowed to regain its original length and the responses to adrenaline or acetylcholine to return to control levels before the next addition of the drug. The effects of the drug on the rhythmic movements of the duodenal segment were also observed. The drug was added to the bath and allowed to remain there for 5 minutes. The bath was washed out by the overflow method at 10 minute intervals until the tissue had regained its normal rhythmic activity.

(c) Experiments on strips of horse carotid artery.

Experiments were performed on isolated strips of horse carotid artery to investigate the effect of drugs (a) directly on arterial smooth muscle and (b) on the responses of the arterial strips to stimulant drugs.

Method

The /

The method adopted was the same as described by Kirpeker and Lewis (1958). Lengths of carotid artery were removed from a freshly killed horse at the slaughter house. Arteries from young horses gave better results than those from older animals. The former were more sensitive to stimulant drugs and quicker to recover from their effects.

A portion of the artery was freed from the fascia and a strip about 4 cm. in length and 2 mm. wide made by cutting it into a spiral, using a pair of iris scissors. Threads were tied to both ends of the strip and it was set up in a 10 ml. organ bath containing oxygenated Tyrode's solution at 36 $\pm$ 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 10 times. The strip was stretched by means of a 10 g. weight for about one hour. Before the experiment was started the additional weight was removed and the lever readjusted. Contractions were induced by addition to the bath of 1.0 to 2.0 µg. per ml. of adrenaline, noradrenaline, 5-hydroxy-tryptamine 1.0 to 2 µg. per ml., acetylcholine 0.01 to 0.05 µg.per ml., histamine 0.1 to 0.2 µg. per ml. and barium chloride 50 to 100 µg. per ml. These were added to the bath as solutions in Tyrode's solution by means of a 1 ml. tuberculin syringe.

Two types of experiments were performed. Stimulant drugs were added to the bath, standard submaximal contractions were recorded for

4 to 5 minutes and the effect of the drugs under investigation was studied on the magnitude of these contractions. Deserpidine and 10-methoxydeserpidine were added 20 minutes before the next addition of the stimulant drug. The time interval between the two responses was 20 to 30 minutes.

In the second group of experiments, a sustained contraction was elicited by adding the stimulant drugs in the same dose range. The drugs under investigation were added to the bath to see whether the contraction was influenced. The preparation was very sensitive to acetylcholine even when stored for four days at 4°C. but did not show the same response to other stimulant drugs. Larger doses of adrenaline or noradrenaline were at times required to induce the desired contractions in the stored arterial tissue.

(d) Experiments on the isolated perfused hindquarters of the rat

Experiments on the isolated perfused hindquarters of the rat were carried out to study the effect of drugs on the tone of isolated blood vessels. The method adopted was essentially the same as that described by Burn (1952) and the outflow was recorded with a Thorpe's drop recorder.

Method

Rats of either sex weighing from 200 to 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 /

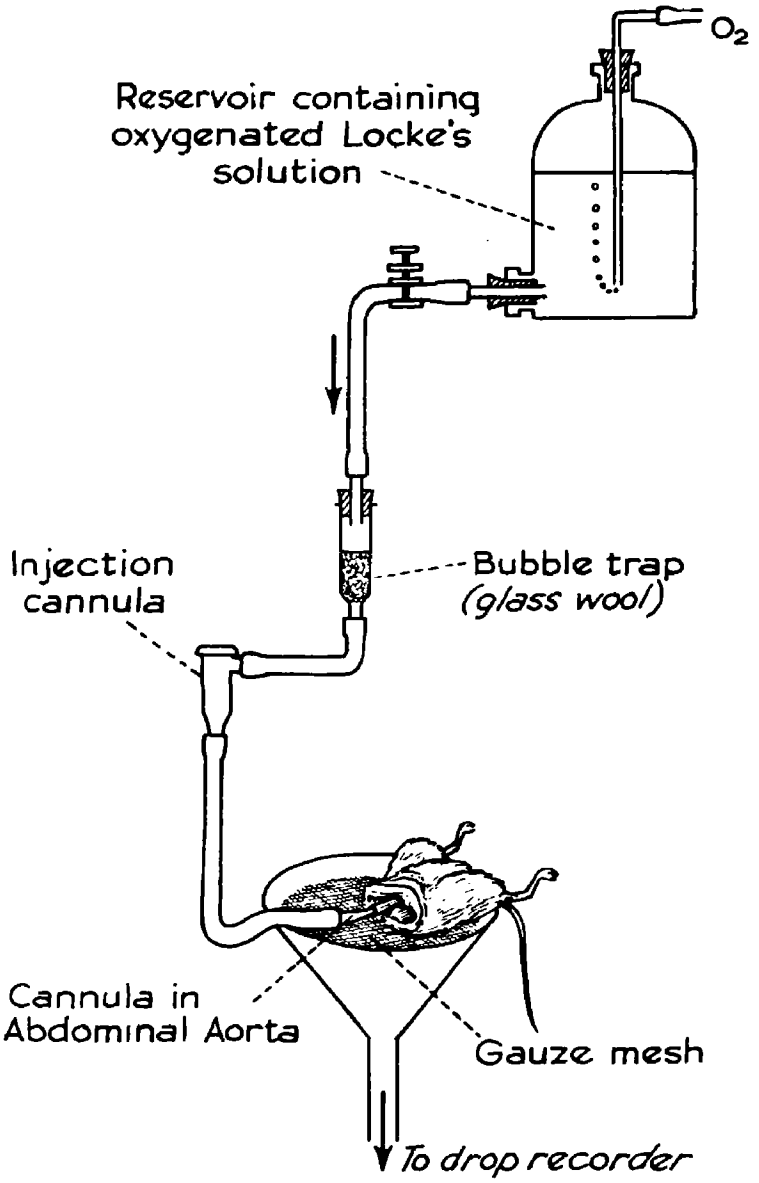

Fig. 7.

Diagram of the apparatus used for perfusion of the isolated rat hindquarters.

incision extending from the sternum downwards. The rectum, the oesophagus and the inferior and superior mesenteric arteries were divided between ligatures and the abdominal viscera removed. The abdominal aorta was cleaned carefully and separated from the inferior vena cava, a loop of thread was made around the aorta, a small incision made with sharp-pointed iris scissors and a small polythene cannula inserted and tied into place. To avoid intravascular clotting of the blood, heparin (10 units in 1 ml. of saline) was injected into the vessels through the cannula using a 1 ml. tuberculin syringe. 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 hindquarters were set up in the apparatus shown in Fig.7, page 45. The reservoir containing the perfusion fluid was connected to the bubble trap and injection cannula by means of rubber tubing. The rate of flow of fluid from the bubble trap to the injection cannula was controlled by means of a screw clip and could be adjusted to a suitable rate at the beginning of each experiment. The hindquarters preparation was placed on a muslin rest lying in a filter funnel. The outflow was led *via* the filter funnel over the contacts of a Thorpe's drop recording assembly.

After setting up the preparation, a uniform outflow record was obtained for at least 15 minutes before the drugs were injected into the injection cannula. Responses to the injection of adrenaline

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(0.1 to 1 µg.), noradrenaline (0.1 to 1 µg.), barium chloride (50 to 100 µg.) and acetylcholine (1 to 5 µg.) were elicited. The quantity of fluid injected into the injection cannula was never more than 0.1 to 0.2 ml. The effects of drugs on the constrictor or dilator responses of the blood vessels were recorded for two or three hours. Some control experiments were also carried out without injecting any drug.

(e) Experiments using the isolated frog rectus abdominis muscle.

The experiments were carried out on the isolated frog rectus abdominis muscle in order to investigate the effect of drugs (a) directly upon the skeletal muscle and (b) on the responses of the skeletal muscle to stimulant drugs.

Method

The procedure adopted for recording the contractions of the isolated frog rectus abdominis muscle was essentially the same as that described by De Jalon (1947). Frogs weighing from 25 to 50 g. were used. An adult frog was stunned by means of a blow on the head, decapitated and pithed. The frog was laid on its back on a dissecting board to which it was pinned. The rectus muscle was exposed by cutting away the skin of the abdomen and then dissected from its origin in the cartilage of the pectoral girdle to insertion in the pelvic girdle. The muscle was freed from the underlying connective tissues, removed from the frog and then suspended in

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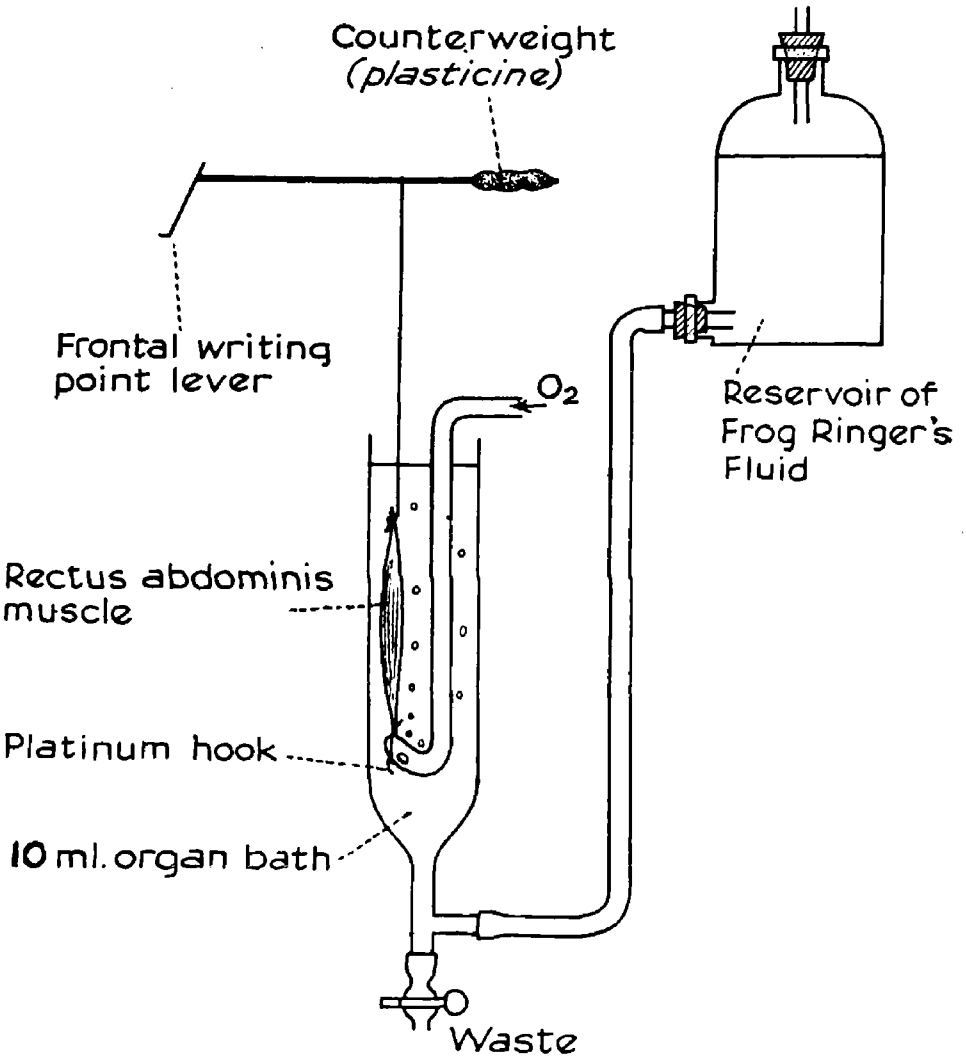

Fig. 8.

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

an organ bath (Fig. 8, page 48) of 10 ml. capacity by means of threads tied to each end of the muscle. A loop was made in the thread at one end in order to fix the muscle to the bent wire in the base of the bath. The long thread on the other end was tied to a modified frontal point writing lever which gave a magnification of from 8 to 10 times.

The bath contained 10 ml. of Ringer's solution (Appendix I, page 452) at room temperature, bubbled continuously with 95% oxygen and 5% carbon dioxide. Acetylcholine, decamethonium or nicotine dissolved in frog Ringer's solution to give the required concentration were added to the bath by means of a 1 ml. tuberculin syringe. Uniform submaximal contractions to the same dose of spasmogen were obtained before the effects of the drugs were studied. The time interval between each dose of spasmogen was six minutes. The resulting contractions were recorded for 90 seconds. With nicotine and decamethonium the time interval between doses was about 15 to 20 minutes. The resulting contractions were recorded for 2 minutes. The effects of drugs on the antagonism of atropine and tubocurarine to acetylcholine were also observed.

(f) Experiments on the isolated auricles of the guinea pig.

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

Method

The procedure adopted was the same as that described by Burn (1952). /

(1952). Adult guinea pigs of either sex were killed by a blow on the head. The throat was cut and blood allowed to drain out. The heart was then 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 in a petri dish upon a piece of cotton wool soaked in Locke's solution. All the extraneous tissue was dissected away until the horse-shoe shaped auricles alone remained. These were then suspended in a 10 ml. organ bath by means of two entomological pins to which fine cotton threads were tied. One thread was tied 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 on a moving smoked surface. After about 30 minutes the beat of the auricles had usually become regular and the experiment was commenced. All drugs were added to the bath in the form of solutions in Locke's solution and by means of a 1 ml. tuberculin syringe. The effects of adrenaline, noradrenaline and acetylcholine were recorded for a period of 60 seconds after which the auricles were washed by replacing and draining the bath fluid three times. Sufficient time was allowed for the auricles to regain a normal regular rhythm and amplitude of beat before the next addition of the drug. 10-methoxydeserpidine and other drugs were added 3 to 5 minutes before each of the stimulant or depressant drugs tested. The bath temperature was maintained at $29 \pm 0.5^{\circ}C$.

(g) Experiments /

(g) Experiments on the isolated perfused rabbit heart.

In order to study the effect of the drugs under investigation upon (a) myocardial tone, amplitude, rate and outflow and (b) responses of the heart due to stimulant and depressant drugs, experiments were carried out on the isolated perfused rabbit heart.

Method

The isolated rabbit heart was perfused according to the method of Langendorff (1895) as described by Burn (1952). This method involves perfusion of the coronary vessels through the aorta. Wegria (1951) 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 was also pointed out that the volume of the coronary perfusate may be increased by a purely mechanical massaging which cardiac muscle - stimulated by a cardiotonic drug - has upon the coronary vessels. Under these circumstances an increase /

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 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 used were within the weight range of 2 to 2.5 kg. The animal was killed by a blow on the head, the throat cut and the blood allowed to drain out. The animal was then placed on 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 oxygenated Locke's solution (Appendix I, page 452) 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 to run through the superior /

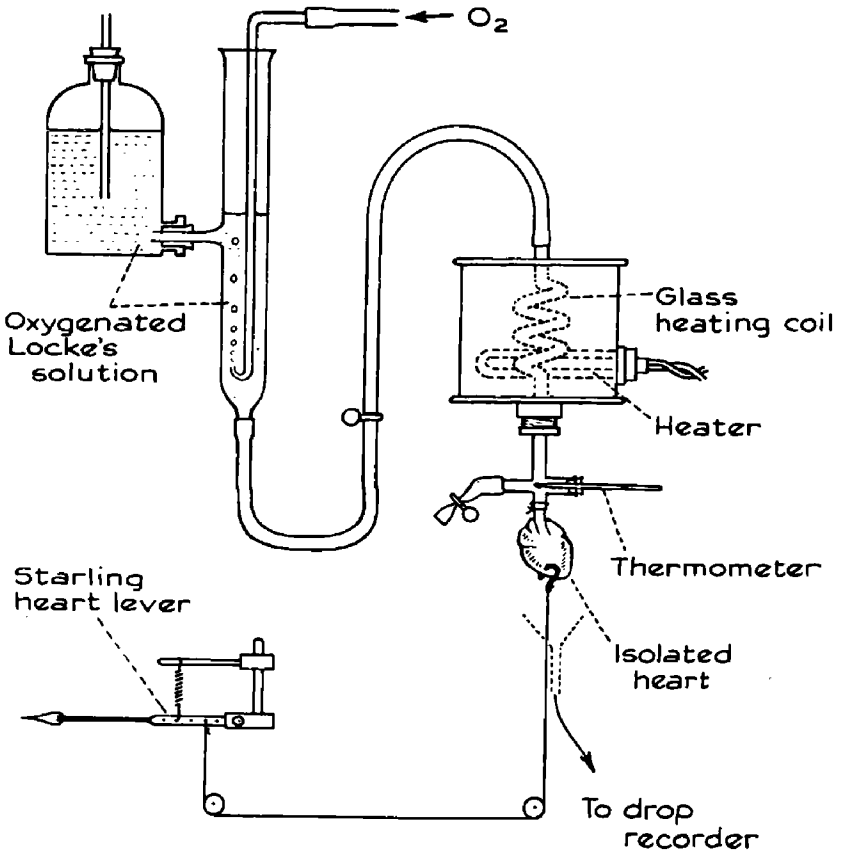

Fig. 9.

Diagram of the apparatus used for recording contractions of the isolated rabbit or guinea pig heart.

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 artery 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 the heart usually started to beat immediately. After about 30 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 reservoir flowed through heating coils situated in a water bath (Fig. 9, page 53) maintained at 37°C. The glass coil was connected to the aortic cannula by a short length of tubing. This length of rubber tubing was kept short so as to avoid a drop in the temperature of fluid flowing from the bath through the coil into the aorta. The temperature drop between the water bath and the cannula was never more than 0.5°C. 10-Methoxydeserpidine and other drugs were injected by /

by means of a 1 ml. tuberculin syringe into the rubber tubing attached to the aortic cannula. The heart rate was counted by direct observation of the movements and the outflow measured by means of a Thorpe's drop recorder.

3. Experiments on the ganglion-blocking activity.

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 60 mg. per kg. of pentobarbitone sodium and trachea and vein cannulae inserted as described previously on page 27. The head was fixed rigidly by passing a brass rod between the jaws and then tying the jaws firmly together with 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 tied firmly into place. The thread was pulled forward and then to one side, thus making an angle of about 30° to the long axis of the cat and then led around pulleys and attached to a frontal point writing lever.

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. /

neck. The cut preganglionic cervical sympathetic chain was then placed on 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 contractions of the nictitating membrane were recorded on a smoked surface. The nerve was stimulated at 3 minute intervals for 10 to 15 seconds. Having obtained standard reproduceable responses of the nictitating membrane by stimulating the nerve trunk, solutions of drugs were injected into the femoral vein or the internal jugular vein one minute before the next period of stimulation. With deserpidine and 10-methoxydeserpidine, the contractions were recorded from 2 to 4 hours after the injection to see if there were any delayed effects. Contractions of the nictitating membrane were also obtained in response to the intravenous administration of adrenaline or noradrenaline (15 to 20 µg. per kg.) and the effects of the drugs were studied on these responses.

4. Determination of the tranquillizing and central nervous system depressant effects.

To investigate the effects of drugs on the central nervous system and upon the general behaviour, two types of experiments were carried out.

(a) Experiments on rats and mice for ptosis, sedation and gastro-intestinal activity.

Reserpine /

Reserpine is reported to have characteristic central nervous system depressant effects on animals as well as on human beings. It has also been shown to increase the gastro-intestinal motility causing loose stools. To evaluate the drugs under investigation and to compare them with reserpine, experiments were performed on rats and mice.

Method

Male albino rats weighing from 200 to 250 g. and mice weighing from 15 to 18 g. were used in these investigations.

Rats in groups of twenty were used; ten out of the twenty were used as controls and the other ten for the drug. Drugs under investigation were injected intraperitoneally and the effects on motor activity, ptotic response and intestinal activity were observed for from six to ten hours.

Mice in groups of twenty were used for the ptotic response; ten members of each group were used as controls and the other ten given the drug.

(b) Experiments on pentobarbitone-induced sleeping time in mice.

Method

The method adopted for the experiment was essentially that described by Cronheim, Brown, Cawthorne, Toekes and Ungari (1954) but using 60 mg. per kg. of pentobarbitone by intraperitoneal injection. The mean sleeping time of a group of 500 male mice weighing from 15 to 16 g. was estimated one week before the drug experiment. The

individual sleeping times were found to vary very widely and the variability of the animals was reduced by putting them into groups of twenty according to their sleeping times. From each group ten were used for the drug and ten for the control. This procedure was repeated five times at each dose level. An intraperitoneal injection of 60 mg. per kg. of pentobarbitone sodium was preceded two hours before by the intraperitoneal injection of the drug or control solution. The period which elapsed between the loss of consciousness and the re-appearance of the righting reflex was noted and called the sleeping time.

5. The determination of acute toxicity in mice.

(a) Determination of the approximate median lethal dose.

Method

The median lethal dose was determined using male mice weighing from 16 to 18 g. and the drugs were administered intraperitoneally to groups of twenty. Ten out of each group were used for control purposes and the other ten were injected with the drug. The dose at which five out of ten died in a period of three hours was taken to be the median lethal dose for that particular group of mice and was expressed in terms of mg. per kg. of body weight. The calculation of the medial lethal dose was carried out according to the method of Miller and Tainter (1944). The difference in the body weight of the mice of any one group was not more than 1 g. The quantity of the drug solution used for intraperitoneal injections was kept constant at 0.15 ml.

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C H A P T E R III

CHAPTER III

RESULTS

Blood pressure of the anaesthetised cat.

10-Methoxydeserpidine at dose levels of 2 to 6 mg. per kg. produced a slow and sustained fall in the arterial blood pressure of the anaesthetised cat. This response was more marked when the initial blood pressure level was high (Fig.10, page 62). In cats with a low blood pressure, (100 mm. Hg. or less) no reduction was noted even up to six hours following the intravenous administration of 10-methoxy-deserpidine. Bradycardia accompanied the reduction of the blood pressure level and was more distinct in the second and third hours of the experiment (Fig.11, page 63).

Deserpidine 2 to 4 mg. per kg. when given intravenously produced an immediate, slight fall followed by a sustained decrease in the arterial blood pressure level of the cat (Fig. 12, page 64). The hypotensive response to deserpidine in the cat was more marked and more clearly defined than that of 10-methoxydeserpidine. The reduction in blood pressure level caused by deserpidine was also accompanied by a significant bradycardia which was more prominent in the second and third hours following the injection of the drug.

Reserpic acid and methyl reserpate in the dose range of 2 to 4 mg. per kg. caused no observable reduction in the blood pressure level of /

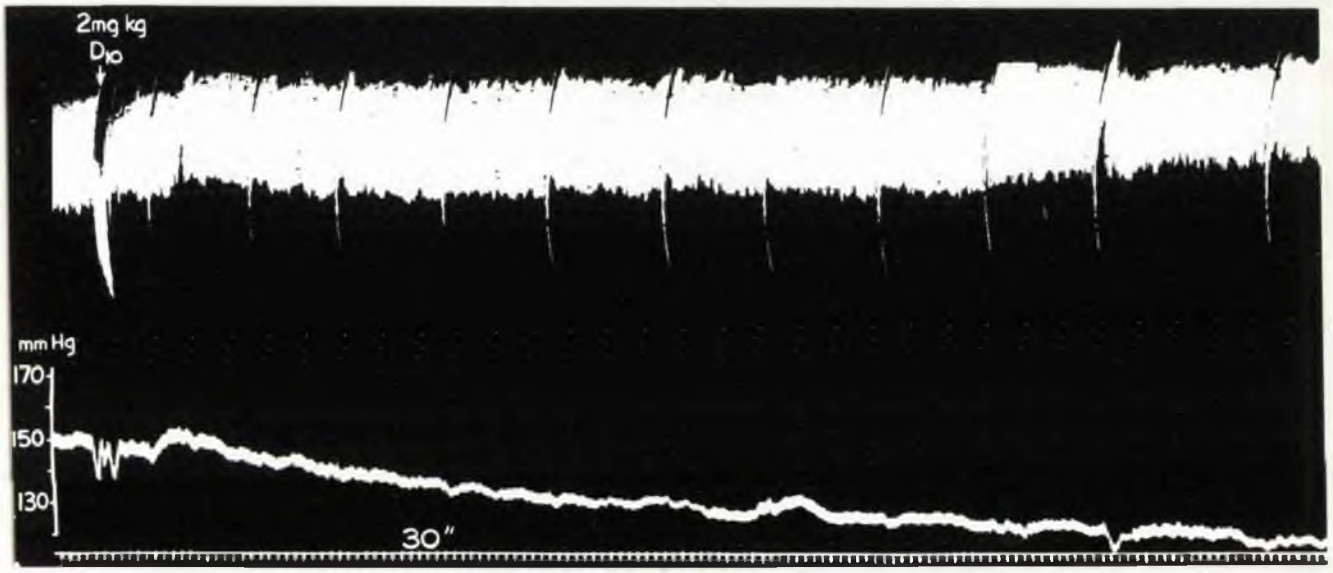

Fig. 10.

Effect of 2 mg. per kg. of 10-methoxydeserpidine (D10) on the respiration (upper record) and blood pressure (lower record) of the pentobarbitone-anaesthetised cat.

Blood pressure record taken from the common carotid artery.

Drugs injected intravenously.

Time interval (lowest trace) = 30 seconds.

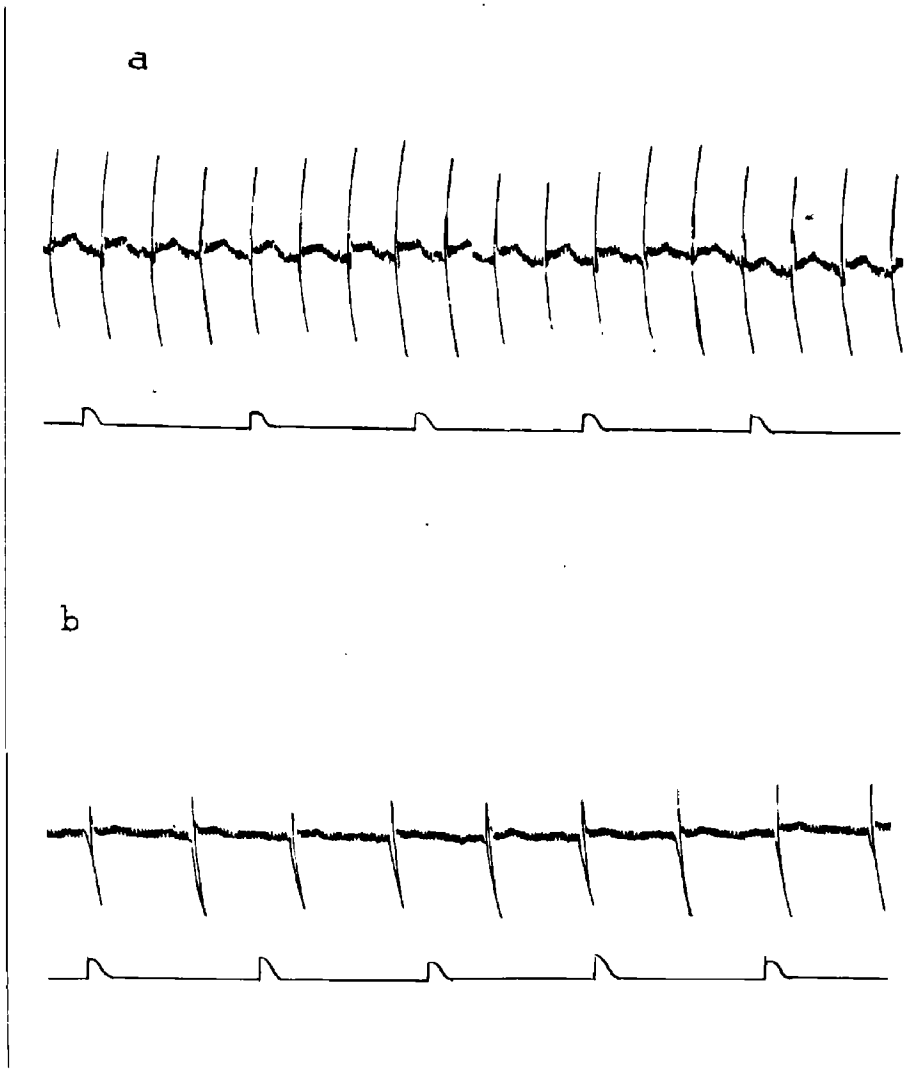

Fig. 11.

The effect of intravenous injection of 2 mg. per kg. of 10-methoxy-deserpidine (D10) on the electrocardiogram of the pentobarbitone-anaesthetised cat.

(a) Before drug injection.
(b) 60 minutes after the drug injection.

Time interval (lower trace) = 5 seconds.

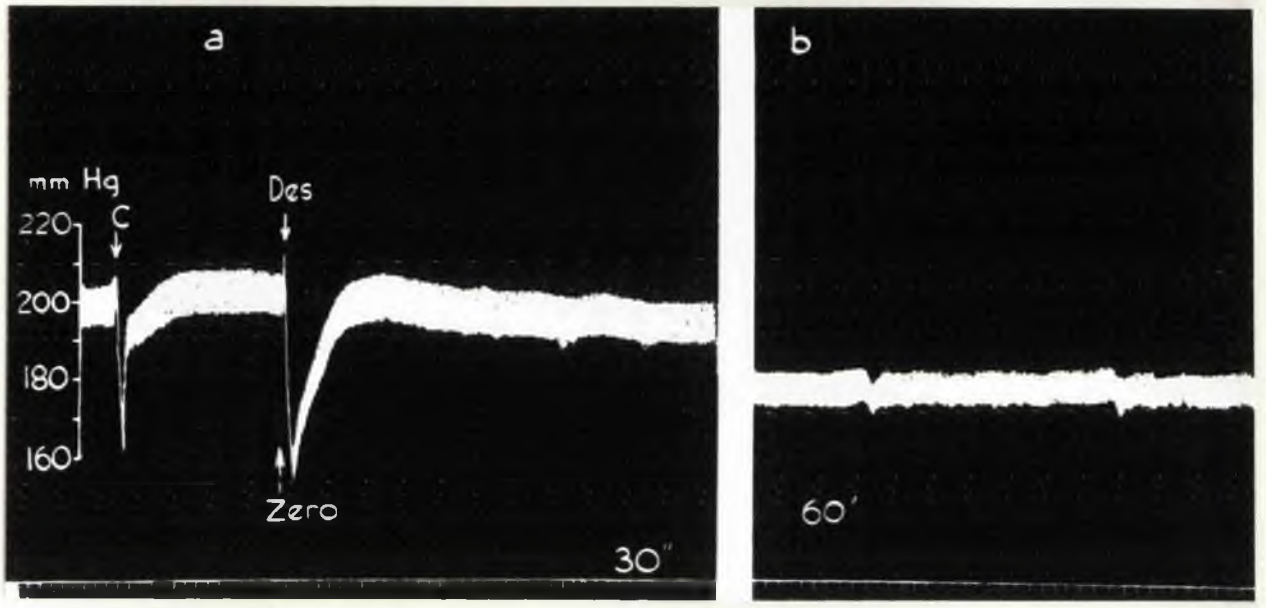

Fig. 12.

Tracing a. Effect of 2 mg. per kg. of deserpidine (Des) on the arterial blood pressure level of the pentobarbitone-anaesthetised cat.
Blood pressure record taken from the common carotid artery.
Drugs administered intravenously.
At C, 1.0 ml. of control solution injected.

Tracing b. Blood pressure level 60 minutes after the drug injection.

Time interval (lower trace) = 30 seconds.

of the anaesthetised cat. A small delayed fall in the blood pressure level was observed when larger doses, of the order of 5 to 10 mg. per kg., were used.

A slight depression of the respiration was observed, following the administration of 2 to 4 mg. per kg. of 10-methoxydeserpidine or 1 to 2 mg. per kg. of deserpidine in the anaesthetised cat.

10-Methoxydeserpidine in the dose range of 2 to 6 mg. per kg. and deserpidine 1 to 2 mg. per kg. did not inhibit the pressor responses elicited by intravenous injections of from 2 to 5 µg. per kg. of adrenaline or noradrenaline. In the same dose range, 10-methoxy-deserpidine and deserpidine produced a characteristic potentiation of the pressor responses (Fig.13, page 66) which was more marked in the second and third hours of the experiment. Methyl reserpate and reserpic acid in dose levels of from 5 to 10 mg. per kg. did not cause an observable modification of the pressor responses to adrenaline and noradrenaline in the cat.

When given intravenously, 10-methoxydeserpidine, 2 to 6 mg. per kg., deserpidine 1 to 2 mg. per kg., and methyl reserpate and reserpic acid, 5 to 10 mg. per kg. did not alter the vasodepressor effects in the anaesthetised cat due to the intravenous injections of from 2 to 5 µg. per kg. of acetylcholine and 2 to 5 µg. per kg. of histamine (Fig.14, page 67).

The pressor response to bilateral occlusion of the common carotid arteries /

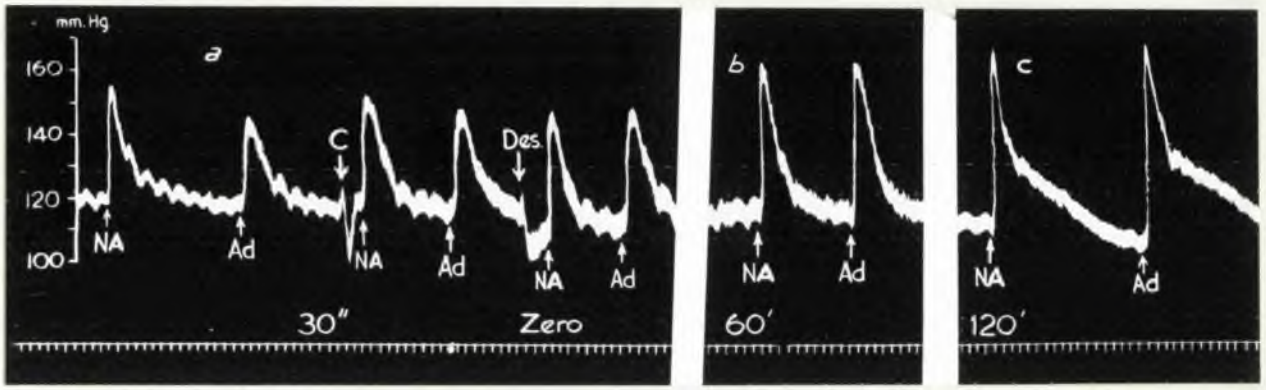

Fig. 13.

Effect of 2 mg. per kg. of deserpidine (Des) on the response of the blood pressure of the pentobarbitone-anaesthetised cat to noradrenaline (NA) or adrenaline (Ad).

At NA, 2 µg. per kg. noradrenaline.
At Ad, 2 µg. per kg. adrenaline.
At C, control solution (1 ml.).

Blood pressure record taken from the common carotid artery.
Drugs injected intravenously.

Tracing b. 60 minutes after deserpidine.

Tracing c. 120 minutes after deserpidine.

Time interval (lower trace) = 30 seconds.

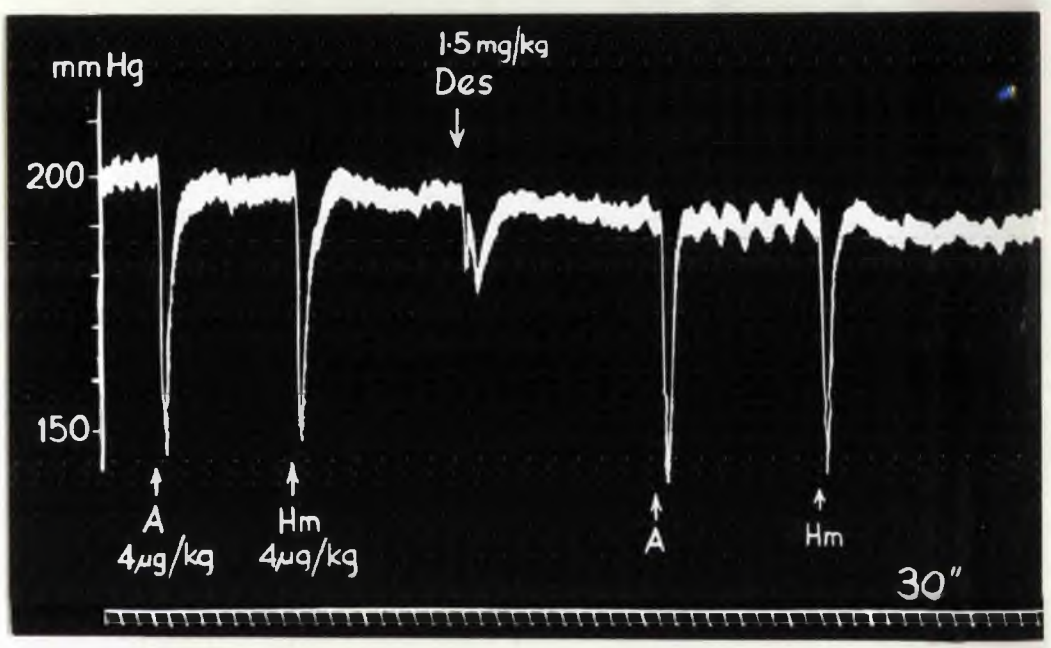

Fig. 14.

Effect of 1.5 mg. per kg. of deserpidine (Des) injected intravenously, on the depressor response of the pentobarbitone-anaesthetised cat to acetylcholine (A) and histamine (Hm).

At A - 4 µg. per kg. of acetylcholine.
At Hm - 4 µg. per kg. of histamine.

Blood pressure recorded from the common carotid artery.

Drugs injected intravenously.

Time interval (lower trace) = 30 seconds.

arteries was markedly reduced following 2 to 4 mg. per kg. of 10-methoxydeserpidine (Fig. 15, page 69), and 1 to 2 mg. per kg. of deserpidine (Fig. 16, page 70). The depression of this response was more marked following the administration of deserpidine than following that of 10-methoxydeserpidine.

Following the intravenous injection of from 2 to 6 mg. per kg. of 10-methoxydeserpidine, pressor responses due to occlusion of the abdominal aorta or stimulation of the cut central end of the left greater splanchnic nerve were not modified (Fig. 17, page 71). Unlike 10-methoxydeserpidine, both the responses were markedly reduced following the administration of from 1 to 2 mg. per kg. of deserpidine but they showed a tendency to recover to normal levels after two to three hours had elapsed (Fig. 18, page 72). No modification of these responses was observed following the administration to the cat of 5 to 10 mg. per kg. of reserpic acid or methyl reserpate.

Ten minutes following the intravenous administration of 4 to 6 mg. per kg. of 10-methoxydeserpidine (Fig. 19, page 73) and 1 to 2 mg. per kg. of deserpidine (Fig. 20, page 74) the magnitude of the pressor response elicited by electrical stimulation of the cut central end of the right vagus was reduced. In some experiments on cats, an actual reversal of this reflex response was observed.

Deserpidine 2 to 5 mg. per kg. caused a slight reduction in the level of the blood pressure which had been raised by continuous infusion /

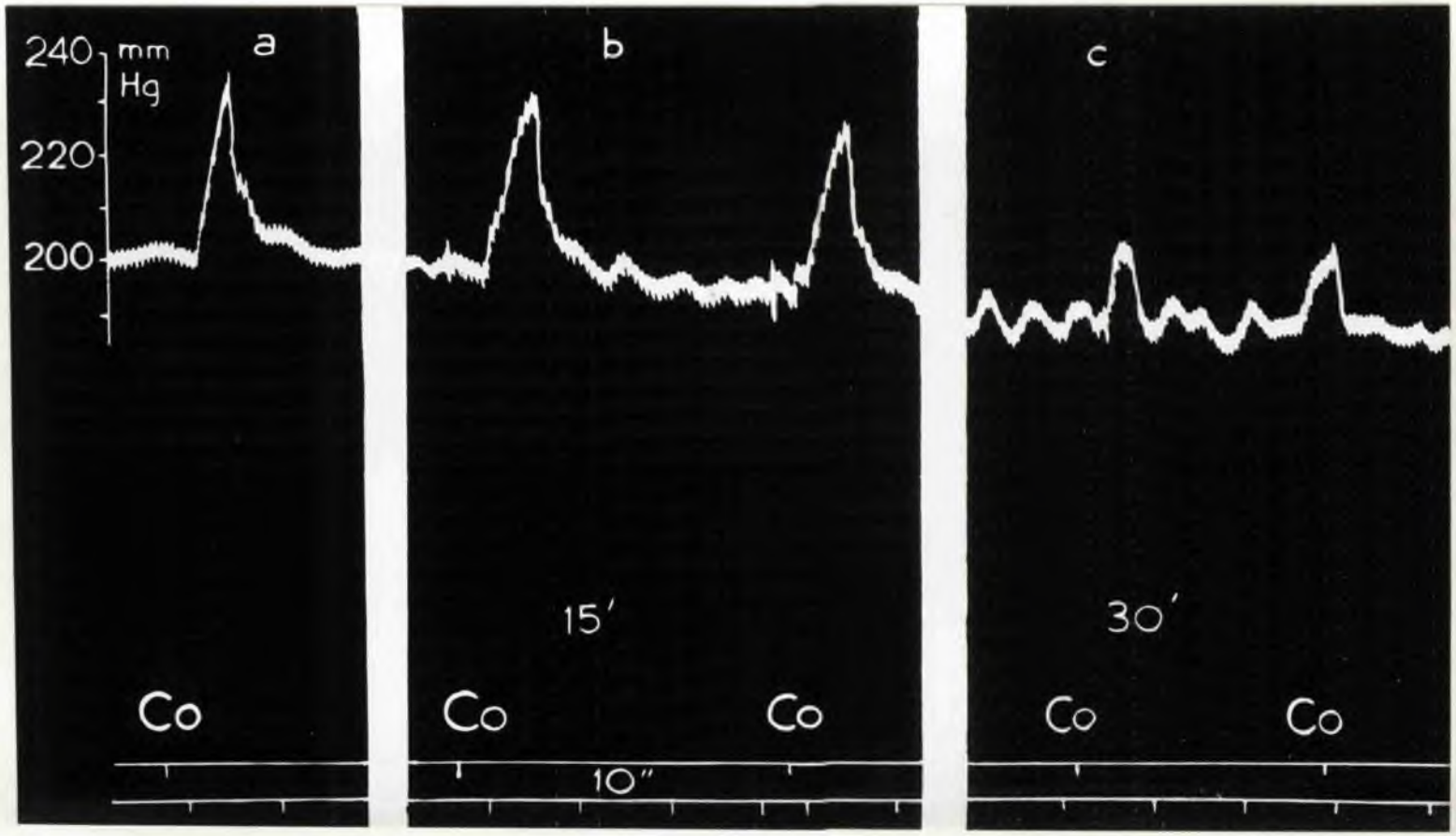

Fig. 15.

Effect of 4 mg. per kg. of 10-methoxydeserpidine upon the response to carotid artery occlusion in the pentobarbitone-anaesthetised cat. 10-methoxydeserpidine given intravenously between a and b.

Tracing a. At CO, both carotid arteries were occluded for 10 seconds.

" b. Fifteen minutes after the drug injection.

" c. Thirty minutes after the drug injection.

Time interval (lower trace) = 30 seconds.

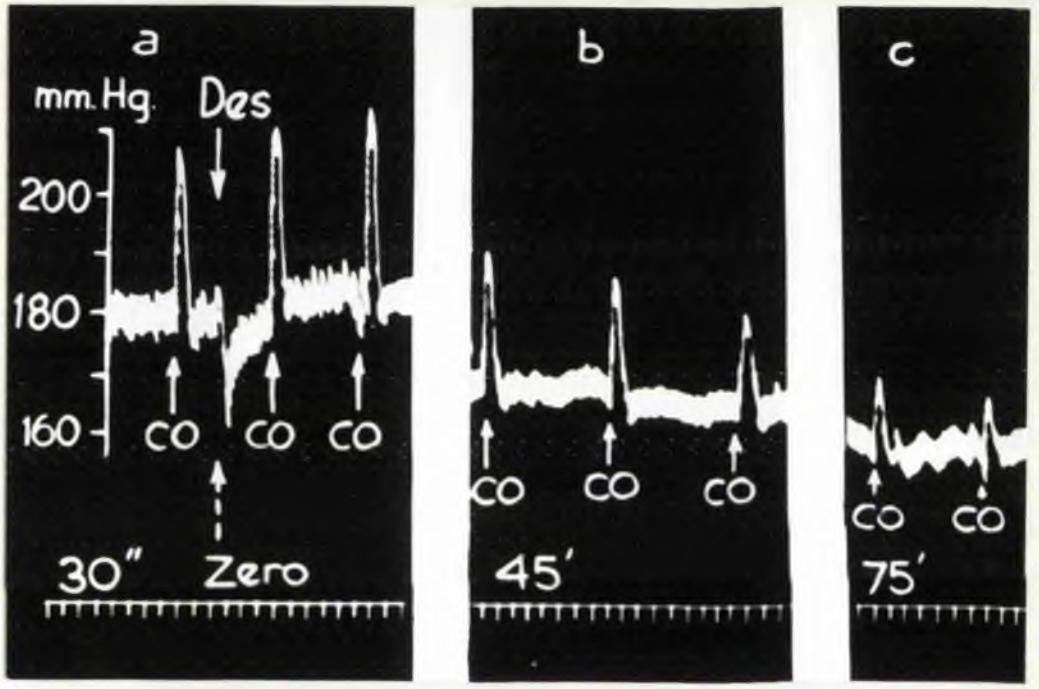

Fig. 16.

Tracing a. Effect of 2 mg. per kg. of deserpidine (Des) on the response to carotid artery occlusion in the pentobarbitone-anaesthetised cat.

At CO both carotid arteries were occluded for 10 seconds.

Tracing b. 45 minutes after the drug injection.

Tracing c. 75 minutes after the drug injection.

At zero time deserpidine was given intravenously.

Time interval (lower trace) = 30 seconds.

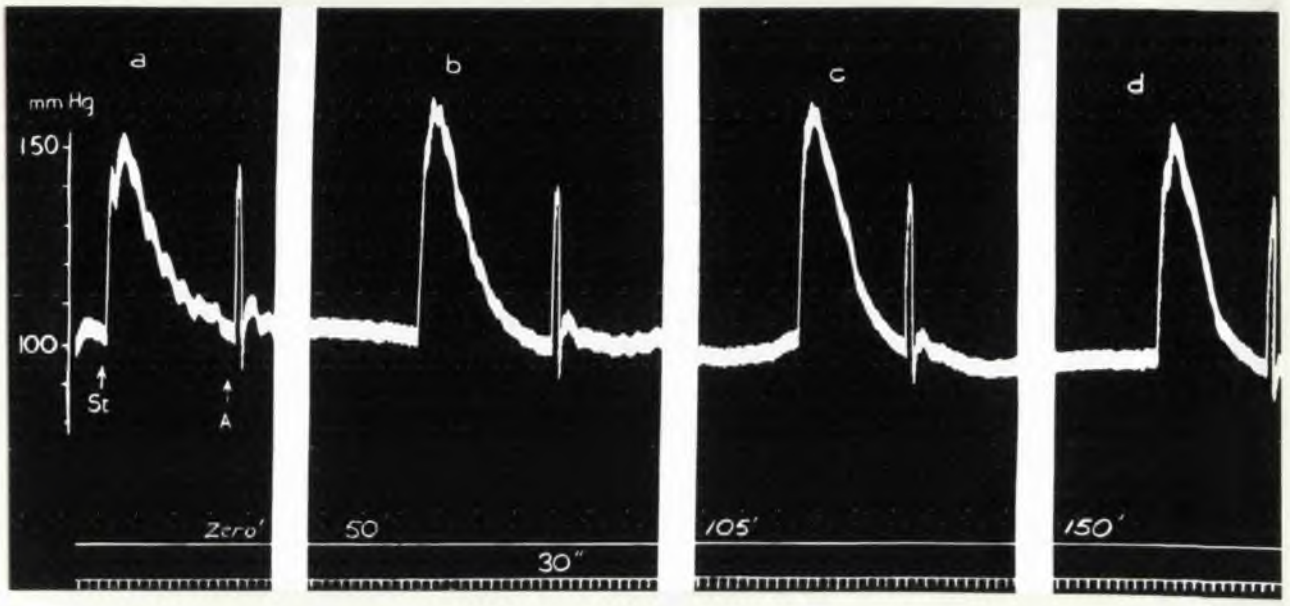

Fig. 17.

Effect of 5 mg. per kg. of 10-methoxydeserpidine on the pressor responses to stimulation of the central end of the left, greater splanchnic nerve and compression of the abdominal aorta in the pentobarbitone-anaesthetised cat.

Tracing a. At St, electrical stimulation of the greater splanchnic nerve (10 volts, 1,000 impulses per minute, pulse width 1.5 m.sec) for 15 seconds.
At A, compression of the abdominal aorta for 10 seconds.
All drugs injected intravenously.
10-methoxydeserpidine injected between (a) and (b).

Tracing b. 50 minutes after the drug injection.

Tracing c. 105 minutes after the drug injection.

Tracing d. 150 minutes after the drug injection.

Time interval (lower trace) = 30 seconds.

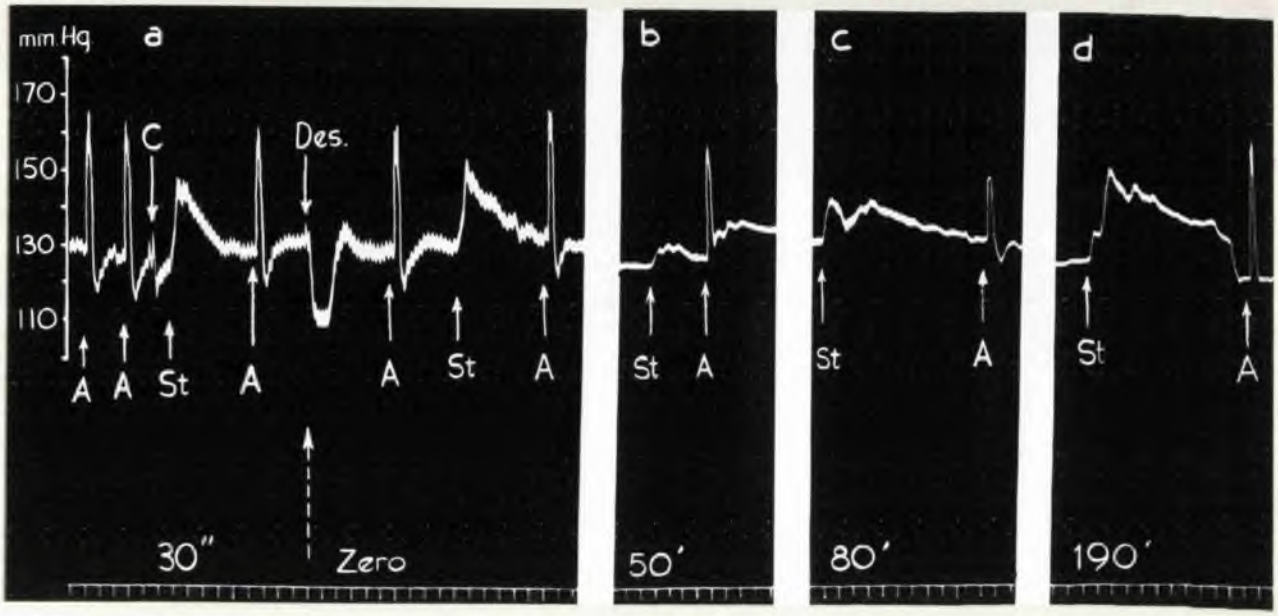

Fig. 18.

Effect of 2 mg. per kg. of deserpidine (Des) on the pressor responses to compression of the abdominal aorta and stimulation of the central end of the left greater splanchnic nerve in a pentobarbitone-anaesthetised cat.

Tracing a. At A, compression of the abdominal aorta for 10 seconds.
At St, electrical stimulation of the greater splanchnic nerve (10 volts, 1,000 impulses per minute, pulse width 1.5 m.sec) for 15 seconds.
All drugs injected intravenously.
At C, control solution (2 ml.) injected.

Tracing b. 50 minutes after the drug injection.

Tracing c. 80 minutes after the drug injection.

Tracing d. 190 minutes after the drug injection.

Time interval (lower trace) = 30 seconds.

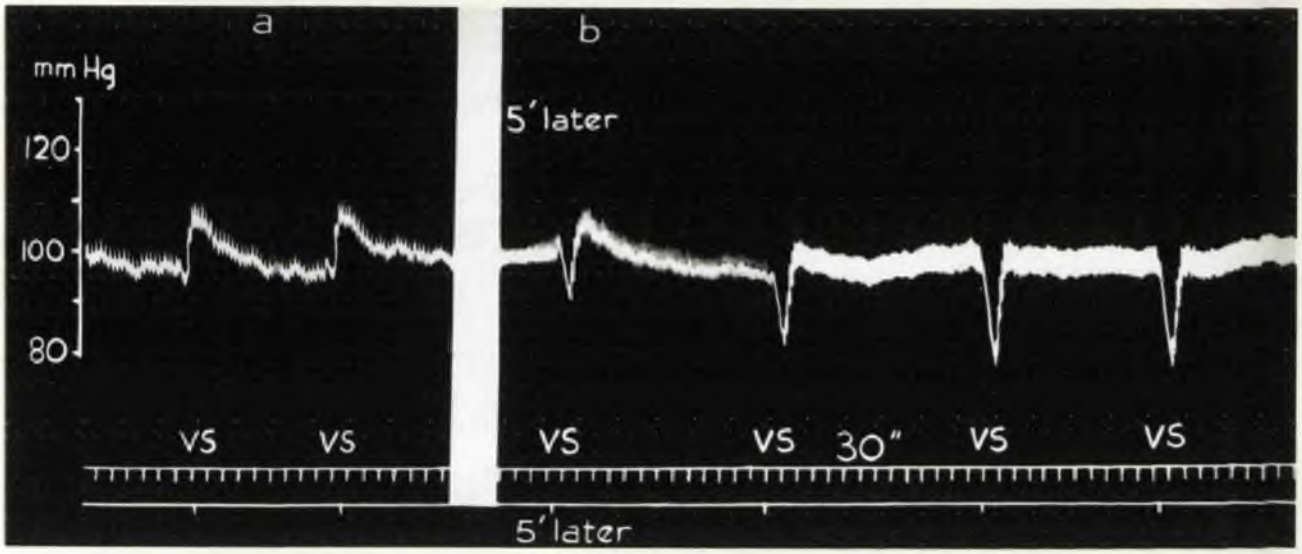

Fig. 19.

Tracing a. Effect of 6 mg. per kg. of 10-methoxydeserpidine on the pressor response to stimulation of the cut central end of the vagus nerve in a pentobarbitone-anaesthetised cat.

At VS, electrical stimulation of the cut central end of the vagus nerve (10 volts, 1,000 impulses per minute, pulse width 1.5 m.sec) for 15 seconds.
10-methoxydeserpidine injected intravenously between (a) and (b).

Tracing b. 5 minutes after the drug injection.

Time interval (lower trace) = 30 seconds.

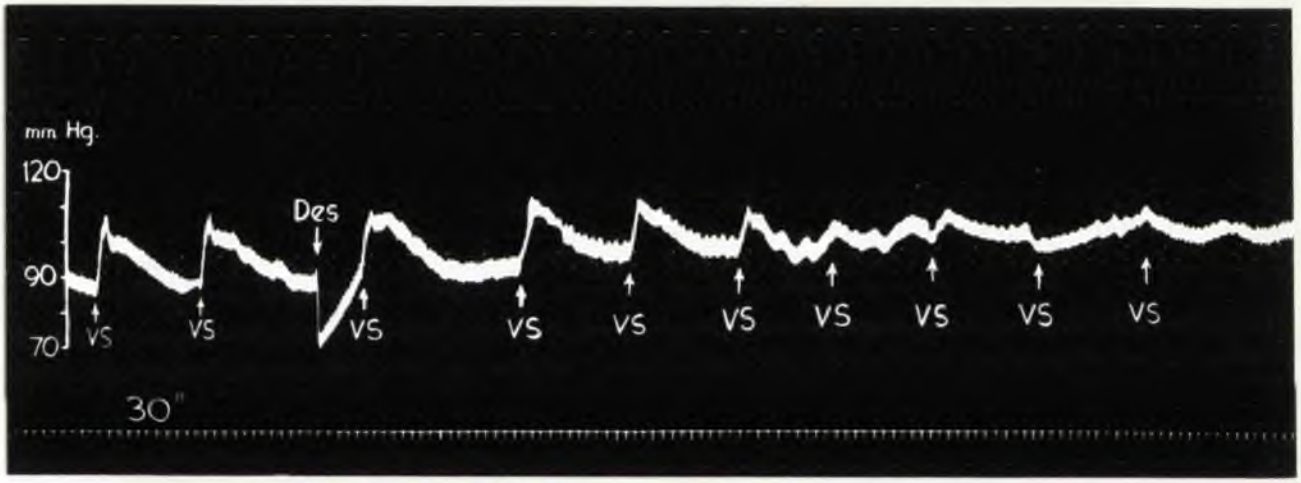

Fig. 20.

Effect of 2 mg. per kg. of deserpidine (Des) on the pressor responses to stimulation of the cut central end of the vagus nerve in a pentobarbitone-anaesthetised cat.

At VS, electrical stimulation of the cut central end of the vagus nerve (10 volts, 1,000 impulses per minute, pulse width 1.5 m.sec) for 15 seconds.

Deserpidine injected intravenously.

Time interval (lower trace) = 30 seconds.

infusion of from 50 to 100 µg. per ml. per minute of adrenaline (Fig. 21, page 76) or noradrenaline (Fig. 22, page 77). No such effect was observed following the injection of from 2 to 6 mg. per kg. of 10-methoxydeserpidine, reserpic acid and methyl reserpate.

Blood pressure of the anaesthetised rat.

A fall in the arterial blood pressure level of the rat was recorded following 1 to 2 mg. of 10-methoxydeserpidine, 0.1 to 0.2 mg. of deserpidine, 2 to 3 mg. of reserpic acid and methyl reserpate. The hypotensive response was more marked with deserpidine and 10-methoxy-deserpidine than with methyl reserpate and reserpic acid. In the same dose range deserpidine, 10-methoxydeserpidine (Fig. 23, page 78), methyl reserpate (Fig. 24, page 79) and reserpic acid (Fig. 25, page 80) when given intravenously to the rat, did not antagonise the hypertensive responses to injections of 1 µg. of adrenaline and 1 µg. of noradrenaline.

Isolated strips of guinea pig ileum.

10-Methoxydeserpidine in bath concentrations of from 20 to 100 mg. per ml. caused an inhibition of the stimulant responses induced by 0.1 to 1 µg. per ml. of acetylcholine (Fig. 26, page 82), 0.01 to 0.1 µg. per ml. of histamine (Fig. 27, page 83), 0.25 to 1 mg. per ml. of barium chloride (Fig. 28, page 84), and 1.0 to 5.0 µg. per ml. of 5-hydroxytryptamine (Fig. 29, page 85).

Deserpidine /

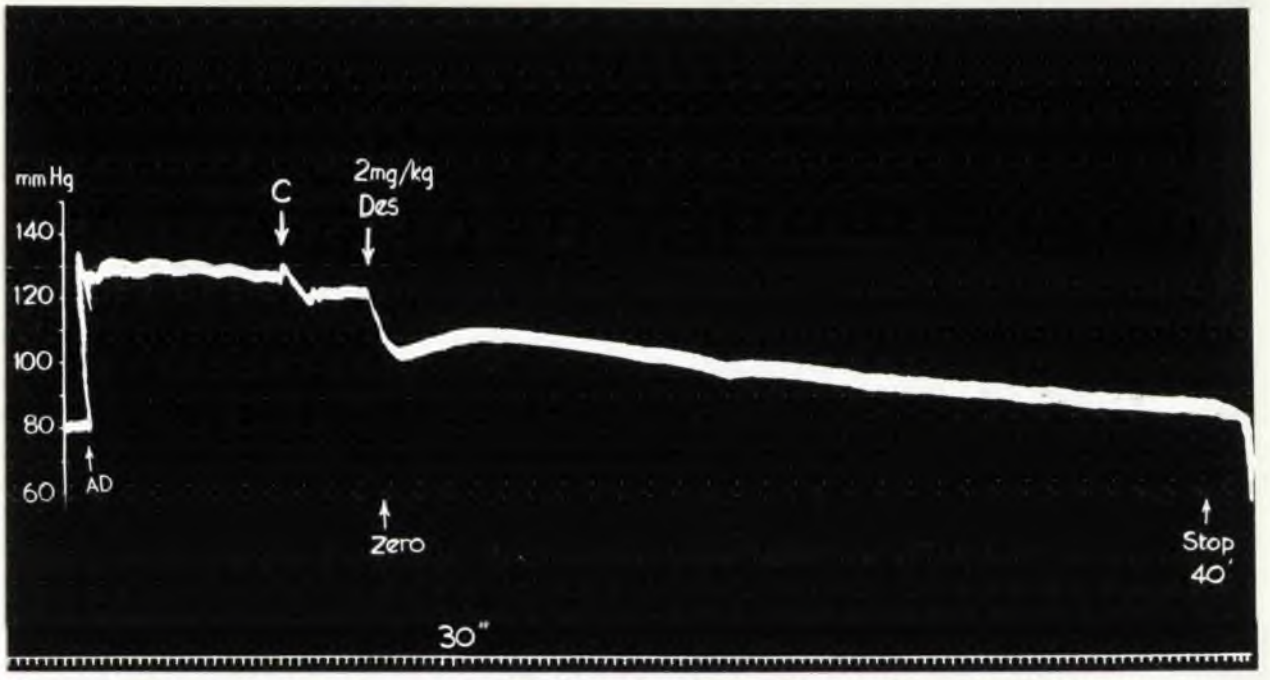

Fig. 21.

Effect of 2 mg. per kg. of deserpidine (Des) injected intravenously on the hypertension caused by slow infusion of adrenaline (Ad).

Adrenaline (Ad) 50 µg. per ml. infused at the rate of 1 ml. per minute using a Palmer's slow injection apparatus.

At C, control solution injected into the vein.
At Zero, drug solution injected into the vein.

Infusion discontinued at "Stop" 40 minutes after the injection of deserpidine.

Time interval (lower trace) = 30 seconds.

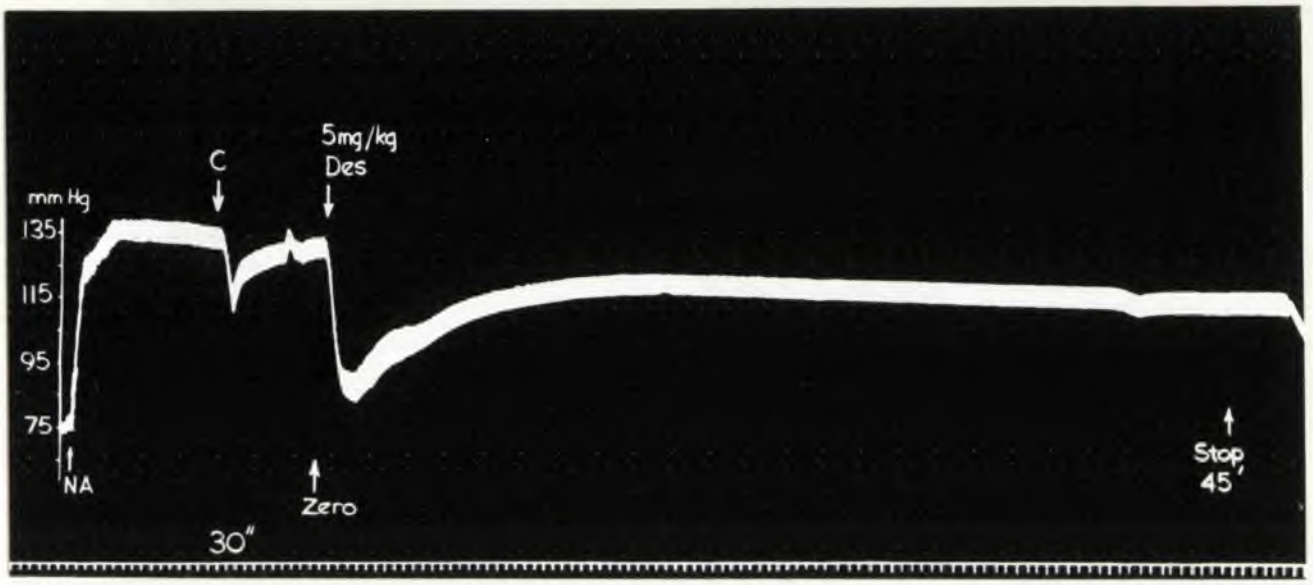

Fig. 22.

Effect of 5 mg. per kg. of deserpidine (Des) injected intravenously on the hypertension caused by slow infusion of noradrenaline (NA).

Noradrenaline (NA) 50 µg. per ml. infused at the rate of 1 ml. per minute using a Palmer's slow injection apparatus.

At C, Control solution injected into the vein.
At Zero, Drug solution injected into the vein.

Infusion discontinued at "Stop" 45 minutes after the injection of deserpidine.

Time interval (lower trace) = 30 seconds.

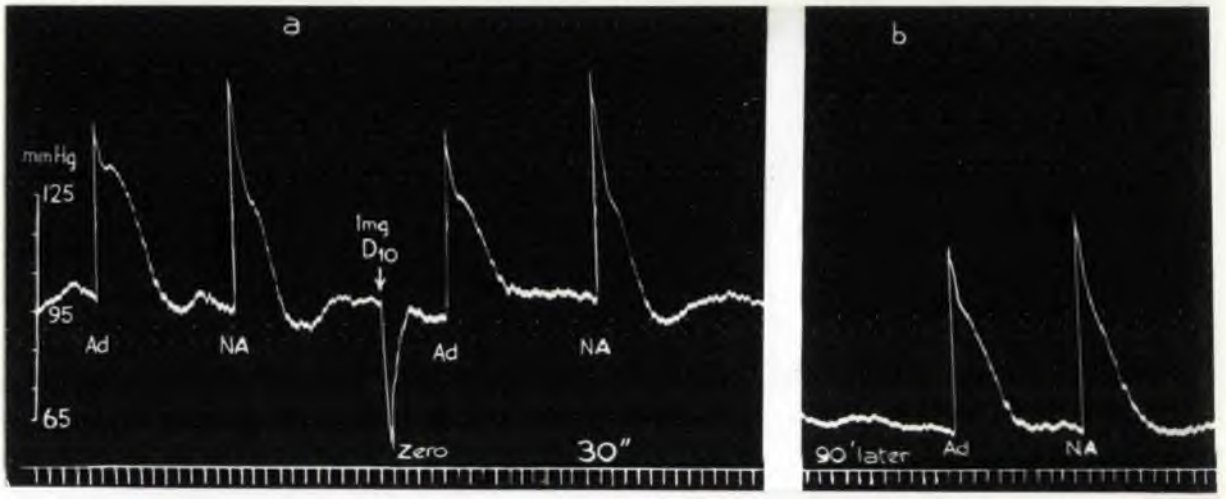

Fig. 23.

Tracing a. Effect of a single dose of 1 mg. of 10-methoxy-deserpidine (D10) upon the responses of the rat blood pressure to adrenaline (Ad) and noradrenaline (NA). Blood pressure recorded from the common carotid artery. Drugs injected into the femoral vein.

At Ad, 1 µg. adrenaline.
At NA, 1 µg. noradrenaline.

Tracing b. 90 minutes after the injection of drug.

Time interval (lower trace) = 30 seconds.

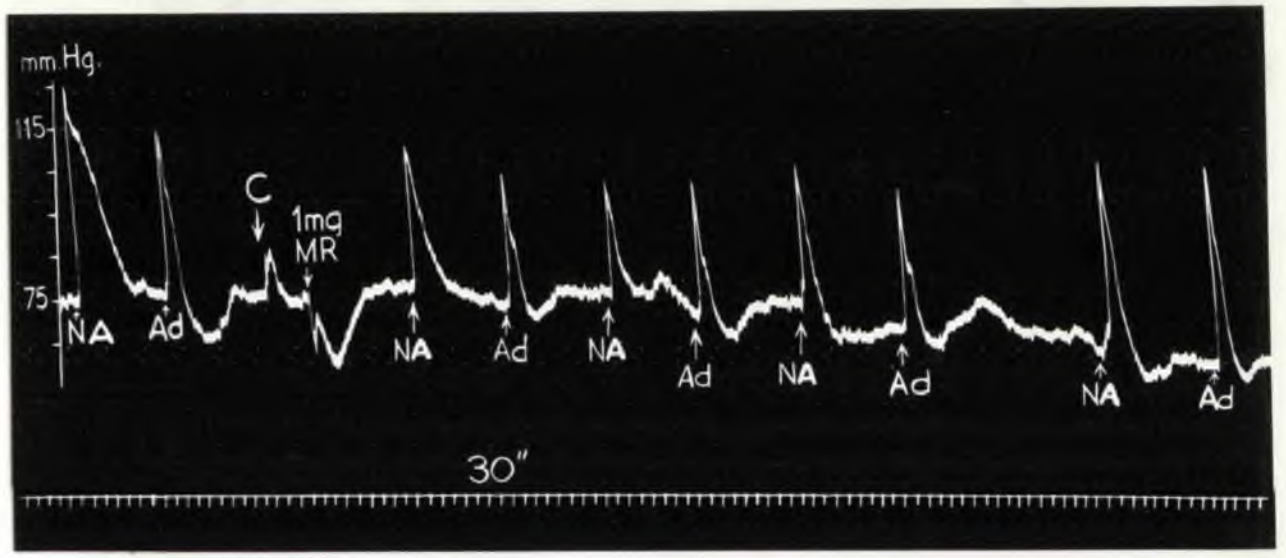

Fig. 24.

Effect of a single dose of 1 mg. of methyl reserpate (MR) on the response of the rat blood pressure to noradrenaline (NA) and adrenaline (Ad).

Blood pressure recorded from the common carotid artery.
Drugs injected into the femoral vein.

At C, 0.1 ml. of control solution.
At NA, 1 µg. noradrenaline.
At Ad, 1 µg. adrenaline.

Time interval (lower trace) = 30 seconds.

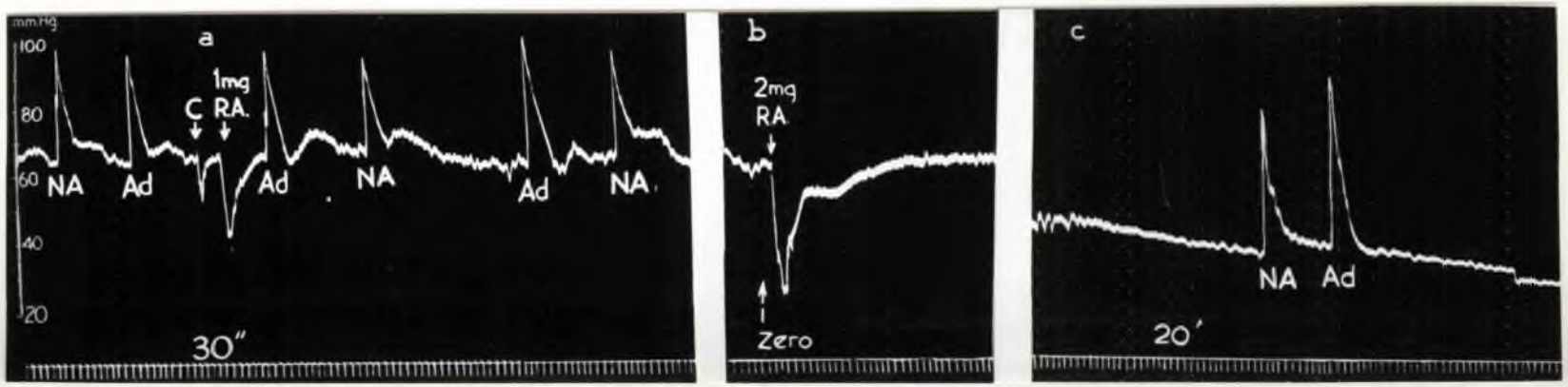

Fig. 25.

Effect of reserpic acid upon the response of the rat blood pressure to noradrenaline (NA) and adrenaline (Ad). Blood pressure recorded from the common carotid artery. All drugs injected into the femoral vein.

Tracing a. At NA, 1 µg. of noradrenaline.
At Ad, 1 µg. of adrenaline.
At C, 0.1 ml. of control solution.
At RA, 1 mg. of reserpic acid.

Tracing b. At RA, 2 mg. of reserpic acid.
Zero indicates the time when the drug was injected.

Tracing c. 20 minutes after the (zero) second injection of the drug.

Time interval (lowest trace) = 30 seconds.

Deserpidine in much lower dose levels (5 to 15 µg. per ml.) produced a more marked and prolonged inhibition of the contractions produced by acetylcholine (Fig. 30, page 86), histamine (Fig. 31, page 87), barium chloride (Fig. 32, page 88) and 5-hydroxytryptamine.

Methyl reserpate 150 to 900 µg. per ml. and reserpic acid 50 to 100 µg. per ml. produced a short-lived inhibition of the responses to acetylcholine (Fig. 33, page 89, and Fig. 34, page 90), histamine (Fig. 35, page 91), barium chloride (Fig. 36, page 92), and 5-hydroxytryptamine (Fig. 37, page 93).

Isolated strips of rabbit duodenum.

10-Methoxydeserpidine, 75 to 100 µg. per ml; deserpidine, 15 to 20 µg. per ml; methyl reserpate, 100 to 200 µg. per ml; and reserpic acid, 50 to 100 µg. per ml., depressed the rhythmic contractions of the isolated rabbit duodenum. The effect of deserpidine (Fig. 38, page 94) was the most prolonged, and a period of from 30 to 40 minutes was required by the gut for the rhythmic activity to return to control levels. 10-Methoxydeserpidine (Fig. 39, page 95) caused a short-lived, depressant effect upon the rhythmic activity of the gut. This returned to control levels in from 10 to 15 minutes.

Methyl reserpate (Fig. 40, page 96) and reserpic acid had a very slight and short-lived effect upon the rhythmic activity of isolated strips of rabbit duodenum.

Contractions of the isolated duodenum following addition to the

bath /

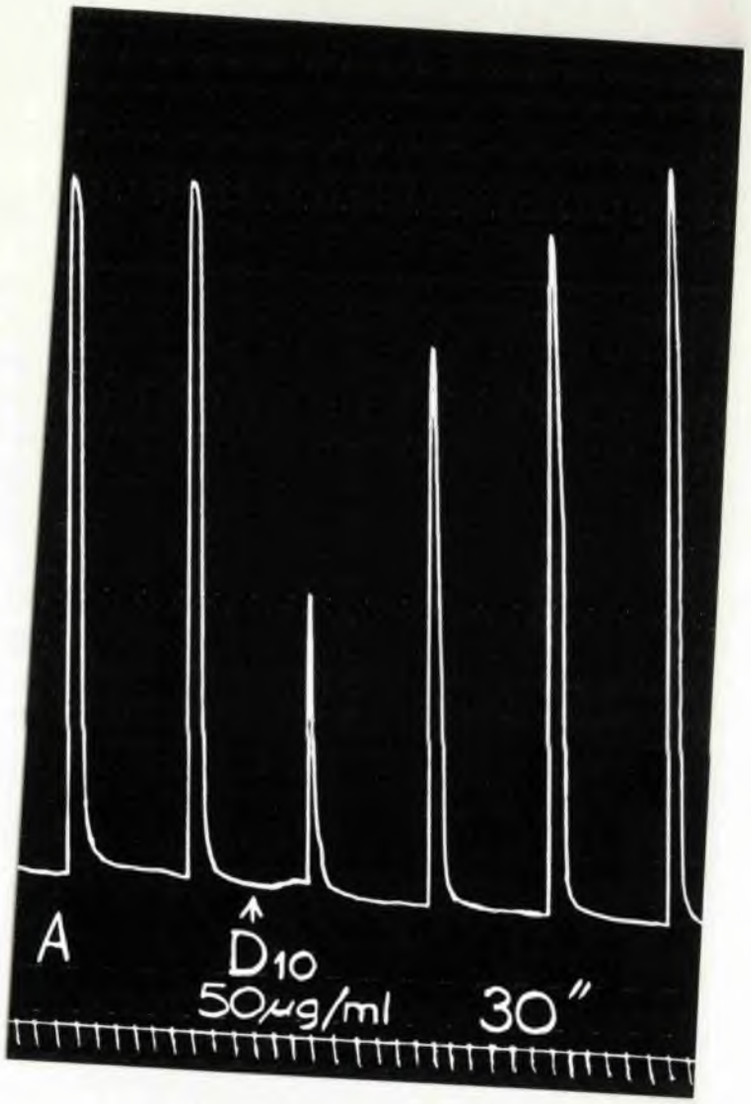

Fig. 26.

Effect of 50 µg. per ml. of 10-methoxydeserpidine (D10) on the response of the isolated guinea pig ileum to acetylcholine (A). All contractions are due to 0.1 µg. per ml. of acetylcholine. Time interval (lower trace) = 30 seconds.

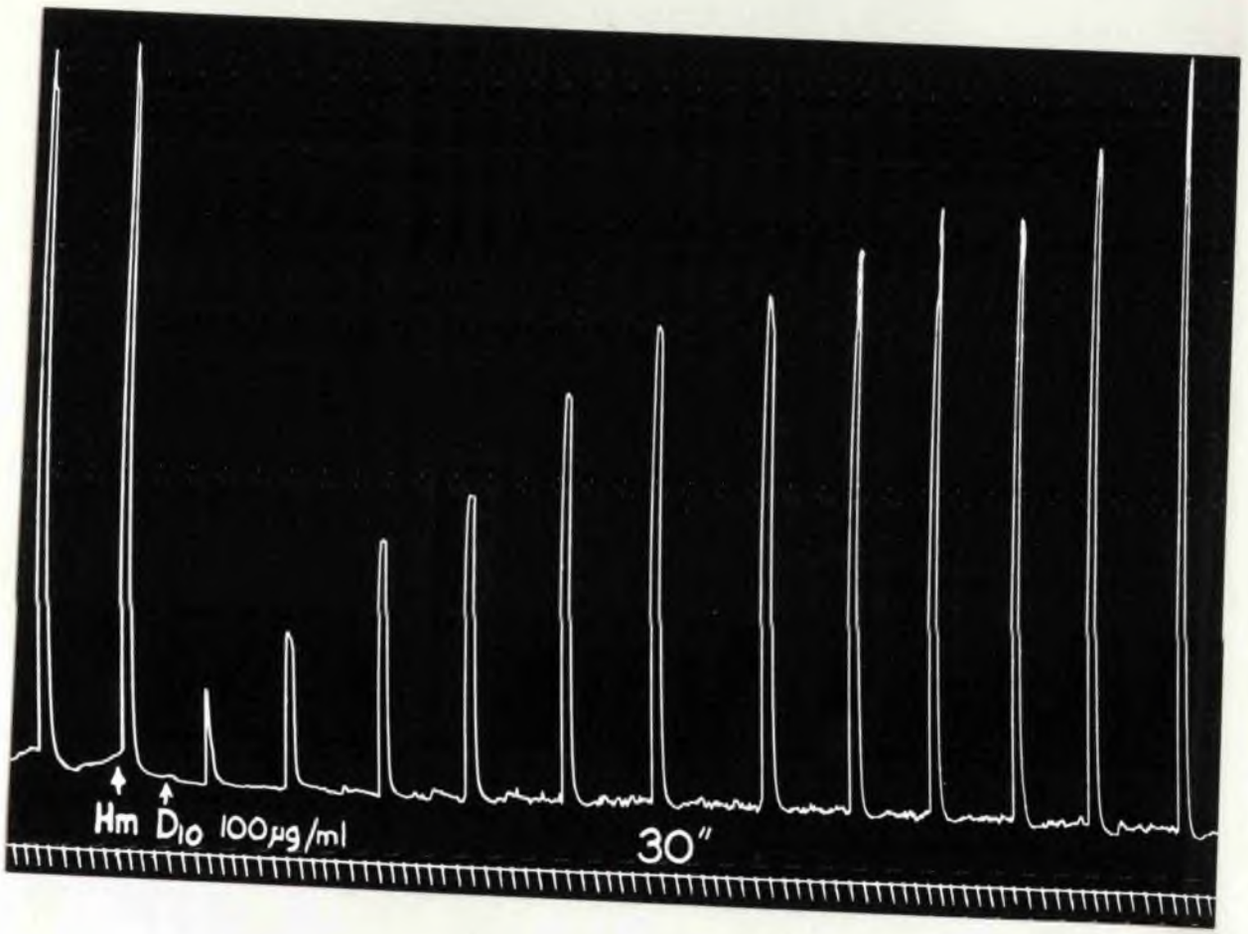

Fig. 27.

Effect of 100 µg. per ml. of 10-methoxydeserpidine (D10) on the response of the isolated guinea pig ileum to histamine (Hm).

All contractions are due to 0.1 µg. per ml. of histamine.

Time interval (lower trace) = 30 seconds.

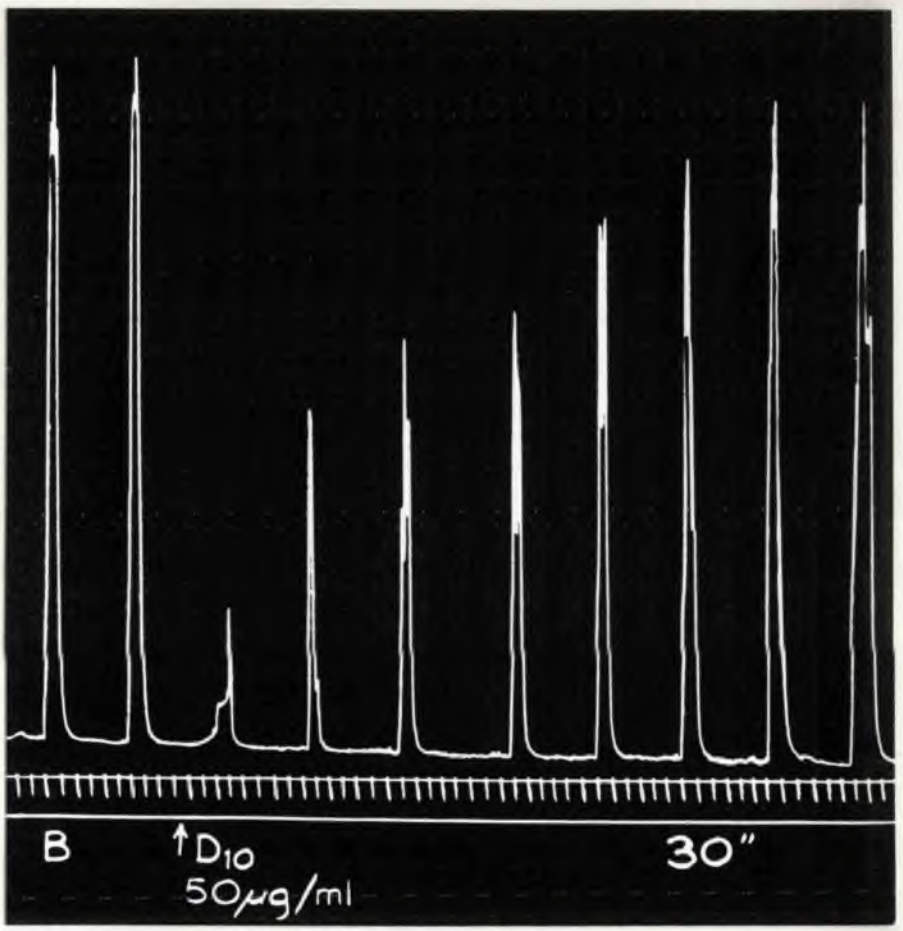

Fig. 28.

Effect of 50 µg. per ml. of 10-methoxydeserpidine on the response of the isolated guinea pig ileum to barium chloride (B).
All contractions are due to 0.25 mg. per ml. of barium chloride.
Time interval (lower trace) = 30 seconds.

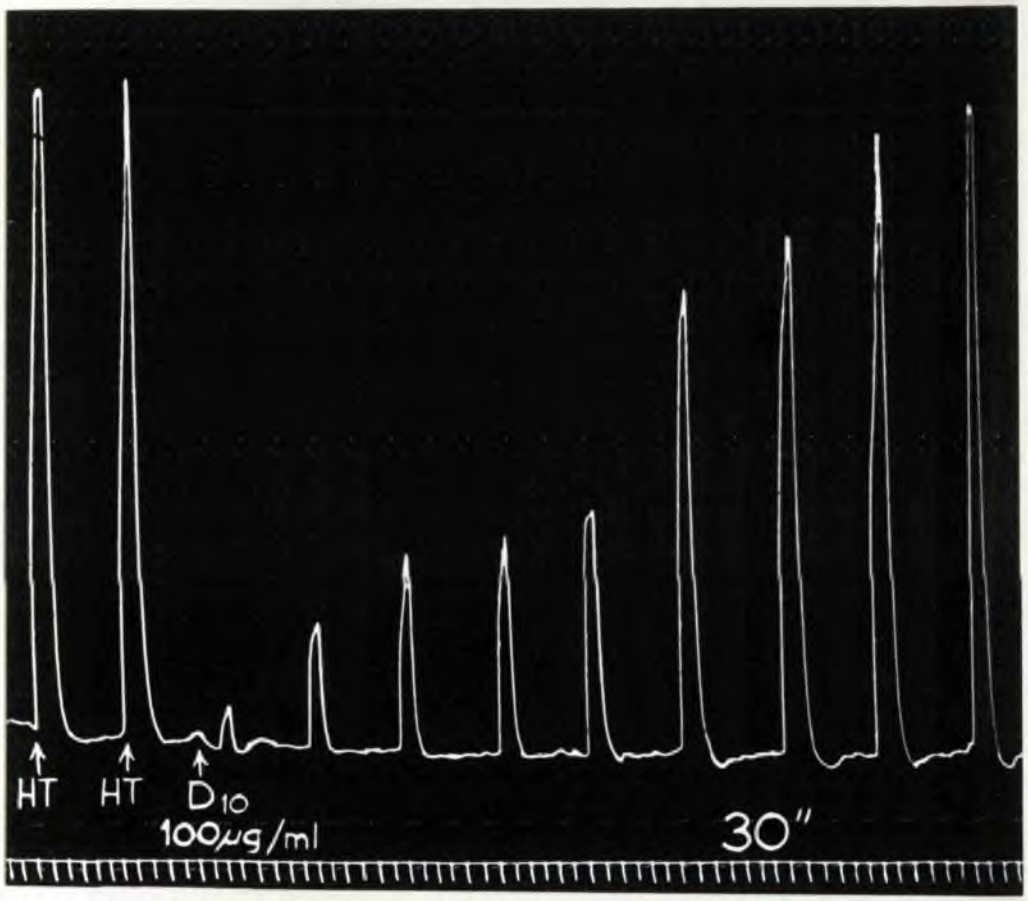

Fig. 29.

Effect of 100 µg. per ml. of 10-methoxydeserpidine (D10) on the response of the isolated guinea pig ileum to 5-hydroxytryptamine (HT).

All contractions are due to 0.5 µg. per ml. of 5-hydroxytryptamine.

Time interval (lower trace) = 30 seconds.

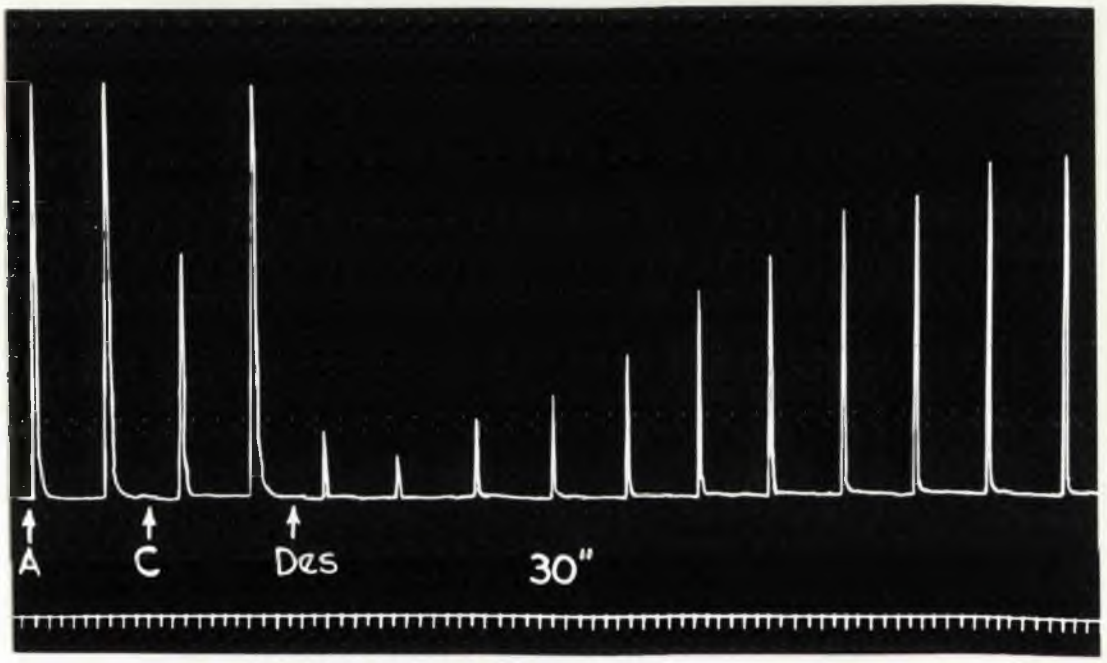

Fig. 30.

Effect of 10 µg. per ml. deserpidine (Des) on the response of the isolated guinea pig ileum to acetylcholine (A).

All contractions are due to 0.02 µg. per ml. of acetylcholine.

At C, 0.1 ml. of control solution added to the bath.

Time interval (lower trace) = 30 seconds.

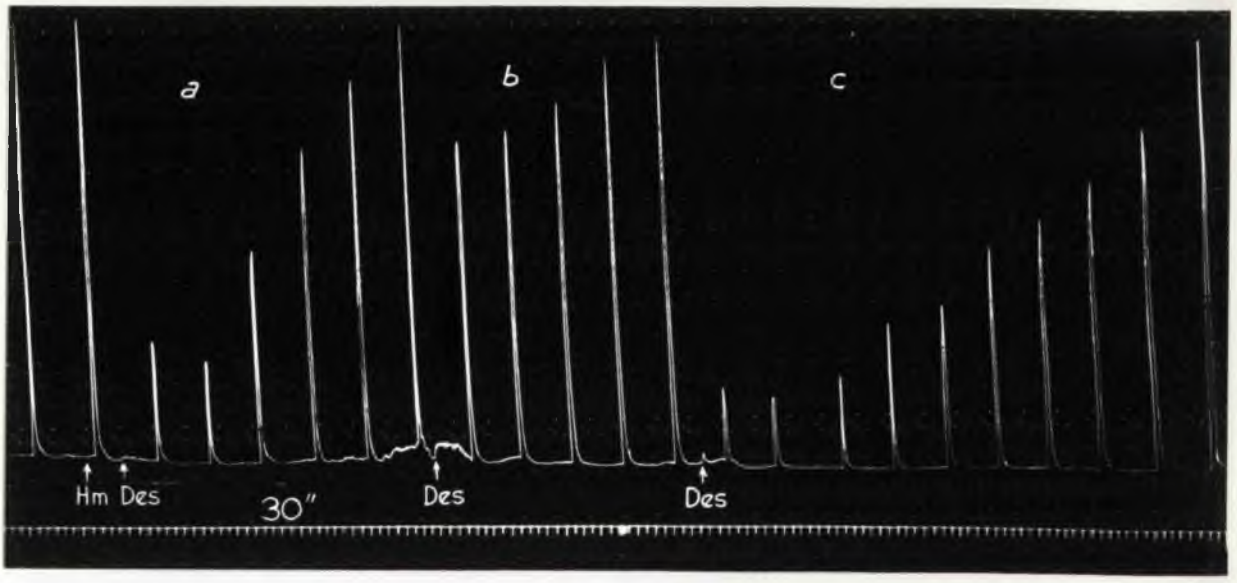

Fig. 31.

Effect of deserpidine (Des) on the response of the isolated guinea pig ileum to histamine (Hm).

All contractions are due to 0.01 µg. per ml. of histamine.

At a, 10 µg. per ml. deserpidine (Des).
At b, 5 µg. per ml. deserpidine (Des).
At c, 15 µg. per ml. deserpidine (Des).

Time interval (lower trace) = 30 seconds.

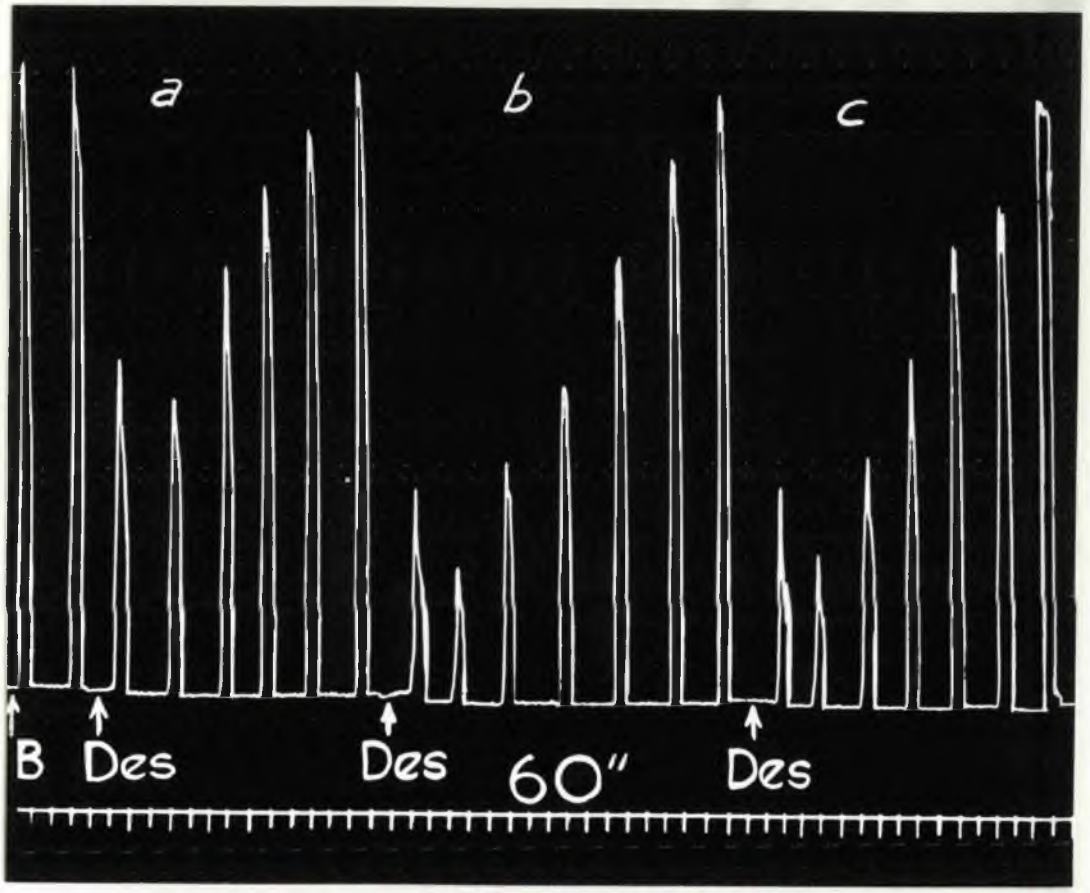

Fig. 32.

Effect of deserpidine (Des) upon the response of the isolated guinea pig ileum to barium chloride (B).

All contractions are due to 50 µg. per ml. of barium chloride.

At a, 7.5 µg. per ml. of deserpidine.
At b and c, 15 µg. per ml. of deserpidine.

Time interval (lower trace) = 60 seconds.

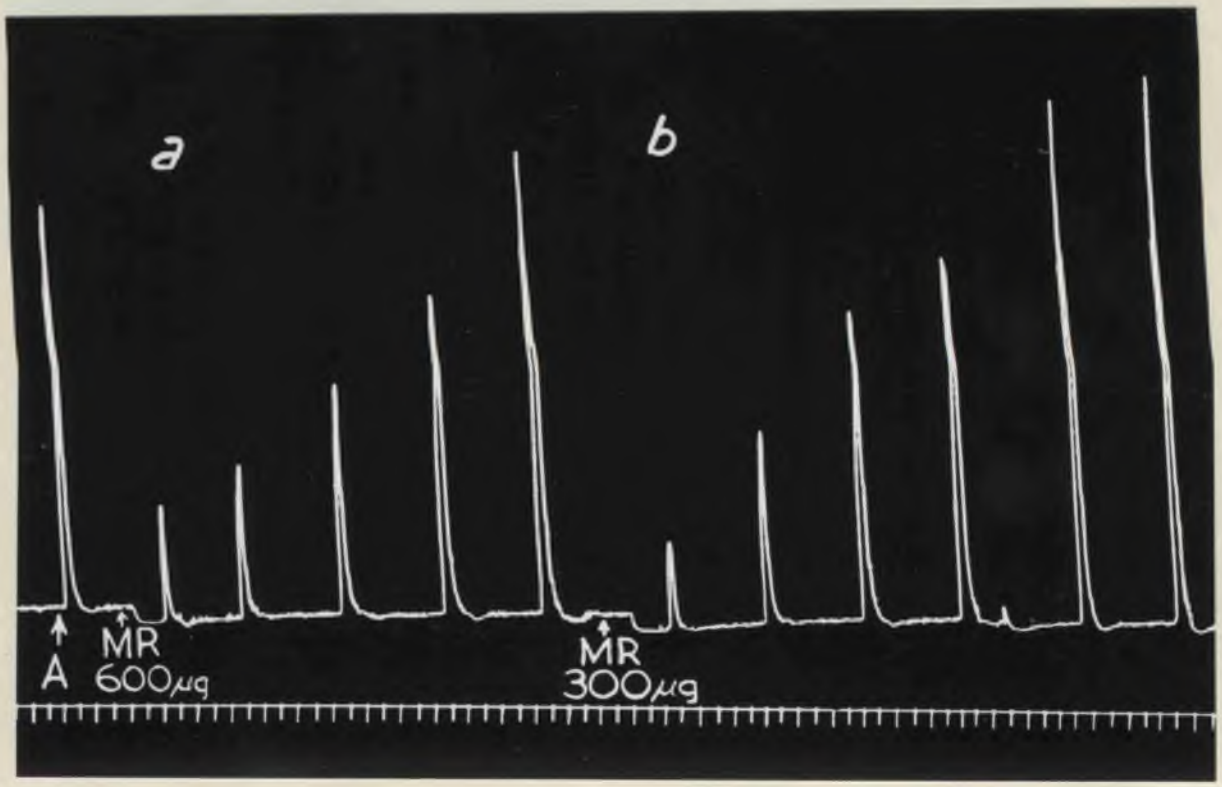

Fig. 33.

Effect of methyl reserpate (MR) on the response of the isolated guinea pig ileum to acetylcholine (A).

All contractions due to 0.2 µg. per ml. of acetylcholine.

At a, 600 µg. per ml. methyl reserpate (MR).
At b, 300 µg. per ml. methyl reserpate (MR).

Time interval (lower trace) = 30 seconds.

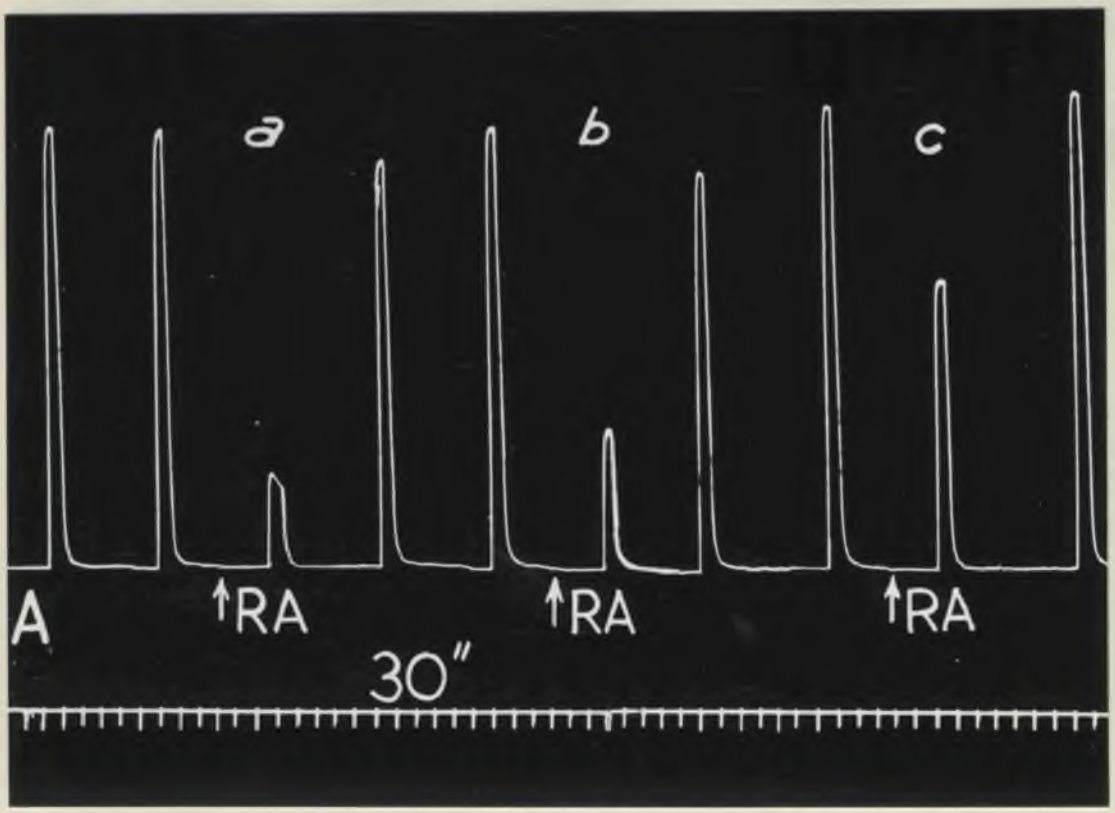

Fig. 34.

Effect of reserpic acid (RA) on the response of the isolated guinea pig ileum to acetylcholine (A).

All contractions are due to 0.1 µg. per ml. of acetylcholine.

At a, 100 µg. per ml. reserpic acid (RA).

At b, 75 µg. per ml. reserpic acid (RA).

At c, 50 µg. per ml. reserpic acid (RA).

Time interval (lower trace) = 30 seconds.

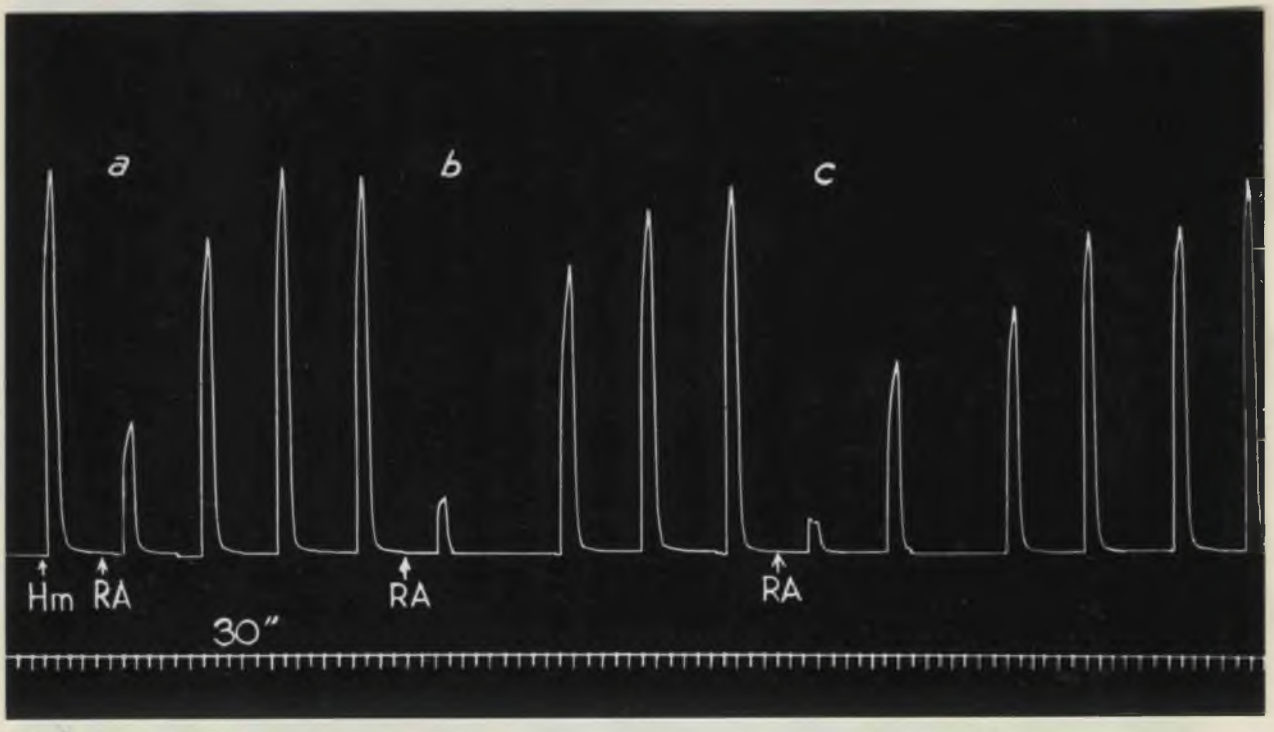

Fig. 35.

Effect of reserpic acid (RA) on the response of the isolated guinea pig ileum to histamine (Hm).

All contractions are due to 0.1 ug. per ml. histamine.

At a, 50 µg. per ml. reserpic acid (RA).

At b, 75 µg. per ml. reserpic acid (RA).

At c, 100 µg. per ml. reserpic acid (RA).

Time interval (lower trace) = 30 seconds.

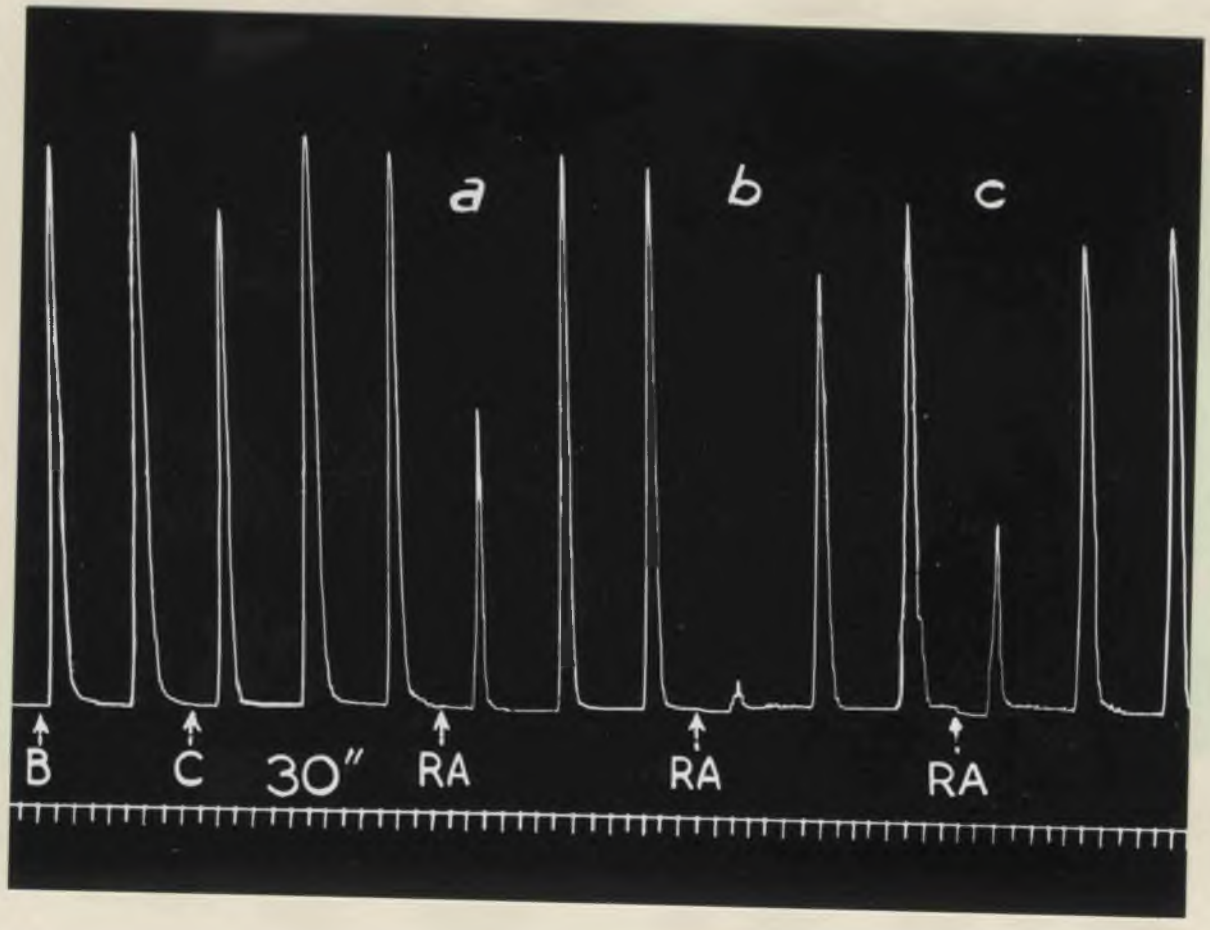

Fig. 36.

Effect of reserpic acid (RA) on the response of the isolated guinea pig ileum to barium chloride (B).

All contractions are due to 0.25 mg. per ml. of barium chloride.

At C, control solution, 0.2 ml. added to the bath.
At a, 45 µg. per ml. reserpic acid (RA).
At b, 90 µg. per ml. reserpic acid (RA).
At c, 65 µg. per ml. reserpic acid (RA).

Time interval (lower trace) = 30 seconds.

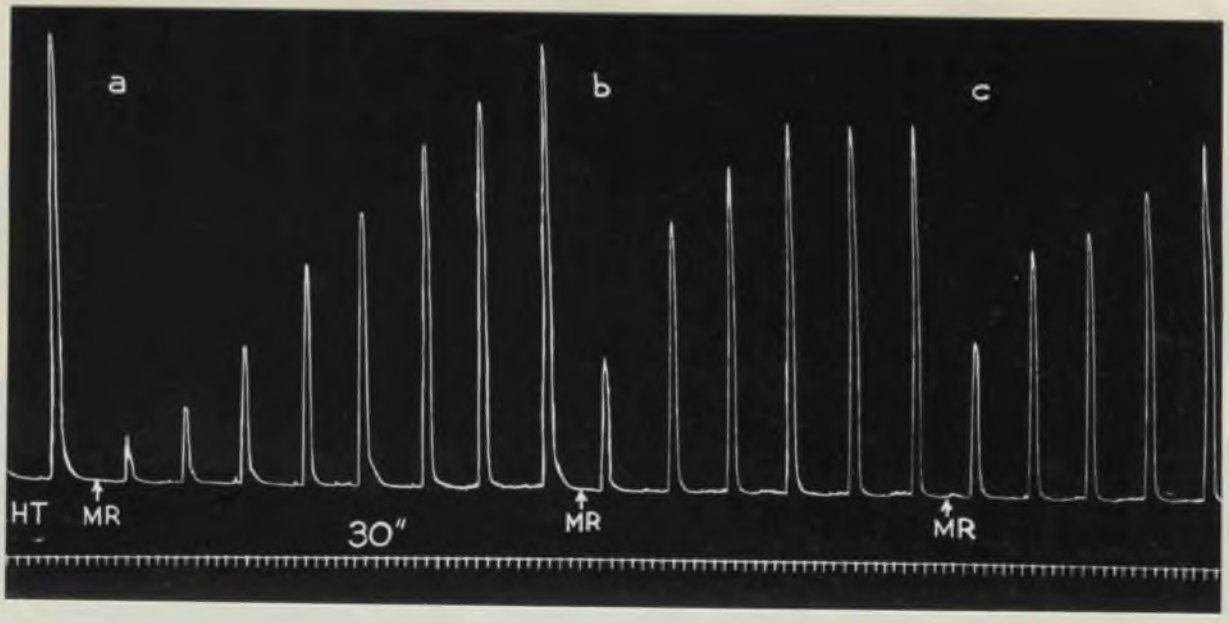

Fig. 37.

Effect of methyl reserpate (MR) on the response of the isolated guinea pig ileum to 5-hydroxytryptamine (HT).

All contractions due to 1 µg. per ml. of 5-hydroxytryptamine.

At a, 600 µg. per ml. methyl reserpate (MR).
At b, 300 µg. per ml. methyl reserpate (MR).
At c, 150 µg. per ml. methyl reserpate (MR).

Time interval (lower trace) = 30 seconds.

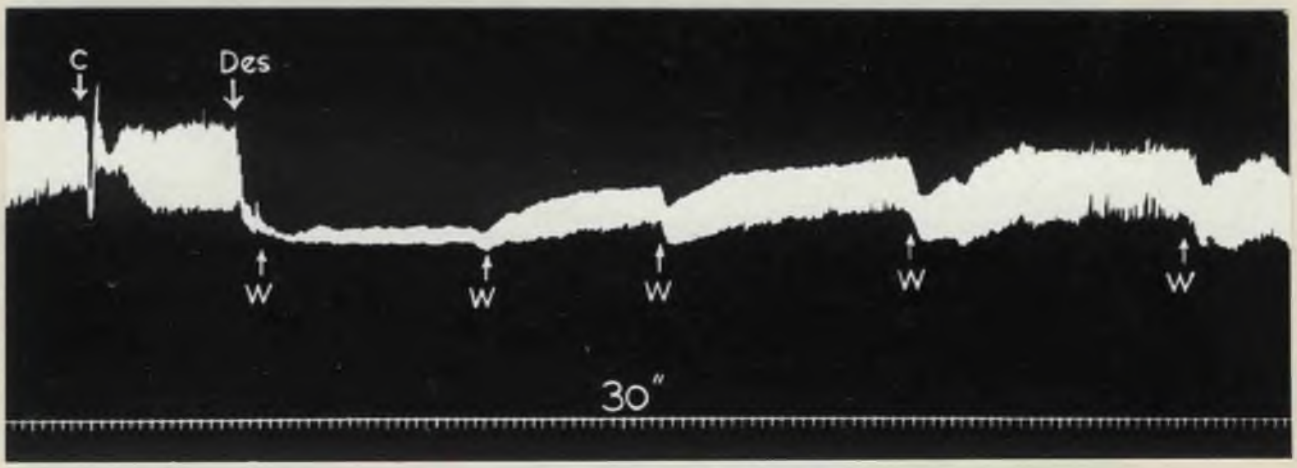

Fig. 38.

Effect of 15 µg. per ml. of deserpidine (Des) upon the peristaltic movements of the isolated rabbit duodenum.

At C, control solution (0.1 ml.) added to the bath.

At W, bath washed out; washing out was repeated at 10 minute intervals.

Time interval (lower trace) = 30 seconds.

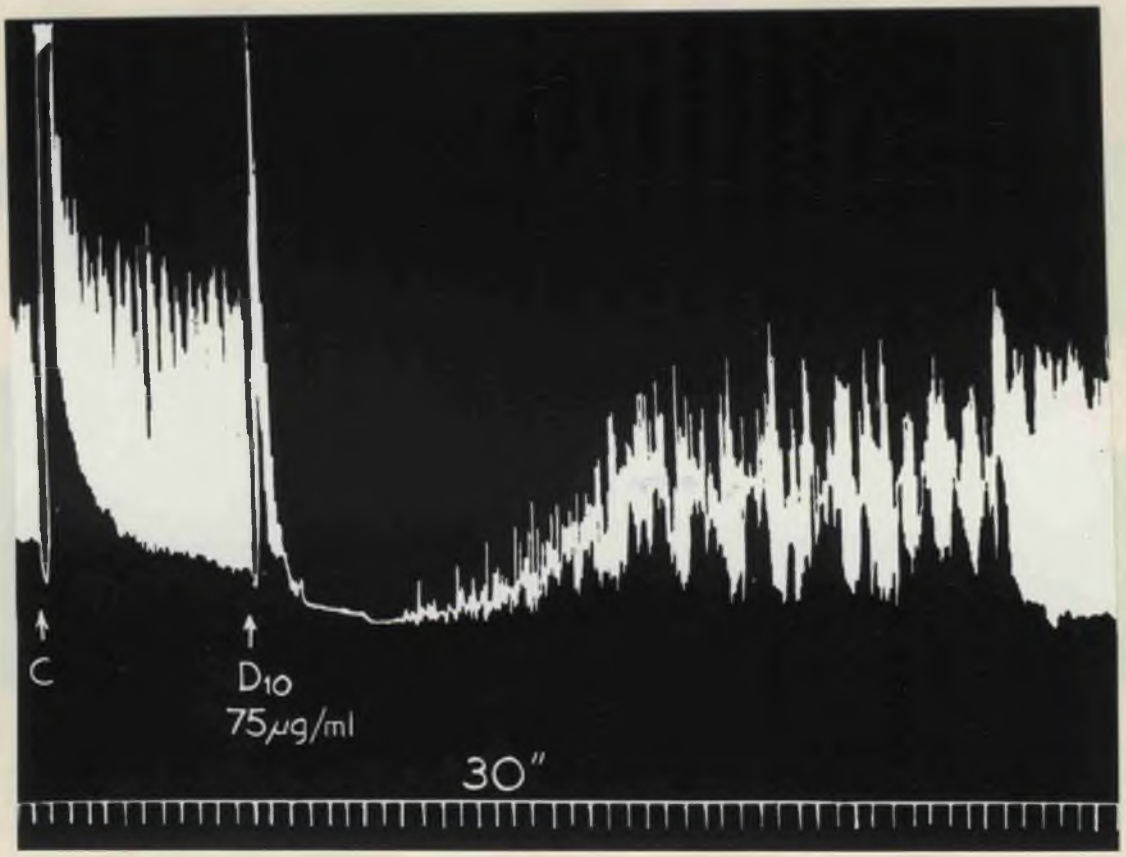

Fig. 39.

Effect of 75 µg. per ml. of 10-methoxydeserpidine (D10) on the peristaltic movements of the isolated rabbit duodenum.

At C, control solution (0.2 ml.) added to the bath.

Time interval (lower trace) = 30 seconds.

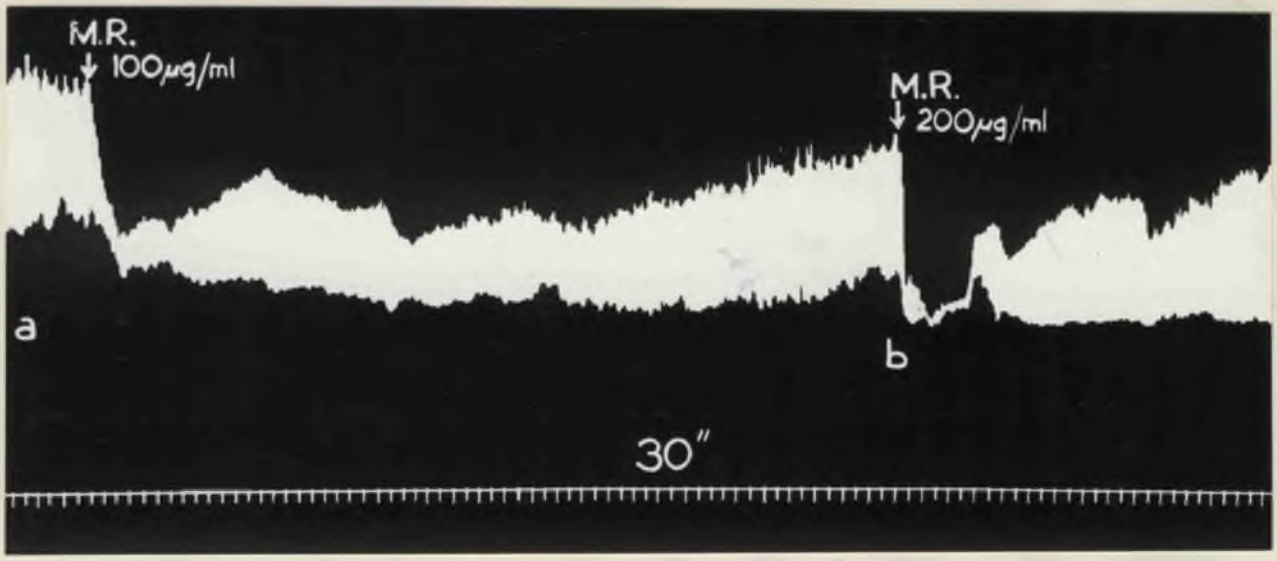

Fig. 40.

Effect of methyl reserpate (MR) on the peristaltic movements of the isolated rabbit duodenum.

At a, 100 µg. per ml. of methyl reserpate (MR).

At b, 200 µg. per ml. of methyl reserpate (MR).

Time interval (lower trace) = 30 seconds.

bath of acetylcholine, 0.01 to 0.1 µg. per ml. and the reduction in the tone produced by 0.01 to 0.05 µg. per ml. of adrenaline were depressed by the addition of 10-methoxydeserpidine and deserpidine (Fig. 41, page 98, and Fig. 42, page 99). The effect of deserpidine was the more prolonged in duration.

Methyl reserpate (Fig. 43, page 100), and reserpic acid (Fig. 44, page 101) at the same dose levels also caused some reduction in the magnitude of the contractions induced by 0.01 to 0.1 µg. per ml. of acetylcholine. They were, however, less potent in this respect than 10-methoxydeserpidine or deserpidine.

In all experiments in which the drugs were dissolved in a solution of acetic acid, the drug-free acid solution was used as the control. It can be seen from inspection of the tracings that this solution was not inert but caused, for example, a brief, reversible fall in tone (Figs. 38, 39, 41 and 44, pages 94, 95, 98, and 101) or a biphasic effect upon tone - which was first reduced and then increased (Fig. 39, page 95 and Fig. 44, page 101). In the latter case, peristalsis was also reduced. The effects of the control solution could be readily distinguished from those of the drug-containing solution.

Isolated strips of horse carotid artery

10-Methoxydeserpidine in the dose range of from 100 to 200 µg. per ml. relaxed sustained contractions of artery strips induced by adrenaline 0.1 to 0.2 µg. per ml. and noradrenaline 0.1 to 0.2 µg. per ml. The degree of relaxation depended upon the magnitude of the dose of 10-methoxydeserpidine used. 10-Methoxydeserpidine also depressed reversibly contractions of the strips induced by adrenaline, 0.01 to 0.02 µg. per ml. (Fig. 45, page 102), noradrenaline, 0.01 to 0.02 µg. per ml. (Fig. 46, page 103), 5-hydroxytryptamine 0.5 µg. per ml., and acetylcholine 0.01 to 0.1 µg. per ml. (Fig. 47, page 104).

Deserpidine, 50 to 200 µg. per ml. also caused relaxation of the sustained contractions induced by adrenaline (Fig. 48, page 105), noradrenaline 0.1 to 0.2 µg. per ml. (Fig. 49, page 106), and 5-hydroxytryptamine 1 to 5 µg. per ml. Like 10-methoxydeserpidine, an inhibition of the responses induced by the addition of 0.01 to 0.02 µg. per /

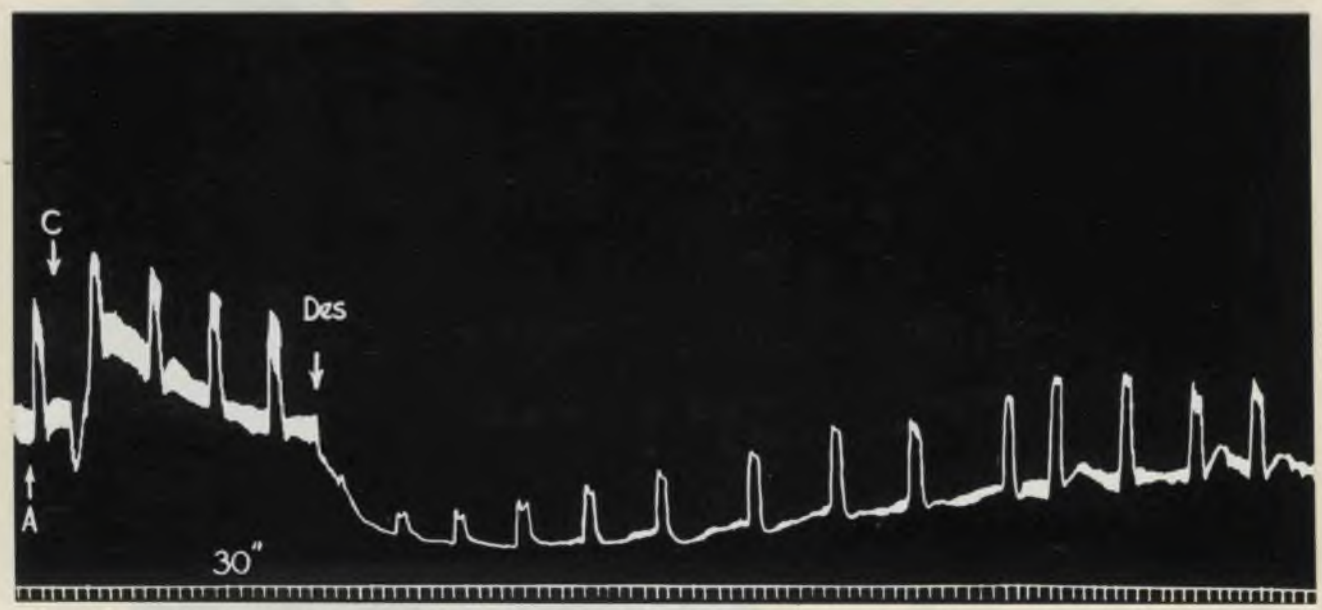

Fig. 41.

Effect of 14 µg. per ml. of deserpidine (Des) on the response of the isolated rabbit duodenum to acetylcholine (A).

All responses are due to 0.01 µg. per ml. of acetylcholine.

At C, control solution 0.2 ml. added to the bath.

Time interval (lower trace) = 30 seconds.

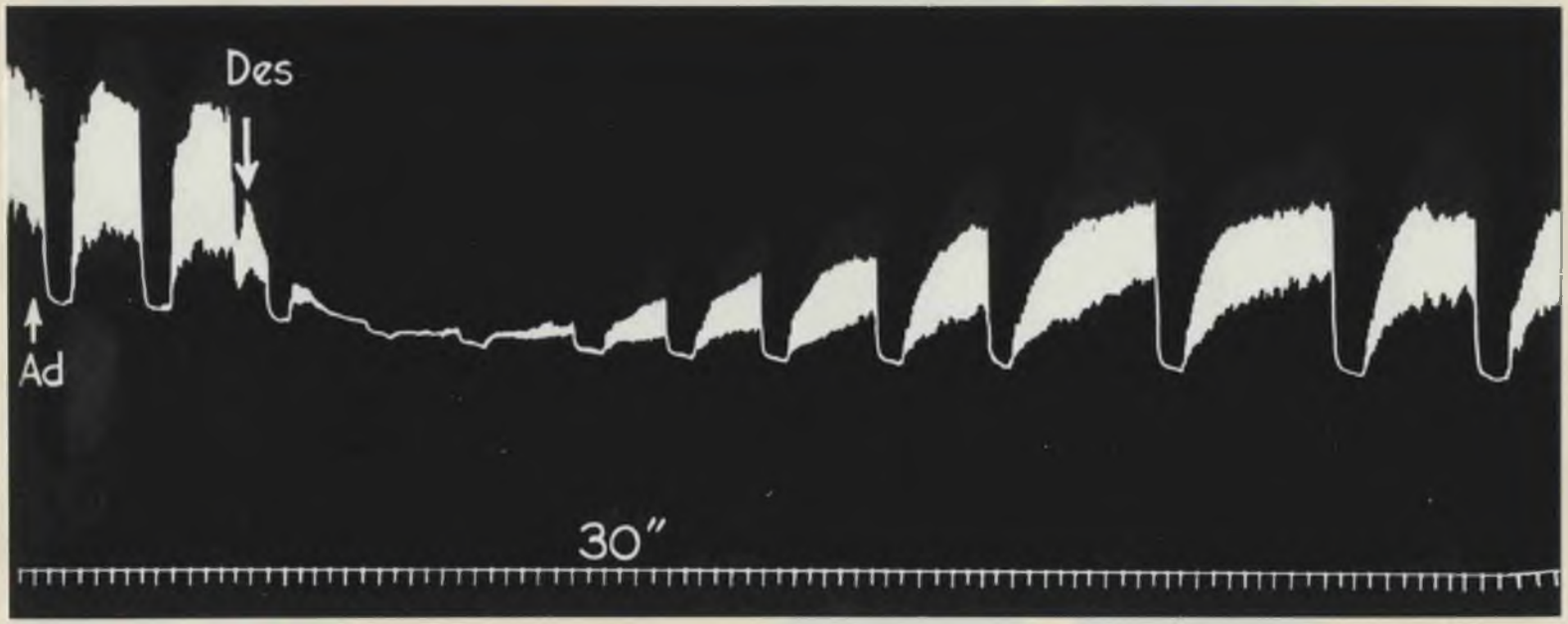

Fig. 42.

Effect of 20 µg. per ml. of deserpidine (Des) upon the responses of the isolated rabbit duodenum to adrenaline (Ad).

All responses are due to adrenaline (Ad) 0.01 µg. per ml.

Time interval (lower trace) = 30 seconds.

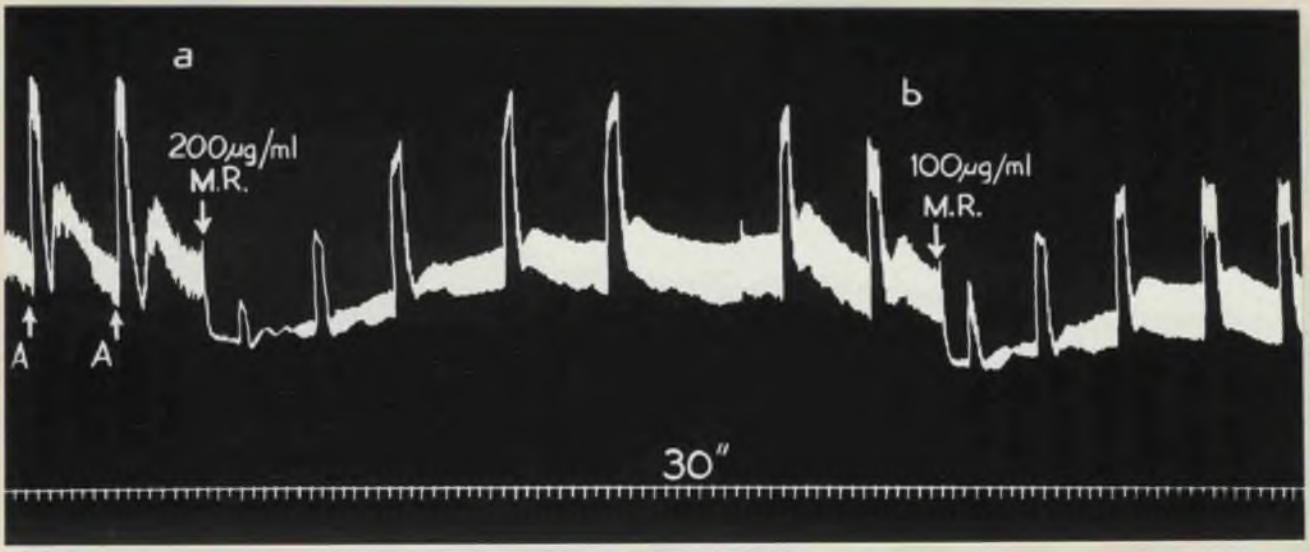

Fig. 43.

Effect of methyl reserpate (MR) on the response of the isolated rabbit duodenum to acetylcholine (A).

All responses are due to 0.1 µg. per ml. of acetylcholine.

At a, 200 µg. per ml. methyl reserpate (MR).

At b, 100 µg. per ml. methyl reserpate (MR).

Time interval (lower trace) = 30 seconds.

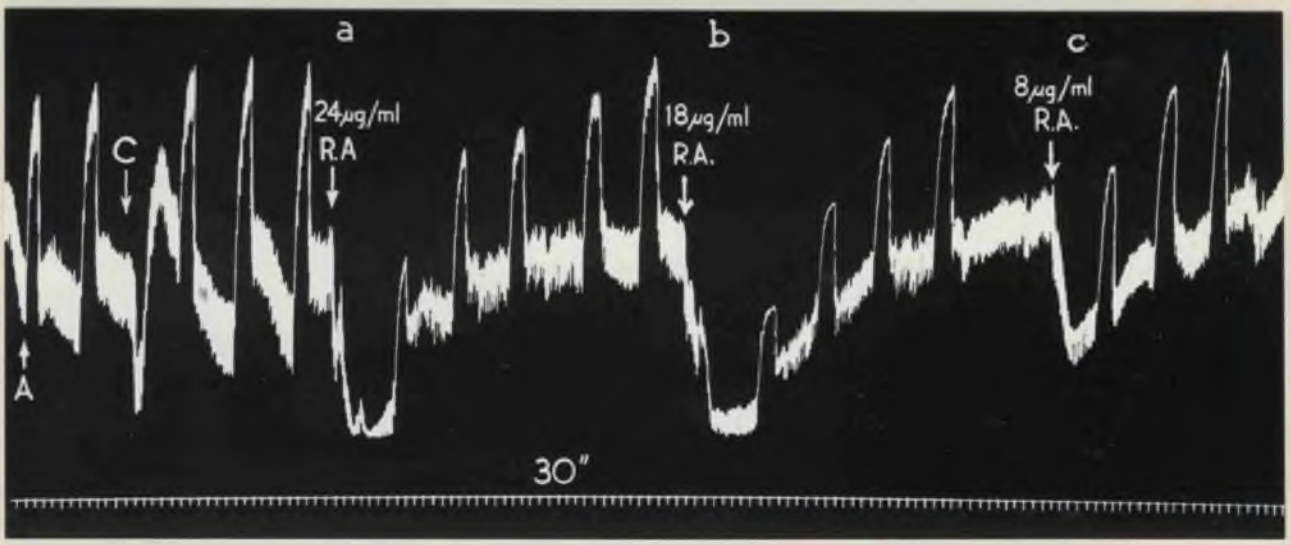

Fig. 44.

Effect of reserpic acid (RA) on the response of the isolated rabbit duodenum to acetylcholine (A). All responses are due to 0.1 µg. per ml. of acetylcholine.

At a, 24 µg. per ml. of reserpic acid (RA).
At b, 18 µg. per ml. of reserpic acid (RA).
At c, 8 µg. per ml. of reserpic acid (RA).
At C, Control solution (0.2 ml.) added to the bath.

Time interval (lower trace) = 30 seconds.

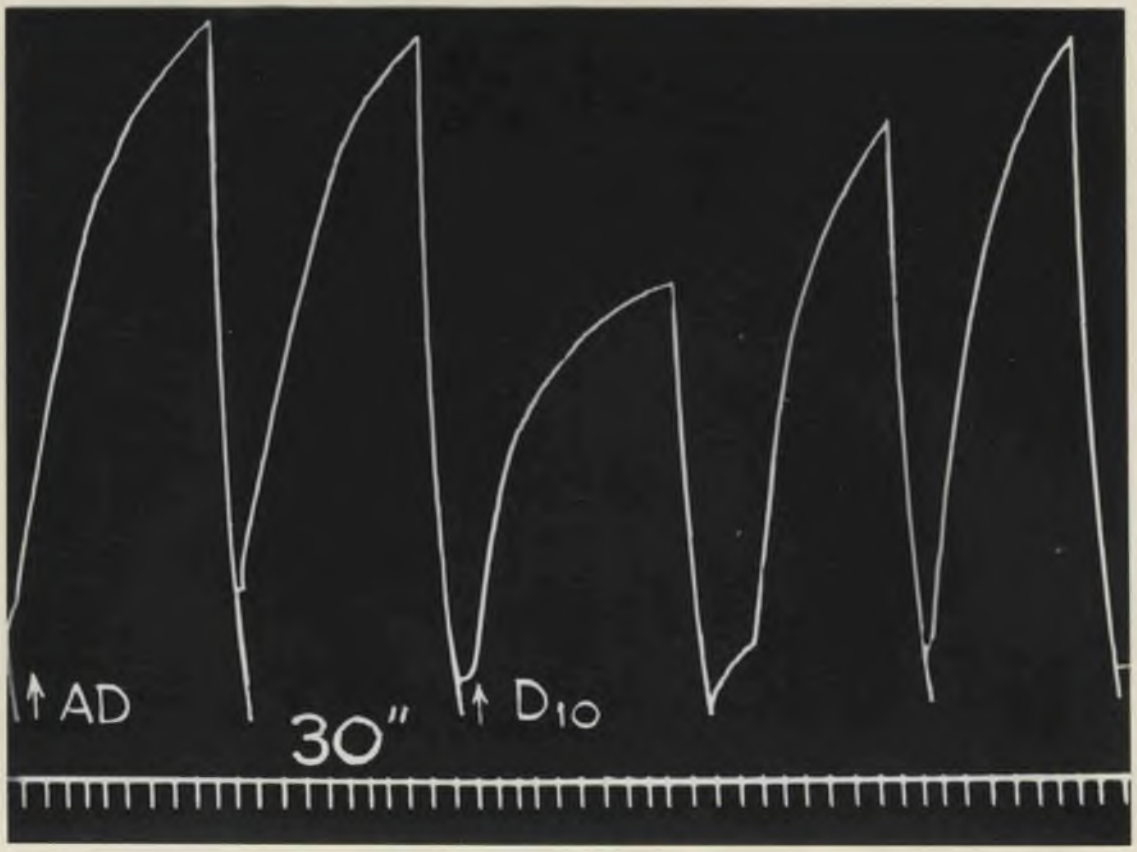

Fig. 45.

Effect of 100 µg. per ml. of 10-methoxydeserpidine (D10) upon the response to adrenaline (Ad) of a strip of horse carotid artery.
All responses due to 0.2 µg. per ml. of adrenaline (Ad).
Time interval (lower trace) = 30 seconds.

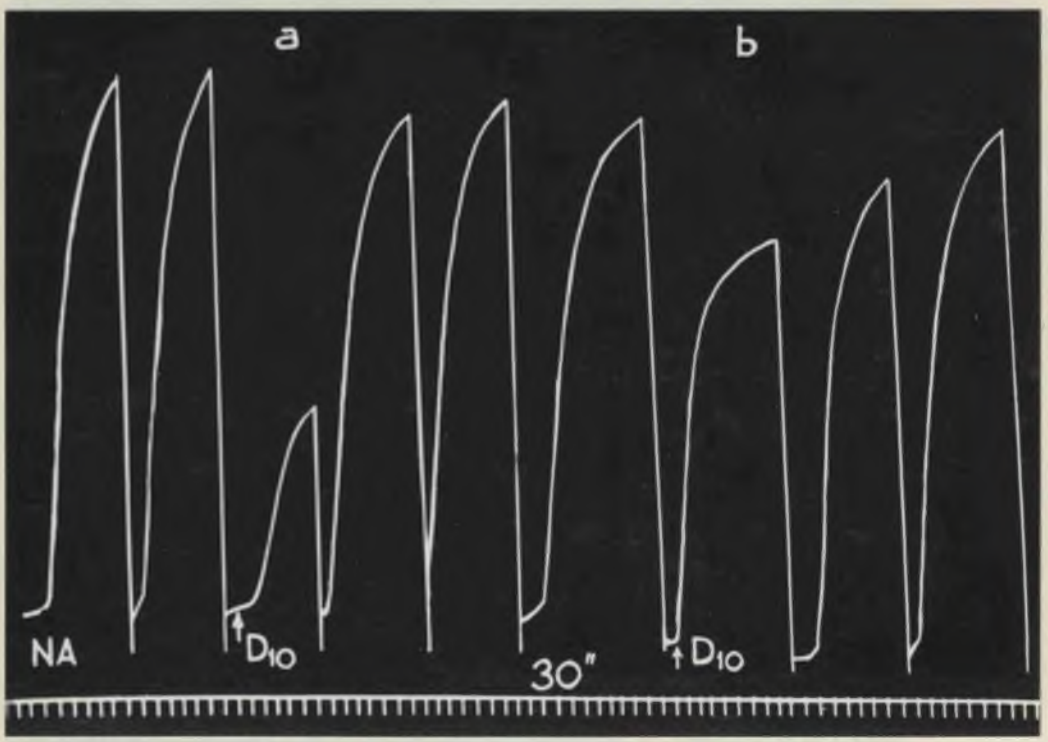

Fig. 46.

Effect of 200 µg. per ml. (at a) and 100 µg. per ml. of 10-methoxydeserpidine (D10) (at b) upon the response to noradrenaline (NA) of a strip of horse carotid artery.

All responses due to 0.2 µg. per ml. of noradrenaline (NA).

Time interval (lower trace) = 30 seconds.

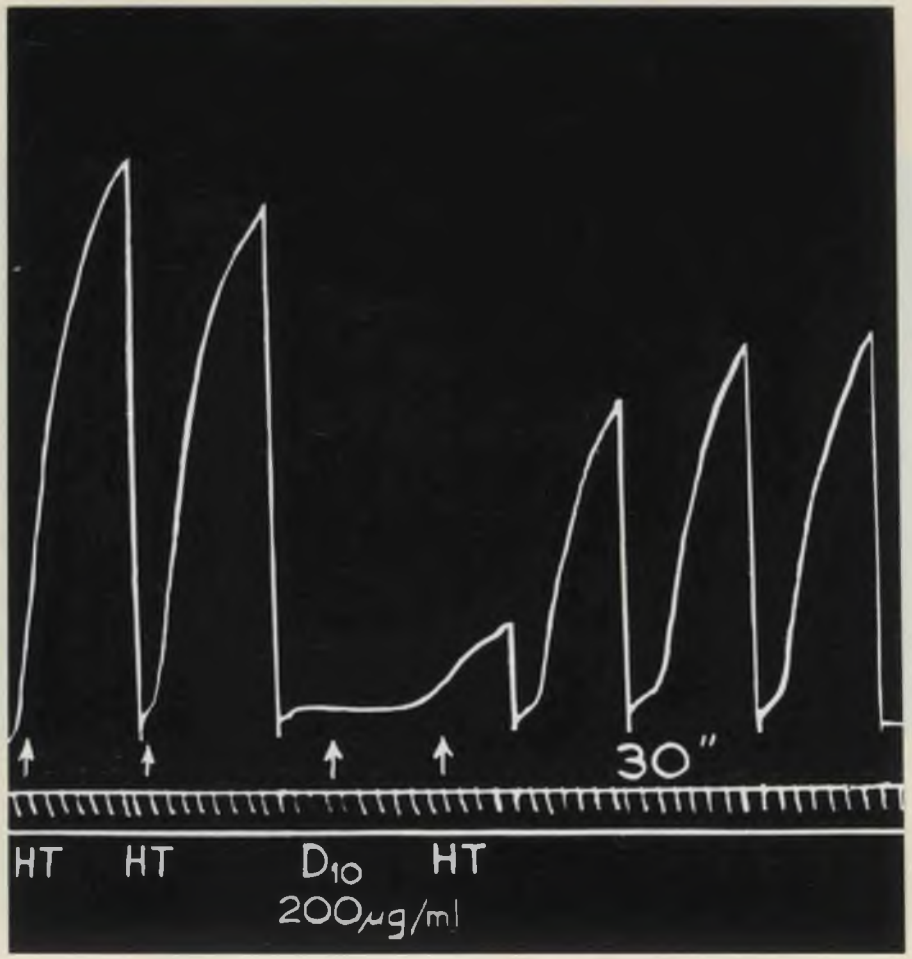

Fig. 47.

Effect of 200 µg. per ml. of 10-methoxydeserpidine (D10) on the response to 5-hydroxytryptamine (HT) of a strip of horse carotid artery.

At HT, 1 µg. per ml. of 5-hydroxytryptamine.

Time interval (lower trace) = 30 seconds.

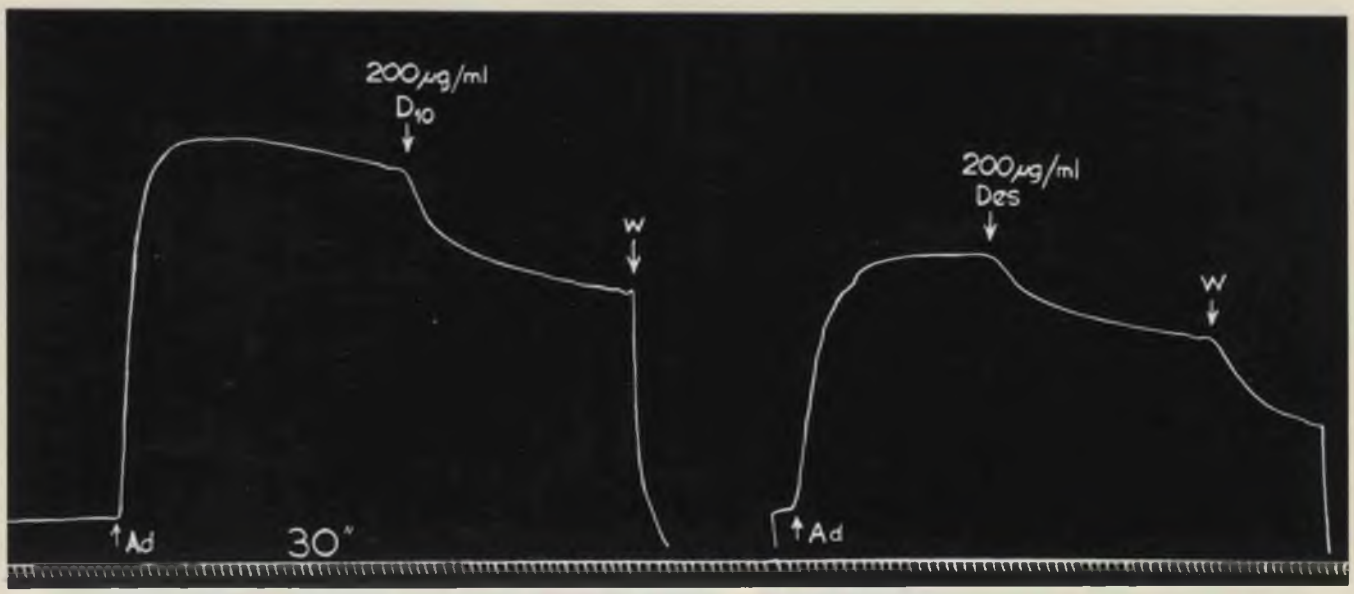

Fig. 48.

Effect of 200 µg. per ml. of 10-methoxydeserpidine (D10) or deserpidine (Des) on the sustained response of a strip of horse carotid artery to adrenaline (Ad).

At Ad, adrenaline 0.5 µg. per ml.

At W, bath washed out.

Time interval (lower trace) = 30 seconds.

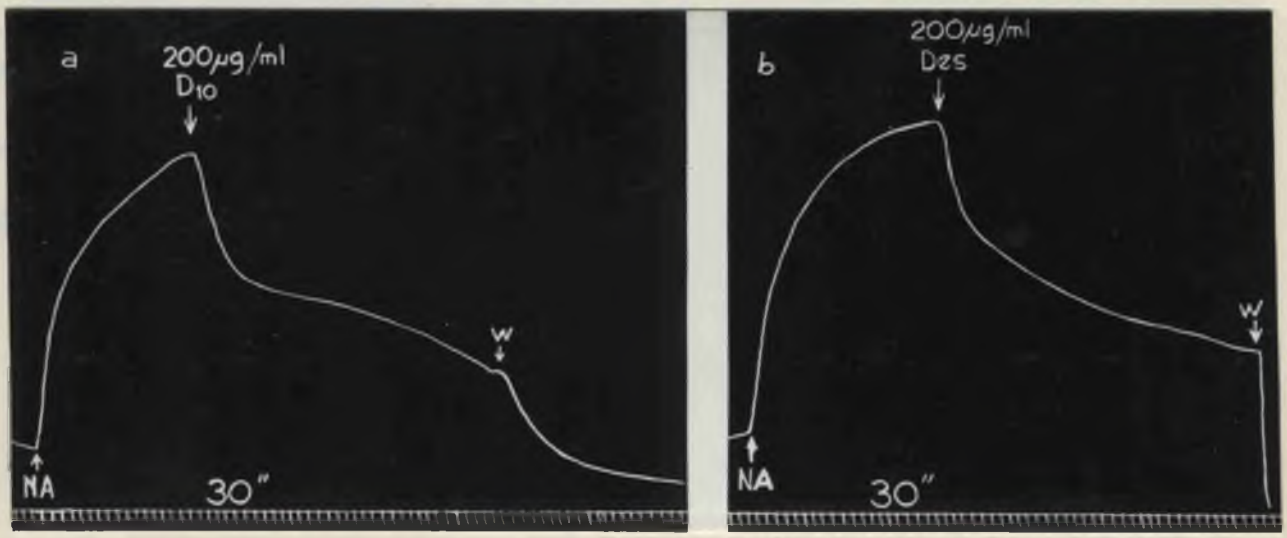

Fig. 49.

Effect of 200 µg. per ml. of (a) 10-methoxydeserpidine (D10) and (b) deserpidine (Des) on the sustained response of a strip of horse carotid artery to noradrenaline (NA).

At NA - Noradrenaline 0.01 µg. per ml.

At W - Bath washed out.

Time interval (lower trace) = 30 seconds.

per ml. of adrenaline and noradrenaline; 0.01 to 0.02 µg. per ml. of acetylcholine, and 1.0 to 5.0 µg. per ml. of 5-hydroxytryptamine was observed. No direct effect was seen with 10-methoxydeserpidine and deserpidine in doses of from 50 to 200 µg. per ml. on the isolated artery strips. Slight reversible inhibition of the responses to adrenaline, noradrenaline, and acetylcholine was noticed with reserpic acid and methyl reserpate in doses of from 0.1 to 0.2 mg. per ml. The control solution had no observable effect on the contractions produced by the stimulant drugs.

Isolated perfused hindquarters of the rat.

When added to the fluid perfusing the isolated hindquarters of the rat, 10-methoxydeserpidine, 0.5 to 2 mg., deserpidine 50 to 200 µg., methyl reserpate 1 to 2 mg., and reserpic acid 1 to 2 mg. did not cause vasodilatation. 10-Methoxydeserpidine, 0.5 to 2 mg. did not antagonise the constrictor responses elicited by adrenaline, 1.0 to 2.0 µg.; noradrenaline, 1.0 to 2.0 µg. (Fig. 50, page 108); barium chloride, 100 to 250 µg. and 5-hydroxytryptamine, 1 to 2 µg. (Fig. 51, page 109). Deserpidine, 50 to 200 µg. produced on the other hand an inhibition of the constrictor responses to adrenaline, 1 to 2 µg. and noradrenaline, 1 to 2 µg. (Fig. 52, page 110). No such effect was observed upon the constrictor responses elicited by barium chloride, 100 to 250 µg. and 5-hydroxytryptamine 1 to 2 µg. The effects of deserpidine were prolonged in duration and irreversible.

Methyl /

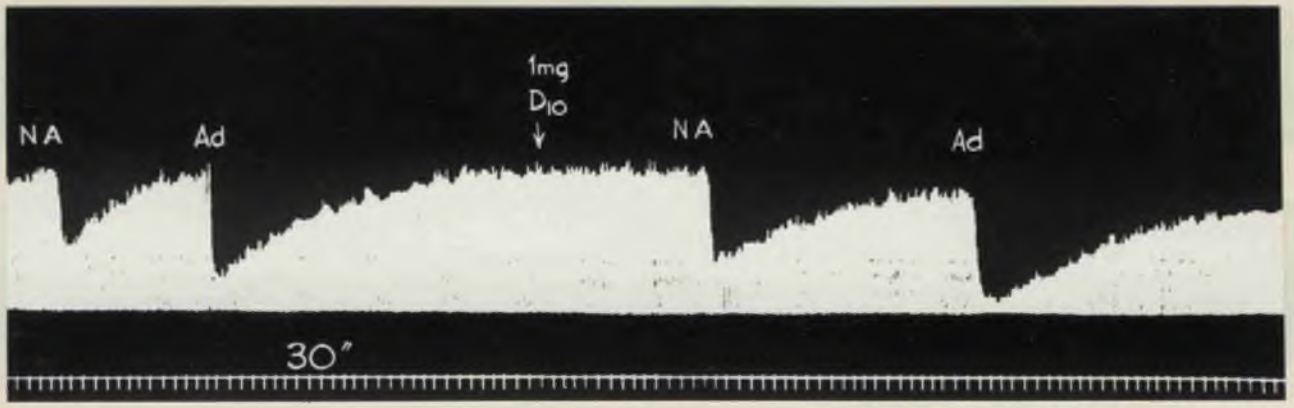

Fig. 50.

Effect of a single dose of 1 mg. of 10-methoxydeserpidine (D10) on the response of the isolated, perfused rat hindquarters to noradrenaline (NA) and adrenaline (Ad).

At NA, 1 µg. of noradrenaline injected into the cannula.

At Ad, 1 µg. of adrenaline injected into the cannula.

Time interval (lower trace) = 30 seconds.

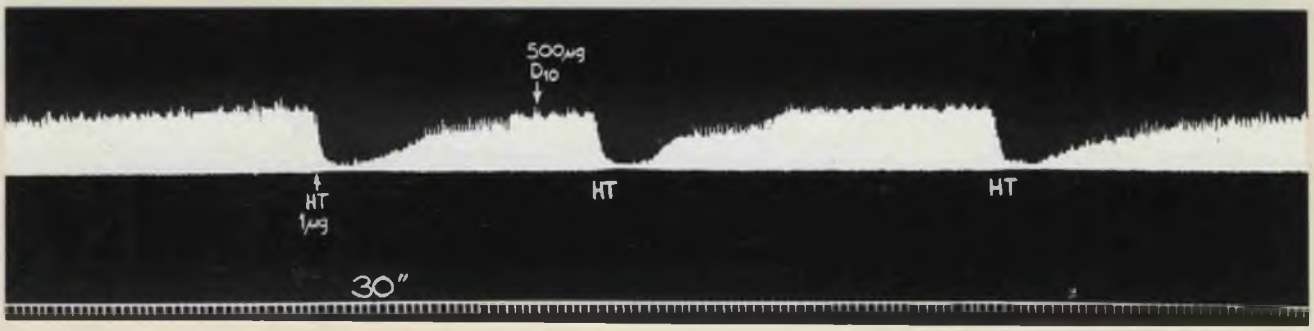

Fig. 51.

Effect of a single dose of 500 µg. of 10-methoxydeserpidine (D10) on the response of the isolated perfused rat hindquarters to 5-hydroxytryptamine (HT).

At HT, 1µg. of 5-hydroxytryptamine injected into the cannula.

Time interval (lower trace) = 30 seconds.

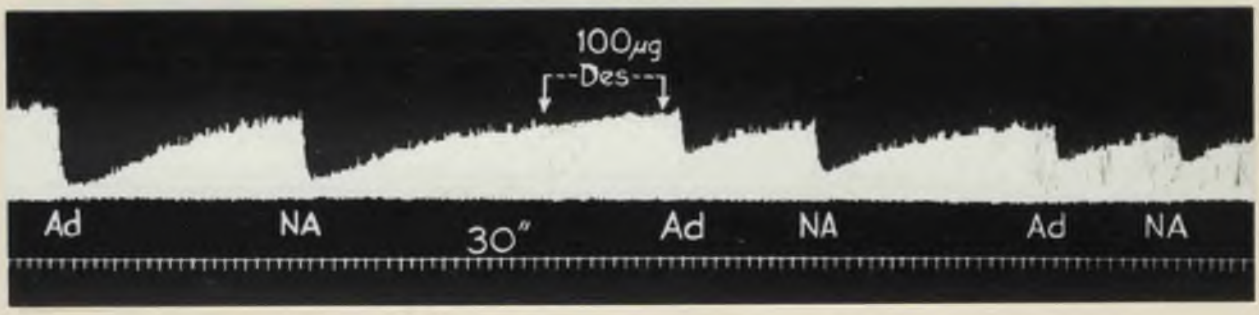

Fig. 52.

The effect of perfusion of the isolated rat hindquarters with a solution of 20 µg. per ml. of deserpidine (Des) for 5 minutes on the response to adrenaline (Ad) and noradrenaline (NA) injected into the cannula.

At Ad, 1 µg. adrenaline injected into the cannula.

At NA, 1 µg. noradrenaline injected into the cannula.

Time interval (lower trace) = 30 seconds.

Methyl reserpate (Fig. 53, page 112) and reserpic acid (Fig. 54, page 113) in dose levels of from 1 to 2 mg. produced a slight but transient inhibition of the responses to 1 to 2 µg. of adrenaline, and noradrenaline. The responses returned to normal levels in about 10 to 15 minutes. No effect was observed on the responses due to 5-hydroxytryptamine, 1 to 2 µg. and barium chloride 100 to 250 µg.

The control solution had no apparent effect upon these responses.

Isolated frog rectus abdominis muscle.

10-Methoxydeserpidine and deserpidine, when used at dose levels of from 80 to 100 µg. per ml. produced a direct stimulant effect upon the isolated frog rectus abdominis muscle. A slow contractural response was observed, the magnitude of which varied from one preparation to the other even when the same dose was used and was not reduced by the previous addition of atropine, 1.0 to 5 µg. per ml. or tubocurarine 5 to 10 µg. per ml. The control solution did not cause any effect.

In the same dose range, deserpidine (Fig. 55, page 114) and 10-methoxydeserpidine (Fig. 56, page 115) antagonised the contractions induced by 1.0 to 2 µg. per ml. of acetylcholine, 2 to 5 µg. per ml. of decamethonium, and 1 to 10 µg. per ml. of nicotine. The contractions of the isolated frog rectus abdominis muscle failed to return to the original level on washing. At small dose levels (10 to 20 µg. per ml.) of deserpidine and 10-methoxydeserpidine, there was no direct stimulant /

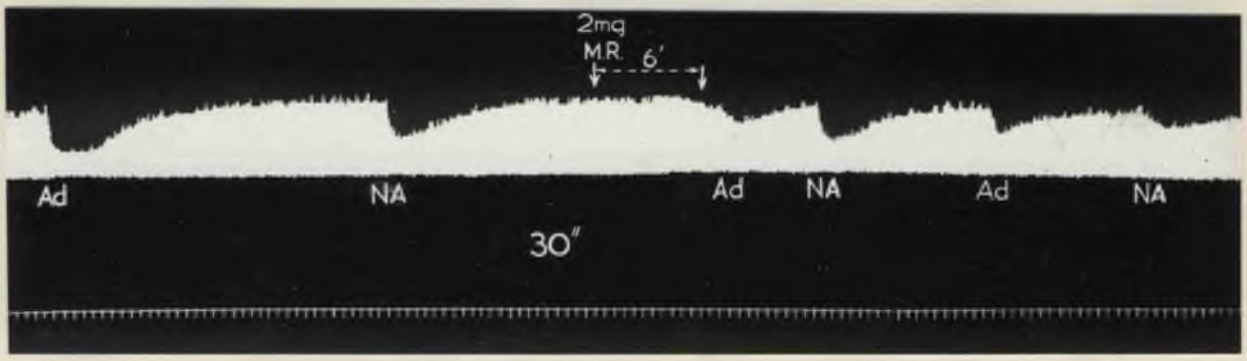

Fig. 53.

Effect of perfusion of the isolated rat hindquarters with a solution of 400 µg. per ml. of methyl reserpate (MR) for 6 minutes on the response to adrenaline (Ad) and noradrenaline (NA).

At Ad, 1 µg. adrenaline injected into the cannula.

At NA, 1 µg. noradrenaline injected into the cannula.

Time interval (lower trace) = 30 seconds.

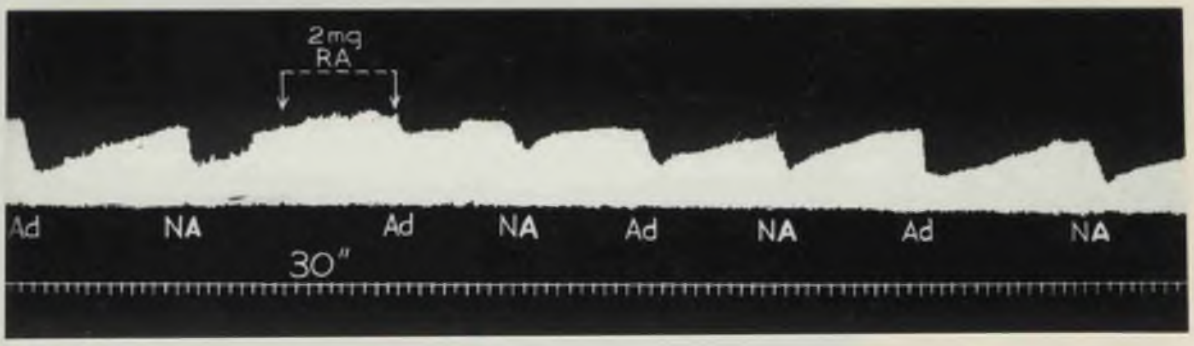

Fig. 54

Effect of perfusion of the isolated rat hindquarters with a solution of 400 µg. per ml. of reserpic acid (RA) for 5 minutes, on the response to adrenaline (Ad) and noradrenaline (NA).

At Ad, 1 µg. adrenaline injected into the cannula.

At NA, 1 µg. noradrenaline injected into the cannula.

Time interval (lower trace) = 30 seconds.

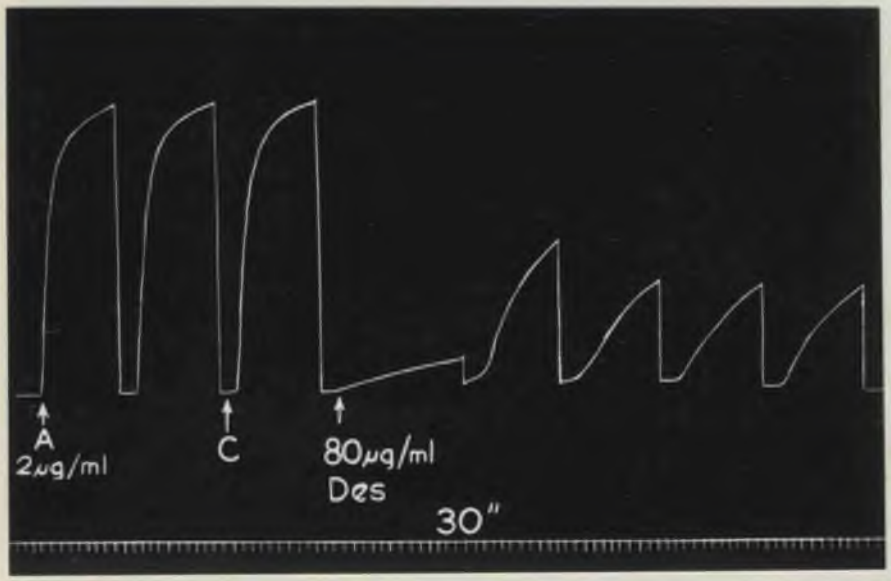

Fig. 55.

Effect of 80 µg. per ml. of deserpidine on the response to acetylcholine (A) of the isolated frog rectus abdominis muscle.

All responses are due to 2 µg. per ml. of acetylcholine (A).

At C, control solution added.

Time interval (lower trace) = 30 seconds.

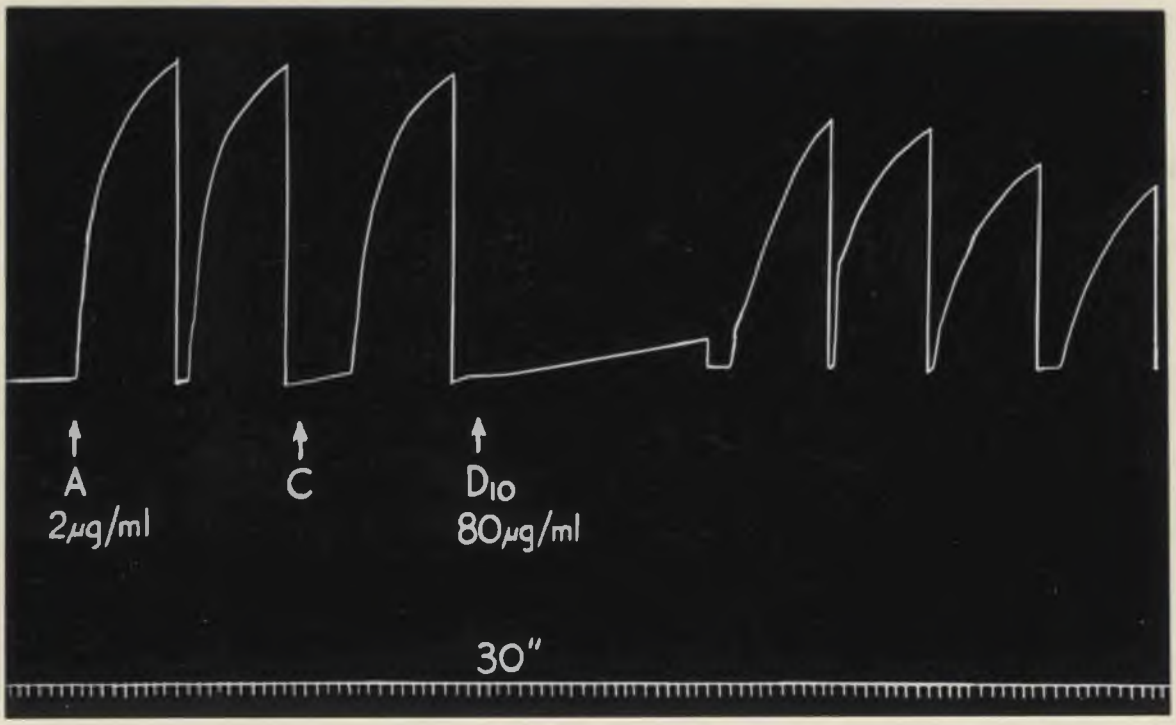

Fig. 56.

Effect of 80 µg. per ml. of 10-methoxydeserpidine (D10) on the response to acetylcholine (A) of the isolated frog rectus abdominis muscle.

All responses are due to 2 µg. per ml. of acetylcholine.

At C, control solution added.

Time interval (lower trace) = 30 seconds.

stimulant effect and no inhibition of the responses to spasmogens was observed.

Isolated guinea pig auricles.

When 10-methoxydeserpidine in a concentration of from 20 to 60 µg. per ml. was added to the bath, no reduction was seen in the rate or amplitude of the contractions of the auricles. In contrast, a marked, but reversible decrease in the rate and amplitude of the contractions of the auricles was noted with doses of from 8 to 16 µg. per ml. of deserpidine. The control solution had no effect. Reserpic acid and methyl reserpate, 10 to 50 µg. per ml. had no depressant effect on the contractions of the auricles.

The increase in the rate and amplitude of the contractions of the auricles induced by adrenaline and noradrenaline, 1 to 2 µg. per ml. was slightly antagonised by 10 to 30 µg. per ml. of 10-methoxydeserpidine (Fig.57, page 117) but the responses returned to normal within a period of 10 minutes. A more marked inhibition of such responses was noted following the addition of 8 to 16 µg. per ml. of deserpidine (Fig. 58, page 118). The responses returned to control levels in from 20 to 30 minutes. Reserpic acid and methyl reserpate, 0.1 to 1 mg. per ml. had a very slight effect upon the responses of the auricles to the addition of 1.0 to 2.0 µg. per ml. of adrenaline and noradrenaline to the bath (Fig.59, page 119). In the same dose range, 10-methoxy-deserpidine, deserpidine (Fig.60, page 120), reserpic acid and methyl reserpate /

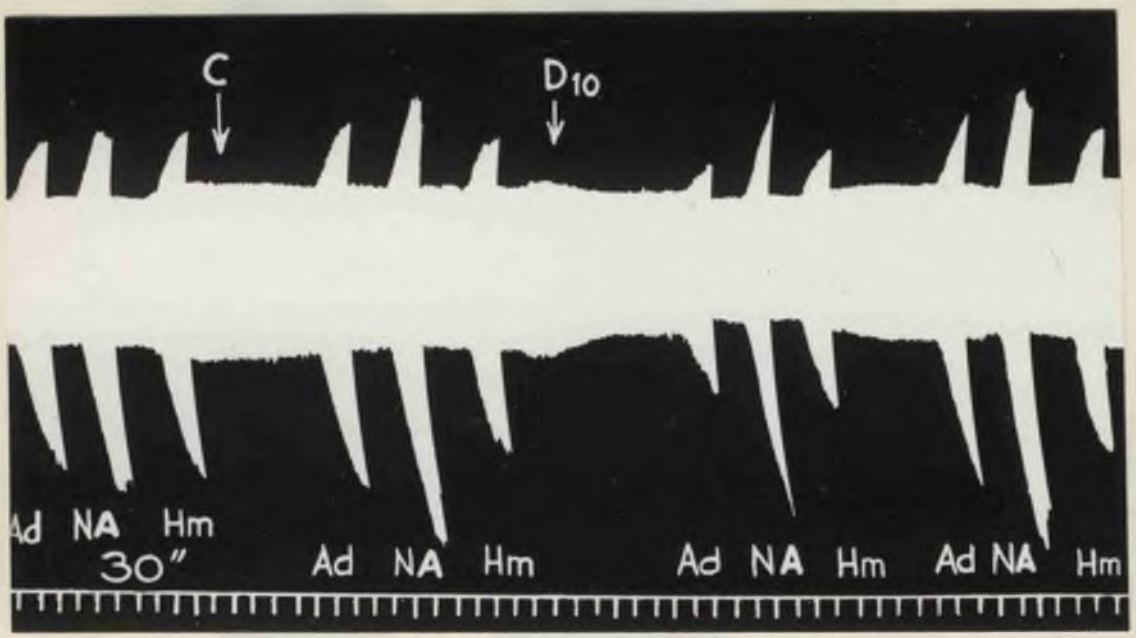

Fig. 57.

Effect of 80 µg. per ml. of 10-methoxydeserpidine (D10) upon the response of the isolated guinea pig auricles to noradrenaline (NA), adrenaline (Ad) and histamine (Hm).

At NA, noradrenaline, 0.1 µg. per ml.
At Ad, adrenaline, 0.1 µg. per ml.
At Hm, histamine, 0.1 µg. per ml.
At C, control solution (0.2 ml.) added to bath.

Time interval (lower trace) = 30 seconds.

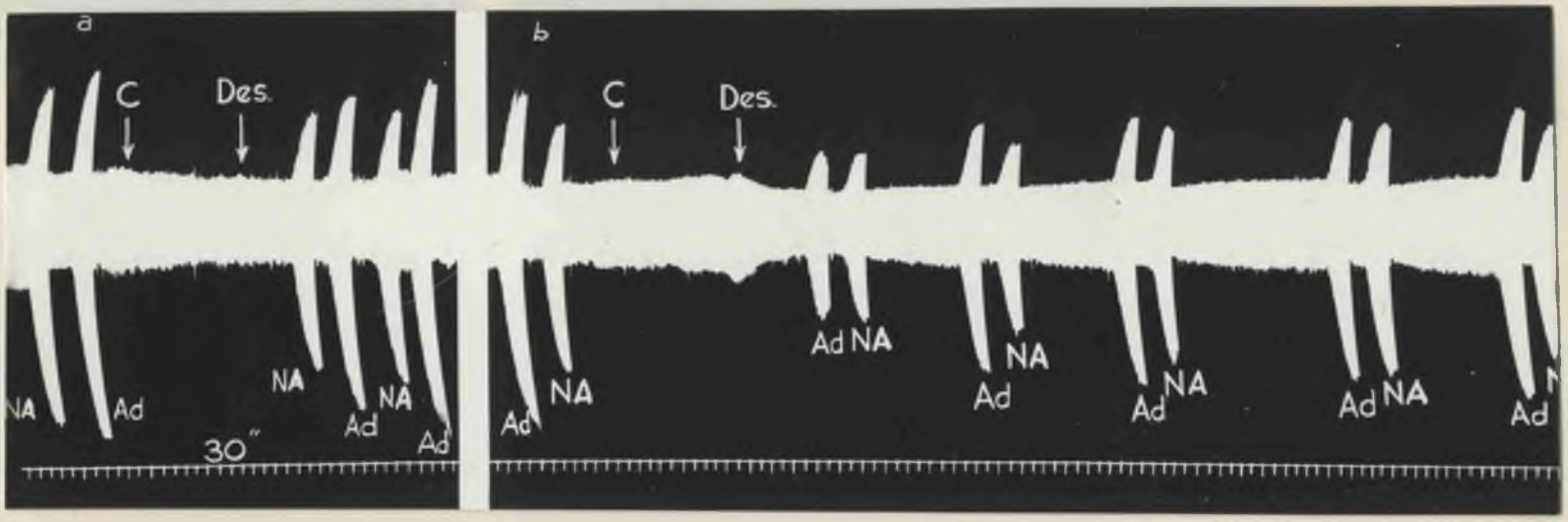

Fig. 58.

Effect of deserpidine on the responses of the isolated guinea pig auricles to alternate administration of adrenaline (Ad) and noradrenaline (NA).

At Ad, adrenaline 0.01 µg. per ml. added to the bath.
At NA, noradrenaline 0.01 µg. per ml. added to the bath.

Tracing a. At C, Control solution added to the bath.
At Des, deserpidine 8 µg. per ml. added to the bath.

Tracing b. At Des, deserpidine 16 µg. per ml. added to the bath.
Time interval (lower trace) = 30 seconds.

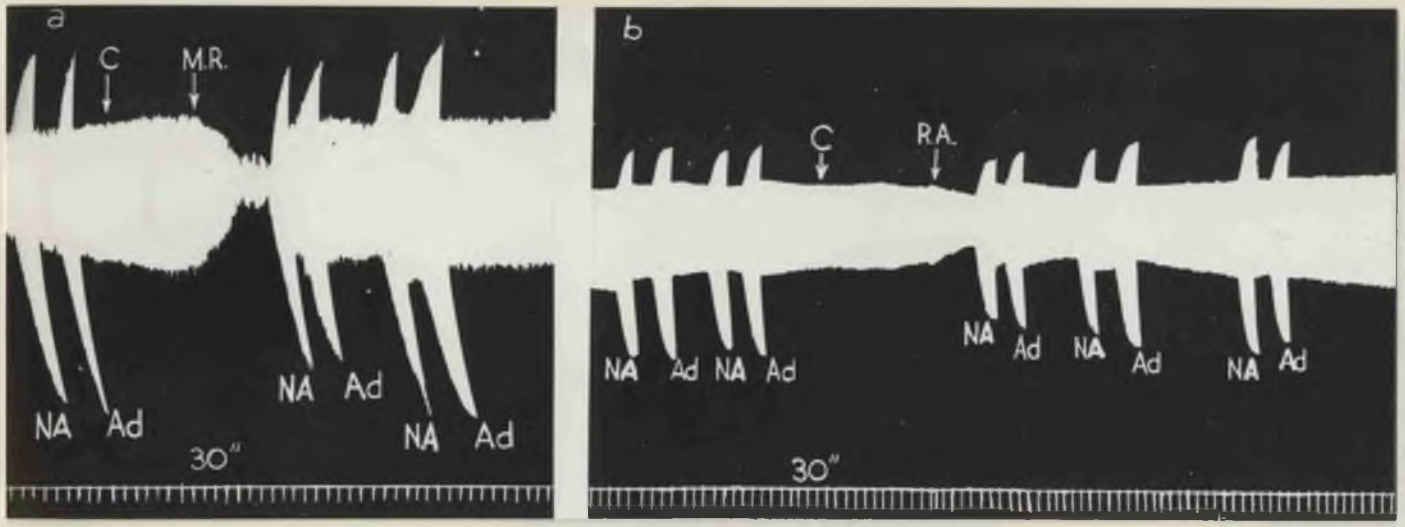

Fig. 59.

Effect of 100 µg. per ml. of (a) methyl reserpate (MR) and (b) reserpic acid (RA) solutions on the responses of the isolated guinea pig auricles to noradrenaline (NA) and adrenaline (Ad).

At Ad, adrenaline 0.1 µg. per ml.

At NA, noradrenaline 0.1 µg. per ml.

At C, control solution (0.2 ml.) added to the bath.

Time interval (lower trace) = 30 seconds.

Fig. 60.

Effect of 10 µg. per ml. of deserpidine (Des) on the response of the isolated guinea pig auricles to acetylcholine.

At Ach, acetylcholine 0.05 µg. per ml. added to the bath.

At W, bath washed out.

Time interval (lower trace) = 30 seconds.

reserpate did not modify the depressor effects of 0.1 to 0.5 µg. per ml. of acetylcholine.

Isolated perfused rabbit heart.

When 18 to 50 µg. of 10-methoxydeserpidine (Fig. 61, page 122) or 10 to 50 µg. of deserpidine (Fig. 62, page 123) were injected into the cannula, a sharp, progressive and irreversible decrease in the rate and amplitude of the contractions of the heart was produced. This effect on myocardial activity was accompanied by a significant increase in the outflow from the heart. When given at the same dose levels, the control solutions had qualitatively similar but quantitatively much weaker actions. Reserpic acid 0.125 to 0.5 mg. per ml. and methyl reserpate 20 to 80 µg. per ml. (Fig. 63, page 124) when perfused, produced a transient but sharp decrease in the rate and amplitude together with a small increase in the outflow from the heart.

Ganglion-blocking activity.

No direct effect upon the nictitating membrane of the anaesthetised cat was observed following the intravenous administration of 4 to 6 mg. per kg. of 10-methoxydeserpidine (Fig. 64, page 125) or 1 to 2 mg. per kg. of deserpidine (Fig. 65, page 126). In the same dose range, these drugs did not antagonise the contractions induced by stimulation of the cervical sympathetic.

10-Methoxydeserpidine 2 to 4 mg. per kg. potentiated the responses of /

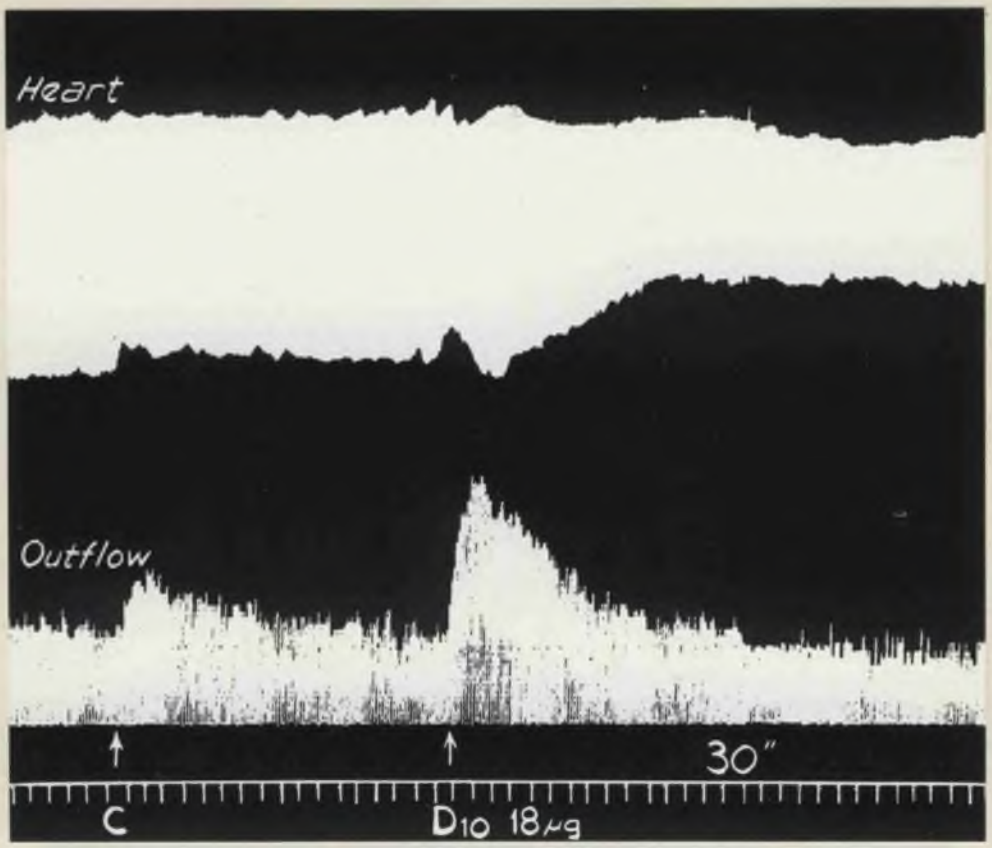

Fig. 61.

Effect of a single dose of 18 µg. of 10-methoxydeserpidine (D10) upon the amplitude, (upper record) and outflow (lower record) of the isolated perfused rabbit heart.

At C, 0.1 ml. of control solution injected into the cannula supporting the heart.

Time interval (lower trace) = 30 seconds.

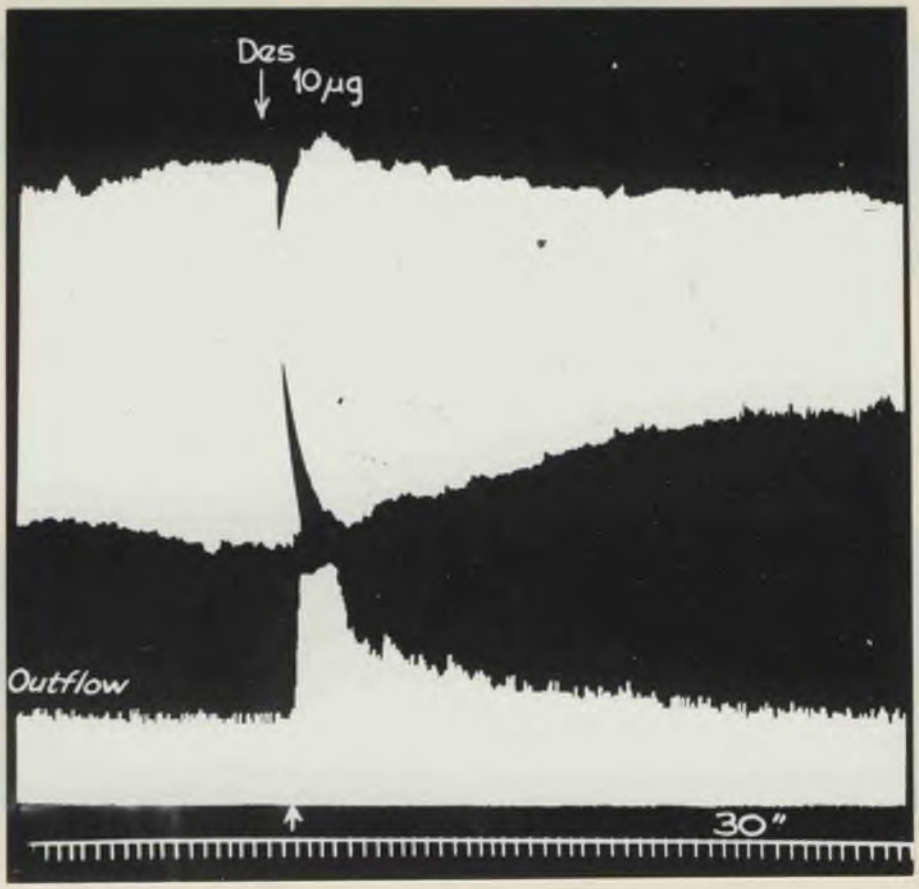

Fig. 62.

Effect of a single dose of 10 µg. of deserpidine (Des) on the amplitude, (upper record) and outflow (middle record) of the isolated, perfused rabbit heart.

Time interval (lowest trace) = 30 seconds.

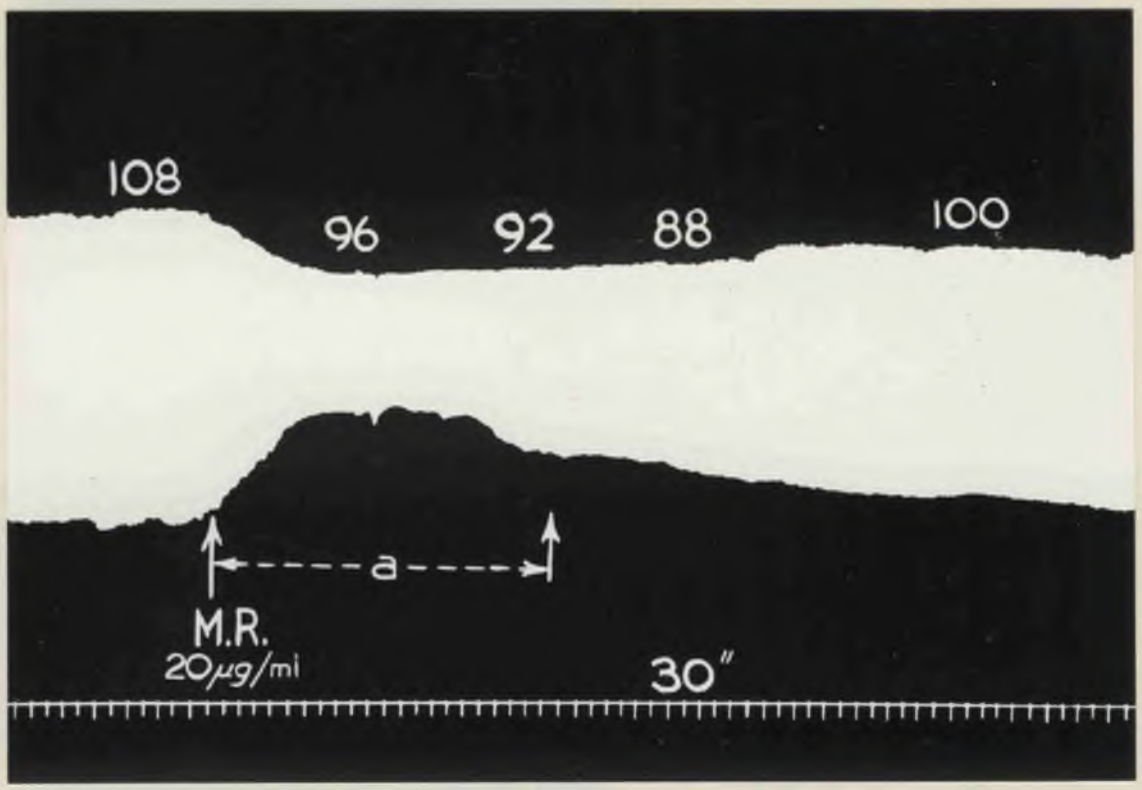

Fig. 63.

Effect of perfusion for 10 minutes of 20 µg. per ml. of methyl reserpate (MR) solution on the rate and amplitude of the beat of the isolated, perfused rabbit heart. Perfusion of the drug solution took place between the vertical arrows.

Numbers above the upper record indicate the heart rate at that time.

Time interval (lower trace) = 30 seconds.

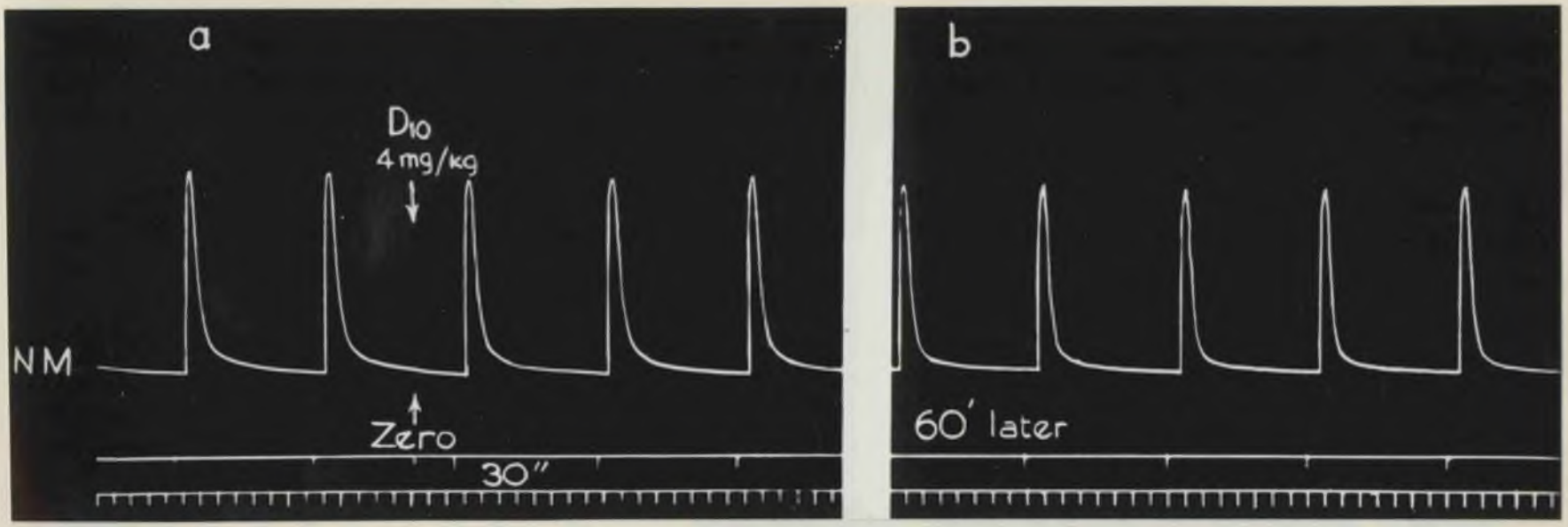

Fig. 64.

Tracing a. Effect of 4 mg. per kg. of 10-methoxydeserpidine (D10) upon the contractions of the nictitating membrane of the pentobarbitone-anaesthetised cat to stimulation of the preganglionic fibres of the cervical sympathetic (10 volts, pulse width 1 m. sec. at a frequency of 1,000 impulses per minute for 10 seconds).

Tracing b. 60 minutes after the drug injection.

Time interval (lower trace) = 30 seconds.

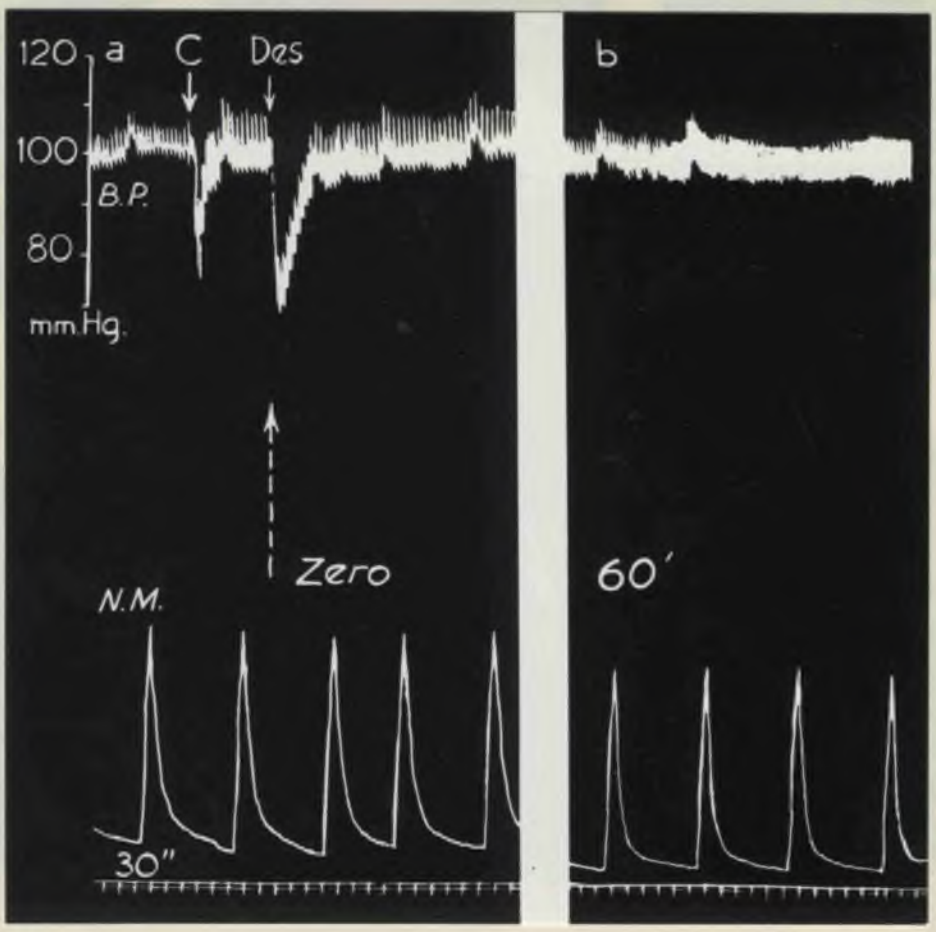

Fig. 65.

Tracing a. Effect of 2 mg. per kg. of deserpidine (Des) on the responses of the arterial blood pressure (upper record) and contractions of the nictitating membrane (lower record) of the pentobarbitone-anaesthetised cat to stimulation of the preganglionic fibres of the cervical sympathetic (10 volts, pulse width 1.5 m.sec at a frequency of 1,000 impulses per minute for 10 seconds).
Blood pressure recorded from the femoral artery.

Tracing b. 60 minutes after the drug injection.
Time interval (lowest trace) = 30 seconds.

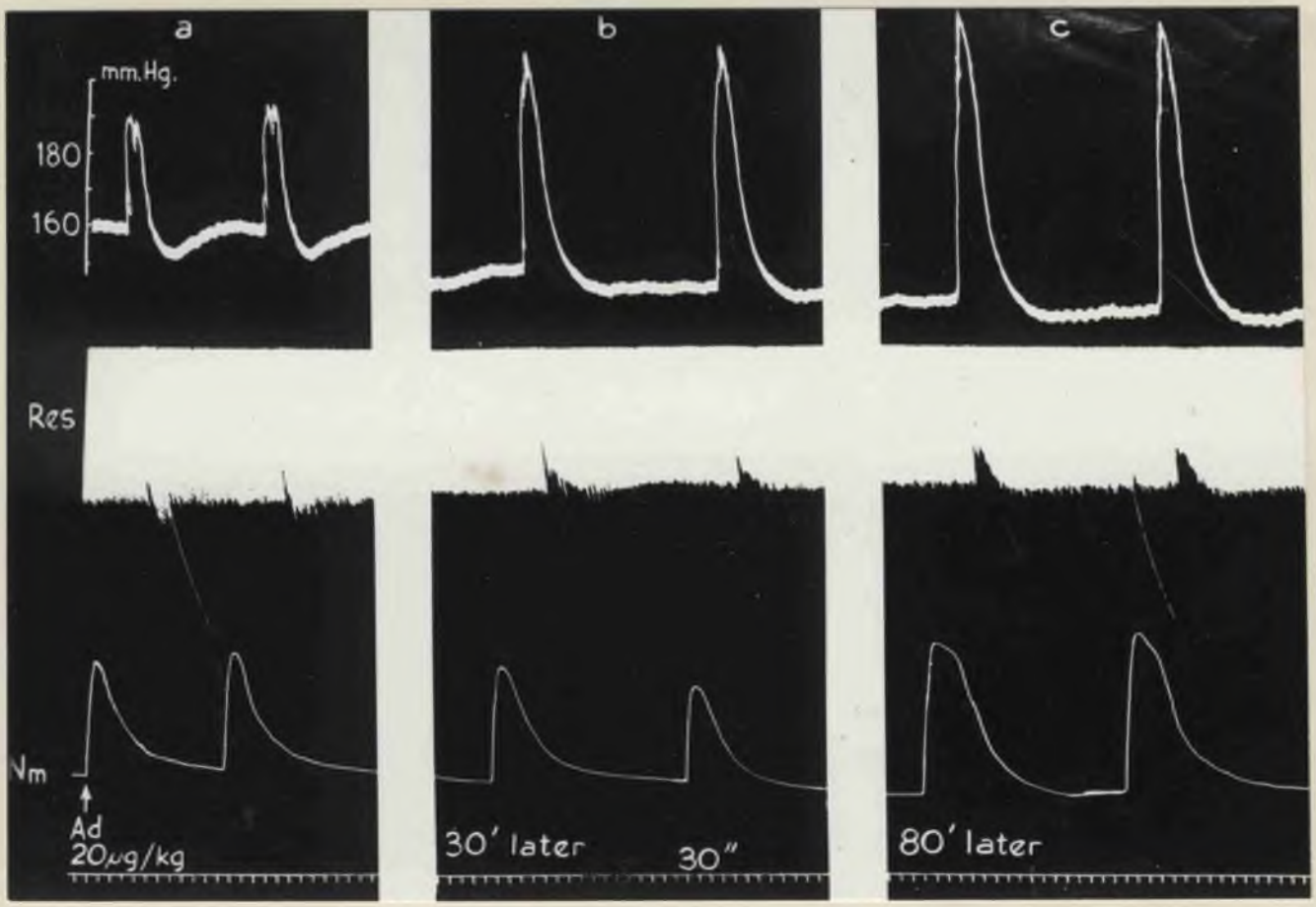

Fig. 66.

Tracing a. Effect of single dose of 4.0 mg. per kg. of 10-methoxy-deserpidine on the response of blood pressure to injection of adrenaline (upper record), on respiration (middle record) and on the response of nictitating membrane to adrenaline (Ad) (lower record).
All responses are due to 20 µg. per kg. of adrenaline (Ad).
10-Methoxydeserpidine was given as a single intravenous dose between (a) and (b).

Tracing b. 30 minutes after the injection of the drug.

Tracing c. 80 minutes after the injection of the drug.

All drugs given intravenously.

Time interval (lowest trace) = 30 seconds.

of the nictitating membrane to injections of 10 to 20 µg. per kg. of adrenaline (Fig. 66, page 127). Reserpic acid and methyl reserpate in doses of the order of 5 to 10 mg. per kg. neither produced any direct effect, nor modified the contractions of the nictitating membrane induced by stimulation of the cervical sympathetic.

Tranquillizing and central nervous system depressant effects.

Effects of 10-methoxydeserpidine and deserpidine on the motility of mice and rats.

When given intraperitoneally, 10-methoxydeserpidine (5 to 40 mg. per kg.) did not produce any observable change in the motor activity of rats or mice. On the other hand, deserpidine in much smaller doses (0.05 to 5 mg. per kg.), produced a marked reduction in motility. In mice, high intraperitoneal doses (40 to 80 mg. per kg.) of 10-methoxy-deserpidine caused a reduction in the motor activity but even at these dose levels, it was much less active than deserpidine or reserpine.

Effects of 10-methoxydeserpidine and deserpidine on gastro-intestinal activity in rats and mice.

Reserpine has been shown to cause increased gastro-intestinal activity in a number of animal species, including the dog, rabbit, rat and mouse.

When given intraperitoneally in the dose range of from 5 to 40 mg. per kg., 10-methoxydeserpidine did not produce diarrhoea or loose stools /

stools in rats or mice whereas in the same species deserpidine in much smaller doses (0.05 to 5 mg. per kg.) produced loose stools.

Effect of 10-methoxydeserpidine on the ptotic response in mice.

10-Methoxydeserpidine in the dose range of from 5 to 60 mg. per kg. did not produce ptosis in mice. On the other hand, deserpidine has been shown to produce marked ptosis in mice (Packman, Abbott and Harrisson, 1956). The absence of the ptotic response in rats and mice and the lack of interference with the motor activity of the animals when 10-methoxydeserpidine was given, indicated an absence of the type of central nervous system depressant activity associated with reserpine-like drugs.

Effects of 10-methoxydeserpidine on pentobarbitone-induced sleep in mice.

10-Methoxydeserpidine at a dose level of 40 mg. per kg. produced an increase of border-line significance in the duration of pentobarbitone-induced sleep ($P = 0.1$). Reserpine and deserpidine in dose levels of 2.5 mg. per kg. produced a significant increase in the sleeping time of mice ($P = 0.05 < 0.1$) (Table 1, page 131).

Prolongation of the sleeping time as compared to the control group, was taken as an indication of an increase in the sedative activity of the drug.

Acute toxicity in mice.

Approximate /

Approximate median lethal dose.

The median lethal dose (LD 50) in mice following the intraperitoneal injection of 10-methoxydeserpidine was found to be 82 ± 2.6 mg. per kg. (Table 2, page 132). The dose sufficient to kill fifty per cent of the drug-injected animals within three hours was considered to be the approximate median lethal dose (LD 50). The intraperitoneal injection of from 40 to 180 mg. per kg. of 10-methoxydeserpidine to mice was followed by a small period of excitation, after which the animals became sluggish and cyanosed. The cyanosis was first noted in the tail and then in the ears and the face. Cyanosis was accompanied by laboured breathing, followed by cessation of respiration and death. The heart continued to beat for some time after the respiration had failed. The results are given in Table 2, page 132 and are shown in Fig.67, page 133.

Table 1. Effect of 10-methoxydeserpidine upon Barbiturate Sleeping Time in Groups of 20 Mice.

Dose (mg. per kg.)	Sleeping Time ± S.D. (mins.)	P.
Control	53 ± 25	-
10	40 ± 28	$< 0.2 > 0.1$
Control	52 ± 23.4	-
20	65 ± 30	$< 0.2 > 0.1$
Control	51 ± 18	-
40	64 ± 27.7	0.1
Control	71 ± 17.28	-
Reserpine 2.5 mg./kg.	105.5 ± 45	$> 0.05 < 0.1$

Table 2. Acute toxity (Median lethal dose in 3 hours (LD 50))

Dose mg. per kg.	percentage mortality	Death of mice in groups of 10 each	Probit	LD 50	
60	0%	$\frac{0}{10} + \frac{0}{10}$	0	83 mg. per kg. intraperitoneal	120 male mice 15-18 g.
70	20%	$\frac{2}{10} + \frac{2}{10}$	4.1584		
75	45%	$\frac{6}{10} + \frac{3}{10}$	4.8743		
90	55%	$\frac{7}{10} + \frac{4}{10}$	5.1257		
105	85%	$\frac{8}{10} + \frac{9}{10}$	6.0364		
120	90%	$\frac{9}{10} + \frac{9}{10}$	6.2816		
40	0%	$\frac{0}{10} + \frac{0}{10}$	0	72 mg. per kg. intraperitoneal	140 male mice 15-18 g.
60	10%	$\frac{1}{10} + \frac{1}{10}$	3.71		
70	70%	$\frac{6}{10} + \frac{8}{10}$	5.52		
80	80%	$\frac{8}{20} + \frac{8}{20}$	5.84		
90	90%	$\frac{9}{10} + \frac{8}{10}$	6.28		
120 180	100%	$\frac{10}{10} + \frac{10}{10}$	6.88 0		
60	10%	$\frac{1}{10} + \frac{1}{10}$	3.718	90 mg. per kg. intraperitoneal	100 male mice 18-20 g.
80	30%	$\frac{2}{10} + \frac{4}{10}$	4.47		
90	50%	$\frac{4}{10} + \frac{6}{10}$	5.00		
100	50%	$\frac{5}{10} + \frac{5}{10}$	5.00		
150	100%	$\frac{10}{10} + \frac{10}{10}$	8.7		

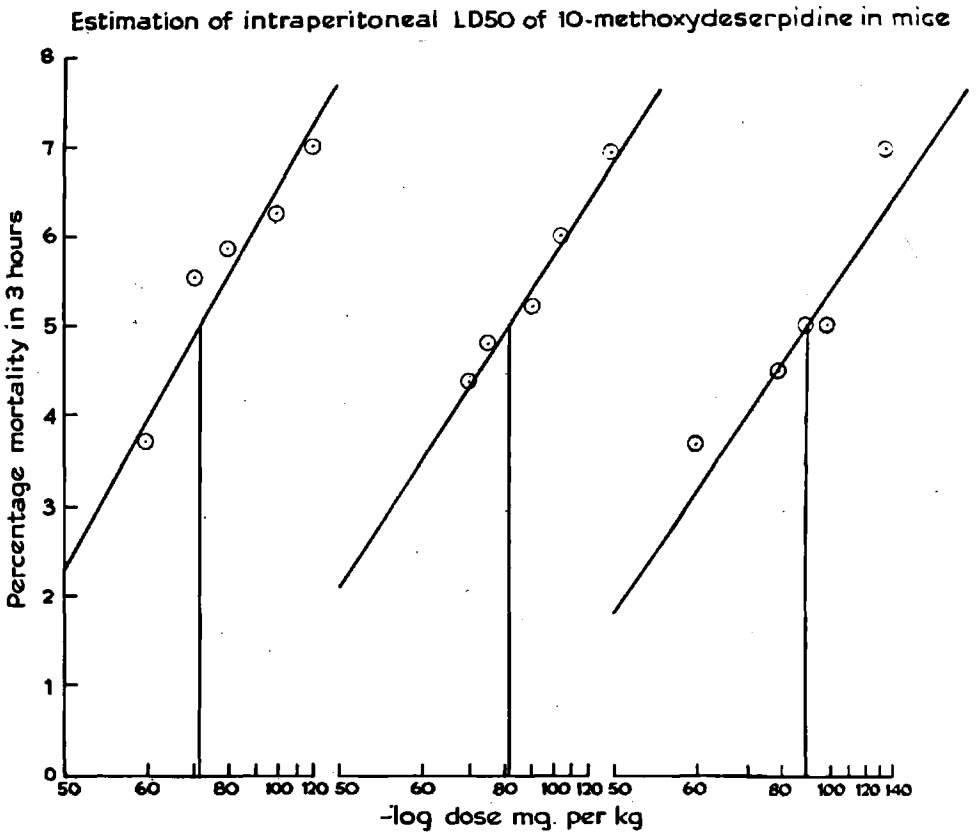
Estimation of intraperitoneal LD50 of 10-methoxydeserpidine in mice
Percentage mortality in 3 hours
0
1
2
3
4
5
6
7
8
50
60
80
100
120
50
60
80
100
120
50
60
80
100
120
140
-log dose mg. per kg

Fig. 67.

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C H A P T E R IV.

CHAPTER IV

DISCUSSION

Deserpidine has been shown to possess pharmacological properties qualitatively identical with those of reserpine (Schneider, Plummer, Earl, Barrett, Rinehart and Dibble, 1955). Reserpine exhibits a complex pattern of activity. Its antihypertensive and sedative actions are regarded as being mainly due to effects on the central nervous system, but there are some which are peripheral in nature (Bein, Gross, Tripod, and Meier, 1953, and Plummer, Earl, Schneider, Trapold and Barrett, 1954). Characteristic features of the action of reserpine are its typical sedative and hypnotic properties which differ from those of other central nervous system depressants such as the barbiturates. Following the administration of reserpine there is observed a fall in the arterial blood pressure level, accompanied by bradycardia, some respiratory inhibition, increased gastrointestinal motility, miosis, ptosis, relaxation of the nictitating membrane and a fall of body temperature.

10-Methoxydeserpidine has antihypertensive actions, qualitatively very similar to those of reserpine and deserpidine, but does not cause ptosis, sedation or increased gastrointestinal activity. To approach the mechanism by which 10-methoxydeserpidine produces a fall in the blood pressure, an account of the pharmacological actions of this compound /

compound and of deserpidine will be given.

10-Methoxydeserpidine and deserpidine both produce a slow, and delayed fall in the arterial blood pressure of the normotensive, anaesthetised cat and rat. Deserpidine is, however, more potent in its hypotensive actions in the rat than 10-methoxydeserpidine. In the cat, hypotension, following the administration of both deserpidine and 10-methoxydeserpidine, is associated with bradycardia. These observations are in agreement with those of Peterfalvi and Jequier, (1960); La Barre, (1960); Slater, Rathbun, Henderson and Neuss, (1955) and Schneider, Plummer, Earl, Barrett, Rinehart and Dibble, (1955).

The characteristic latency in the onset of action of reserpine (Bein, 1953) was also noted in the cat and rat following the use of 10-methoxydeserpidine and deserpidine.

Methyl reserpate and reserpic acid had little effect on the blood pressure level of the cat and rat except when doses 5 to 10 times greater than those of deserpidine were used. These observations support the views of earlier workers, that removal of the ester group caused almost complete loss of the typical reserpine-like hypotensive and sedative actions (Plummer, Barrett and Rutledge, 1954 and Bein, 1956). 10-Methoxydeserpidine and deserpidine both potentiated the hypertensive responses to adrenaline and noradrenaline. The augmented response of the nictitating membrane to adrenaline following the administration /

administration of 10-methoxydeserpidine noted by the author of this thesis, does not confirm the observations of Peterfalvi and Jequier, (1960).

The observations that deserpidine and 10-methoxydeserpidine did not inhibit contractions of the nictitating membrane elicited either by adrenaline or by electrical stimulation of the preganglionic fibres of the cervical sympathetic is an indication that these drugs do not reduce the blood pressure level by a depressant action upon sympathetic ganglia.

The response to bilateral carotid artery occlusion was also reduced by 10-methoxydeserpidine. This effect was not, however, abolished or reversed as claimed by Peterfalvi and Jequier (1960). Deserpidine markedly depressed the pressor response to bilateral occlusion of the common carotid arteries.

The reflex rise in the blood pressure following occlusion of the abdominal aorta or stimulation of the central end of the greater splanchnic nerve was not affected by 10-methoxydeserpidine; deserpidine, on the other hand, caused a prolonged reduction of both reflexes. Bein (1953), using reserpine, observed similar effects in cats anaesthetised with dial-urethane, but in contrast to deserpidine, and like 10-methoxydeserpidine, reserpine did not cause inhibition of the pressor response to stimulation of the afferent splanchnic nerves. Bein (1953) considered that reserpine acted upon /

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. The pressor response to the stimulation of the cut central end of the vagus was abolished or reversed following the administration of deserpidine and 10-methoxy-deserpidine.

Bein (1955) using reserpine, made a detailed analysis of the effects of reserpine upon centrally mediated reflex activity in the cat and concluded that the actions of reserpine were mainly central in origin. Following the intravenous administration of from 10 to 20 µg. per kg. of reserpine to the anaesthetised cat, the carotid sinus pressor reflex was depressed but the activity of the carotid sinus pressor-receptors was not inhibited. When the brain stem was sectioned at a point just lower than the inferior corpora quadrigemina, these small doses of reserpine were ineffective. The reflex responses which had been inhibited with reserpine returned to normal levels after the brain stem was sectioned. He pointed out that a physiological contact between the medulla oblongata and the mid-brain was essential for the characteristic effects of reserpine. When the brain stem was severed, much larger doses of reserpine were required to inhibit the pressor reflex than when it was intact. Bein (1955) suggested that reserpine may act on central regulatory mechanisms which integrate autonomic and somatic functions. The similarity in the effects of deserpidine /

deserpidine and 10-methoxydeserpidine on the blood pressure and vasomotor reflexes of the cat may indicate that in some respects 10-methoxydeserpidine has a qualitatively similar mechanism of action to deserpidine or reserpine but it is puzzling to find that they resemble one another in this respect only.

Depression of the rhythmic activity of preparations of isolated cardiac muscle was observed following the use of deserpidine and 10-methoxydeserpidine. The rate and amplitude of the contractions of the isolated rabbit heart were decreased and cardiac outflow was markedly increased. In these preparations the drug seems to act directly upon the cardiac muscle. On the other hand, the characteristic stimulant action of adrenaline and noradrenaline on the isolated heart was slightly reduced following the use of deserpidine whereas 10-methoxydeserpidine had no effect. A slight depression of the spontaneous activity of the isolated auricles was noted. The responses of the auricles to adrenaline and noradrenaline were slightly depressed whereas the depressant effects of acetylcholine were unaltered.

No vasodilator effect was observed in the isolated rat hindquarters preparation following perfusion by deserpidine or 10-methoxydeserpidine. McQueen and Blackman (1955) using deserpidine, however, observed a vasodilatation in the perfused rat hindquarters in which vasomotor tone had been increased by infusion of noradrenaline, vasopressin or barium chloride.

Deserpidine /

Deserpidine markedly depressed the vasoconstrictor responses to injections of adrenaline and noradrenaline, but had no observable effect upon those due to injections of 5-hydroxytryptamine. 10-Methoxydeserpidine did not modify any of the vasoconstrictor responses to adrenaline, noradrenaline or 5-hydroxytryptamine in the isolated perfused hindquarters of the rat. Methyl reserpate and reserpic acid both reduced these responses reversibly but only when doses about 10 to 20 times larger than those of deserpidine were used.

In experiments performed using isolated strips of horse carotid artery, deserpidine and 10-methoxydeserpidine caused no direct relaxant effect but produced relaxation of the contractions of artery strips which had been elicited by adrenaline, noradrenaline, 5-hydroxytryptamine and acetylcholine. Prior addition of deserpidine and 10-methoxydeserpidine also reduced the stimulant responses to these drugs.

In the isolated guinea pig ileum, 10-methoxydeserpidine and deserpidine antagonized the stimulant action of acetylcholine, histamine, barium chloride and 5-hydroxytryptamine. The inhibition of the stimulant responses of the isolated guinea pig ileum was also observed with methyl reserpate and reserpic acid but the effect was very shortlived. A slow contraction of the frog rectus abdominis muscle was seen following the use of 10-methoxydeserpidine and deserpidine. This effect was followed by a reduction in the magnitude of acetylcholine-induced /

induced contractions of the isolated rectus abdominis muscle, which was irreversible in nature, showing that a disturbance in the cellular activity of the muscle had taken place. Gillis and Lewis (1956), using reserpine, noted a similar effect on the isolated frog rectus abdominis muscle but the doses used were much smaller (10 µg. per ml.) than those used in investigation of deserpidine and 10-methoxydeserpidine. The isolated tissue preparations used in this investigation differ widely in their sources, structure and properties. From the data presented above, certain characteristic properties of the reserpine-like hypotensive agents on isolated tissue preparations and intact animals can be demonstrated. These actions are the general depressant effects on the increased tone and motility of preparations of smooth or skeletal muscle produced by the use of stimulant drugs and the non-specific nature of these antagonistic effects.

10-Methoxydeserpidine seems to lack the characteristic sedative and tranquillizing effects of reserpine and deserpidine. When used in doses 10 to 30 times larger than deserpidine, 10-methoxydeserpidine did not produce sedation or inertia in mice or rats; this is evidence of the lack of a typical feature associated with reserpine-like central nervous system depressants. These observations confirm the results of Peterfalvi and Jequier (1960) but do not agree with those of La Barre (1960) who presented evidence of tranquillizing activity in rats following the intramuscular injection of 4 to 6 mg. per kg. of 10-methoxydeserpidine. /

methoxydeserpidine.

Unlike reserpine and deserpidine, gastrointestinal activity was not increased in rats or mice following intraperitoneal injection of 10-methoxydeserpidine, yet a reserpine-like inhibition of the rhythmic contractions of the isolated rabbit duodenum was observed. This observation may indicate that there is a difference in the mechanism by which 10-methoxydeserpidine acts upon the intact intestine in situ from that which is involved in its action upon isolated intestinal tissue. The lack of reserpine-like sedative and tranquillizing actions which is associated with 10-methoxydeserpidine may be attributed to properties conferred on the molecule by the change in the position of the methoxy group from position 11 to position 10 in ring A. More marked central activity is found in reserpine and deserpidine. In the former there is a 11-methoxy group which is absent from the latter. This may mean that the 10-methoxy group in 10-methoxydeserpidine either hinders the fit of the molecule on to the central receptor sites or perhaps prevents the drug from reaching them. The hypotensive actions of reserpine are thought by some to be due to effects upon stores of catechol amines in the heart, arteries and at various other peripheral sympathetic neuro-effector sites. The fact that the antihypertensive actions of reserpine are linked with an action upon peripheral stores of noradrenaline is suggested by the observations of Burn and Rand (1958). On the other hand, 10-methoxy-deserpidine /

deserpidine has hypotensive actions but no demonstrable sedative effects, yet does not deplete stores of catechol amines in the brain or heart (Leroy and Schaepdryver, 1961). It is not possible to suggest a definite site or mechanism of action which can account for all of the effects of 10-methoxydeserpidine observed in the intact animal and in isolated tissues. While 10-methoxydeserpidine has no marked effects upon the central nervous system, it appears from a study of its effects upon isolated tissue preparations that it may have a peripheral site of action.

Many of the observations may, however, be explained by assuming that 10-methoxydeserpidine, like reserpine or deserpidine, acts by depressing the ability of the smooth muscle to contract. This implies that a process common to contraction - whether myogenic or drug-induced - is influenced by the drug. From the data presented it is felt that these non-specific effects of 10-methoxydeserpidine are due to an interference with metabolic processes in the normal cell.

The experimental work to be described in the next chapter deals with attempts to investigate in more detail the possibility of a site of action of 10-methoxydeserpidine at cellular level. 10-Methoxy-deserpidine may lower the level of the labile high energy phosphate compounds present in different tissues. Interference at the cellular level may also be reflected in the oxygen uptake of various tissues in the presence of 10-methoxydeserpidine. It may, for example, inhibit tissue /

tissue respiration, as does cyanide, or it may stimulate it, as does 2,4-dinitrophenol. In the next chapter the in vitro effects of 10-methoxydeserpidine on the respiration, ATP-ase activity and anaerobic glycolysis of rat brain, liver and skeletal muscle are described.

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C H A P T E R V.

Biochemical investigations.

CHAPTER V

Biochemical Investigations

1. Determination of the effects of 10-methoxydeserpidine on tissue respiration

Method

The experiments were carried out by Warburg's direct method (Warburg, 1930).

Male rats weighing from 175 to 200 g. were used. The animals were killed by a blow on the back of the neck. The brain and liver were rapidly removed and transferred to ice-cold oxygenated Krebs-Ringer phosphate solution (Appendix I, page 452). Tissue slices of 0.26 mm. thickness were prepared using a McIlwain tissue slicer. One hundred mg. wet weight of tissue slices were added to 2.5 ml. of Krebs-Ringer phosphate solution contained in the main chamber of the flask. The centre well of the flask contained 0.2 ml. of 20 per cent $^{W}/_{V}$ potassium hydroxide solution. In the side arm of the flask was placed 0.3 ml. of drug solution, control solution or distilled water. After equilibration for 10 minutes at $37^{\circ} \pm 0.5^{\circ}C.$, the contents of the side arm were tipped. Readings were taken at ten minute intervals for one hour. An atmosphere of air was used and the rate of shaking the flasks was 105 per minute. The total volume in the flask was 3 ml. 10-methoxydeserpidine at dose levels /

levels of 100 and 200µg. per ml. were used for both liver and brain tissues.

(a) Potassium stimulated respiration in rat liver and brain slices

Method

The procedure adopted was the same as that described above, with the addition to the contents of the main chamber of 0.1 ml. of 0.1 molar potassium chloride solution (Ashford and Dixon, 1935).

2. Determination of the effects of 10-methoxydeserpidine on the adenosinetriphosphatase activity in rat brain, liver and skeletal muscle.

Method

Adenosinetriphosphatase (ATP-ase) activity was measured by the method of Lardy and Wellman (1953). The amount of inorganic phosphate liberated from adenosinetriphosphate (ATP) in the presence of a suspension of brain, liver or skeletal muscle was used to measure the ATP-ase activity. Inorganic phosphate was measured by the method of Furchgott and de Gubareff (1956).

Rats weighing from 150 to 200 g., which had been fasted overnight to reduce the tissue glycogen level, were used. They were killed by a blow on the back of the neck and the brain and liver removed rapidly and transferred /

transferred to ice-cold 0.25 molar sucrose solution. The tissues were removed from the sucrose solution, blotted dry, weighed and, using an M.S.E. homogeniser, a $33\frac{1}{3}$ per cent $^{W}/_{V}$ homogenate in 0.25 molar sucrose solution was prepared. 0.3 ml. of this homogenate containing 100 mg. wet weight of tissue was used in each experiment. The reaction was carried out in Warburg flasks. The main chamber contained 0.1 ml. of 0.15 molar potassium chloride solution, 0.1 ml. of 0.1 molar adenosintriphosphate and the drug solution, control solution or distilled water. The total volume of the flask was adjusted to 1 ml. with distilled water. The pH of the flask contents was 7.4. The flasks were stoppered and placed in a water bath at 25°C. and shaken at a rate of 105 per minute for 5 minutes to attain equilibrium. The reaction was then started by tipping the contents of the side arm into the main chamber and the flasks shaken for a period of 10 minutes. The reaction was then stopped by the addition of 1 ml. of 10 per cent $^{W}/_{W}$ of perchloric acid. Inorganic phosphate liberated in the blank flask was estimated in the same manner.

In experiments using rat skeletal muscle the homogenate was prepared in a manner similar to that described on page 150.

3. Determination of the effects of 10-methoxydeserpidine on anaerobic glycolysis in rat liver, brain and skeletal muscle.

Method

The method used was similar to that of Warburg (1923) but flasks with /

with two side arms were used (Umbreit, Burris and Stauffer, 1957).

Male rats weighing from 175 to 200 g. were fasted overnight to reduce the glycogen content of the tissues. They were killed by a blow on the back of the neck and the brain and liver rapidly removed and transferred to ice-cold Krebs-Ringer bicarbonate solution (Appendix I, page 452). The chilled tissues were sliced by means of a McIlwain tissue slicer giving slices of 0.26 mm. thickness. One hundred mg. wet weight of these were put into the main chamber of the flask which contained 1.6 ml. of Krebs-Ringer bicarbonate solution. 0.2 ml. of the drug solution was placed in one side arm and 0.2 ml. of glucose solution containing 10 μmoles in the other. Control flasks were set up in which one side arm contained 0.2 ml. of distilled water or 0.2 ml. of the solvent used to prepare the drug solution. The final flask volume was 2 ml. Each flask was gassed for 5 minutes with a mixture of 95 per cent nitrogen and 5 per cent carbon dioxide. The flasks were stoppered, placed in a water bath at 37 $\pm$ 0.5°C., and shaken for 5 minutes to allow equilibration to take place. After tipping the side arm contents, the reaction was stopped at zero time, 30 minutes and 60 minutes by the addition of 4 ml. of 0.3 M. barium hydroxide solution and 4 ml. of 5 per cent solution of zinc sulphate (M. Somogyi, cited by Nelson, 1944). Glucose was estimated by the method of Nelson (1944). In both brain and liver experiments 200 μg. per ml. of 10-methoxydeserpidine was used.

(a) <u>Anaerobic glycolysis in rat skeletal muscle</u>

The /

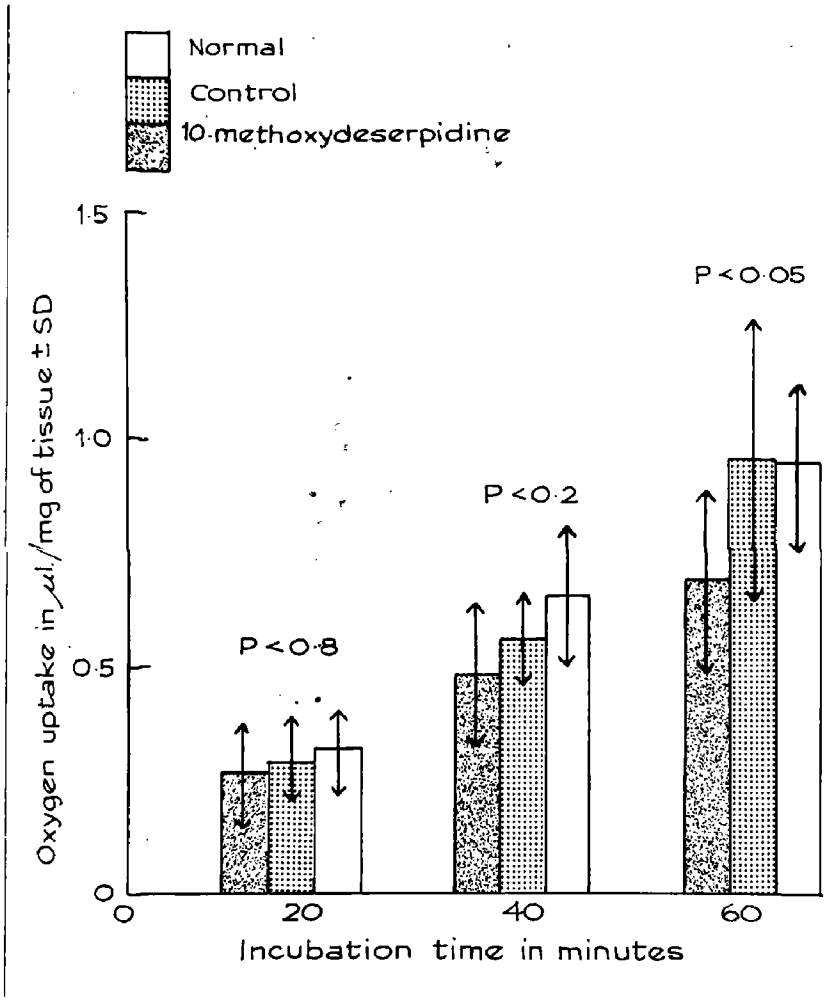

Fig. 68.

Effect of 100 µg. per ml. of 10-methoxydeserpidine on the <u>in vitro</u> respiration of rat brain slices.

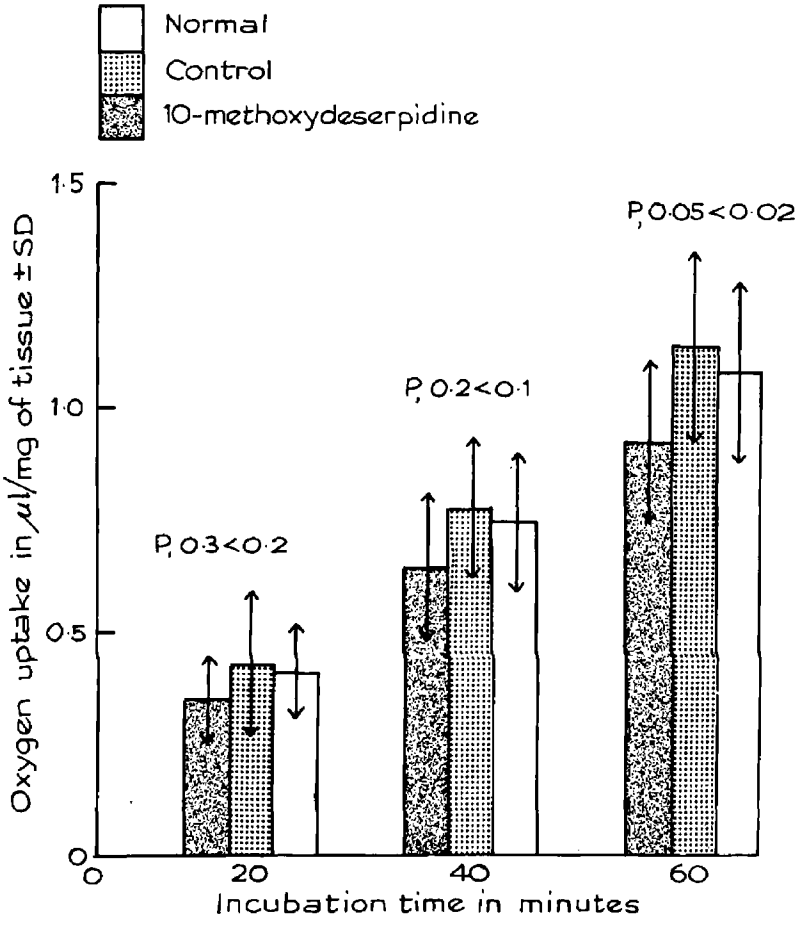

Fig. 69.

Effect of 100 µg. per ml. of 10-methoxydeserpidine on the *in vitro* respiration of rat liver slices.

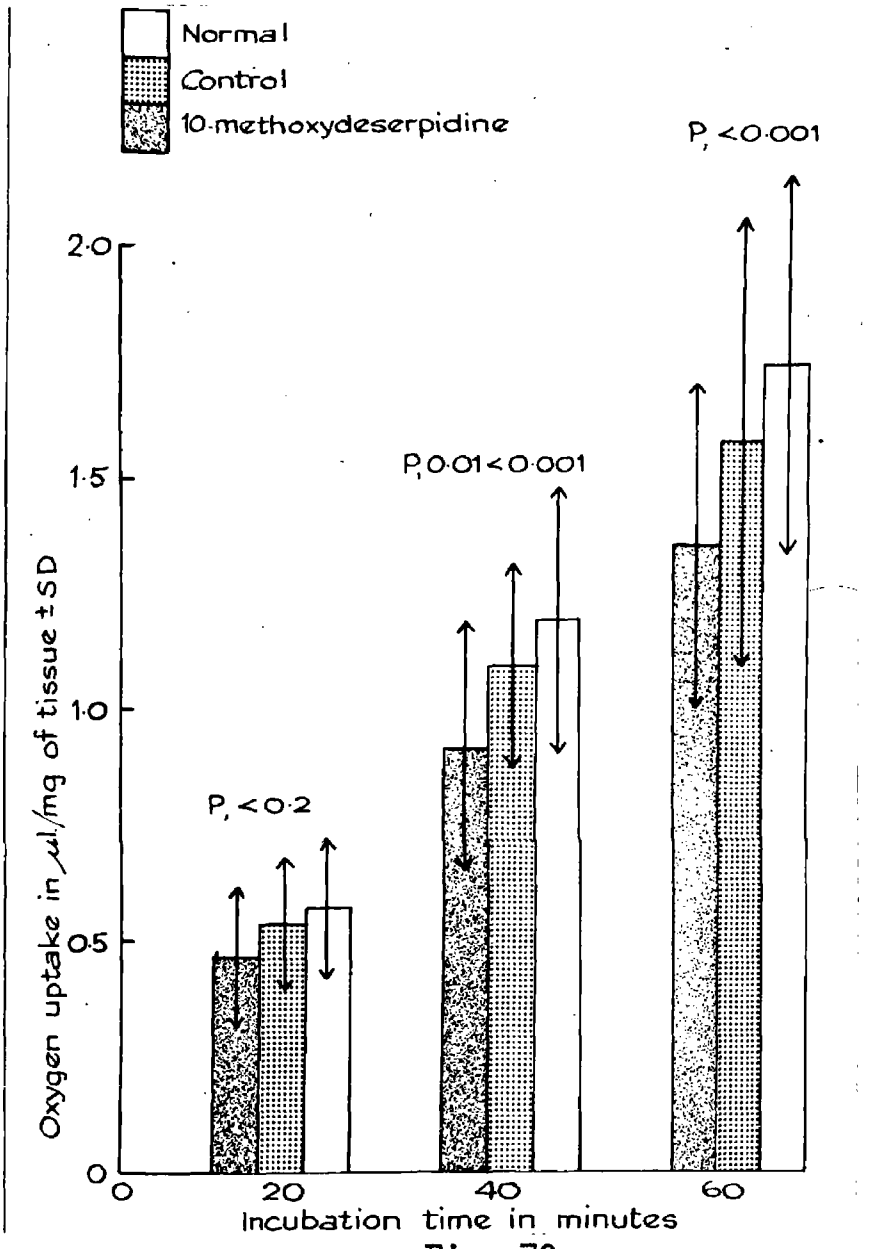

Fig. 70.

Effect of 100 µg. per ml. of 10-methoxydeserpidine on the *in vitro* potassium-stimulated respiration of rat brain slices.

The method used was similar to that described for liver and brain but after killing the rat, the hind leg muscles were quickly removed and dropped into liquid nitrogen. The frozen tissue was powdered by grinding in a pestle and mortar. Five g. of the powder was weighed and mixed thoroughly with 20 ml. of Krebs-Ringer bicarbonate solution. 0.5 ml. of this suspension was placed in the main chamber of the flask which also contained 1.1 ml. of Krebs-Ringer bicarbonate solution.

Results

Tissue respiration in vitro.

10-Methoxydeserpidine at a dose level of 100 µg. per ml. produced a significant depression (P, = 0.05) of the respiration of rat brain slices (Fig. 68, page 151) and of liver slices (Fig. 69, page 152) after a period of 60 minutes. In the potassium-stimulated respiration of the brain in vitro, this effect was more marked during a period of from 20 to 60 minutes (Fig. 70, page 153). At the same dose level no effect was observed on the potassium-stimulated respiration of liver slices (Fig. 71, page 155).

When used in doses of 200 µg. per ml., 10-methoxydeserpidine caused a significant depression ($P = 0.05 < 0.001$) of both potassium-stimulated and non-stimulated respiration of rat brain slices (Fig.72, page 156, and Fig. 73, page 157) and rat liver slices (Fig. 74, page 158, and Fig. 75, page 159).

Anaerobic glycolysis in vitro.

10-Methoxydeserpidine /

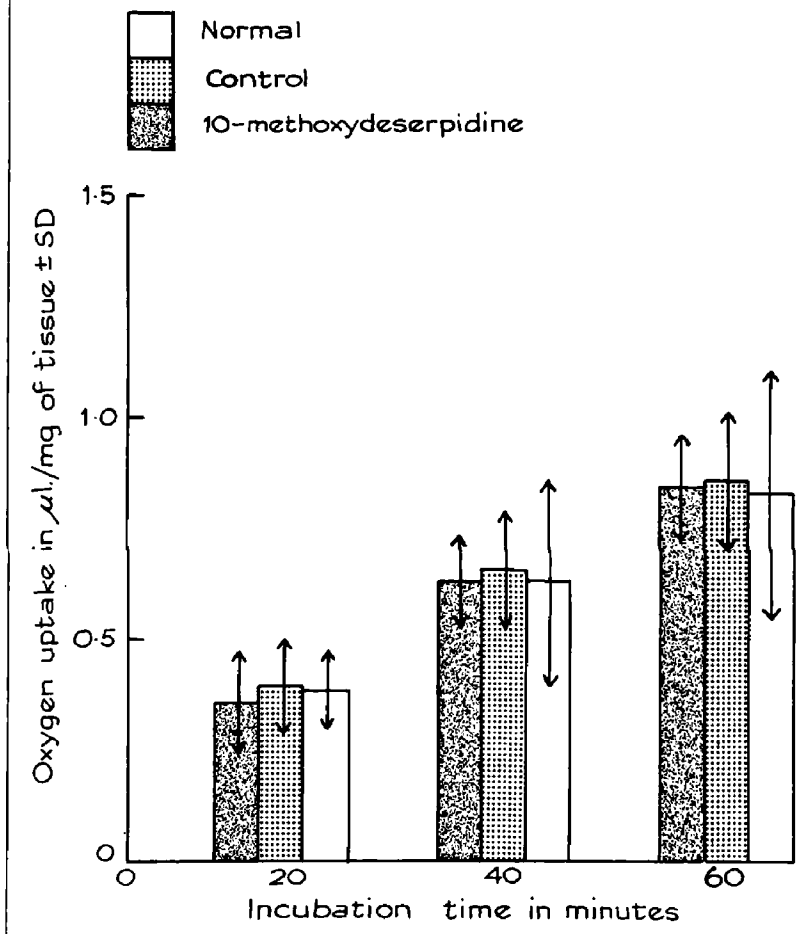

Fig. 71.

Effect of 100 µg. per ml. of 10-methoxydeserpidine on the *in vitro* potassium-stimulated respiration of rat liver slices.

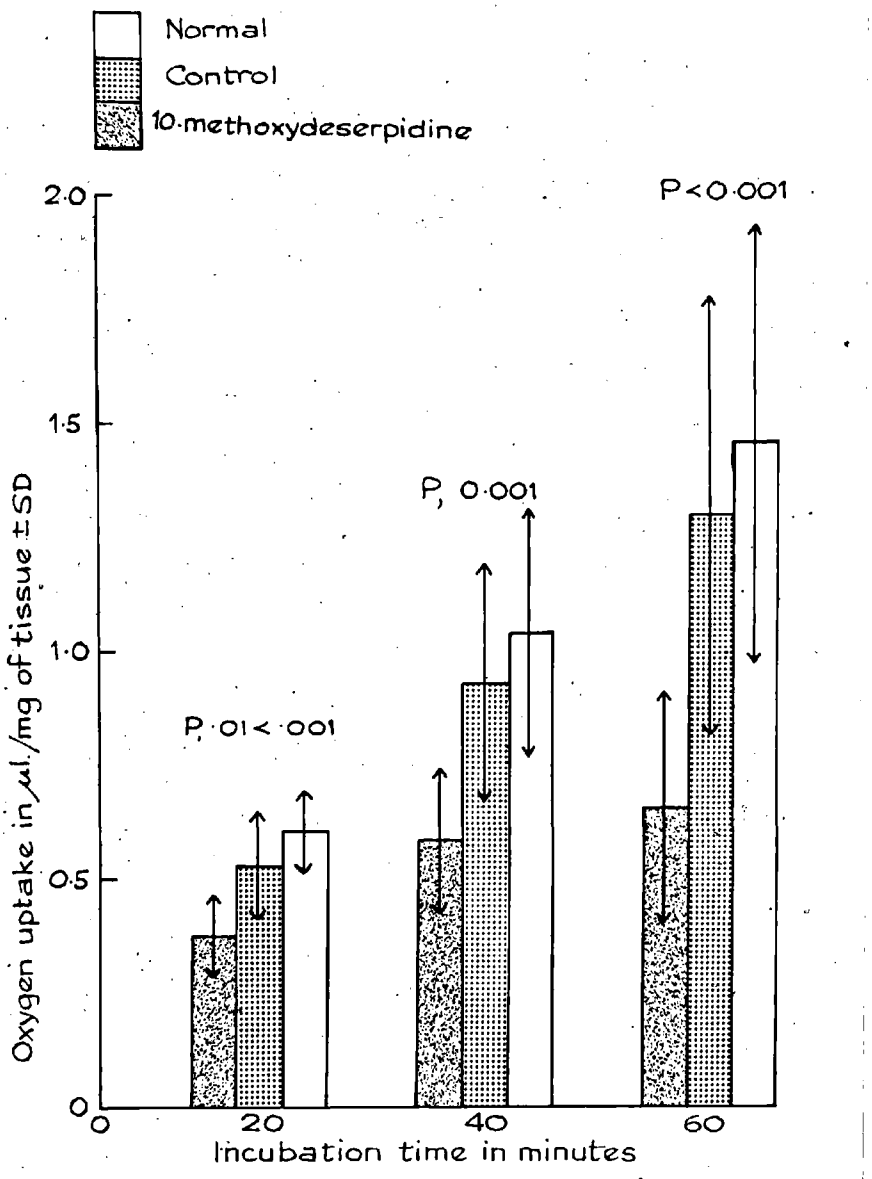

Fig. 72.

Effect of 200 µg. per ml. of 10-methoxydeserpidine on the *in vitro* potassium-stimulated respiration of rat brain slices

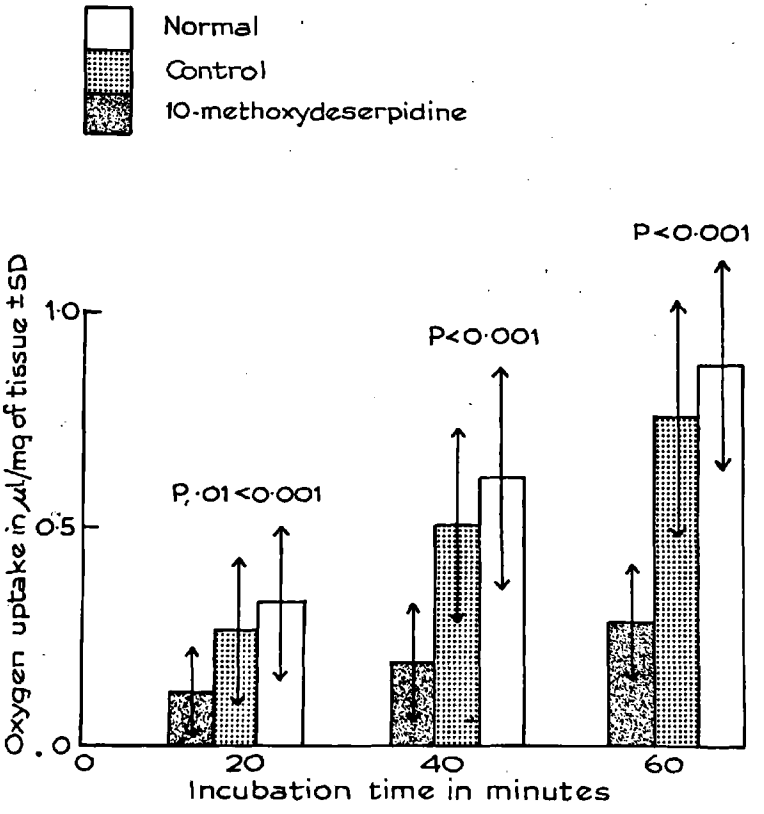

Fig. 73.

Effect of 200 µg. per ml. of 10-methoxydeserpidine on the <u>in vitro</u> respiration of rat brain slices.

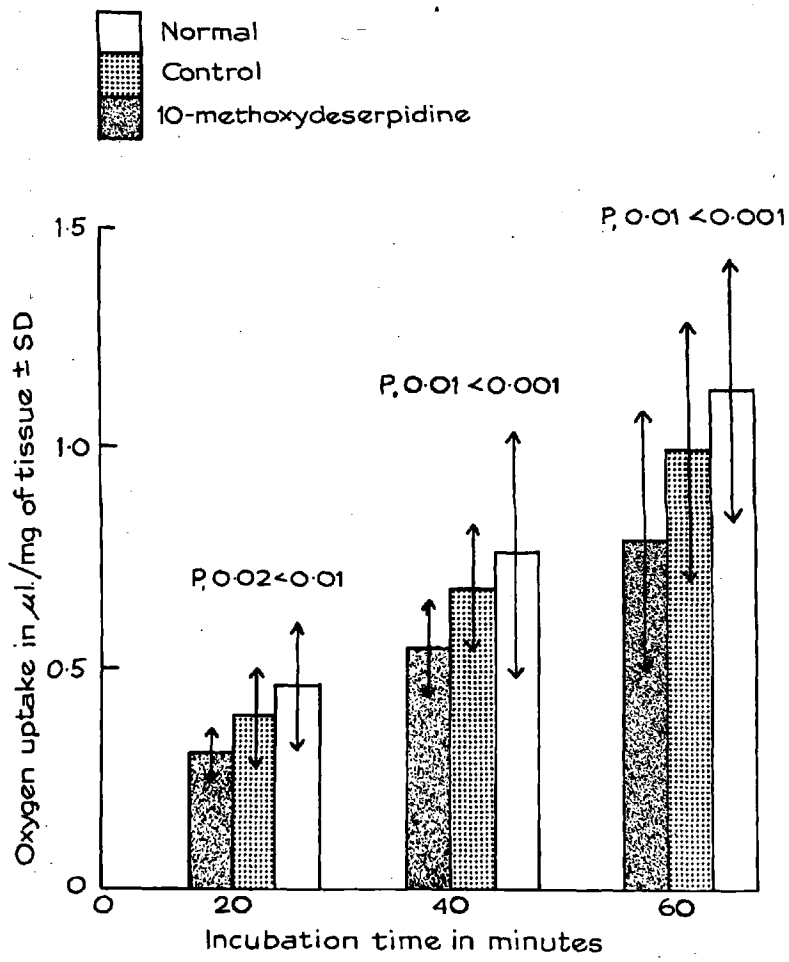

Fig. 74.

Effect of 200 µg. per ml. of 10-methoxydeserpidine on the in vitro respiration of rat liver slices

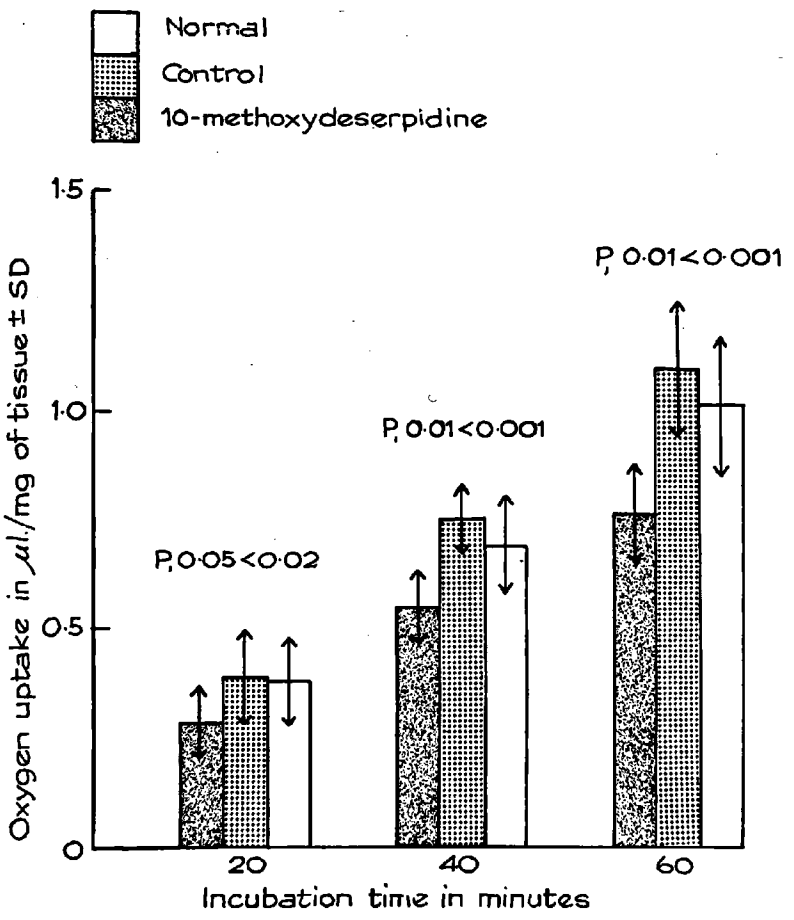

Fig. 75.

Effect of 200 µg. per ml. of 10-methoxydeserpidine on the *in vitro* potassium-stimulated respiration of rat liver slices

10-Methoxydeserpidine 200 µg. per ml. did not alter anaerobic glycolysis in homogenates of rat liver (Fig. 76, page 161), brain (Fig. 77, page 162) and skeletal muscle (Fig. 78, page 163).

Adenosinetriphosphatase-activity (ATP-ase activity) in vitro.

The ATP-ase activity of rat skeletal muscle was significantly depressed ($P, = 0.02 < 0.05$) following the use of 200 µg. per ml. of deserpidine (Fig. 79a, page 164). At the same dose level of 10-methoxy-deserpidine, no effect was observed upon the in vitro ATP-ase activity of homogenates of rat brain (Fig. 80b, page 165) and liver (Fig. 81b, page 166). When a larger dose, 600 µg. per ml., of 10-methoxy-deserpidine was used, a significant depression of the liver ATP-ase activity was noted (Fig. 81a page 166) whereas that of brain remained unaltered (Fig. 80a page 165).

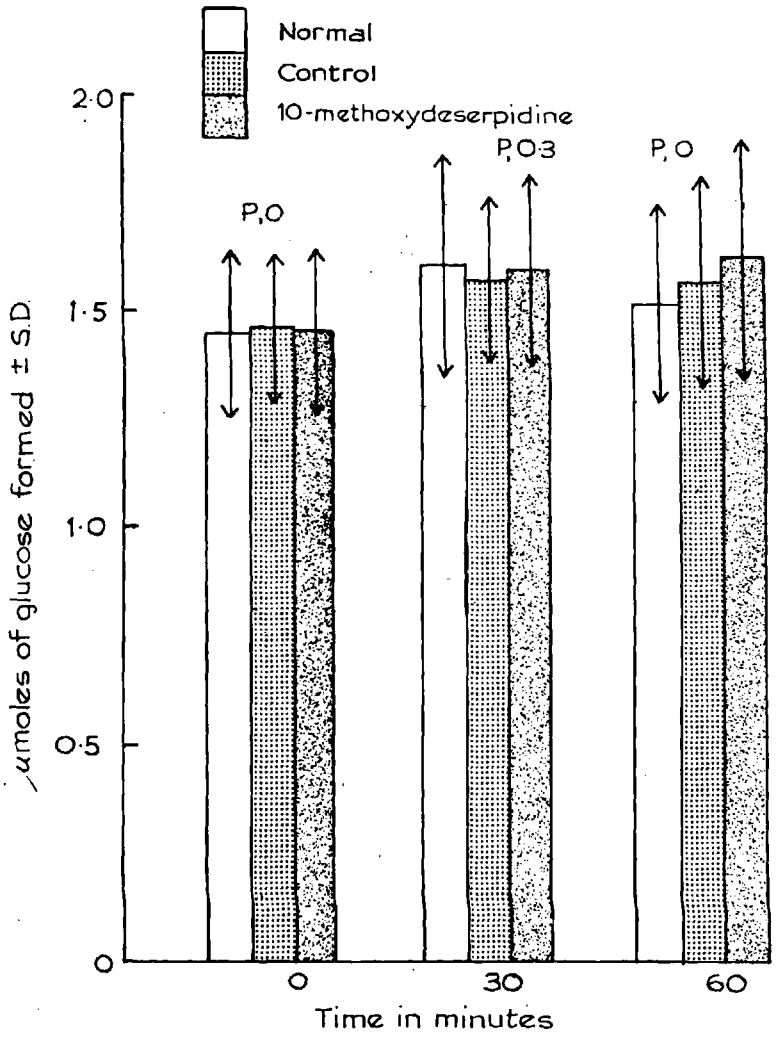

Fig. 76.

Effect of 200 µg. per ml. of 10-methoxydeserpidine on the *in vitro* anaerobic glycolysis of rat liver homogenate

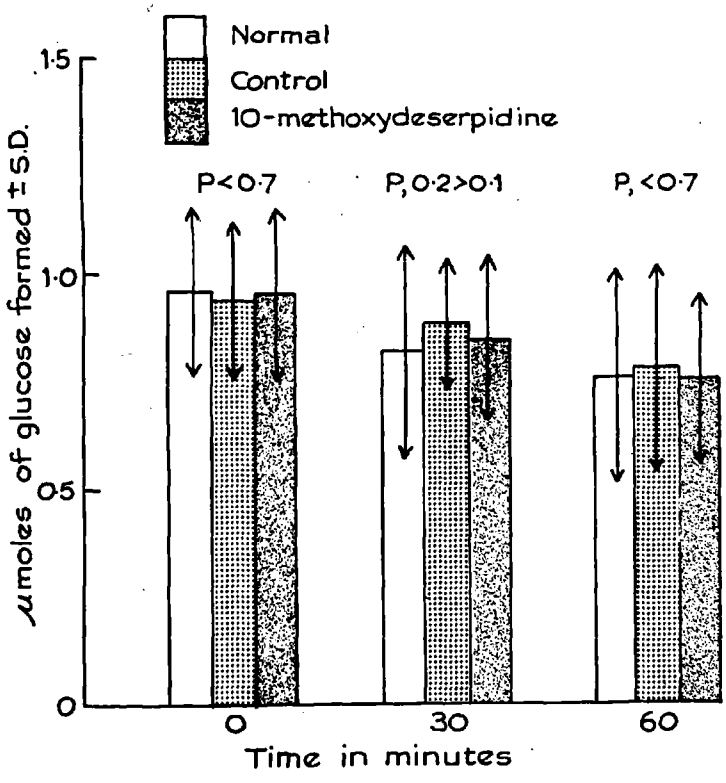

Fig. 77.

Effect of 200 µg. per ml. of 10-methoxydeserpidine on the *in vitro* anaerobic glycolysis of rat brain homogenate

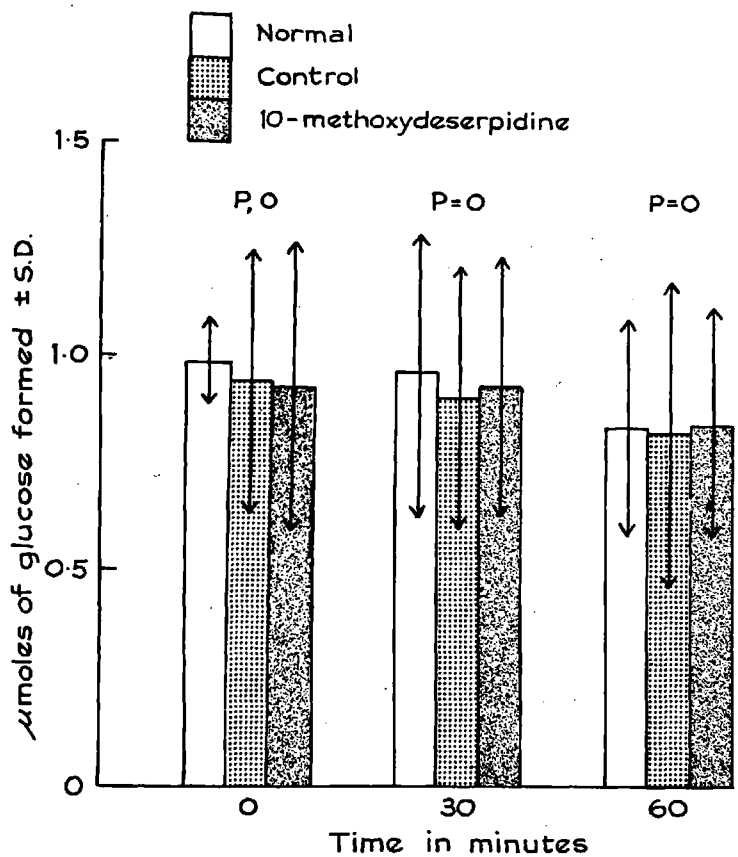

Fig. 78.

Effect of 200 µg. per ml. of 10-methoxydeserpidine on the *in vitro* anaerobic glycolysis of rat skeletal muscle homogenate.

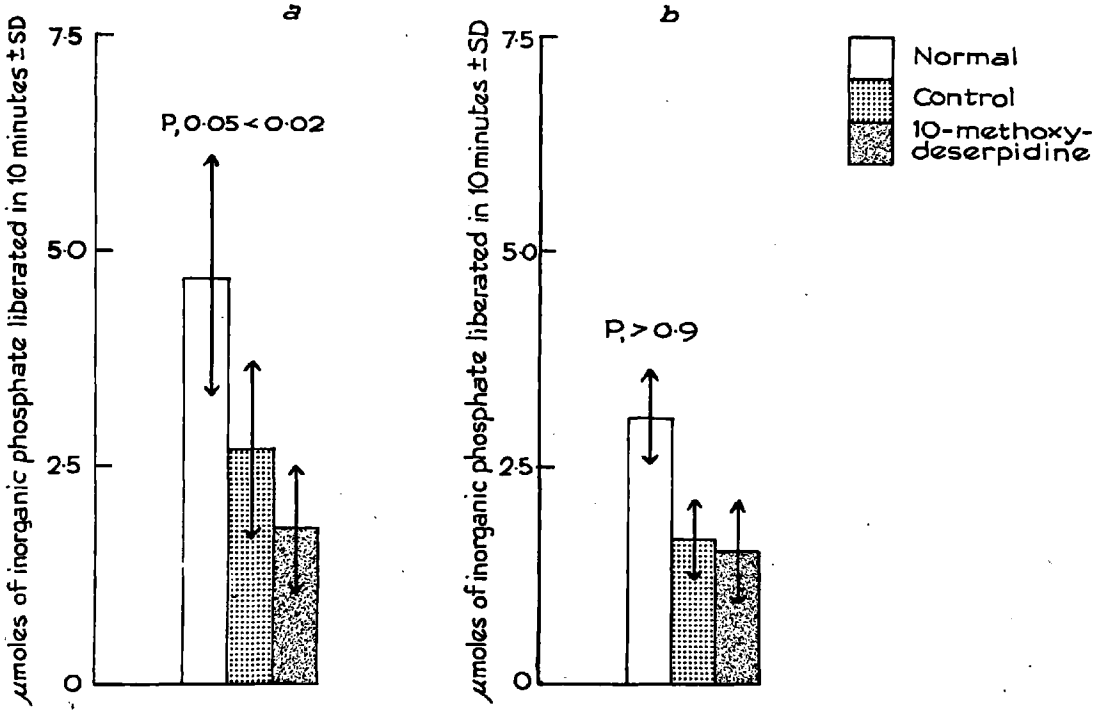

Fig. 79.

Effect of 200 µg. per ml. (at a) and 100 µg. per ml. (at b) of 10-methoxydeserpidine on the in vitro adenosine triphosphatase activity of rat skeletal muscle.

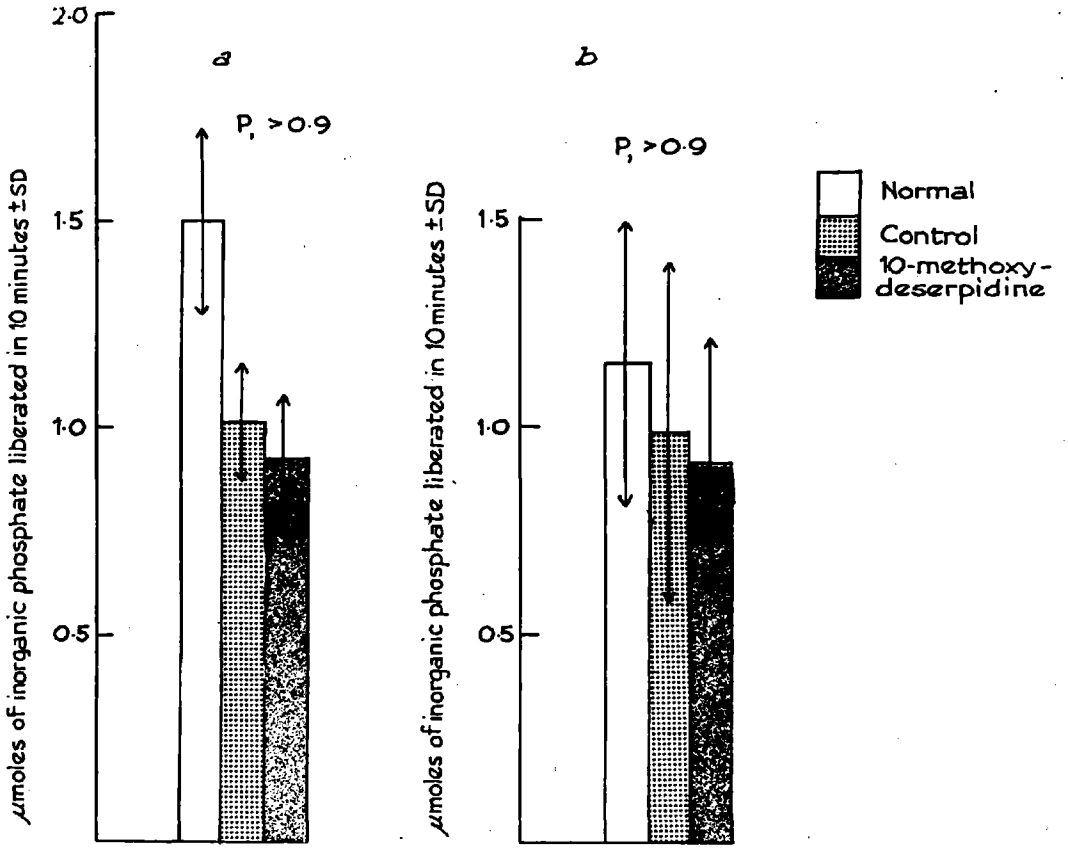

Fig. 80.

Effect of 600 µg. per ml. (at a) and 200 µg. per ml. (at b) of 10-methoxydeserpidine on the in vitro adenosine triphosphatase activity of rat brain homogenate.

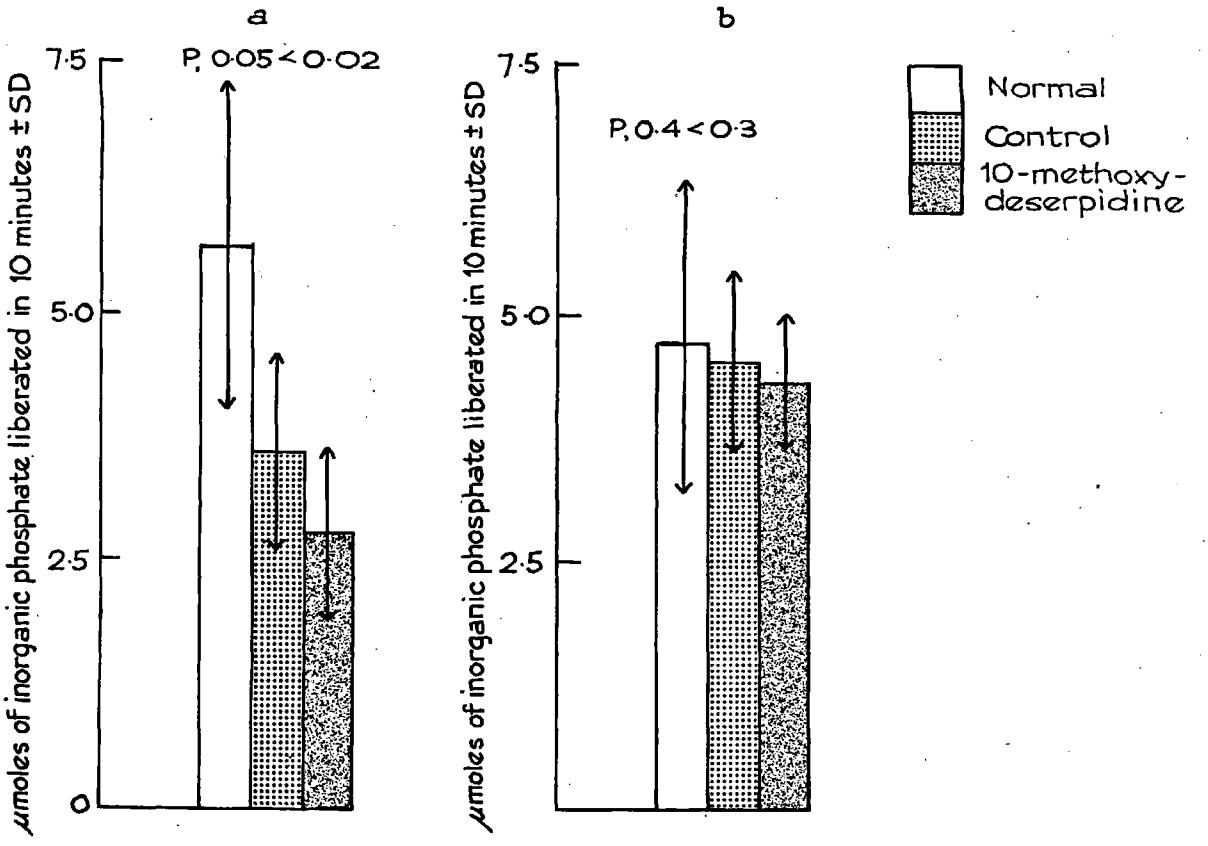

Fig. 81

Effect of 600 µg. per ml. (at a) and 200 µg. per ml. (at b) of 10-methoxydeserpidine on the in vitro adenosine triphosphatase activity of rat liver homogenate.

Table 3.

No. of observations	Dose level (µg. per ml.)	The effect of 10-methoxydeserpidine on the respiration of rat brain slices (oxygen uptake in µl./mg. wet weight of tissue ± SD).		
		Incubation time in minutes		
		20'	40'	60'
11	200	0.124 ± 0.104	0.184 ± 0.138	0.285 ± 0.134
11	Control	0.265 ± 0.172	0.511 ± 0.252	0.759 ± 0.282
11	Blank	0.334 ± 0.191	0.615 ± 0.237	0.880 ± 0.247
		P = 0.05 > 0.02	P = 0.001	P = > 0.001
9	100	0.267 ± 0.106	0.480 ± 0.155	0.699 ± 0.200
9	Control	0.279 ± 0.080	0.564 ± 0.093	0.960 ± 0.306
9	Blank	0.303 ± 0.088	0.653 ± 0.159	0.941 ± 0.190
		P = 0.8	P = 0.2	P = 0.05

P = Significance of difference between drug and control treated tissue.

Table 4.

No. of observations	Dose level (µg. per ml.)	The effect of 10-methoxydeserpidine on the respiration of rat liver slices (oxygen uptake in µl/mg. wet weight of tissue ± SD).		
		Incubation time in minutes		
		20'	40'	60'
16	200	0.301 ± 0.060	0.542 ± 0.109	0.790 ± 0.286
16	Control	0.390 ± 0.096	0.680 ± 0.140	0.998 ± 0.284
16	Blank	0.456 ± 0.130	0.761 ± 0.284	1.137 ± 0.286
		P, 0.02 > 0.01	P, 0.01 > 0.001	P, 0.05 > 0.001
10	100	0.348 ± 0.09	0.647 ± 0.160	0.927 ± 0.180
10	Control	0.424 ± 0.160	0.770 ± 0.160	1.147 ± 0.210
10	Blank	0.401 ± 0.109	0.744 ± 0.150	1.078 ± 0.200
		P = 0.2	P = 0.1	P = 0.02

P = Significance of difference between drug and control treated tissue.

Table 5.

No. of observations	Dose level (µg. per ml.)	The effect of 10-methoxydeserpidine on the potassium-stimulated (0.1M.Kcl) respiration of rat brain slices (oxygen uptake in µl./mg. wet weight of tissue ± SD).		
		Incubation time in minutes		
		20'	40'	60'
12	200 µg.	0.370 ± 0.087	0.590 ± 0.156	0.653 ± 0.248
12	Control	0.525 ± 0.13	0.938 ± 0.268	1.342 ± 0.460
12	Blank	0.604 ± 0.08	1.049 ± 0.268	1.464 ± 0.460
		P = 0.01 < 0.001	P = > 0.001	P = 0.01 < 0.001
10	100	0.465 ± 0.155	0.926 ± 0.260	1.369 ± 0.340
10	Control	0.541 ± 0.140	1.119 ± 0.220	1.584 ± 0.480
10	Blank	0.572 ± 0.155	1.206 ± 0.290	1.753 ± 0.800
		P = 0.3 < 0.2	P = 0.001	P = 0.001

P = Significance of difference between drug and control treated tissue.

Table 6.

No. of observations	Dose level (µg. per ml.)	The effect of 10-methoxydeserpidine on the potassium-stimulated (0.1M.Kcl) respiration of rat liver slices (oxygen uptake in µl. per mg. wet weight of tissue ± SD).		
		Incubation time in minutes		
		20'	40'	60'
12	200	0.283 ± 0.075	0.547 ± 0.078	0.766 ± 0.109
12	Control	0.390 ± 0.109	0.749 ± 0.084	1.094 ± 0.150
12	Blank	0.382 ± 0.079	0.683 ± 0.109	1.014 ± 0.150
		P = 0.02 > 0.05	P > 0.001	P > 0.001
9	100	0.366 ± 0.088	0.632 ± 0.095	0.841 ± 0.110
9	Control	0.392 ± 0.109	0.632 ± 0.095	0.858 ± 0.150
9	Blank	0.388 ± 0.109	0.629 ± 0.220	0.823 ± 0.280
		P = 0.6	P = 0.6	P = 0.6

P = Significance of difference between drug and control treated tissue.

Table 7.

No. of observations.	Tissue used	Dose Level (µg. per ml.)	The effect of 10-methoxydeserpidine upon anaerobic glycolysis in rat skeletal muscle, brain and liver. (glucose (µmoles) Mean ± SD).								
			Incubation time in minutes								
			0'			30'			60'		
			Blank	Control	Drug	Blank	Control	Drug	Blank	Control	Drug
8	skeletal muscle	200	0.988± 0.110	0.940 ± 0.330	0.929± 0.340	0.962± 0.330	0.905 ± 0.300	0.931± 0.302	0.837± 0.250	0.823 ± 0.350	0.849± 0.260
				P = 0			P = 0			P = 0	
8	brain	200	0.960± 0.191	0.940 ± 0.172	0.952± 0.192	0.818± 0.241	0.885 ± 0.141	0.842± 0.190	0.757± 0.241	0.777 ± 0.231	0.750± 0.189
				(P = 0.7)			(P, 0.2 < 0.1)			(P = 0.7)	
9	liver	300	1.448± 0.120	1.460 ± 0.150	1.453± 0.111	1.610± 0.270	I.570 ± 0.190	1.598± 0.210	1.522± 0.220	1.576 ± 0.230	1.634± 0.270
				P = 0			P = 0.3			P = 0	

P = Significance of difference between drug and control treated tissue.

Table 8.

No. of observations	Tissue used	Dose (µg.per ml.)	The effect of 10-methoxydeserpidine upon ATP-ase activity in rat skeletal muscle, brain and liver homogenates.		
			Inorganic phosphate (µmoles) released in 10 minutes (Mean ± SD).		
			Blank	Control	Drug
9	skeletal muscle	100	2.908 ± 0.630	1.650 ± 0.540	1.500 ± 0.690
9		200	4.703 ± 1.420	2.714 ± 1.010	1.810 ± 0.780
				(P, = 0.05 > 0.02)	
9	brain	200	1.150 ± 0.340	0.993 ± 0.420	0.907 ± 0.288
11		600	1.402 ± 0.270	0.809 ± 0.378	0.854 ± 0.180
				(P, = < 0.9)	
12	liver	200	4.779 ± 1.058	4.568 ± 0.980	4.268 ± 0.660
11		600	5.676 ± 1.210	3.607 ± 1.01	2.793 ± 0.813
				(P, = 0.05 > 0.02)	

P = Significance of difference between drug and control treated tissue.

Specimen protocol of group of experiments to investigate the effect of 200 µg. per ml. of 10-methoxydeserpidine on the <u>in vitro</u> ATP-ase activity of homogenate of rat skeletal muscle.

Contents of Warburg Flask (1 ml.) = { 0.1 ml. of 0.15 molar potassium chloride solution. 0.1 ml. of 0.1 molar adenosine triphosphate solution. 0.1 ml. of drug, control solution or distilled water. 100 mg. wet weight of tissue. Distilled water to make the contents of the flask up to one ml.

pH = 7.4

Incubation time - 10 minutes.

Temperature - 25°C.

No. of Exp.	BLANK	NORMAL			DRUG			CONTROL		
	optical density	optical density	true optical density	umoles of inorganic phosphate	optical density	true optical density	umoles of inorganic phosphate	optical density	true optical density	umoles of inorganic phosphate
1	0.790	1.650	0.860	7.250	1.050	0.260	1.975	1.230	0.440	3.550
2	0.840	1.550	0.710	5.900	1.020	0.180	1.250	1.190	0.350	2.750
3	0.790	1.550	0.760	6.350	1.200	0.410	3.275	1.250	0.460	3.725
4	0.690	1.150	0.460	3.725	0.990	0.300	2.325	1.150	0.460	3.725
5	0.780	1.350	0.570	4.700	1.090	0.310	2.400	1.240	0.460	3.725
6	0.750	1.200	0.450	3.625	0.995	0.245	1.825	0.990	0.240	1.800
7	0.518	1.018	0.500	4.075	0.720	0.202	1.450	0.791	0.273	2.075
8	0.492	0.915	0.423	3.400	0.655	0.163	1.100	0.655	0.163	1.100
9	0.458	0.870	0.412	3.300	0.580	0.122	0.750	0.709	0.251	1.975
	Mean ± SD	4.703 ± 1.42			1.810 ± 0.780			2.714 ± 1.01		

Specimen protocol of a group of experiments to investigate the effect of 200 µg. per ml. of 10-methoxydeserpidine on the <u>in vitro</u> anaerobic glycolysis of homogenate of rat skeletal muscle.

Contents of Warburg Flask with two arms (2 ml.)
- 1.6 ml. of Krebs-Ringer bioarbonate solution.
- 0.2 ml. (10 µmoles) of glucose solution.
- 0.2 ml. of drug, control solution or distilled water.
- 100 mg. wet weight of tissue.

Eaoh flask was gassed with 95 per oent nitrogen and 5 per cent oarbon dioxide for 5 minutes.

pH. = 7.8 Temperature 37 ± 0.5° C.

No. of Expt.	Incubation time in minutes								
	0 time			30 minutes			60 minutes		
	Normal	Drug	Control	Normal	Drug	Control	Normal	Drug	Control
1	1.410	1.440	1.390	1.410	1.320	1.190	1.040	1.160	1.300
2	1.370	1.320	1.330	1.390	1.350	1.390	1.260	1.250	1.390
3	1.220	1.190	1.260	1.210	1.170	1.180	1.060	1.010	0.940
4	0.890	0.825	0.820	0.850	0.790	0.830	0.740	0.800	0.660
5	0.650	0.700	0.640	0.655	0.590	0.640	0.640	0.640	0.390
6	0.730	0.790	0.815	0.745	0.610	0.560	0.700	0.690	0.710
7	0.720	0.600	0.740	0.695	0.800	0.710	0.550	0.710	0.690
8	0.540	0.570	0.525	0.745	0.820	0.740	0.710	0.530	0.510
Mean ± SD	0.988± 0.110	0.929± 0.340	0.940± 0.330	0.962± 0.330	0.931± 0.302	0.905± 0.300	0.837± 0.250	0.849± 0.260	0.823± 0.350

Specimen protocol of a group of experiments to investigate the effect of 200 µg. per ml. of 10-methoxydeserpidine on the <u>in vitro</u> respiration of rat brain slices.

Contents of Warburg flask (3 ml.)
- 2.5 ml. of Krebs-Ringer phosphate solution.
- 0.3 ml. of drug, control solution or distilled water.
- 100 mg. wet weight of tissue slices.
- 0.1 ml. of 0.1 molar potassium chloride solution.

pH = 7.2

Temperature = 37 ± 0.5° C.

0.2 ml. of 20 per cent potassium hydroxide solution was placed in the centre well of the flask.

No. of Expt.	Uptake of oxygen in ul./mg. wet weight of tissue.								
	20 minutes			40 minutes			60 minutes		
	Normal	Drug	Control	Normal	Drug	Control	Normal	Drug	Control
1	0.577	0.436	0.512	1.021	0.639	0.896	1.539	0.899	1.264
2	0.483	0.462	0.551	0.993	0.709	0.779	1.476	1.006	1.311
3	0.602	0.462	0.632	1.131	0.616	0.911	1.631	0.600	1.058
4	0.585	0.345	0.390	1.021	0.486	0.630	1.440	0.486	0.787
5	0.656	0.192	0.541	1.248	0.272	0.800	1.872	0.352	1.525
6	0.817	0.323	0.262	1.577	0.475	0.606	2.375	0.589	2.050
7	0.717	0.346	0.475	1.060	0.709	1.004	1.372	1.023	1.130
8	0.495	0.421	0.705	0.709	0.795	1.361	0.907	1.154	1.095
9	0.488	0.303	0.455	0.710	0.483	0.800	0.828	0.676	1.965
10	0.648	0.266	0.446	1.007	0.414	0.800	1.200	0.547	0.735
11	0.770	0.455	0.630	1.344	0.785	1.290	1.935	1.020	1.082
12	0.410	0.431	0.705	0.672	0.708	1.380	1.000	0.893	2.102
Mean ± SD	0.604± 0.08	0.370± 0.087	0.525± 0.13	1.049± 0.268	0.590± 0.156	0.938± 0.268	1.464± 0.460	0.653± 0.248	1.342± 0.460

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C H A P T E R VI.

CHAPTER VI

DISCUSSION

When the results obtained from experiments on isolated tissue preparations are considered, it seems that,in general, 10-methoxy-deserpidine acts by depressing the ability of the smooth muscle cell to contract.

The energy required by muscle for contraction or for the maintenance of tone, is known ultimately to be derived from chemical reactions taking place within the cell (Needham, 1960). It is also generally assumed that the enzymatic splitting of adenosine triphosphate provides energy for the musoular contraction (Perry, 1956, and Gelfan, 1958). Acoording to Szent-Gyorgyi(1960) "muscle contraction is an interaction of adenosine triphosphate, actin and myosin, an interaction which entails violent changes in colloidal state and involves a shortening of the fibrous particles of the protein complex, actomyosin. The free energy which enabled the shortening fibres to lift a weight and do work is supplied by adenosine triphosphate, the terminal bond of which is hydrolyzed in the process." Munch-Petersen (1953) and Lange (1955) obtained direct evidence that the contracting muscle contained less adenosine triphosphate than the resting muscle. They concluded that the bond energy of adenosine triphosphate was utilised during the process /

process of muscular contraction. It is also thought, that the metabolic processes of the cell, such as respiration and glycolysis which indirectly support the muscular activity, do so by virtue of the synthesis of adenosine triphosphate. The high energy phosphate bond of adenosine triphosphate is synthesized by the oxidative reactions of carbohydrate metabolism. These are, firstly, the anaerobic conversion of glycogen to lactic acid; secondly, the oxidation of lactic acid to pyruvate by the mitochondrial system; and thirdly, the reaction of adenosine diphosphate (ADP) with inorganic phosphate to form adenosine triphosphate. This is also known as Lohmanns' reaction and can be shown as follows:-

(1) Creatine phosphate + Adenosine diphosphate $\xrightarrow{\text{Creatine phosphokinase}}$ Creatine + Adenosine triphosphate

(2) Adenosine triphosphate $\xrightarrow{\text{ATPase}}$ Adenosine diphosphate + H_3PO_4

Lohmann (1934) was the first to demonstrate the significance of these reactions. He postulated that before creatine-phosphate breakdown could yield the energy required for muscular contraction, hydrolysis of adenosine triphosphate must have occurred. This reaction was the energy-yielding reaction and took place close to the time of the actual muscular contraction.

As with striated muscle, the immediate source of energy for contraction in smooth muscle is probably the high energy phosphates such

as /

as adenosine triphosphate and creatine phosphate. Furchgott (1955) has shown that determination of these compounds in rabbit thoracic aorta, freed of extraneous fat and connective tissue and incubated in oxygenated Krebs' glucose solution at 37°C. for a period of 45 minutes, gave average values of about 0.2 mM. per g. of phosphocreatine and 0.7 mM. per g. of adenosine triphosphate and adenosine diphosphate.

Detailed investigations by Csapo (1948, 1950 and 1960) suggest that the uterine muscle of the rabbit is qualitatively similar in its biochemical activity to that of striated muscle. Using rabbit uterus, Csapo (1955) repeated the early observations of Lundsgaard (1934) and obtained identical results. Lundsgaard (1934) demonstrated that when treated with iodoacetic acid under anaerobic conditions, the isolated frog rectus abdominis muscle could produce only a limited number of contractions and that the contractions ceased when the stores of high energy phosphates (creatine phosphate and adenosine triphosphate) in the muscle were exhausted.

The biochemical properties of the contractile proteins of vascular smooth muscle have not been investigated. From the smooth muscle of the uterus Csapo (1950) obtained less actin and myosin than from the skeletal muscle. He also found the actin to myosin ratio to be lower in the uterine smooth muscle than that on skeletal muscle. On the addition of adenosine triphosphate, actomyosin threads from uterine muscle contracted more slowly and to smaller extent than those from skeletal muscle.

When /

When 10-methoxydeserpidine was used in larger doses (200 µg. per ml.) it significantly depressed the *in vitro* respiration of rat brain and liver slices. When it was used in lower concentrations (100 µg. per ml.), however, it produced, after a delay of from 40 to 60 minutes, an inhibitory effect upon the *in vitro* respiration of these tissue preparations (Chapter V, page 154). Kirpekar and Lewis (1960) have shown that reserpine (100 µg. per ml.) produced a significant depression of the respiration of rat brain homogenates, whereas the respiration of rat liver homogenates remained unaffected. Earlier observations of Gillis and Lewis (1957) indicated that reserpine (50 µg. per ml.) was capable of depressing the respiration of the isolated smooth muscle of the rabbit intestine. From their observations, Gillis and Lewis pointed out that the effects of reserpine upon spontaneous or drug-induced tone were similar in many aspects to those of experimentally-induced anoxia. They concluded that reserpine appeared to make the tissue anoxic by virtue of its ability to interfere with the energy-producing reactions of carbohydrate metabolism.

The energy required for muscular contraction is derived both from aerobic and anaerobic processes, the former probably contributing the greater amount of energy. It has been known for many years (Gross and Clark, 1923, and Garry, 1928) that the contractile properties of isolated smooth muscle alter reversibly when the supply of oxygen or substrate is stopped.

Gross /

Gross and Clark (1923), using the isolated intestine of the rabbit or rat, found that the substitution of oxygen by nitrogen in the bath fluid led to an immediate loss of tone in the isolated intestine and that the tissue became insensitive to the application of adrenaline or pilocarpine. No diminution in the response of the gut to direct muscle stimulants (potassium chloride and barium chloride) was observed. Garry (1928), using the isolated intestine and uterus of various animal species including the guinea pig, rabbit, cat and dog, observed that lack of oxygen in the bathing fluid resulted in a significant loss of tone. Prasad (1935 a and b) carried out detailed investigations upon the contractile activity and carbohydrate metabolism of the isolated rabbit ileum and isolated cat colon. He estimated the quantity of glucose consumed by the muscle during activity and resting periods under aerobic and anaerobic conditions. The effects of anoxia and metabolic inhibitors such as iodoacetic acid on the glycolysis and tone of the muscle were also determined.

Prasad concluded that the loss in the tone of the isolated gut was due to anoxia which further depressed the carbohydrate metabolism of the cell. He also pointed out that when treated with iodoacetic acid under anaerobic conditions, isolated intestinal smooth muscle retained very little contractile activity. Under aerobic conditions it was not much affected. He suggested that the reservoir of available anaerobic energy was very small. West, Hadden, and Farah (1951) also considered that /

that the amount of available anaerobic energy was only a small fraction of that available during aerobic activity.

The normal spontaneous activity of smooth muscle requires the steady production of energy from readily available sources. Under aerobic conditions, the metabolism can easily meet the requirements, but anoxia eliminates the most efficient sources of energy, namely oxidative metabolism (Lipmann, 1942).

The effects of anoxia, of substrate deficiency (Feldberg and Solandt, 1943) and of metabolic inhibitors such as cyanide, have been attributed to an interruption of the normal supply of chemical energy to the muscle. Such an interruption might show itself as a fall of the concentrations of metabolic high-energy phosphate compounds such as adenosine triphosphate and creatine phosphate which are generally thought to be the immediate sources of chemical energy for muscular activity.

Anoxia has been shown to cause a reduction in the adenosine triphosphate content of intestinal smooth muscle (Furchgott and Shorr, 1948). It has also been demonstrated by Born (1956), using the isolated taenia coli of the guinea pig, that when the muscle is deprived of oxygen or glucose it loses its spontaneous tone and motility. It also loses the ability to maintain increased tension during stimulation. Those changes developed more rapidly in the absence of oxygen than in the absence of glucose and they were accompanied by a significant decrease /

decrease (50 to 80 per cent) in the creatine phosphate level of the tissue. The adenosine triphosphate content of the taenia coli did not change markedly. Born (1956) concluded that a correlation existed between the active tension and the creatine phosphate concentration of isolated smooth muscle in the absence of glucose or oxygen.

In more recent investigations, Furchgott and de Gubareff (1958) have demonstrated that when guinea pig atria were suspended in Krebs' bicarbonate medium which was gassed with 95 per cent nitrogen and 5 per cent carbon dioxide, they lost their contractile activity. There was reduction in the amplitude of the electrically-induced contractions of the isolated atria and within 15 to 20 minutes this was reduced to 10 per cent of the initial level. This effect was associated with a marked reduction in creatine phosphate and adenosine triphosphate levels, the former being the more affected. Furchgott and de Gubareff confirmed the suggestion of Greiner (1952) that the decrease in the adenosine triphosphate level might not be the cause of the depression of the contractile activity of the cardiac muscle under anoxic conditions but could be due to other intracellular changes resulting from oxygen lack.

It has been observed (Chapter III, Fig.49, page 106) that the ability of arterial smooth muscle to maintain drug-induced tone was markedly reduced in the presence of 10-methoxydeserpidine. Under the influence of this drug, the muscle lost tone. Similar observations

were recorded using reserpine by Gillis and Lewis (1957) and by Kirpekar and Lewis (1958). These workers also employed the isolated guinea pig ileum and isolated strips of horse carotid artery. The effects of reserpine upon drug-induced tone were similar to those of experimentally produced anoxia. Using reserpine, Kirpekar and Lewis in 1959 estimated the adenosine nucleotide levels of different tissues of the rat inoluding the liver, heart, skeletal muscle and brain. It was shown that reserpine significantly depressed adenosine triphosphate levels in brain and liver with a corresponding increase in adenosine diphosphate levels. Reserpine did not, however, cause any change in the adenosine triphosphate content of the heart and skeletal muscle. From their in vivo studies, they concluded that reserpine inhibited oxidative phosphorylation. It has also been shown by Abood and Romanchek (1957) that in in vitro oxidative phosphorylation, reserpine acts as an "uncoupling agent". Uncoupling agents can disturb the metabolism of the cell by depressing the formation of high energy phosphate bonds without depressing, or even slightly stimulating, the oxygen consumption of the system. This dissociation of oxidation from phosphorylation is termed "uncoupling", and the agents which do this are known as "uncoupling agents". (Brody, 1955). 2,4-dinitrophenol is a typical example of this type of compound.

It has also been shown by Kirpekar and Lewis (1958) that when isolated horse carotid artery strips are rendered anoxic, the drug-induced /

induced contractions are reduced in magnitude but never completely eliminated.

Since oxidation proceeds both aerobically and anaerobically, it is possible that the small response obtained after rendering the tissue anoxic is due to the energy supplied by anaerobic glycolysis. 10-Methoxydeserpidine does not produce an in vitro inhibition of anaerobic glycolysis in the rat brain, liver and skeletal muscle (Chapter V, page 160). Gillis and Lewis (1957), using reserpine, observed similar effects upon anaerobic glycolysis in isolated intestinal smooth muscle of the rabbit. Since 10-methoxydeserpidine and reserpine resemble one another in their actions upon the anaerobic glycolysis and respiration of isolated tissues, it is possible that 10-methoxydeserpidine, like reserpine, inhibits aerobic oxidative processes more effectively than anaerobic ones.

During the present investigation, it was observed that 10-methoxydeserpidine (200 µg. to 600 µg. per ml.) caused a significant in vitro inhibition of the adenosine triphosphatase activity of homogenates of rat liver and skeletal muscle. No such effect was observed upon the adenosine triphosphatase activity of the rat brain, even when very large doses of 10-methoxydeserpidine (600 µg. per ml.) were used. These observations are in agreement with those of Kirpekar and Lewis (1960) who, using reserpine, obtained a depression of the in vitro adenosine triphosphatase activity of rat liver homogenates.

The /

The dose used to produce inhibition of the adenosine triphosphatase activity of rat liver homogenates was very high (600 µg. per ml.) but 10-methoxydeserpidine at the same dose level was incapable of producing an effect upon the adenosine triphosphatase activity of rat brain homogenate. This may show that the mechanisms in the rat liver and skeletal muscle are more sensitive than those in the rat brain.

It has been shown (Carlsson and Hillarp, 1956) that adenosine triphosphate plays an important role in the storage and release of catechol amines from the adrenal medulla. These authors observed that the depletion of the adrenal medulla of catechol amines by morphine and insulin was accompanied by a parallel depletion of adenosine triphosphate. Reserpine has been shown to cause depletion of noradrenaline and serotonin from brain and other tissues (Holzbauer and Vogt, 1956, and Pletscher, Shore and Brodie, 1955). These observations have been extended by Kirpekar, Goodlad and Lewis (1958) who found that reserpine depleted the rat adrenal medulla, both of its catechol amines and adenosine triphosphate. It has also been suggested (Sulser and Brodie, 1960) that the alterations in catechol amine levels of the brain are linked with the central nervous system depressant effects of reserpine. It is not, however, certain that these changes are the cause of its pharmacological actions, nor is it clear that the primary point of action of the drug is upon the mechanisms which influence or control binding, uptake, release or metabolism of the amines. There is also

some /

some evidence (Leroy and Schaepdryver, 1961) that analogues of reserpine which lack its characteristic central nervous system depressant actions do not modify catechol amine levels in the brain. These authors found that in mice, 5 mg. per kg. of syrosingopine had no effect upon brain noradrenaline levels although it reduced them in the heart. 10-Methoxydeserpidine (25 mg. per kg.) had no effect upon the catechol amine levels of brain or heart. Neither compound possessed reserpine-like central nervous system depressant actions yet both were capable of lowering the blood pressure level. These effects can be looked upon as an indication that the antihypertensive properties of compounds of this type are not necessarily linked with a depletion of cardiovascular stores of catechol amines. The sedative activity is however linked with an effect of this kind in the brain (Sanan and Vogt, 1962).

The fact that the central nervous system depressant actions of reserpine are linked with the depletion of the brain of its noradrenaline and serotonin is strengthened by the observations of Garattini, Lamesta, Mortari and Valzelli (1961) who investigated the antihypertensive and sedative actions of certain analogues of reserpine including 10-methoxydeserpidine. These compounds possessed antihypertensive activity but were not sedative and did not, even at very high dose levels, cause depletion of serotonin or noradrenaline from the rat brain. On the other hand, Orlans, Finger and Brodie (1960) found that antihypertensive drugs such as syrosingopine were much more potent in /

in releasing peripheral noradrenaline than brain noradrenaline - an effect which was associated with the induction of bradycardia and hypotension and with a reduction in the responsiveness of certain pressor reflexes and in that to ganglion stimulants. It was not associated with sedation. They concluded that the tranquillizing actions of reserpine were due to an effect on the central nervous system and that the antihypertensive actions were due to its peripheral effects.

The mechanism by which 10-methoxydeserpidine lowers the blood pressure level seems to be different from that of syrosingopine. The similarity between the actions of 10-methoxydeserpidine and reserpine on cellular metabolic processes may indicate a partial resemblance in the properties of these two drugs.

The evidence presented supports the view that 10-methoxydeserpidine has a peripheral site of action. 10-Methoxydeserpidine may exert its actions by interfering with tissue metabolism, probably that part which supplies the energy for muscular contraction. It is also considered possible that 10-methoxydeserpidine depresses the aerobic metabolic activity of the cell more effectively than the one under anaerobic conditions. By doing so, 10-methoxydeserpidine may affect the oxidative reactions of carbohydrate metabolism which are involved in the synthesis of adenosine triphosphate. It is suggested that in vitro adenosine triphosphatase activity of the rat liver and the skeletal muscle is

more /

more sensitive to the presence of 10-methoxydeserpidine than that of the brain. The observations of Garattini, Lamesta, Mortari and Valzelli (1961) support the view that 10-methoxydeserpidine possesses antihypertensive actions due to an action at peripheral sites and has no central nervous depressant activity.

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C H A P T E R VII

CHAPTER VII.

SUMMARY OF PART I (a)

In the introductory chapter, a brief review of the literature upon the pharmacological properties of 10-methoxydeserpidine, deserpidine, reserpic acid and methyl reserpate together with a short historical account of the discovery and properties of reserpine are presented.

In Chapter II, the experimental techniques used in the investigation of the general pharmacological properties of 10-methoxydeserpidine, deserpidine, reserpic acid and methyl reserpate are described.

In Chapter III, the results of the investigation upon the properties of 10-methoxydeserpidine, deserpidine, methyl reserpate and reserpic acid are described. 10-Methoxydeserpidine was found to possess hypotensive actions qualitatively similar to those of reserpine and deserpidine but not to exhibit similar central nervous system depressant actions.

10-Methoxydeserpidine caused a fall in the blood pressure level of the anaesthetised cat and rat but was less potent in the rat. It caused a generalised depression of preparations of isolated cardiac muscle, vascular or intestinal smooth muscle and skeletal muscle. 10-Methoxydeserpidine also antagonized the contractile responses of these tissues elicited by different spasmogens. Reserpic acid and

STUDIES ON THE MODE OF ANTIHYPERTENSIVE ACTION OF CHLOROTHIAZIDE AND SOME ALLIED COMPOUNDS

PART I (b)

C H A P T E R I.

C H A P T E R I

INTRODUCTION

Early in 1935 the German pharmacologist Domagk published his observations on the antibacterial properties of p-aminobenzene sulphonamide (sulphanilamide). This discovery revolutionised the treatment of a group of bacterial infections including puerperal sepsis, erysipelas and many other diseases due to streptococcal, meningococcal and gonococcal infections.

The introduction of sulphanilamide (Fig. 82, page 200) as a chemotherapeutic agent was soon followed by the observations of Southworth (1937) and Strauss and Southworth (1938) that it was capable of causing a metabolic acidosis in human subjects. While treating patients with sulphanilamide these authors observed a drop in the carbon dioxide-combining capacity of the plasma with a definite increase in the urine flow and in the renal excretion of sodium and potassium ions. A fall in the carbon dioxide content and pH of the plasma, coupled with a rise in the pH of the urine following the administration of sulphanilamide was also observed in dogs by Marshall, Cutting and Emerson in 1938. These authors also suggested that sulphanilamide depressed the tubular reabsorption of bicarbonate and thus tended to cause a metabolic acidosis. These findings were confirmed by Beckman, Rossmeisl, Pettengill /

Pettengill and Bauer (1940) in their studies on human subjects. They demonstrated a sharp rise in the urinary excretion of sodium and bicarbonate ions during the first day of administration of sulphonamides with a corresponding decrease of sodium and bicarbonate levels in the blood. The potassium and chloride excretion was less affected. The significance of these observations was not fully appreciated at that time, but in 1940 Mann and Keilin discovered that sulphonamides which possessed unsubstituted sulphonamido groups, for example, benzylsulphanilamide, pyridine-3-sulphonamide and aminonaphthalenesulphonamide exerted a marked inhibitory effect on carbonic anhydrase activity. Carbonic anhydrase is the enzyme which catalyses the reaction between water and carbon dioxide to form carbonic acid and is a source of hydrogen ions in the body.

$$H_2O + CO_2 \rightleftharpoons {}^{C}A \rightleftharpoons H_2CO_3 \rightleftharpoons {}^{C}A \rightleftharpoons H^+ + HC\bar{O}_3$$

The presence of carbonic anhydrase in the body was first reported by Meldrum and Roughton in 1932. The renal tubules (Davenport and Wilhelmi, 1941) like the erythrocytes (Meldrum and Roughton, 1933), the gastric mucosa (Davenport and Fisher, 1938), the lens and the retina of the eye (Bakker, 1941) contain substantial amounts of this enzyme. Carbonic anhydrase occurs in lower, but still significant, concentrations in the brain (Ashby, 1943 and Ashby, 1944), liver, pancreas and spleen (Goor, 1940).

The importance of carbonic anhydrase in the physiology of the kidney /

kidney was understood from the early observations on the rise in urinary pH, the loss of sodium and the acidosis which accompanied the administration of sulphanilamide. The first experimental exploration of the effects of carbonic anhydrase inhibitors on renal function was that of Höber in 1942 who showed that sulphanilamide and a series of other unsubstituted sulphonamides caused the urine of the perfused frog kidney to become alkaline. Höber (1942) concluded that carbonic anhydrase was involved in the reabsorption of bicarbonate by the kidney. The theory of urine acidification by bicarbonate reabsorption at that time implied that the acid excreted was derived largely from 'carbonic acid' in the glomerular filtrate. In 1945 an important change took place in the concept of renal tubular function when Pitts and Alexander showed that in acidotic dogs infused with, and excreting large amounts of buffer, the amount of titratable acid excreted was several times that which could be derived from the filtered carbonic acid by reabsorption of the filtered bicarbonate. They, therefore, concluded that the urine must be acidified by the secretion of acid and proposed that this secretion was actually an exchange of hydrogen ions from the tubule cells for sodium ions from the lumen of the tubules. Demonstrating that the excretion of titratable acid in mildly acidotic dogs could be partially suppressed by sulphanilamide, these authors postulated that carbonic acid was the immediate source of the secreted hydrogen ions and that carbonic anhydrase was involved in the conversion

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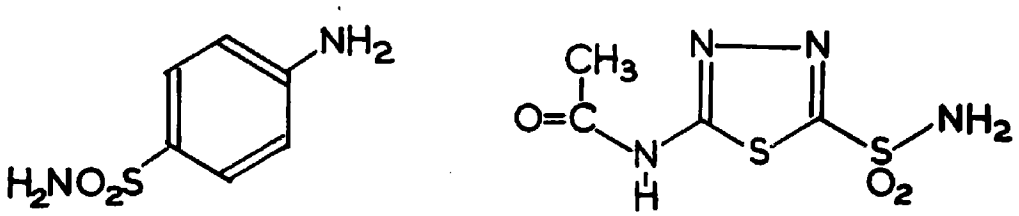

Sulphanilamide Acetazolamide

Chemical formulae of Sulphanilamide and Acetazolamide

Fig. 82

of metabolic carbon dioxide to carbonic acid. It was assumed at that stage that the reduction in the rate of excretion of titratable acid, when the enzyme was inhibited by sulphanilamide, represented the effect of a reduction in the rate of hydration of carbon dioxide (Beckman, Rossmeisl , Pettengill and Bauer, 1940). The significance of these findings has been fully reviewed by Berliner and Orloff (1956).

Schwartz in 1949, taking advantage of the saluretic property of sulphanilamide, administered it to patients suffering from congestive heart failure but he found it too toxic to be used as a natriuretic and diuretic agent.

From the investigations referred to above, it was now apparent that an inhibitor of carbonic anhydrase more potent and more specific than sulphanilamide might be a valuable therapeutic agent.

A study of the structure-activity relationships in the sulphonamides resulted in the synthesis of more highly potent carbonic anhydrase inhibitors. Thus in 1950 Roblin and Clapp synthesised a number of unsaturated sulphonamides, including acetazolamide (Fig. 82, page 200). Miller, Dessert and Roblin in 1950 demonstrated that acetazolamide was 300 times as potent as sulphanilamide in inhibiting carbonic anhydrase in vitro. Doses of acetazolamide could, therefore, be given which would bring about almost complete inhibition of carbonic anhydrase in vivo. The rise in urinary pH and increase in bicarbonate excretion which followed the administration of sulphanilamide, were observed more strikingly /

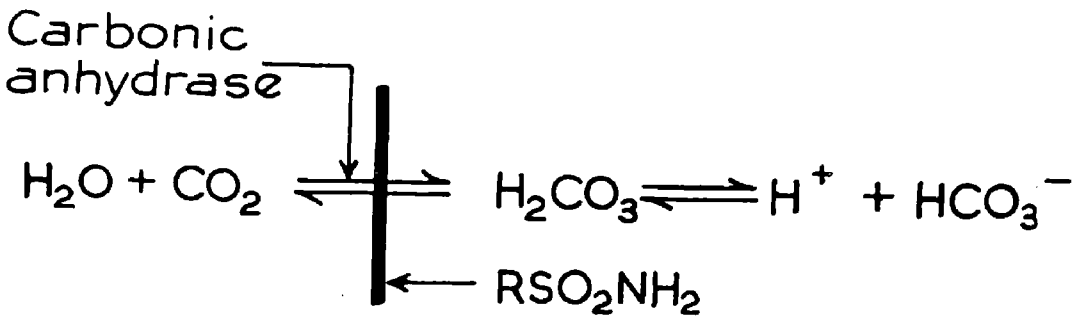

Fig. 82a

Diagram showing the point of action of sulphonamides which inhibit carbonic anhydrase activity.

strikingly after the use of acetazolamide. The effects of acetazolamide have been studied in detail in the dog (Berliner, Kennedy and Orloff, 1951 and Berliner, 1952), the rat (Maren, Wadsworth, Yale, Alonso, 1954), the rabbit (Holtmeier, Spuhler, Buhlmann and Labhart, 1953), in man (Counihan, Evans and Milne, 1954; Friedberg and Halpern, 1952; Friedberg, Taymor, Minor and Halpern, 1953; and Taymor, Minor and Friedberg, 1954), as well as in other species, including fish (Hodler, Heinemann, Fishman and Smith, 1955), the alligator (Hernandez and Coulson, 1954; Coulson and Hernandez, 1957; and Coulson, Hernandez and Beebe, 1957) and the chicken (Wolbach, 1955).

Administration of a single therapeutic dose of acetazolamide to the dog was followed by a marked increase in the urinary excretion of sodium, potassium and bicarbonate, accompanied with an increase in the urine flow and a decrease in the excretion of ammonia and titratable acid. The excretion of chloride was not, however, significantly changed (Berliner, Kennedy and Orloff, 1951). Except for the increase in potassium excretion these changes could be attributed to an interference with the hydrogen-sodium exchange mechanism. The direct effects of the diminished exchange would be the decrease in the reabsorption of sodium and bicarbonate and consequently a failure to acidify the urine. The increase in the excretion of potassium ions in the urine was attributed to the suppression of hydrogen ion transport. In the alligator, in contrast to the results obtained from mammals, /

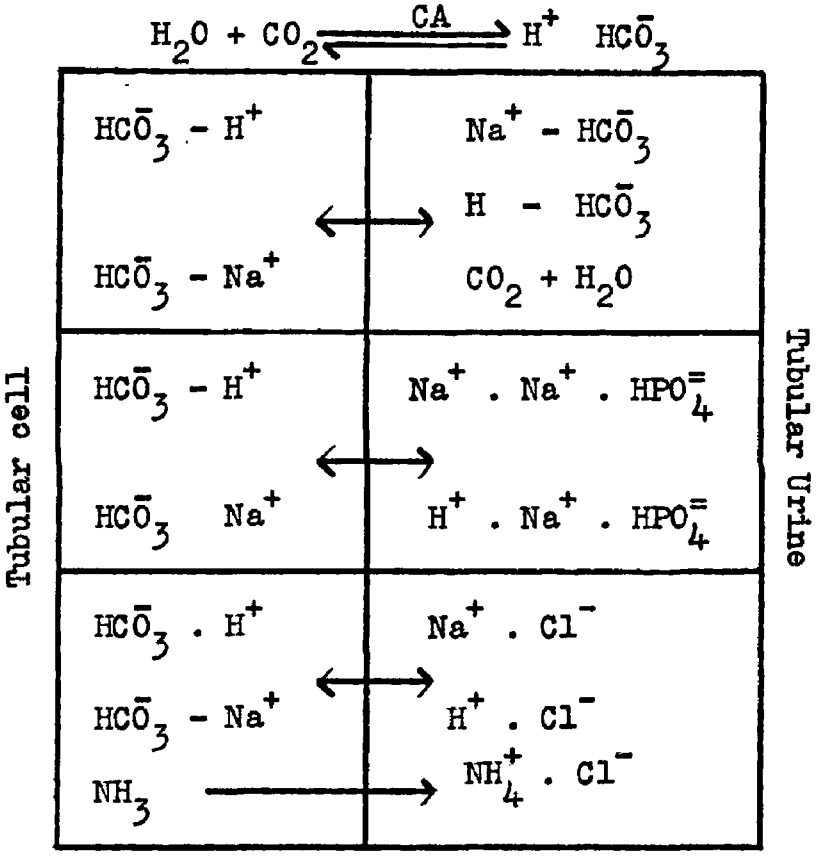

Fig. 83

The role of $H^+ - Na^+$ exchange in the reabsorption of bicarbonate, the acidification of the urinary buffers, and the excretion of ammonia.

mammals, acetazolamide increased excretion of chloride without increasing that of sodium bicarbonate, thus causing a systemic alkalosis (Coulson and Hernandez, 1957).

Berliner and his associates (1951) also observed that following the administration of acetazolamide, as much as 50 per cent of the filtered bicarbonate appeared in the bladder urine, showing that proximal, as well as distal tubular reabsorption of bicarbonate, was dependent on the rate of hydration of carbon dioxide. It was, therefore, suggested by Gilman (1958) that a single underlying mechanism of hydrogen ion-sodium ion (H^+-Na^+) exchange, could account for the reabsorption of bicarbonate along the length of the nephron. He also suggested that there were three important renal mechanisms responsible for maintaining the levels of extracellular bicarbonate and these were (a) reabsorption of bicarbonate ions, (b) acidification of urinary buffers and (c) exchange of urinary sodium with ammonia. Gilman (1958) has explained the role of the H^+-Na^+ exchange in the reabsorption of bicarbonate, the acidification of the urinary buffers and excretion of ammonia in the way shown in Fig. 83, page 203. He considered that the rate of hydrogen ion transport was responsible for the renal control of acid-base metabolism. When hydrogen ion transport went on at the maximum rate the reabsorption of bicarbonate was complete, the urinary buffers were maximally acidified and the ammonia mechanism worked efficiently. The bicarbonate ions, derived from the

hydration of carbon dioxide, were returned to the extracellular fluid in combination with the fixed cation sodium exchanged for hydrogen ions.

Conversely when the rate of hydrogen ion transport was reduced, the reabsorption of bicarbonate was complete, there was no acidification of the urinary buffers and ammonia disappeared from the urine; sodium bicarbonate was mobilised from the extracellular fluid and excreted together with water.

Although the detailed studies of Maren, Wadsworth, Yale and Alonso (1954) and Maren, Mayer and Wadsworth (1954) defined the pattern of the renal effects of acetazolamide, they did not enable an evaluation of its potential usefulness as a diuretic to be made. The dog, when given acetazolamide in oral doses of 5 mg. per kg. once daily, responded with a diuresis but this was followed by a recovery phase which was marked by the complete retention of bicarbonate and sodium ions and by increased amounts of titratable acid and ammonia, together with small quantities of potassium in the urine. This recovery phase lasted until the metabolic balance for sodium and bicarbonate ions was restored. They also reported that this refractory state was not due to an absence of inhibition of carbonic anhydrase but was presumably due to reduction of the plasma bicarbonate concentration. These workers also observed that following administration of acetazolamide, hypopotassaemia developed and persisted for as long as the administration of the drug continued. These findings suggested that acetazolamide, even in the refractory state, /

state, caused the usual rise in cellular pH. They also observed that fatal potassium depletion occurred in two dogs following the administration of 1,000 mg. per kg. of body weight of acetazolamide daily for a period of three days.

The mechanism of the development of the refractory state following the repeated use of acetazolamide was clearly explained by Gilman (1958), who showed that the repeated use of a carbonic anhydrase inhibitor might result in the excretion of an acid urine due to the diminution of the bicarbonate levels of the plasma. The use of acetazolamide in a severe metabolic acidosis might, therefore, have no effect on the diuresis and little effect on the titratable acidity of the urine. From these observations it was clear that when the bicarbonate load in the system was low, the reabsorption of bicarbonate ions could take place without the catalytic hydration of carbon dioxide. On the other hand, in bicarbonate-loaded subjects, the inhibition of carbonic anhydrase would reduce the hydrogen ion-transport which was directed towards bicarbonate reabsorption. This is the reason that the carbonic anhydrase inhibitor, acetazolamide, is effective only in subjects with normal bicarbonate levels and ineffective in a metabolic acidosis. It is also ineffective in an acidosis induced by the administration of ammonium chloride (Maren, 1956).

Although in normal animals the diuretic effects of acetazolamide have been found to compare favourably with those of the mercurial diuretics /

diuretics (Moyer, Spurr and Ford, 1954; and Cardillo, Mullin, Schiffer and Lyons, 1954), investigators who have used acetazolamide in patients severely ill with cardiac disease have generally found it far less effective than the mercurial diuretics (Counihan, Evans and Milne, 1954; and Relman, Leaf and Schwartz, 1954).

There are very few pharmacodynamic agents whose mechanism of action is so well understood as that of acetazolamide. As a result it has not only proved useful therapeutically but has also been of great value in the study of the mechanism of formation of the urine (Berliner, Kennedy and Orloff, 1951; and Pitts, 1952), the formation of the gastric juice (Janowitz, Colcher and Hollander, 1952) and the formation of the ocular fluids (Westrand, 1952).

Since acetazolamide has a tendency to cause hypopotassaemia it is undesirable to use this drug in the treatment of conditions such as congestive heart failure, where prolonged treatment may cause serious potassium loss. Acetazolamide can, however, be employed as a diuretic in short term therapy, for example in the oedema of premenstrual tension and toxaemia of pregnancy (Assali, Monk, Ullrich, Voskian and Singh, 1955). Its clinical usefulness is seen also in a reduction of the intraocular pressure in glaucoma - an effect due to inhibition of ciliary carbonic anhydrase (Becker, 1954a, Becker, 1954b) and its use in epilepsy in children (Bergstrom, Carzoli, Lombroso, Davidson and Wallace, 1952).

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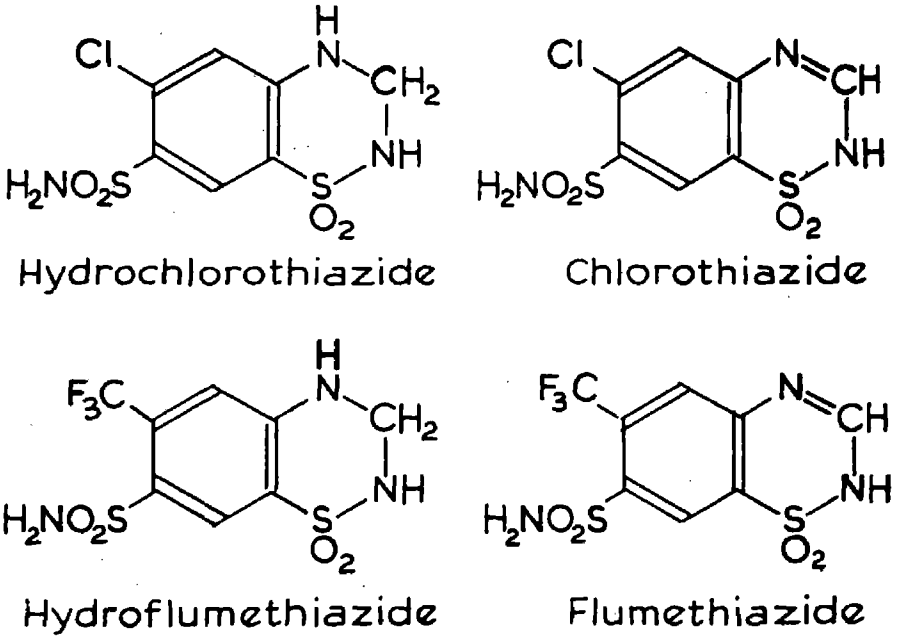
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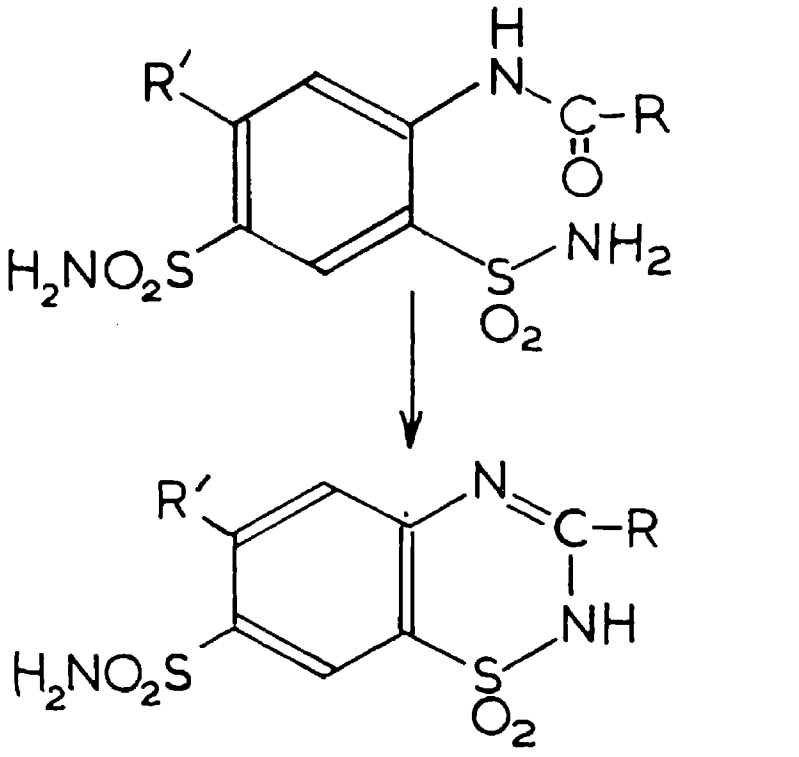
Fig. 84.

Due to the limitations of acetazolamide as a diuretic agent, an intensive search was carried out with the object of producing a better, but a chemically related, diuretic. As a result of careful chemical and pharmacological studies, Novello and Sprague in 1957 reported the discovery of chlorothiazide (6-chloro-7-sulphamyl-1, 2, 4-benzothiadiazine-1, 1-dioxide) (Fig. 84, page 208). This can be regarded as being formed from an aromatic sulphonamide by ring closure in the manner shown in Fig. 85, page 210.

The pharmacological actions of chlorothiazide have been studied by a number of groups of workers using different species including man (Beyer, Baer, Russo and Haimbach, 1957; Russo, Baer, Noll and Beyer, 1957; Baer, Leidy and Brooks, 1957; and Ford, Moyer and Spurr, 1957).

Chlorothiazide is a stronger carbonic anhydrase inhibitor <u>in vitro</u> than acetazolamide but does not retain this activity <u>in vivo</u> except when used in larger and repeated doses (Beyer, Baer, Russo and Haimbach, 1957). It is an orally effective diuretic which causes an increased excretion of sodium and chloride with a weaker, but definite effect on potassium and bicarbonate excretion (Ford, Moyer, and Spurr, 1957). The enhanced elimination of sodium and chloride is accompanied by osmotic amounts of water.

A voluminous literature on the pharmacology and clinical uses of chlorothiazide which contains publications from all parts of the world has appeared. The practical importance of this type of drug led to an /



R=H, R'=Cl, Chlorothiazide

Fig. 85.

an intensive search by a number of workers for improved and more potent diuretics. New and more active compounds of the benzothiadiazine series have appeared, including hydrochlorothiazide (De Stevens, Werner, Halamandaris and Ricca, 1958); flumethiazide and hydroflumethiazide (Holdrege, Babel and Cheney, 1959); benzothiazide (P'An, Scriabine, McKersie and Melamore, 1960) and trichloromethiazide (Taylor and Winbury, 1960). Compounds such as salamid (5-chloro-2,4-bis-sulphonamido aniline) - actually an intermediate in the synthesis of chlorothiazide (Lund and Störling, 1959) - and a benzophenone derivative, hygroton (Stenger, Wirz and Pulver, 1959) are also reported to have more potent diuretic actions than chlorothiazide.

All these benzothiadiazine derivatives are probably carbonic anhydrase inhibitors to a greater or lesser degree. The general pharmacological properties and the pattern of electrolyte excretion exhibited by all the benzothiadiazine derivatives are qualitatively similar to those of chlorothiazide.

Effects of chlorothiazide on renal function

All the benzothiadiazine derivatives in clinical use cause increased excretion of sodium, chloride, potassium, bicarbonate and water. They are inhibitors of carbonic anhydrase, yet their saluretic action is more like that of the organo-mercurial diuretics than that of acetazolamide. The renal tubular reabsorption of sodium and chloride ions is inhibited in nearly equimolar amounts by chlorothiazide and these /

these are eliminated in the urine with osmotic equivalents of water. In large and repeated doses, the action of chlorothiazide becomes more like that of the specific carbonic anhydrase inhibitors such as acetazolamide. Thus at high dose levels chlorothiazide increases the excretion of potassium and bicarbonate, resulting in an alkaline urine and a corresponding decrease of systemic potassium and bicarbonate levels (Baer, Russo and Beyer, 1959).

Hydrochlorothiazide (Fig. 84, page 208) is a more potent diuretic and saluretic agent than chlorothiazide. The slight structural modification resulting from saturation of the double bond in the heterocyclic ring has thus resulted in a marked increase of its diuretic potency as compared with chlorothiazide (Barrett, Rutledge, Sheppard and Plummer, 1959; and Renzi, Chart and Gaunt, 1959). Hydrochlorothiazide appears to have less tendency to cause excretion of bicarbonate (Bartorelli, Gargano and Zanchetti, 1959) and potassium, but the relatively greater chloride excretion caused by this compound may result in a hypochloraemic alkalosis in contrast to the metabolic acidosis of acetazolamide.

Although the natriuretic response to hydrochlorothiazide in human subjects is about twenty times greater than that of chlorothiazide, the qualitative pattern of electrolyte excretion remains the same (Richterich, 1958). Renzi, Chart and Gaunt in 1959 in their experiments on fluid-loaded rats have shown that hydrochlorothiazide had from 7 to 20 times /

times the relative potency of chlorothiazide. They also demonstrated that hydrochlorothiazide modified the antidiuretic effects caused by vasopressin, reserpine and syrosingopine in normal animals. In adrenalectomized animals it enhanced sodium but not water excretion. It also antagonised the sodium-retaining effect of aldosterone and desoxycorticosterone and enhanced the natriuretic response caused by prednisolone.

Poutsiaka, Piala, Smith, Burke and Thomas (1960) have shown that intravenous administration of flumethiazide to alkalotic or acidotic dogs enhanced diuresis, causing an increased excretion of sodium and chloride. In normal dogs excretion of sodium and chloride was more marked, with little observable effects on potassium and bicarbonate excretion.

Chlorothiazide can thus retain its diuretic activity both in conditions of experimental alkalosis and acidosis (Beyer, 1958). Following the administration of large amounts of sodium chloride, chlorothiazide caused a substantial increase in both sodium and chloride excretion, with a much lesser effect on the excretion of potassium and bicarbonate ions and upon the urinary pH. In an ammonium chloride-induced acidosis in dogs, chlorothiazide caused a greater excretion of chloride ions than did sodium, whereas in an alkalosis due to administration of sodium bicarbonate there was a greater increase in the elimination of bicarbonate than of chloride ions. /

ions. No alteration was noted in the saluretic response to chlorothiazide in dogs maintained for several days on a low salt diet.

The salt-retaining action of steroids is reversed by chlorothiazide and excretion of potassium is increased, indicating that chlorothiazide is not a true antagonist to the effects of steroids on kidney function (Beyer, 1958).

The antidiuretic hormone (vasopressin), when given parenterally, produced a marked depression in the excretion of electrolytes and water. Chlorothiazide has been shown to reverse this effect in dogs (Beyer, 1958) and in the rat (Kovacs, David and Horvath, 1959).

Chlorothiazide at therapeutic dose levels has no effect on the glomerular filtration rate, renal blood flow, glucose reabsorption or tubular secretion of p-aminohippurate. It is a weak competitive inhibitor of carbonic anhydrase and can, in very high doses, depress the clearance of phenol red and the renal tubular secretory capacity for p-aminohippurate in dogs (Baer, Beyer, Russo and Titus, 1958).

The physiological disposition of chlorothiazide in the dog has been clearly discussed by Baer, Leidy and Brooks (1957) and Baer, Leidy, Brooks and Beyer (1959) and in human beings by Brettel, Gordon and Aikawa, (1959).

When chlorothiazide (10 mg. per kg.) was given intravenously to dogs, 67 to 76 per cent of the dose was excreted within the first hour and 86 to 99 per cent was excreted within six hours. The

clearance /

clearance of chlorothiazide from the plasma occurred with corresponding rapidity. Four hours following the intravenous administration of 20 mg. per kg. of 3-Cl4 labelled chlorothiazide to a bilaterally nephrectomised dog, the plasma level was 26.3 mg./litre and some 40 per cent of the dose was eliminated in the bile and another 10 per cent was found in the intestinal tract. Small amounts of chlorothiazide were found in the liver, lung and spleen. Little or none was detected in muscle, fat, brain and the cerebrospinal fluid. No accumulation of the drug could be demonstrated in the erythrocytes or other tissues following its intravenous administration.

Probenecid, at a dose level that completely inhibited the tubular secretion of penicillin, partially depressed the renal tubular secretion of chlorothiazide. This indicates that elimination of chlorothiazide is carried out by the renal tubules, at the site which is known to be responsible for the secretion of penicillin, p-aminohippurate and phenol red.

3-Cl4 labelled chlorothiazide has been used as a tracer to study the metabolism of chlorothiazide in human subjects. In normal individuals, following an oral dose of 0.5 g. of chlorothiazide, a maximum serum concentration of 4 µg. per ml. was obtained at two hours. Excretion rate was greatest during the first five hours. When 0.5 g. of chlorothiazide was given intravenously, 90 per cent of the dose was excreted within two hours, with a complete disappearance of the drug from the blood within 24 hours (Brettell, Gordon and Aikawa, 1959).

Hydrochlorothiazide /

Hydrochlorothiazide and flumethiazide are handled by the kidneys in a similar manner to chlorothiazide. Oral administration of tritium-labelled hydrochlorothiazide to water-loaded rats was followed by the excretion of 52 per cent of the dose in 24 hours. A greater proportion of the dose was retained in animals when larger amounts of the drug were administered. In dogs, after oral administration of 0.31 mg. per kg. of hydrochlorothiazide, blood levels were found to be extremely low, as were the levels in the brain and spleen but liver and kidney showed much higher concentrations of the tritium-labelled drug. These levels were achieved one hour after drug administration (Sheppard, Bowen, Mowles and Plummer, 1959).

Hydrochlorothiazide disappears somewhat more slowly from the plasma of nephrectomised dogs than does chlorothiazide. This indicates that extrarenal degradation of hydrochlorothiazide, or its elimination in the bile, proceeds more slowly than that of chlorothiazide (Baer, Russo and Beyer, 1959).

The renal clearance studies on chlorothiazide indicate that it is excreted rapidly and completely in the urine and is secreted by the renal tubules. The tubular secretory component is believed to be inhibited by probenecid.

Except for a depression in the glomerular filteration rate that is produced by large intravenous doses of chlorothiazide and hydrochlorothiazide, these compounds appear to have no adverse effects on renal /

renal function. Histopathological studies following the administration of large doses of chlorothiazide to dogs for many weeks, did not reveal lesions which could be attributed to these drugs (Peck, McKinney, Baer, McManus and Beyer, 1958).

The diuretic response to hydrochlorothiazide was depressed when it was given intravenously at a dose level of 800 mg. per day to human subjects, thus indicating that high doses exerted toxic effects on renal tubular function (Moyer, Fuchs, Irie and Bodi, 1959). The toxicity of the benzothiadiazine derivatives is, however, very low and this was demonstrated by Peck, McKinney, Baer, McManus and Beyer (1958) in their investigations on different species, including the dog. In mice the acute oral and intravenous LD50 of 8.5 and 1.1 g. per kg. respectively, indicated good oral absorption and an excellent margin of safety. Chronic oral administration of dose levels of up to 600 mg. per kg. per day to dogs for six months and 500 to 1,000 mg. per kg. per day intravenously for periods of three weeks, without apparent signs of toxicity provided added evidence of its safety as a diuretic.

Mode of diuretic action of chlorothiazide

The mode of diuretic action of chlorothiazide has been discussed by Beyer (1958) who compared its effects on kidney function with those of the carbonic anhydrase inhibitor, acetazolamide and the organo-mercurial diuretics. So far as it is known the influence of chloro-thiazide /

chlorothiazide on the transport mechanisms of the nephron is directed specifically towards the transfer of electrolytes. This effect is said to be primary and is not dependent upon extrarenal drug effects. Lavender and Pullman (1958) collected urine samples separately from the two kidneys of the same dog following the unilateral renal arterial infusion of chlorothiazide. They found an increased excretion of water, sodium, chloride and, to a lesser degree, of potassium in the experimental kidney. The effects were independent of alterations in glomerular filtration rate. A secondary effect upon the contralateral kidney was observed as the drug gained access to this through the systemic circulation.

The nature of the diuretic activity of chlorothiazide and its mode of action can also be approached by a consideration of the effects of other diuretic agents. The pattern of sodium, chloride, potassium and water excretion of chlorothiazide is similar to that of the organomercurial diuretics but a marked increase of urinary pH is not typical of the latter. The increased pH and natriuretic effects resemble those of acetazolamide, but this compound does not induce a notable chloruresis (Maren, Wadsworth, Yale, and Alonso, 1954).

The known sensitivity of certain enzymes to heavy metals has made it attractive to speculate that enzymatic inhibition is responsible for the diuretic activity of the organic mercurials. Compounds of mercury have a high affinity for thiol enzymes. Because of their ability to combine /

combine with thiol groups the increased electrolyte and water excretion induced by mercurials can be antagonised by the administration of dimercaprol (Brunner, 1959).

The onset of diuresis with the mercurial diuretics is relatively slow, reaching a maximum in from one to two hours and this persists for some hours. The diuresis consists of an increase in sodium, chloride and water excretion, with minimum effects on potassium and bicarbonate levels. The response to mercurial diuretics is enhanced by the co-administration of acidifying salts such as ammonium chloride (Axelrod and Pitts, 1952) and is decreased in an alkalosis (Levy, Weiner and Mudge, 1958).

Mercurial agents are gastro-intestinal irritants and so cannot be used in acute abdominal conditions or in chronic kidney disease. Maren (1956) reported that the concurrent administration of acetazolamide and mercurial diuretics decreases, but does not abolish, the response of dogs to the mercurial diuretics. Riggs and Berkson (1958) however, have shown that the chloruretic and diuretic responses of dogs to 1 to 2 mg. per kg. of a mercurial diuretic given intravenously was almost completely suppressed by acetazolamide (20 mg. per kg. per hour) given as a continuous intravenous infusion. They concluded that there was a good inverse correlation between the urinary pH and the response to organo-mercurial agents. Vargas and Cafruny in 1958 demonstrated that mercurial diuretics produced renal cortical ischaemia and increased /

increased the wet weight of dog and rat kidneys. A similar effect was not noticed when the animals were treated with chlorothiazide.

Mechanism of the diuretic action of chlorothiazide

The precise mechanism of action of the benzothiadiazine diuretics is unknown. Histochemical and chemical data from in vitro studies (Cafruny and Farah, 1956; Mann and Keilin, 1940 and Davenport and Wilhelmi, 1941) indicate that the mercurials and sulphonamides may inhibit specific enzyme systems which are required for the activation of the ion exchange mechanisms of the kidney, especially those for sodium reabsorption. A comparison of the actions of different types of diuretics has made it clear that the behaviour of chlorothiazide differs from that of other classes of diuretics in a number of important ways. The increased urinary pH with enhanced potassium excretion, observed with higher doses of chlorothiazide, is almost certainly attributable to inhibition of carbonic anhydrase (Beyer, 1958).

Pitts, Kruck, Lozano, Taylor, Heidenreich and Kessler (1958) in their experiments on anaesthetised dogs have shown that blockade of sodium and chloride reabsorption is additive when chlorothiazide and chlormerodrine are given respectively in maximal doses. These authors suggest that chlorothiazide either blocks a different reabsorptive mechanism from that influenced by the mercurial diuretics, or more probably, interferes with the different enzymatic reactions which supply energy /

energy to a single reabsorptive mechanism. They suggested that there are not less than three biochemically distinct mechanisms or sources of energy concerned with the reabsorption of sodium and chloride ions, one of which was sensitive to chlorothiazide and one or more resistant to both drugs. The mechanisms responsible for bicarbonate reabsorption, including the mechanism for sodium, hydrogen and potassium ion exchanges were all affected qualitatively in a similar manner by chlorothiazide and acetazolamide - presumably as a consequence of their carbonic anhydrase inhibiting activities. Acetazolamide was the more potent.

Ford (1957) and Ford and Rochelle (1959) in their clinical studies have carefully evaluated the mechanism of diuretic action of chlorothiazide by comparing its actions with those of other diuretics. Mercurials, acetazolamide and chlorothiazide were administered alone and in various combinations in order to determine the effects on the excretion of various electrolytes. They found that after a maximum increase in the sodium excretion, following the injection of a mercurial diuretic (meralluride), the administration of acetazolamide produced another significant increase in the sodium excretion thus indicating that two non-competitive mechanisms for the tubular reabsorption of sodium had been inhibited. At the peak of this second increase and while meralluride and acetazolamide were still being infused, the administration of chlorothiazide was observed to produce a third significant increase over the previous rate of sodium excretion. This observation /

observation indicated that a third mechanism or receptor site was affected, resulting in an increased inhibition of the mechanism for sodium reabsorption. When acetazolamide followed the administration of chlorothiazide no augmentation of sodium excretion was observed. As part of the mode of action of chlorothiazide involved carbonic anhydrase inhibition these authors concluded that it acted upon this enzyme by competition with acetazolamide. On the other hand, chlorothiazide has other actions, for example an effect upon sodium excretion which cannot be blocked following the administration of acetazolamide. When administration of mercurial diuretics was followed by the administration of chlorothiazide, no augmentation in sodium excretion was observed but the converse was not true. Chlorothiazide appears, therefore, to affect the same mechanism for sodium excretion as the mercurials.

Ford and Rochelle (1959) suggested that the mercurial diuretics shared a mechanism of action with chlorothiazide. They also suggested that acetazolamide shared a mechanism with chlorothiazide and that chlorothiazide also appeared to act through a third, non-competitive mechanism.

The enhanced excretion of chloride ions does not fit the classic picture of renal sodium reabsorption which has been put forward by a number of workers to explain the mechanism of urinary acidification (Pitts and Alexander, 1945; Pitts, Lotspeich, Schiess and Ayer, 1948 and /

and Berliner and Orloff, 1956). The chloruretic action of chlorothiazide may merely reflect the absence of interference with the reabsorption of filtered bicarbonate.

Beyer (1958) has differentiated between an agent which inhibits the sodium-hydrogen ion exchange in the nephron from drugs such as acetazolamide which cause carbonic anhydrase inhibition elsewhere, for example in the erythrocytes. Compounds with actions similar to those of acetazolamide, by depressing the rate of transfer of carbon dioxide from the kidney, might increase the bicarbonate excretion as well as inhibit the sodium-hydrogen ion exchange. The reciprocal relation between chloride and bicarbonate excretion has been explained in more detail by Hilton, Capeci, Kiss, Kruesi, Glaviano and Wegaria in 1956.

The site of action of chlorothiazide

Information on the site of action of chlorothiazide is mainly derived from studies using the 'stop flow' techniques of Malvin, Wilde, Vander and Sullivan (1958). These authors investigated the mechanism of sodium transport along the renal tubule using the 'stop flow' analysis. During varying degrees of osmotic diuresis in dogs the ureter of one kidney was occluded for $2\frac{1}{2}$ to 8 minutes. Following occlusion, final urine samples were collected and analysed for sodium, creatinine and para-aminohippurate or glucose. Using the urinary creatinine concentration as an index of water movements, the mass of sodium and water reabsorbed from the proximal segments during occlusion /

occlusion was calculated.

Vander, Malvin, Wilde and Sullivan (1959) have performed a series of experiments upon dogs using the 'stop flow' technique. They suggested on the basis of their results that chlorothiazide, like the mercurial diuretics, diminished the reabsorption of electrolytes in the proximal tubular system. They also indicated that the secretion of potassium in the distal tubules was greatly increased by chlorothiazide, whereas the potassium reabsorption in the distal tubules was slightly decreased. On the other hand, Kessler, Hierholzer, Gurd and Pitts (1959) reported depression of sodium and potassium reabsorption in the distal tubule, accompanied by an increase in the pH of the distal tubular urine. The effect on electrolyte and fluid composition in the proximal tubules confirmed the observations of Vander and his associates (1959) and Baer and his co-workers (1959) that chlorothiazide was secreted by the renal tubules and that this secretion could be inhibited by probenecid. They concluded that the mechanism for the elimination of chlorothiazide coincided with that which was responsible for the secretion of penicillin, para-aminohippurate and phenol red. Kessler and his associates (1959) have localised the tubular secretion of chlorothiazide to the proximal segment of the nephron.

<u>The general pharmacology of the benzothiadiazine derivatives.</u>

<u>Cardiovascular system.</u>

Chlorothiazide /

Chlorothiazide and hydrochlorothiazide in small doses had no effect on the function of the isolated guinea pig or rabbit heart, whereas high doses appeared to have a slight transient depressant effect upon myocardial function. Responses of the isolated heart and auricles to adrenaline and noradrenaline were not altered by chlorothiazide or hydrochlorothiazide. No electrocardiographic changes were observed in rats and rabbits even at high dose levels. (Preziosi, Bianchi, Loscalzo, Schaepdryver, 1959; and Barrett, Rutledge, Sheppard and Plummer, 1959).

In the normotensive cat, dog and rabbit these compounds do not alter the heart rate, blood pressure levels or the respiratory pattern but in a proportion of vagotomised dogs a slight hypotensive effect was seen by Preziosi and his associates (1959). In dogs, chlorothiazide does not modify the responses of the blood pressure to adrenaline or noradrenaline and local application of chlorothiazide to the region of the carotid sinus has no direct effect on the blood pressure or on the carotid sinus pressor reflex. No effect on acute neurogenic hypertension in dogs was observed. In the same species, chlorothiazide had no anticholinergic or ganglion-blocking activity and had little or no effect on the responses to carotid occlusion or to stimulation of the peripheral or central ends of the vagus. It appeared, however, to prolong the depressor action of an intravenous injection of acetylcholine.

Beavers and Blackmore (1958) suggested that chlorothiazide had a direct /

direct action on vascular smooth muscle. In their experiments on anaesthetised dogs they found that the pressor responses to adrenaline and noradrenaline and the depressor effects of isoprenaline were decreased. It was suggested that chlorothiazide might exert its anti-hypertensive effects by reducing the responsiveness of the blood vessels to agents which alter vascular tone. Barac (1958) had previously demonstrated that chlorothiazide did not cause vasodilatation in the blood vessels of the kidney perfused with fresh blood and that it did not antagonise renal vasoconstriction in the kidney perfused with stored blood.

Hydrochlorothiazide potentiated the hypotensive action of hydralazine and increased the depressor response to histamine in dogs (Barrett, Rutledge, Sheppard and Plummer, 1959). It did not alter the hypertensive condition caused by chronic administration of hydrocortisone to rats. Hydrochlorothiazide when given over a long period of time, significantly inhibited the blood pressure rise associated with adrenal regeneration hypertension. No similar effect was noted following acute treatment with hydrochlorothiazide. The hypotensive effect of this compound was related to a marked decrease in the appetite of the treated animals for sodium chloride solution (Renzi, Chart and Gaunt, 1959). These workers suggested that hydrochlorothiazide lowered blood pressure only when sodium intake was reduced or when this drug enhanced the sodium loss - effects which are associated with a decrease in /

in the volume of plasma or extracellular fluid.

Hydrochlorothiazide had no effect on the heart rate, blood pressure or the cardiovascular responses to adrenaline and noradrenaline in the anaesthetised dog.

Chlorothiazide and hydrochlorothiazide potentiate the antihypertensive effects of reserpine in the anaesthetised cat. This effect was noticed when these compounds were given intravenously prior to the injection of reserpine. This observation indicates the possibility that the antihypertensive effect of the benzothiadiazines is due, at least in part, to a specific pharmacodynamic action, independent of the alterations in the electrolyte pattern and the reduction of plasma volume (Preziosi, Marmo and Miele, 1961).

In a more recent publication Preziosi, Schaepdryver, Marmo and Miele (1961) have shown that hydrochlorothiazide had no effect on the carotid sinus baroreceptors, the carotid body chemoreceptors or on the vasomotor centre. Small doses of hydrochlorothiazide inhibited the pressor responses following the stimulation of adrenergic fibres without influencing the vascular responses to catechol amines and synthetic hypertensine, whereas in high dose levels it decreased the vascular responses to adrenaline and noradrenaline. They also observed a marked decrease of the adrenaline and noradrenaline content of the liver, heart, spleen and adrenals following a single intravenous dose of 10 mg. per kg. of hydrochlorothiazide in dogs and mice.

Book and Gross (1960) using normal dogs have shown that the oral administration /

administration of 5 mg. per kg. daily of hydrochlorothiazide for one week significantly reduced the pressor responses to various doses of adrenaline, noradrenaline and angiotensine. They also observed that the antihypertensive activity of chlorisondamine was enhanced when given in combination with hydrochlorothiazide.

Merrill, Guinand-Baldo and Giordano in 1958 have already demonstrated that chlorothiazide reduced the vasopressor response of patients to noradrenaline in the presence of ganglion-blocking agents. They suggested that chlorothiazide may exert its hypotensive action in part by blocking the response of the blood vessels to increased circulating pressor amines, or by inhibiting the release of these at the neuro-effector sites. It may be significant that a low salt diet also decreases the responsiveness of patients to adrenaline and noradrenaline (Raab, Humphreys, Makous, De Grandpre and Gigee, 1952).

Poutsiaka, Piala, Smith, Burke and Thomas in 1960, using flumethiazide in normotensive dogs, have shown that this compound had negligible effects on the blood pressure, the pulse rate, the responses of the blood pressure to adrenaline and acetylcholine, the response to occlusion of the common carotid artery and the pressor responses to stimulation of the cut peripheral and central ends of the vagus nerve.

A decrease in the tone of the isolated rabbit gut, with no change in the peristaltic movements was also noted following low doses of chlorothiazide (Preziosi, Bianchi, Loscalzo and Schaepdryver, 1959) and /

and hydrochlorothiazide (Preziosi, Schaepdryver, Marmo and Miele, 1961).

The contractions of the isolated pregnant uterus of the mouse induced by synthetic oxytocin were slightly inhibited by low doses of hydrochlorothiazide.

Clinical applications of chlorothiazide.

The natriuretic and diuretic properties of chlorothiazide have greatly facilitated the management of hypertension, particularly when there is oedema formation due to salt and water retention. Oedema is found in many disease states in which there is interference with electrolyte balance. This results in an accumulation of sodium with osmolar amounts of water in the extracellular tissue spaces. Almost all types of fluid retention of a systemic or local nature, which can be attributed to the retention of sodium, have responded very well to chlorothiazide therapy. Total elimination of oedema fluid in such conditions as congestive heart failure, nephrosis and portal cirrhosis has been achieved and maintained by the administration of chlorothiazide (Laragh and Demartini, 1957; Sherlock, Read, Laidlaw and Haslam, 1958; Burch and White, 1959; and Eskwith, 1959).

In cases of advanced cirrhosis of the liver, hepatic coma can be precipitated by the increased excretion of potassium caused by chlorothiazide. This can be avoided by adding supplements of potassium during the period of treatment.

Digitalis intoxication may be induced by the loss of excessive potassium /

potassium (Lown, Salzberg, Ensflberg and Weston, 1951) during prolonged chlorothiazide treatment in cases of congestive heart failure. This can, however, be prevented by using digitalis im smaller doses or by giving potassium.

Chlorothiazide has been used in fluid retention due to toxaemias of pregnancy (Finnerty, Buchholz and Tuckman, 1958; Assali, Judd, Mondz and Dasgupta, 1958 and Landesman, Ollstein and Quinton, 1959) and premenstrual tension (Jungek, Barfield and Greenblatt, 1959).

Other therapeutic uses include the treatment of the anginal syndrome (Marshall, 1959), fluid retention due to steroid therapy (Warshaw, 1959), obesity (Landes and Peters, 1958) and in local oedema (Barker, Carey and Brough, 1959). Due to its safety and effectiveness chlorothiazide has attained a world wide clinical popularity.

The advent of chlorothiazide constituted an important advance in the management of certain types of arterial hypertension. It has proved its value as an adjunct to other antihypertensive agents, including reserpine and ganglion-blocking agents; (Tapia, Dustan, Schneckloth, Corcoran and Page, 1957; Freis and Wilson, 1957; Rochelle, Bullock and Ford, 1958; Heider, Dennis and Moyer, 1958; Barnett and Marshall, 1958; Dustan, Cumming, Corcoran and Page, 1959; Moser and Macauley, 1959 and Smirk, McQueen and Morrison, 1960) and in a proportion of cases when it is administered alone (Wilkins, 1957; Hall and Owen, 1959; Freis, Wanko, Wilson and Parrish, 1958; and Juel-Jensen and Pears, 1960).

The mechanism of antihypertensive action of chlorothiazide.

Although much effort has been expended in attempts to evaluate the mechanisms underlying the antihypertensive action of chlorothiazide, these are still obscure. Arguments have been advanced to indicate that chlorothiazide has a unique antihypertensive action which is not totally dependent upon its diuretic effects. Suggestions include that the hypotensive effect is related to a decrease in plasma and extracellular fluid volumes (Wilson and Freis, 1959; Macleod, Dustan and Schneckloth, 1959; and Macleod, Dustan and Page, 1960), a fall in cardiac output (Dustan, Cumming, Corcoran and Page, 1959 and Crosley, Castillo, Freeman, White and Rowe, 1958), dehydration of the arteriolar walls (Lauwers and Conway, 1960), deoreased arteriolar responsiveness to substances which act on vascular smooth muscle (Beavers and Blackmore, 1958 and Merrill, Guinand-Baldo and Giordano, 1958), depression of vascular smooth muscle (Preziosi, Bianchi, Loscalzo and Schaepdryver, 1959), reduction in the sodium content of the arteriolar walls (Aleksandrow, Wysznacka and Gajewski, 1959) and interference with the action of aldosterone (Hollander, Chobanian and Wilkins, 1959).

A relationship between the occurrence of elevated blood pressure and a high salt intake has been suggested by a number of workers (McQuarrie, Thompson and Anderson, 1936; Lenel, Katz and Rodbard, 1948; Sapirstein, Brandt and Drury, 1950; and Meneely, Tucker, Darby and Auerbach, 1953).

Meneely /

Meneely and his associates and Sapirstein and his co-workers in their experiments on rats have shown that administration of hypertonic saline with or without the use of sodium-retaining hormones such as desoxycorticosterone acetate, produced a hypertensive state. This was similar in most respects to human hypertension. This correlation between blood pressure elevation and salt intake in human subjects has recently been stressed by Dahl and Love (1957) who also suggested that the level of sodium intake was the primary aetiological factor in the development of essential hypertension. The observation that salt restriction enhances the hypotensive action of diuretics is additional evidence that this effect may be due to increased sodium loss, which may further result in shrinkage of the extracellular fluid space and a reduction of plasma volume (Freis, Wanko, Wilson and Parrish, 1958).

There is no doubt that chlorothiazide increases the urinary excretion of sodium, potassium, chloride and bicarbonate and there is some evidence that antihypertensive potency and sodium depletion are linked but the source of the sodium eliminated, whether it comes from the extracellular spaces or elsewhere is not known, nor is the point in the cell at which the drug acts.

The work described in this part of the thesis was undertaken to investigate certain aspects of the mode of antihypertensive action of chlorothiazide. The earlier part of this section of the thesis is devoted to a study of the pharmacological properties of chlorothiazide and some allied compounds. It was felt that interpretation of the data /

data obtained in such experiments would give some information as to the action of chlorothiazide at the cellular level. It appears, however, that the fundamental action of chlorothiazide must ultimately involve the electrolyte metabolism of the cell. With this possibility in mind the behaviour of sodium and potassium ion movements in the cell under the influence of chlorothiazide has also been investigated. The fact that some energy-yielding mechanism is necessary for the movements of sodium ions from extracellular spaces to the cell has led to some experiments being made on the adenosinetriphosphatase activity of rat skeletal muscle under the influence of chlorothiazide.

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C H A P T ER II.

C H A P T E R II

A. MATERIALS

The drugs used in the investigation which is described in this section of the thesis, together with their shortened names are as follows:-

1. Acetylcholine chloride is described as acetylcholine.
2. (-)-Adrenaline bitartrate is described as adrenaline.
3. (-)-Noradrenaline bitartrate is described as noradrenaline.
4. Histamine acid phosphate is described as histamine.
5. 5-Hydroxytryptamine creatinine sulphate is described as 5-hydroxy-tryptamine.

The benzothiadiazine compounds investigated were:-

Chlorothiazide (Merck, Sharp and Dohme) and described as CT.

Hydrochlorothiazide (Ciba) described as HCT.

Flumethiazide (Glaxo) described as FCT.

Benzothiadiazine derivatives are sparingly soluble in water. A one per cent solution of chlorothiazide was prepared freshly each day so as to avoidhydrolysis. One gram of chlorothiazide was dissolved in 3.4 ml. of N.sodium hydroxide solution and made up to 100 ml. with distilled /

distilled water, the pH of the solution being 8 to 8.2. The solutions were mixed with the appropriate physiological saline before use.

When concentrations of drugs are mentioned in the text then, unless otherwise stated, these refer to the final bath concentrations in terms of weight in volume.

The conventional abbreviations for volumes and weight of the metric system are used throughout this part of the thesis. The statistical method employed to calculate the experimental findings is described in Appendix I, page 451.

B. EXPERIMENTAL

A number of benzothiazide derivatives have been reported to possess diuretic (Ford, Moyer and Spurr, 1957) and antihypertensive properties (Hollander and Wilkins, 1957). Very little information is available about the effects of chlorothiazide on the cardiovascular system of the cat or on isolated tissues and organs.

In order to evaluate the pharmacology of chlorothiazide and allied compounds in more detail, work has been carried out on intact animals and isolated tissues with special reference to their antihypertensive properties. The object at this stage was to determine whether any part of the hypotensive response to chlorothiazide was due to effects other than upon electrolyte balance.

Experiments were carried out in the following order:-

1. Experiments on the arterial blood pressure level and vasomotor reflexes of normotensive anaesthetized cats and rats.

2. /

2. Experiments on the responses of isolated tissue preparations taken from organs containing smooth, cardiac, vasoular or skeletal muscle.

   (a) Isolated strips of guinea pig ileum.

   (b) Isolated strips of rabbit duodenum.

   (c) Isolated strips of horse carotid artery.

   (d) Blood vessels of the isolated perfused rat hindquarters in situ.

   (e) Isolated cardiac muscle, including the isolated guinea pig auricles and the isolated, perfused rabbit heart.

3. Studies on the influence of chlorothiazide on ion fluxes in isolated strips of the thoracic aorta of the rabbit and isolated sartorius muscle of the frog.

   (a) Uptake of potassium-42.

   (b) Efflux of potassium-42.

   (c) Uptake of sodium-24.

   (d) Efflux of sodium-24.

4. Biochemical investigations.

   (a) Studies on the adenosine triphosphatase aotivity of rat skeletal muscle.

(i) Experiments on the blood pressure of the anaesthetised cat.

The experimental procedure used in this investigation was similar to the one described previously (page 26). Chlorothiazide was given in /

in the form of a slow intravenous infusion at a rate of 1 ml. per minute. The doses of chlorothiazide administered were from 50 mg. to 200 mg. per kg. of body weight.

The effect of intravenous administration of chlorothiazide (50 to 200 mg. per kg.) upon the vasopressor responses to adrenaline or noradrenaline (10 to 20 µg. per kg.) were studied (page 31). Using similar doses of adrenaline and noradrenaline the effects of chlorothiazide (50 to 200 mg. per kg.) given by intravenous injection upon the contractions of the nictitating membrane were also observed.

(ii) Experiments on the blood pressure of the anaesthetised rat.

The experimental procedure was the same as that described previously (page 35). Drugs were administered intravenously into the femoral vein and the effects of intravenous administration of 5 to 10 mg. chlorothiazide on the pressor responses to adrenaline, 1 to 2 µg. and noradrenaline, 1 to 2 µg. were studied.

(iii) Experiments on the isolated, perfused hindquarters of the rat.

Experiments on the isolated, perfused hindquarters of the rat were carried out to study the effects of the drugs under investigation on an isolated vascular bed. The experimental procedure was the same as /

as that described previously (page 44). Chlorothiazide in dose levels of from 5 to 20 mg. was infused for 5 to 10 minutes and the effects were studied on the vasoconstrictor responses elicited by injections of adrenaline, 1 to 2 µg., noradrenaline 1 to 2 µg., 5-hydroxytryptamine, 1 to 5 µg., and barium chloride from 50 to 250 µg.

(iv) Experiments on isolated strips of horse carotid artery.

Experiments were carried out on isolated strips of horse carotid artery to determine the direct effects of 0.125 to 1 mg. per ml. of chlorothiazide and hydrochlorothiazide on the arterial smooth muscle and on the responses elicited by 0.01 to 0.1 µg. per ml. of acetylcholine, 1 to 2 µg. per ml. 5-hydroxytryptamine, 1 to 2 µg. per ml. of adrenaline and 1 to 2 µg. per ml. of noradrenaline. The experimental procedure was the same as that described already (page 42).

(v) Experiments on the isolated, perfused rabbit heart.

Experiments were carried out on the isolated, perfused rabbit heart to observe the effects of chlorothiazide, hydrochlorothiazide and flumethiazide on the rate, tone and outflow of this organ. Chlorothiazide in doses of 1 to 10 mg., hydrochlorothiazide and flumethiazide in doses of 0.1 to 0.25 mg. were injected into the cannula supporting the heart. The method adopted was the same as that previously described (page 51).

(vi) Experiments on isolated guinea pig auricles.

The experimental procedure adopted was the same as that described elsewhere (page 49). Chlorothiazide, 1 to 2 mg. per ml., hydrochlorothiazide /

hydrochlorothiazide 0.1 to 0.2 mg. per ml., and flumethiazide 0.1 to 0.2 mg. per ml. were used. The effects of the drugs on the rate and the amplitude of the auricles and also upon the responses of the auricles to adrenaline, 0.1 to 1.0 µg. per ml., noradrenaline, 0.1 to 1.0 µg. per ml., and acetylcholine, 1.0 to 2.0 µg. per ml. were studied.

(vii) Experiments on isolated strips of rabbit duodenum.

The procedure adopted to perform these experiments was the same as that described already on page 41 . Chlorothiazide in doses of 1 to 2 mg. per ml., hydrochlorothiazide 0.1 to 1 mg. per ml., and flumethiazide 0.1 to 1 mg. per ml., were used and their effects on the rhythmic activity of the isolated, duodenal strips studied.

(viii) Experiments on isolated strips of guinea pig ileum.

The experimental procedure was the same as that described previously (page 39). The effect of chlorothiazide 1 to 2 mg. per ml., hydrochlorothiazide 0.1 to 1 mg. per ml., and flumethiazide, 0.05 to 0.2 mg. per ml. on the tone of the gut and also on its responses to acetylcholine, 0.1 to 1 µg. per ml., histamine 0.1 to 1 µg. per ml., 5-hydroxytryptamine 1 to 5 µg. per ml., and barium chloride 0.25 to 0.5 µg. per ml. were observed.

(ix) Studies on the influence of chlorothiazide on ion fluxes in the isolated, vasoular smooth muscle of the rabbit and isolated, skeletal muscle of the frog.

To /

To study the effects of chlorothiazide on ion movements across the cell membrane, experiments were conducted using isolated strips of rabbit aorta and frog sartorius muscle. An investigation was made on the effects of chlorothiazide on the output and uptake of sodium-24 and potassium-42.

(a) Experiments on isolated strips of rabbit aorta.

Uptake of potassium-42.

Adult male rabbits weighing from 2.0 to 2.5 kg., were killed by a blow on the head and bled out. About 5 to 6 cm. of thoracic aorta was carefully removed and transferred rapidly into the ice-cold Krebs-Ringer solution (Appendix I , page 452).

The piece of the aorta was opened longitudinally using sharp-pointed iris scissors and divided transversely in the middle into two strips. The strips were weighed on the torsion balance and adjusted to be approximately equal. One strip was used as a control and the other for the experiment. The paired strips of aorta were blotted dry and hooks made from No. 20 hypodermic needles were attached to each end. The strips were suspended from one end, from the barrel of the hypodermic syringe into two centrifuge tubes, each containing 9 ml. of Krebs-Ringer solution at room temperature and which was oxygenated continuously throughout the experiment. Part of the stable potassium in the bathing fluid had been replaced by isotonic 42 KCl solution which contained 1.- to 1.2 mc. per 10 ml. at the time of dispensing. The volume /

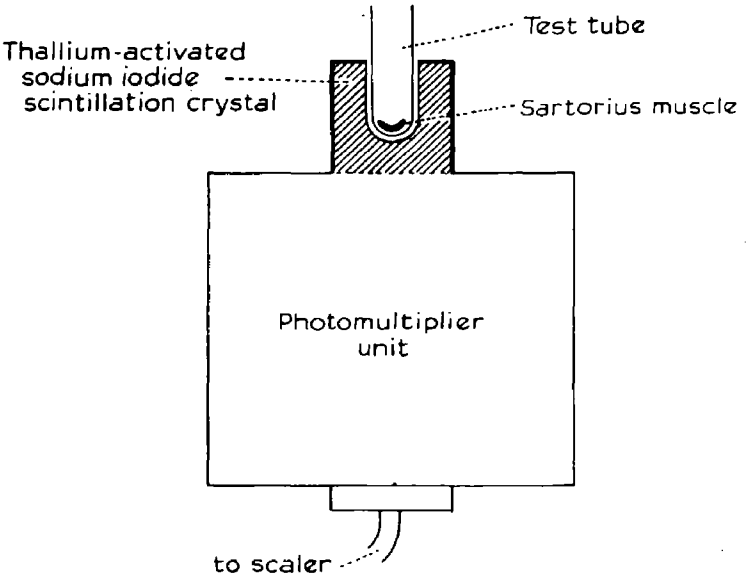

Fig. 86.

Diagram of the apparatus used for measuring radioactivity in muscle.

volume usually added was 0.25 ml.

One centrifuge tube contained chlorothiazide, from 1 to 2 mg. per ml., and the other contained the same volume of the control solution. Before immersing the muscle in the tube the radioactive Krebs-Ringer solution was counted by placing the tube in the central well of a thallium-activated, sodium iodide, scintillation crystal (Ekco type N.597) (Fig. 86, page 264) linked through a photomultiplier unit to an automatic scaler (Ekco type N.530D).

Each strip of aorta was exposed to the drug or control solution for 30 minutes after which the muscle was removed from the tube and washed with a stream of normal, non-radioactive Krebs-Ringer solution for 5 seconds, blotted dry and counted in a well type scintillation counter for a period of one minute. This procedure was repeated at 30 minute intervals until a steady state was reached. This was usually from 2 to 3 hours. The total radioactive counts left in the muscle were measured at the end of the experiement. Each of the muscles was dissolved in 2 ml. of concentrated nitric acid by warming it slightly on an electric hot plate. This mixture was adjusted to 5 ml. and counted by resting the test tube in the central well of a thallium-activated, sodium iodide, scintillation crystal linked through a photo-multiplier unit to an automatic scaler.

Corrections for decay were not made in each series of experiments as parallel control experiments were performed under identical conditions. /

conditions. Corrections were made for background and the counts were expressed as counts per minute.

(b) Potassium-42 efflux.

Rabbits weighing from 2 to 2.5 kg. were injected intraperitoneally with 1 ml. per kg. of isotonic solution of 42-KCl which contained 1.0 to 1.20 mc. in 10 ml. at the time of dispensing.

The animal was left for 2 hours to attain equilibration and then killed by a hard blow on the head and bled out. The aorta was removed in the way described previously (page 263) and two strips of approximately the same weight were prepared as before. Two series each containing nine 10 ml. centrifuge tubes were set up in parallel. Each tube contained 10 ml. of non-radioactive, Krebs-Ringer solution at room temperature which was continuously oxygenated throughout the experiment. One series of tubes was used for the control and the other for the drug. Each of the strips of aorta was suspended from one end by means of a steel hook made from an entomological pin and stretched by a weight made out of a No. 20 hypodermic needle which was attached to the lower end of the aortic strip. The paired strips were suspended, one in the first member of the control series of tubes and the other in the corresponding tube in the test series. After a period of 10 minutes in the first tube the muscles were transferred to the second in the series, and so on. The fluid remaining in the previous tubes was counted using an

M6 counter linked to an automatic scaler (Ekco type N530D). In the test series the drug was present in the fourth, fifth and sixth tubes and the control solution in the corresponding tubes of the other series. After immersing the aortic strips in the ninth tube of the series the muscle was removed and digested in 2 ml. of concentrated nitric acid; the volume was adjusted to 10 ml. with Krebs-Ringer solution and counted in the usual manner. Chlorothiazide in doses of from 1 to 2 mg. per ml. was used.

(c) Uptake of sodium-24.

These experiments were carried out in a similar manner to that described in the investigation of potassium-42 uptake (page 263) but an isotonic solution of 24-NaCl was used in place of 42-KCl. Of this solution 0.2 ml. was added to 30 ml. of Krebs-Ringer solution and 9 ml. of this solution was used in each series of test and control experiments.

(d) Sodium-24 efflux.

These experiments were carried out in the same manner as that described in the investigation of potassium-42 efflux (page 266) but an isotonic solution of 24-NaCl was used in place of 42-KCl. Two ml. of this solutionwere injected intravenously 2 hours before the removal of the aorta. The radioactive solution was counted by resting the tube in the central well of the thallium-activated, sodium iodide scintillation crystal (Ekco type N.597) linked through a photomultiplier unit to an automatic scaler (Ekco type N530D).

Experiments /

Experiments investigating the efflux and uptake of potassium-42 and sodium-24 on frog sartorius musole were performed in the manner described previously (page 263 and 266).

(x) Biochemical investigations

Experiments on the adenosinetriphosphatase activity of rat skeletal muscle.

Method. The procedure adopted for estimation of the adenosinetriphosphatase activity of rat skeletal muscle was that described previously (page 148), but chlorothiazide (1 to 2 mg. per ml.) was used in place of 10-methoxydeserpidine.

C. RESULTS

Blood pressure and respiration of the anaesthetised cat.

Chlorothiazide in dose levels of from 50 to 200 mg. per kg. had no observable effect on the blood pressure and the respiration of the normotensive anaesthetised cat (Fig. 86,page 269). No bradycardia was observed during or after the administration of the drug.

The responses to small doses of adrenaline, 2 µg. per kg. were depressed after or during the infusion of 166 to 200 mg. per kg. of chlorothiazide, whereas responses to 2 µg. per kg. of noradrenaline were not modified.

The pressor response to bilateral occlusion of the common carotid artery was also reduced following infusion of chlorothiazide (total dose /

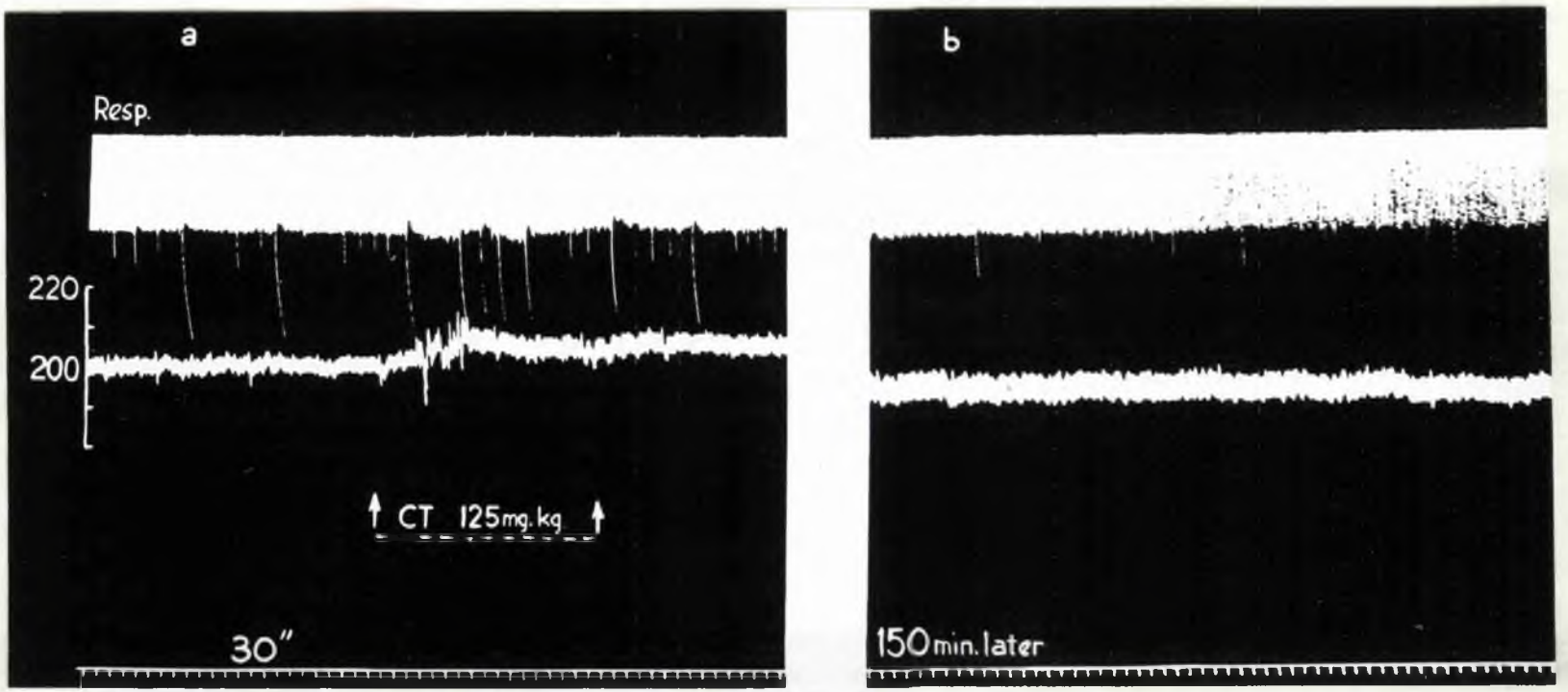

Fig. 87.

Tracing a. Effect of the infusion of 125 mg. per kg. of chlorothiazide (CT) over 10 minutes upon the respiration (upper record) and arterial blood pressure level (middle record) of the pentobarbitone-anaesthetised cat. The blood pressure record was taken from the common carotid artery.

Drug solution was infused between the two vertical arrows.

Tracing b. Blood pressure level after 150 minutes of drug infusion. Time interval (lower trace) = 30 seconds.

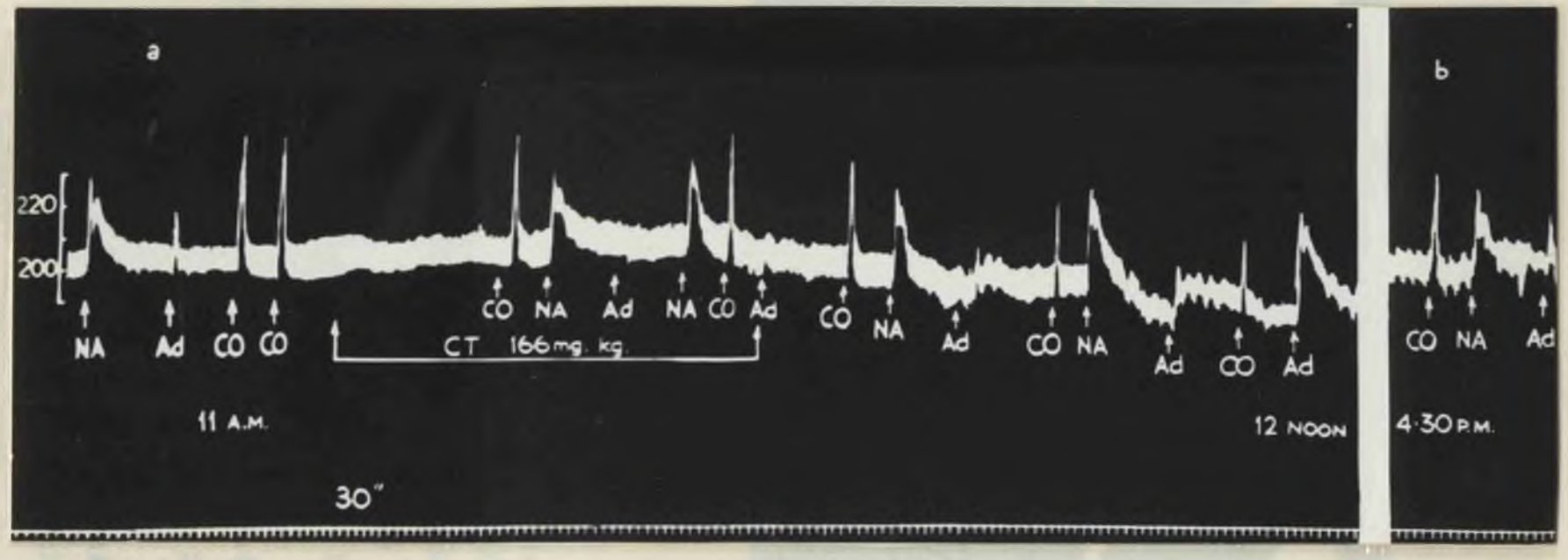

Fig. 87.

Tracing a. Effect of the infusion of 166 mg. per kg. of chlorothiazide (CT) over a period of 25 minutes upon the responses of the blood pressure to noradrenaline (NA), adrenaline (Ad) and to bilateral occlusion of the common carotid arteries (CO) in a pentobarbitone-anaesthetised cat. All drugs given intravenously.

At NA, 1 µg. per kg. of noradrenaline.
At Ad, 1 µg. per kg. of adrenaline.
At CO, bilateral occlusion of the common carotid arteries for 10 seconds.

Tracing b. 300 minutes after the end of infusion.

Time interval (lower trace) = 30 seconds.

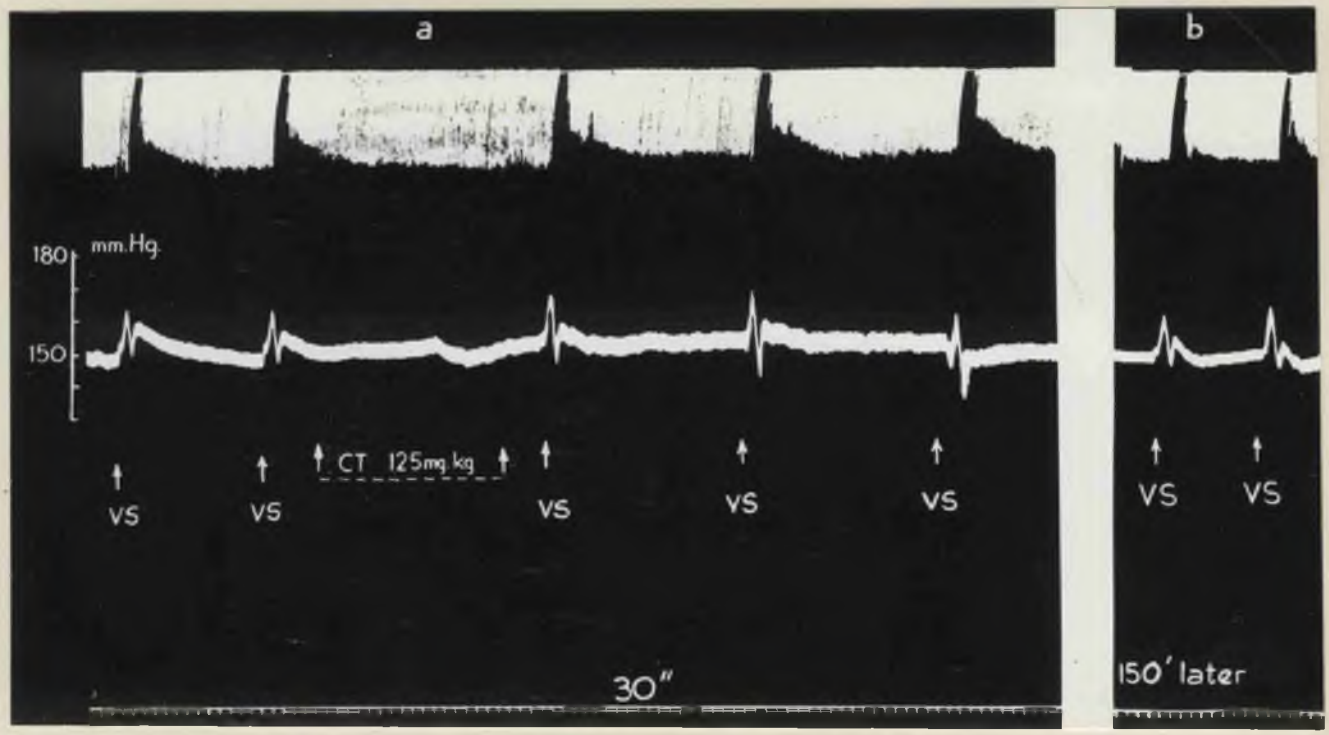

Fig. 88.

Tracing a. Effect of the infusion of 125 mg. per kg. of chlorothiazide (CT) over a period of 10 minutes upon the respiration (upper record) and the pressor responses to electrical stimulation of the cut central end of the right vagus (lower record) in a pentobarbitone-anaesthetised cat.

At VS, electrical stimulation of the cut central end of the right vagus (10 volts, 1,000 impulses per minute, pulse width 1.5 msec.) for 15 seconds.

Tracing b. 150 minutes after the drug infusion. Chlorothiazide was infused between the two vertical arrows.
Time interval (lowest trace) = 30 seconds.

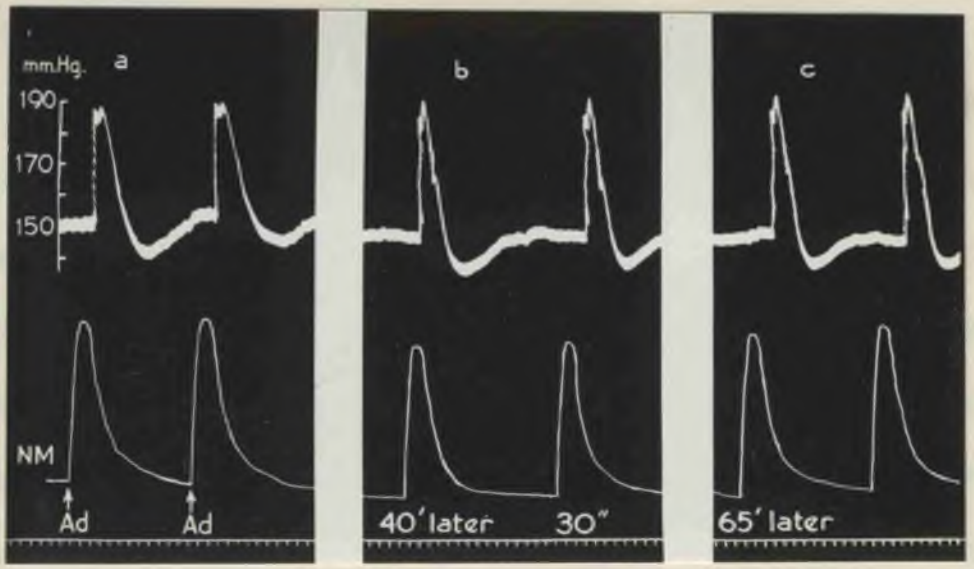

Fig. 89.

Tracing a. Effect of a single dose of 200 mg. per kg. of chlorothiazide upon the pressor responses to adrenaline (Ad, upper record) and the response of the nictitating membrane to adrenaline (lower record).
All responses are due to 20 µg. per kg. adrenaline (Ad).
Chlorothiazide was given as a single intravenous injection between (a) and (b).

Tracing b. 40 minutes after the injection of the drug.

Tracing c. 65 minutes after the injection of the drug.

All drugs given intravenously.
Time interval (lowest trace) = 30 seconds.

dose of 150 to 200 mg. per kg.). This effect was reversible after about 3 to 5 hours (Fig. 87, page 270).

Following the infusion of chlorothiazide (total dose of 100 to 200 mg. per kg.) the pressor response due to the stimulation of the central end of the vagus nerve was not modified (Fig. 88, page 271). Single intravenous injections of chlorothiazide in dose levels of 100 to 200 mg. per kg. did not reduce the responses of the blood pressure and nictitating membrane to high doses of adrenaline (10 to 20 µg. per kg.) and also had no effect on the responses of the nictitating membrane to electrical stimulation of preganglionic sympathetic fibres (Fig. 89, page 272).

Blood pressure of the anaesthetised rat.

Chlorothiazide in dose levels of from 5 to 10 mg. when injected intravenously into the femoral vein of the rat had no effect on the blood pressure but after 15 to 20 minutes reduced the responses of the blood pressure to adrenaline 1 µg. and noradrenaline 1 µg. (Fig. 90, page 275).

Isolated perfused hindquarters of the rat.

No vasodilatation was observed following the infusion of 5 to 20 mg. of chlorothiazide over a period of 5 to 10 minutes. There was a slight reduction observed in the constrictor responses to adrenaline 1 µg. and noradrenaline 1 µg. but only when chlorothiazide was given in large doses, i.e., 10 to 20 mg. (Fig. 91, page 276). Responses to the /

the injection of 1 to 5 µg. of 5-hydroxytryptamine were not modified (Fig. 92, page 277).

Isolated strips of horse carotid artery.

Chlorothiazide in doses of 0.125 to 1 mg. per ml. reduced the responses of arterial strips to acetylcholine 0.1 to 1 µg. per ml. (Fig.93, page 278), adrenaline 0.2 to 1 µg. per ml. (Fig.94, page 279), noradrenaline from 0.2 to 2 µg. per ml. (Fig.95, page 280) and 5-hydroxytryptamine 0.5 µg. per ml. (Fig.96, page 281). Hydrochlorothiazide 1 mg. per ml. reduced the responses to noradrenaline and adrenaline, 0.2 to 2 µg. per ml.

Isolated perfused rabbit heart.

Chlorothiazide in doses of from 1 to 10 mg. had no effect on the tone, rate, amplitude and outflow of the rabbit heart (Fig.97, page 282). Hydrochlorothiazide 0.5 to 2 mg. also showed no effect (Fig. 98, page 283).

Isolated guinea pig auricles.

Chlorothiazide 0.1 to 0.2 mg. per ml. and hydrochlorothiazide 0.1 to 0.2 mg. per ml. did not influence the rate and amplitude of the contractions (Fig.99, page 284). When large doses of the order of 1 to 2 mg. per ml. of chlorothiazide were used a transient depressant effect on the normal contractions was noticed (Fig.100, page 285). No antagonism of responses to adrenaline 0.1 to 1 µg. per ml. and noradrenaline 0.1 to 1 µg. per ml. was observed.

Isolated strips of rabbit duodenum.

No /

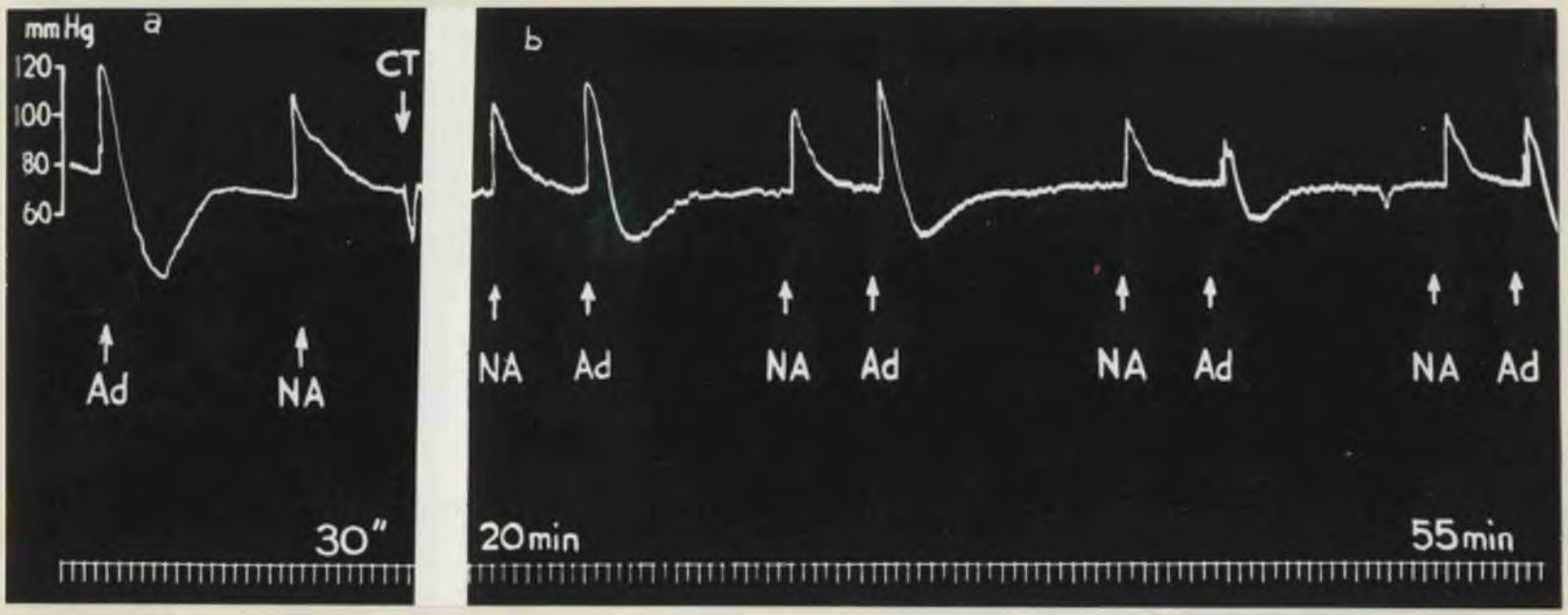

Fig. 90.

Tracing a. Effect of single dose of 10 mg. of chlorothiazide (CT) upon the responses of the rat blood pressure to adrenaline (Ad) and noradrenaline (NA). Blood pressure recorded from the common carotid artery. Drugs injected into the femoral vein.

At Ad, 1 µg. adrenaline.
At NA, 1 µg. noradrenaline.
At CT, 10 mg. chlorothiazide.

Tracing b. 20 minutes after the drug administration.

Time interval (lower trace) = 30 seconds.

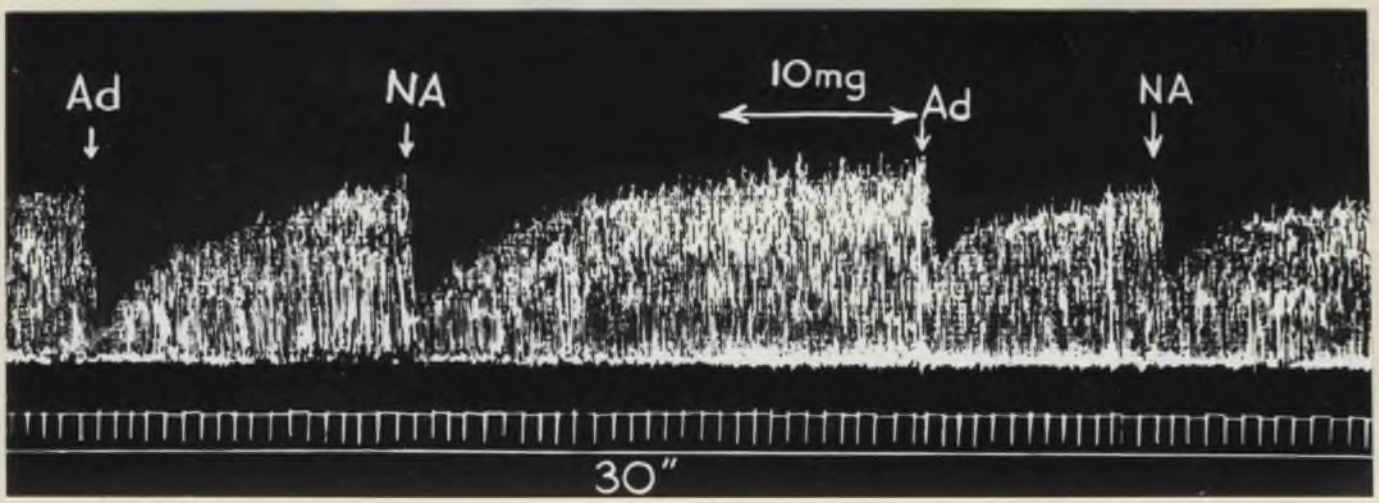

Fig. 91.

Effect of perfusion of the isolated rat hindquarters with a solution of 2 mg. per ml. of chlorothiazide for 5 minutes on the response to adrenaline (Ad) and noradrenaline (NA).

At Ad, 1 µg. of adrenaline injected into the cannula.
At NA, 1 µg. of noradrenaline injected into the cannula.

Time interval (lower trace) = 30 seconds.

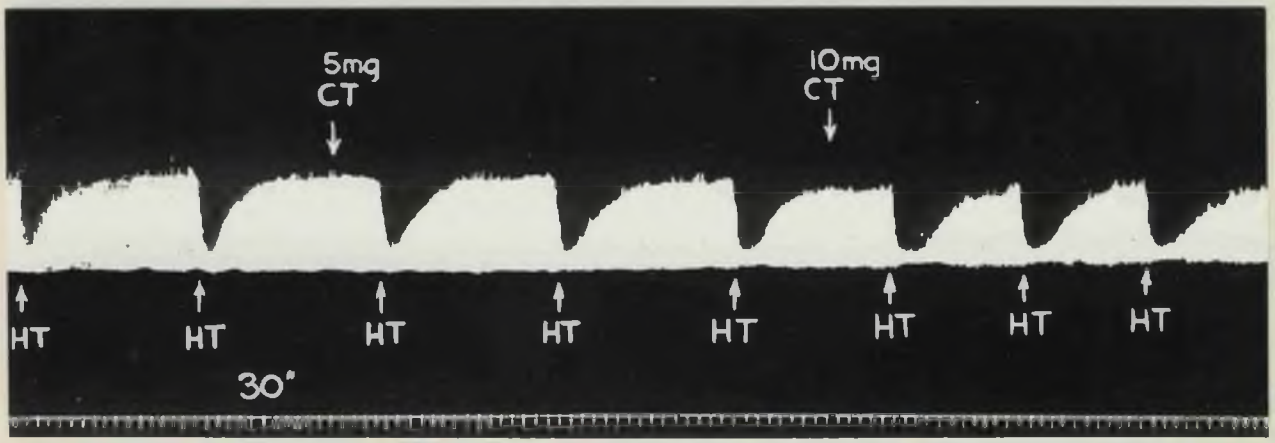

Fig. 92.

Effect of injecting 5 mg. and 10 mg. of chlorothiazide on the responses of the isolated perfused rat hindquarters to 5-hydroxytryptamine (HT).

At HT, 1 µg. 5-hydroxytryptamine injected into the cannula.

Time interval (lower trace) = 30 seconds.

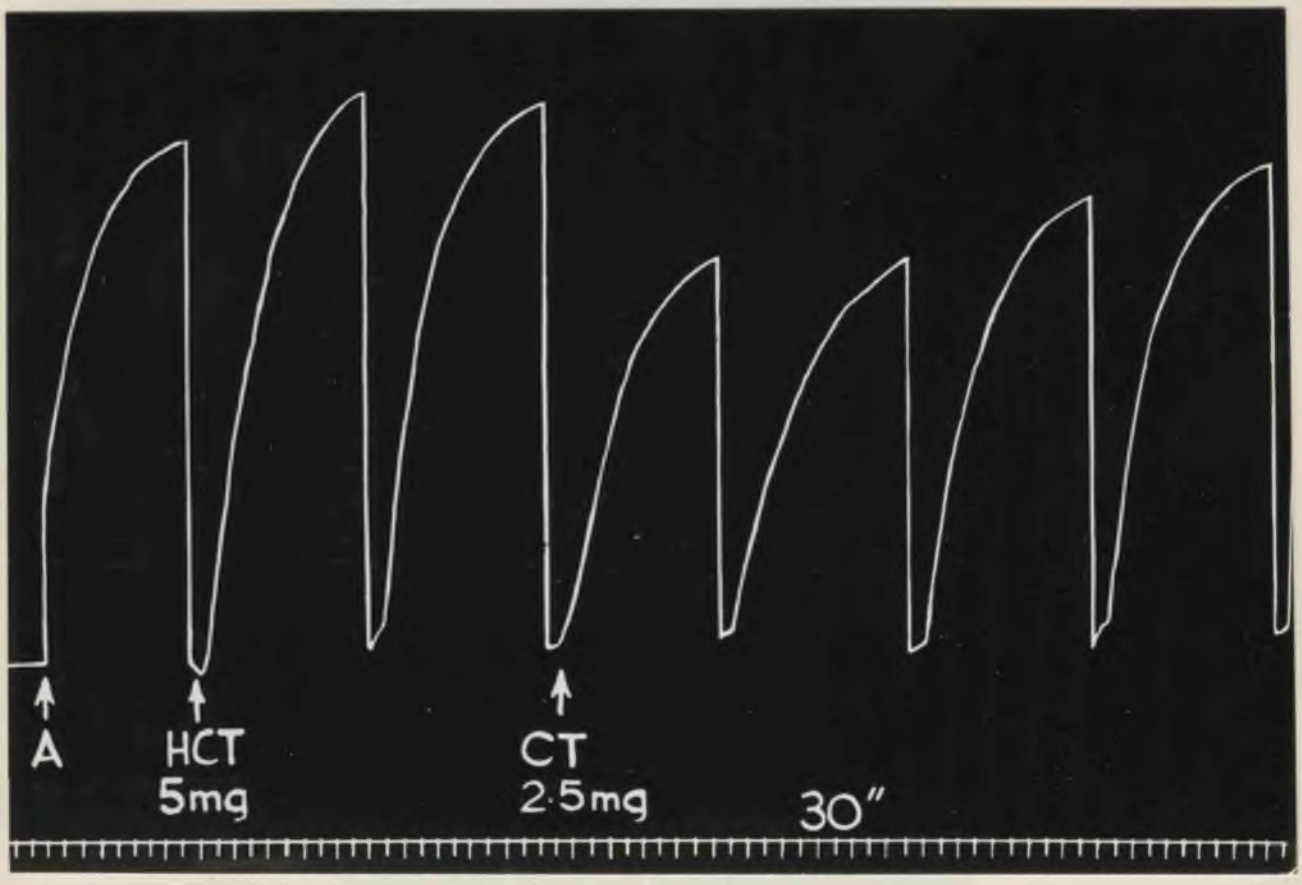

Fig. 93.

Effect of 0.5 mg. per ml. of hydrochlorothiazide (HCT) and 0.25 mg. per ml. of chlorothiazide (CT) on the response to acetylcholine (A) of an isolated strip of horse carotid artery.

All contractions are due to 0.1 µg. per ml. of acetylcholine (A).

Time interval (lower trace) = 30 seconds.

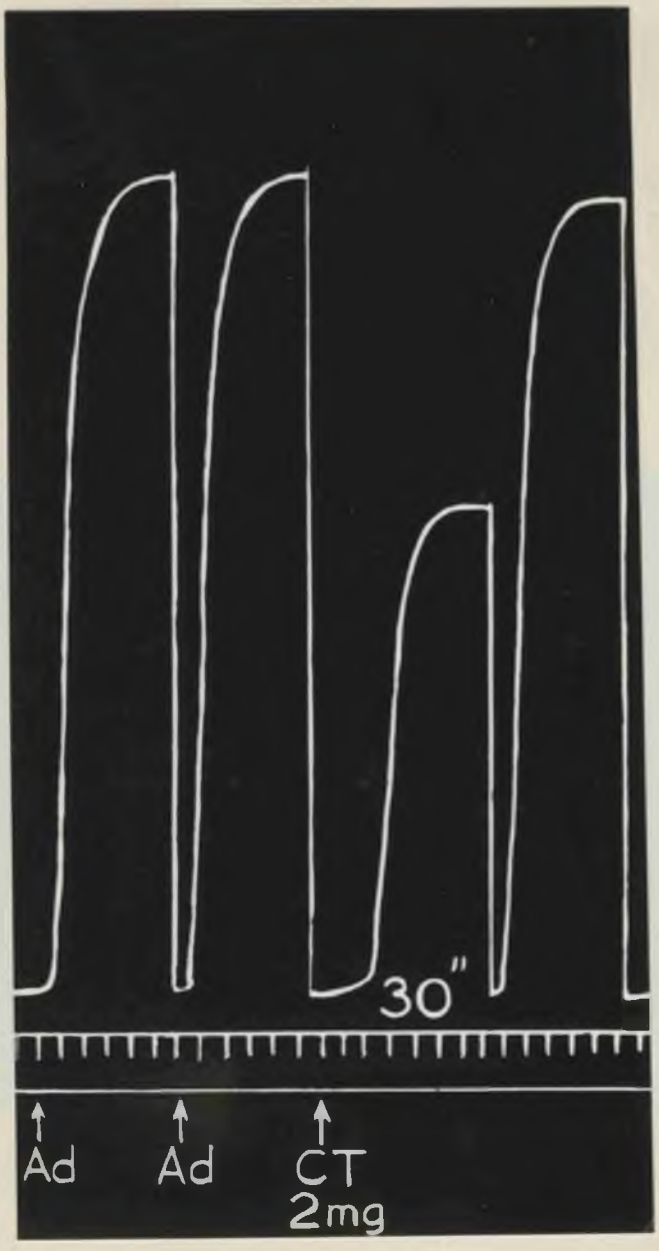

Fig. 94.

Effect of 0.2 mg. per ml. of chlorothiazide (CT) on the response to adrenaline (Ad) of an isolated strip of horse carotid artery.

All contractions are due to 0.1 µg. per ml. of adrenaline (Ad).

Time interval (lower trace) = 30 seconds.

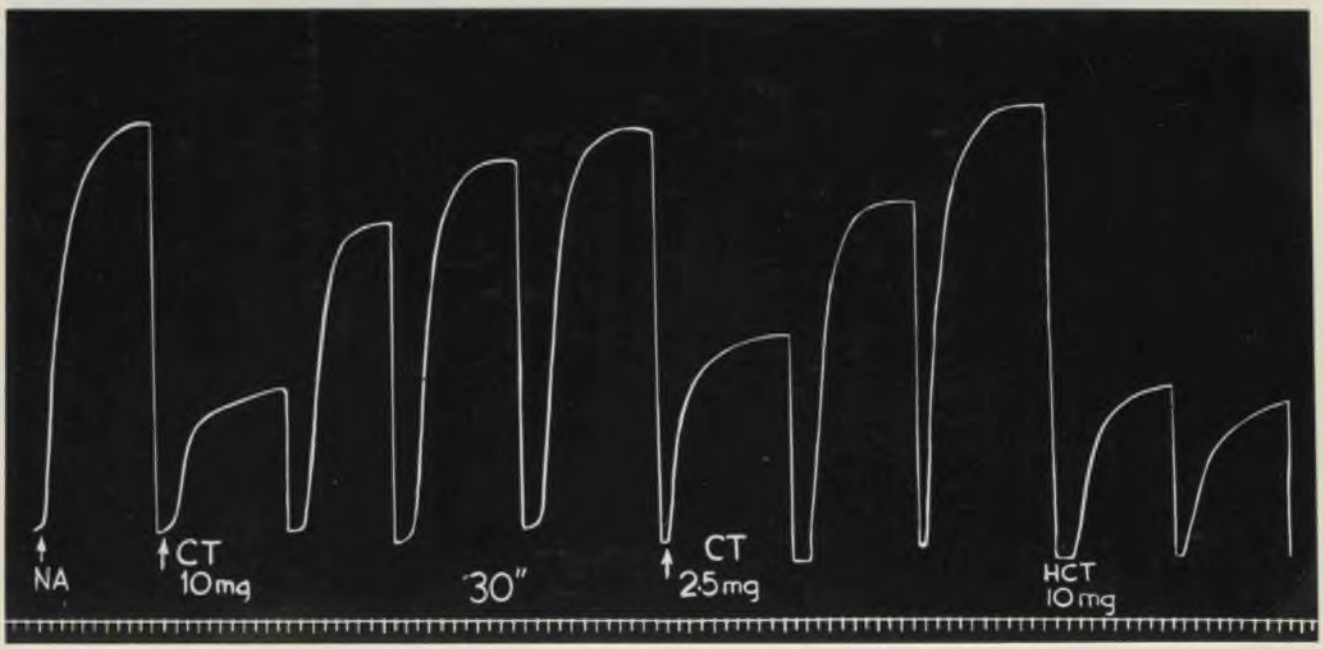

Fig. 95.

Effect of chlorothiazide (CT) and hydrochlorothiazide (HCT) on the response to noradrenaline (NA) of isolated strips of horse carotid artery.

All contractions are due to 0.1 µg. per ml. of noradrenaline.
Time interval (lower trace) = 30 seconds.

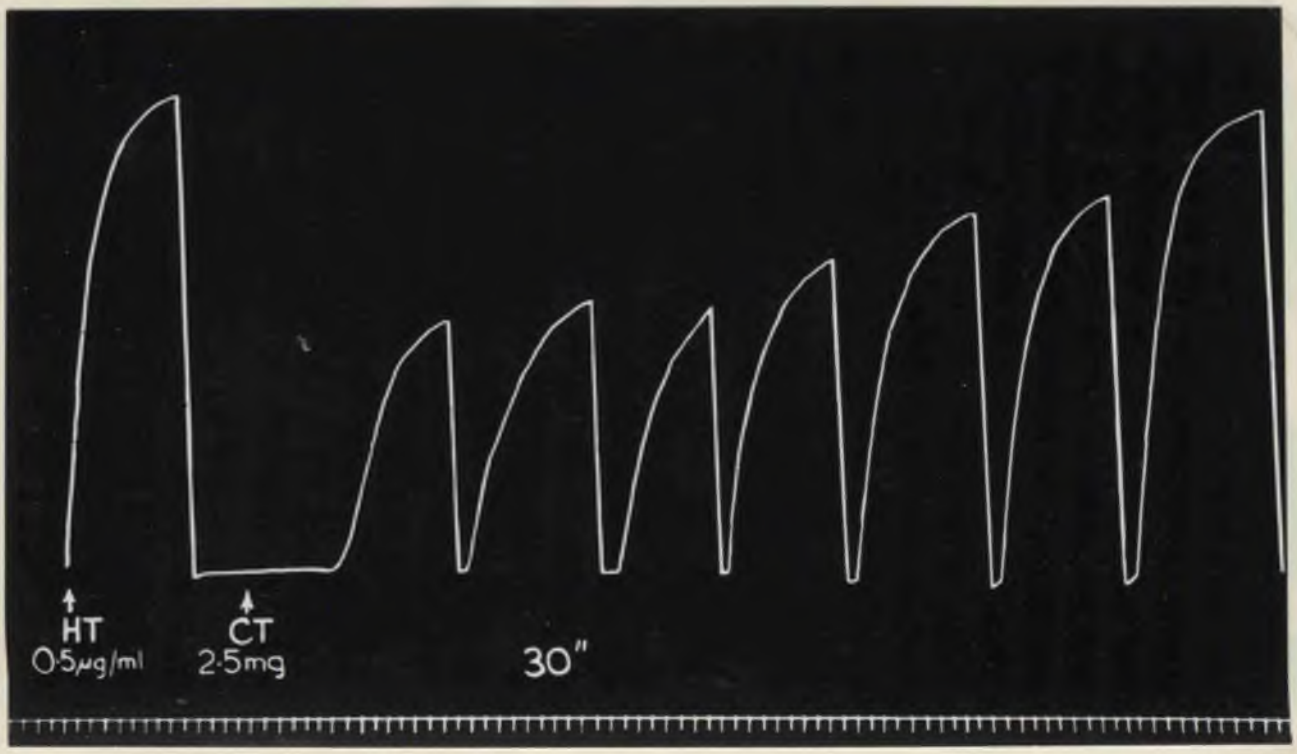

Fig. 96.

Effect of 0.25 mg. per ml. of chlorothiazide (CT) on the response to 5-hydroxytryptamine of an isolated strip of horse carotid artery.

All contractions are due to 0.5 µg. per ml. of 5-hydroxytryptamine.

Time interval (lower trace) = 30 seconds.

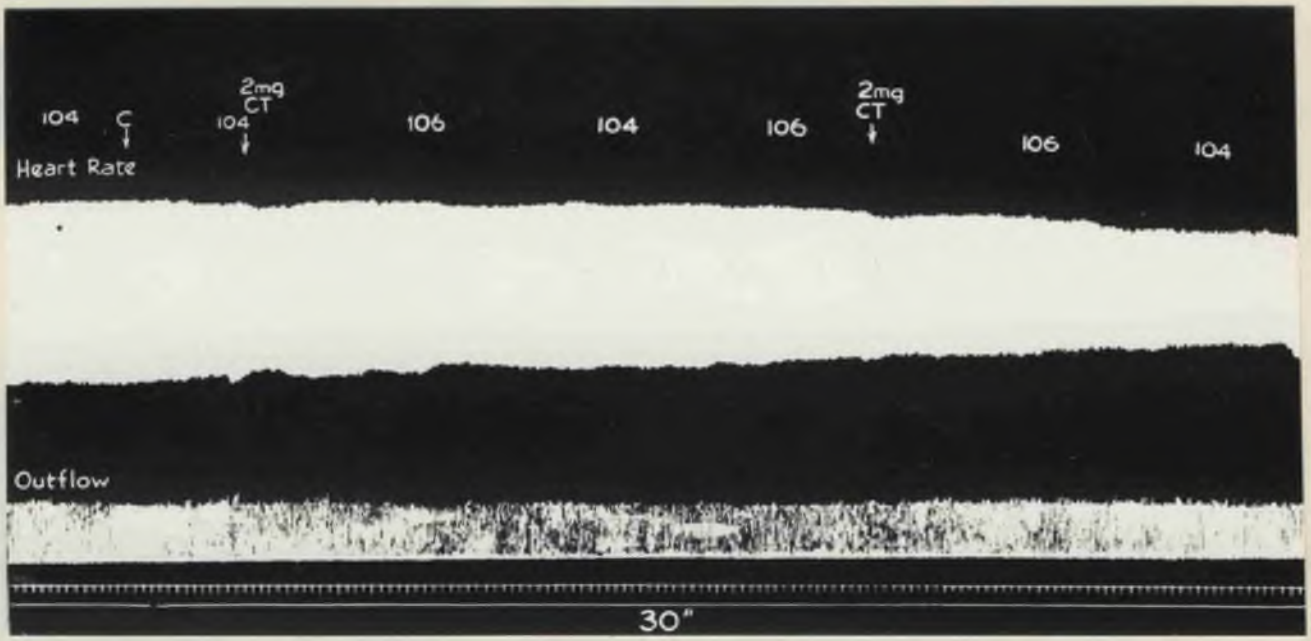

Fig. 97.

Effect of 2 mg. of chlorothiazide upon the amplitude and rate (upper record) and outflow (lower record) of the isolated perfused rabbit heart.

At C, control solution injected into the cannula.

The heart rate is indicated above the upper tracing.

Time interval (lowest trace) = 30 seconds.

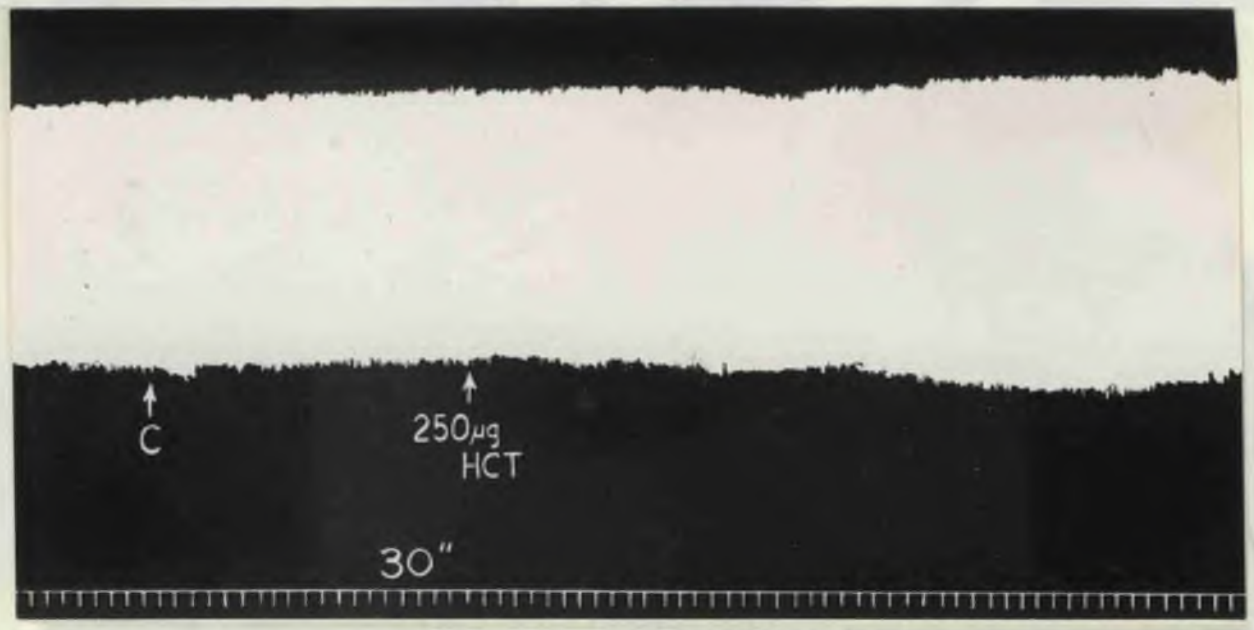

Fig. 98.

Effect of a single dose of 250 µg. of hydrochlorothiazide (HCT) upon the amplitude and tone of the isolated perfused rabbit heart.

At C, control solution injected into the cannula.

Time interval (lower trace) = 30 seconds.

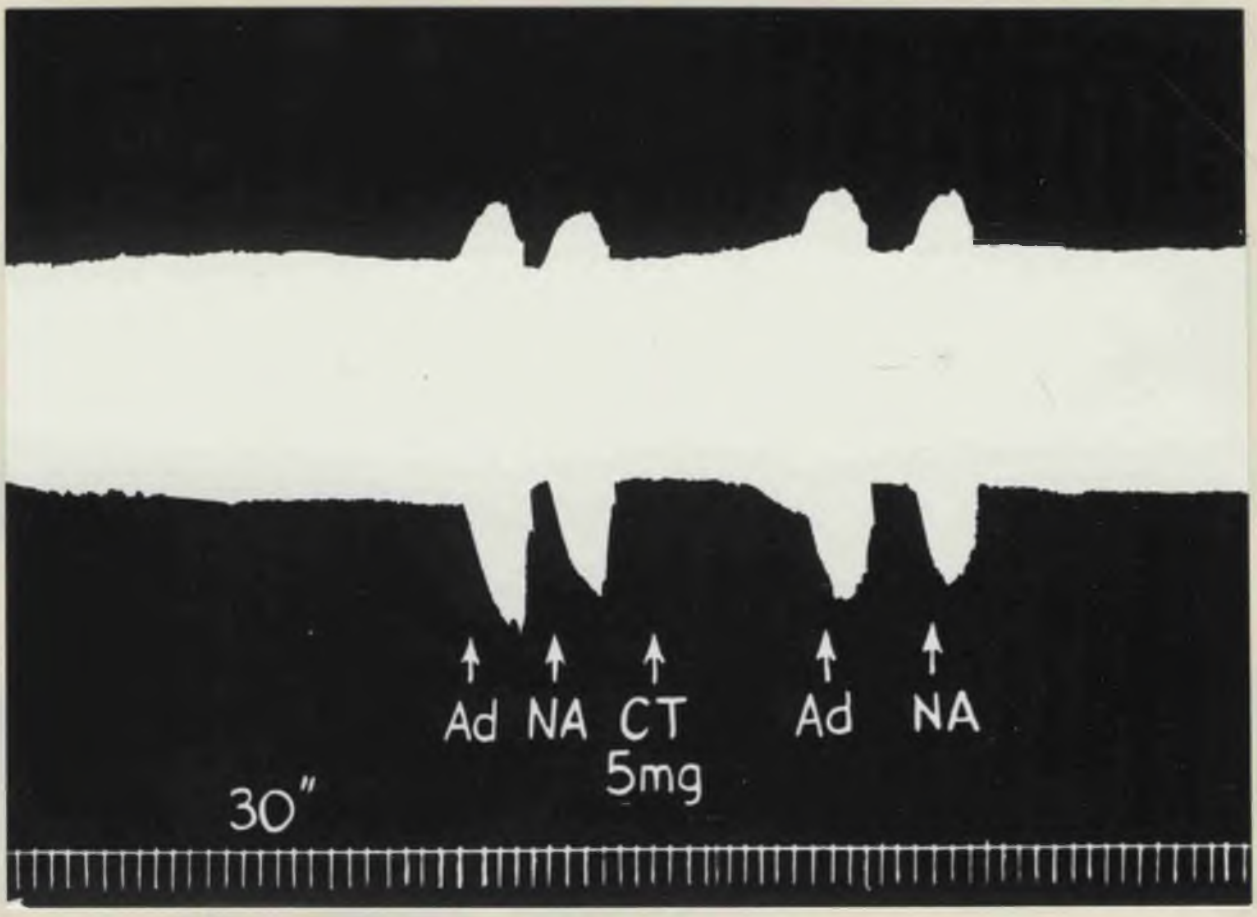

Fig. 99.

Effect of 0.5 mg. per ml. of chlorothiazide (CT) on the response of the isolated guinea pig auricles to adrenaline (Ad) and noradrenaline (NA).

At Ad, adrenaline, 0.1 µg. per ml.
At NA, noradrenaline, 0.1 µg. per ml.

Time interval (lower trace) = 30 seconds.

Fig. 100.

Effect of 1 mg. per ml. of chlorothiazide (CT) upon amplitude and tone of the isolated guinea pig auricles.

At C, control solution added to the bath.

Time interval (lower trace) = 30 seconds.

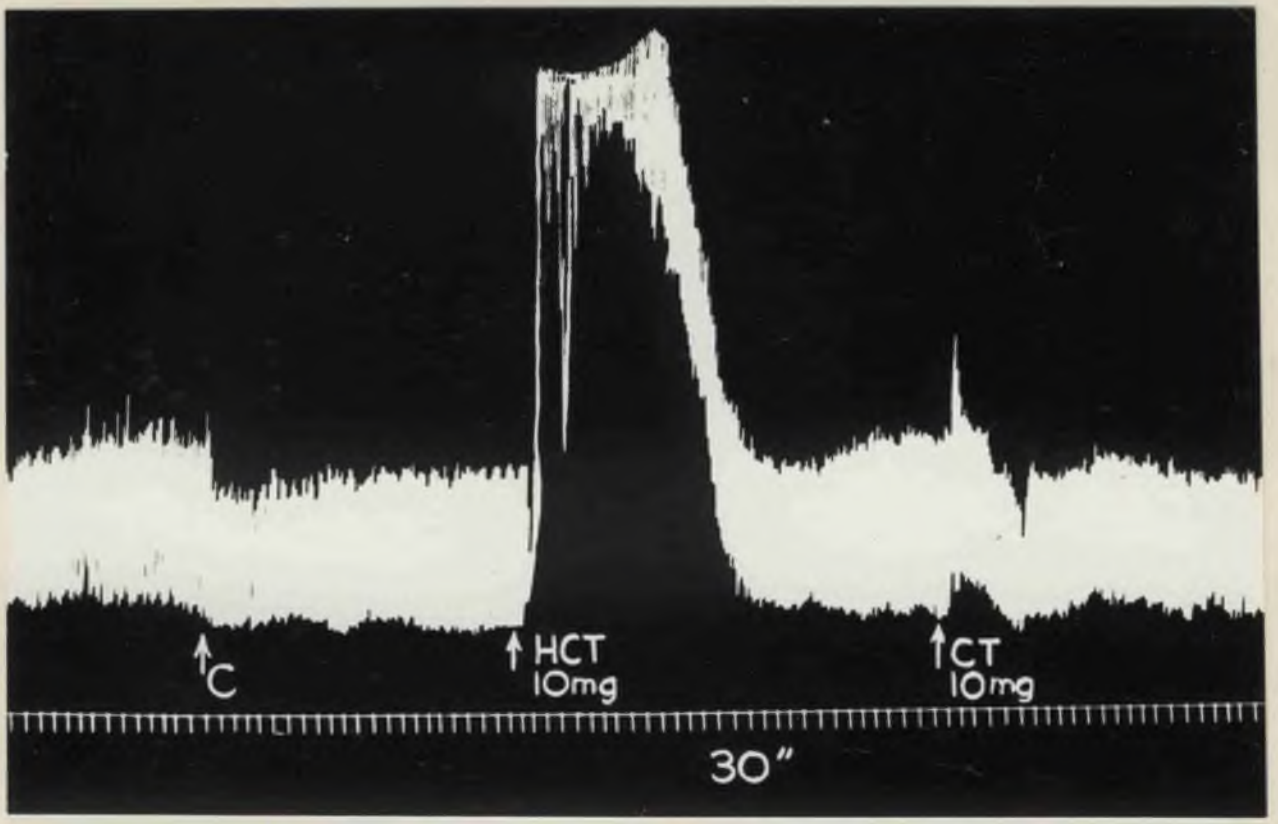

Fig. 101.

Effect of hydrochlorothiazide (HCT) and chlorothiazide (CT) on the peristaltic movements and tone of the isolated rabbit duodenum.

At C, control solution added to the bath.
At HCT, 10 mg. of hydrochlorothiazide added to the bath.
At CT, 10 mg. of chlorothiazide added to the bath.

Time interval (lower trace) = 30 seconds.

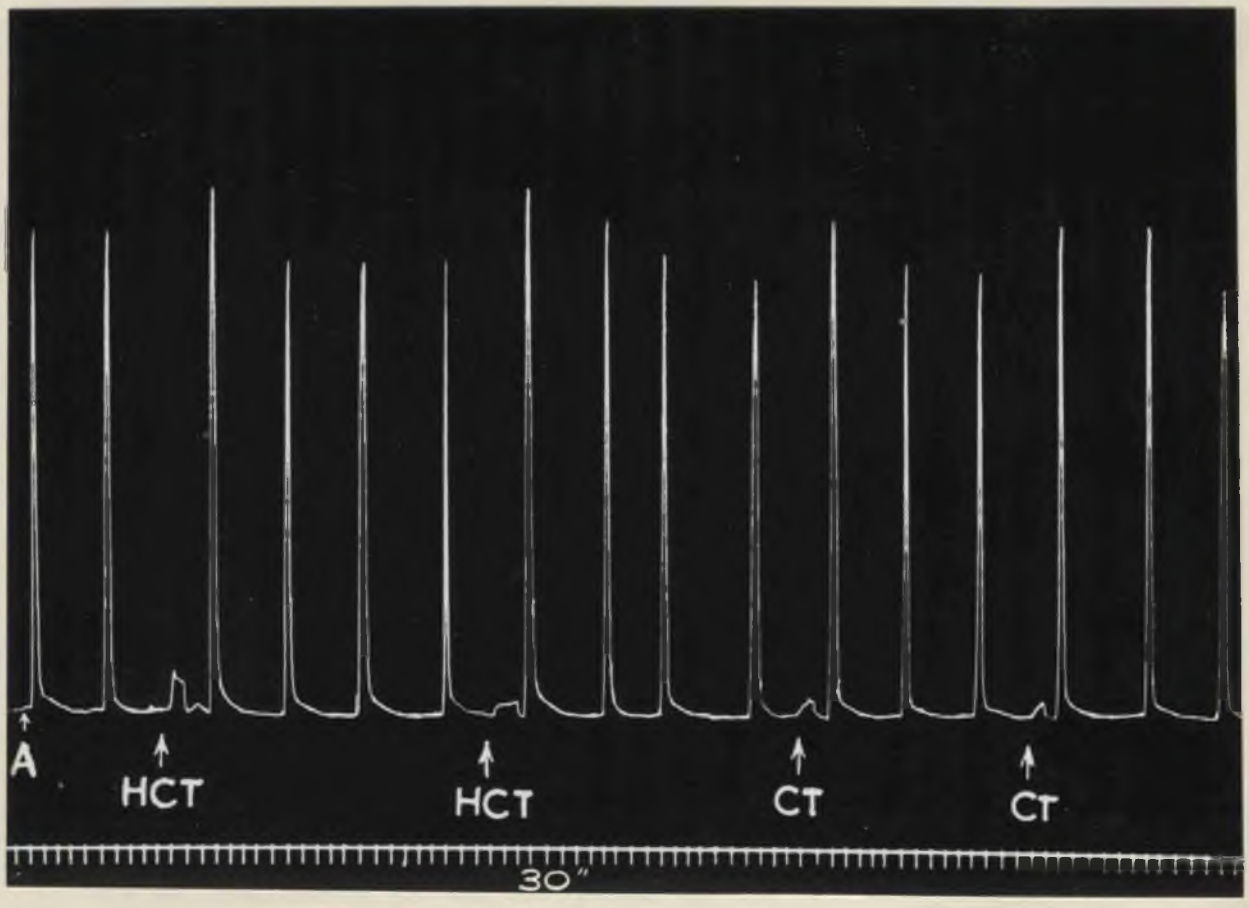

Fig. 102.

Effect of 2 mg. per ml. of hydrochlorothiazide (HCT) and 2 mg. per ml. of chlorothiazide (CT) on the responses of the guinea pig ileum to acetylcholine (A).

All contractions are due to 0.1 µg. per ml. of acetylcholine.

Time interval (lower trace) = 30 seconds.

No effect was observed on the rhythmic activity of the rabbit duodenum following doses of 0.5 to 1 mg. per ml. of chlorothiazide. Hydrochlorothiazide 0.1 to 1 mg. per ml. increased the tone of the gut and decreased the rhythmic activity of the duodenum (Fig. 101, page 286).

Isolated strips of guinea pig ileum.

Chlorothiazide from 1 to 2 mg. per ml. and hydrochlorothiazide 0.1 to 1 mg. per ml. increased the response to acetylcholine and also increased the tone of the gut. No antagonism was observed to responses elicited by acetylcholine 0.1 to 1 µg. per ml., 5-hydroxytryptamine 1 to 5 µg. per ml., histamine 0.01 to 1 µg. per ml. and barium chloride 0.15 to 0.25 µg. per ml. (Fig. 102, page 287).

Uptake of sodium-24.

(a) Isolated strips of rabbit thoracic aorta.

Chlorothiazide in dose levels of 1 to 2 mg. per ml. depressed uptake of sodium-24 in the sections of rabbit thoracic aorta. The depression in the uptake of sodium-24 was very significant ($P = 0.001$) as compared to that of the control experiments (Fig. 103, page 289, Table 10, page 298).

(b) Isolated frog sartorius muscle.

Chlorothiazide in dose levels of 1 to 2 mg. per ml. depressed ($P = 0.05$) uptake of sodium-24 in the isolated frog sartorius muscle (Fig. 104, page 290, Table 10, page 298). The depression in the uptake of sodium-24 in this tissue was not as significant as that observed in the /

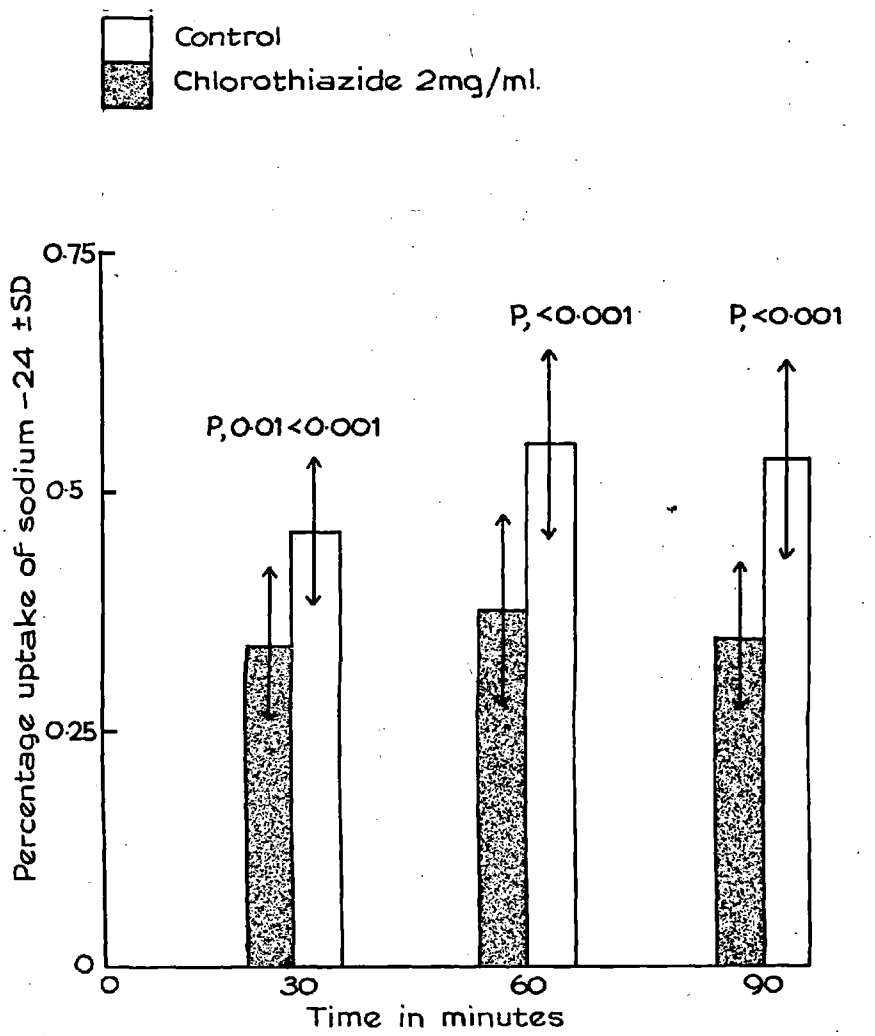

Fig. 103.

Uptake of sodium-24 by isolated strips of rabbit aorta.

The vertical bars signify the standard deviation of the mean.

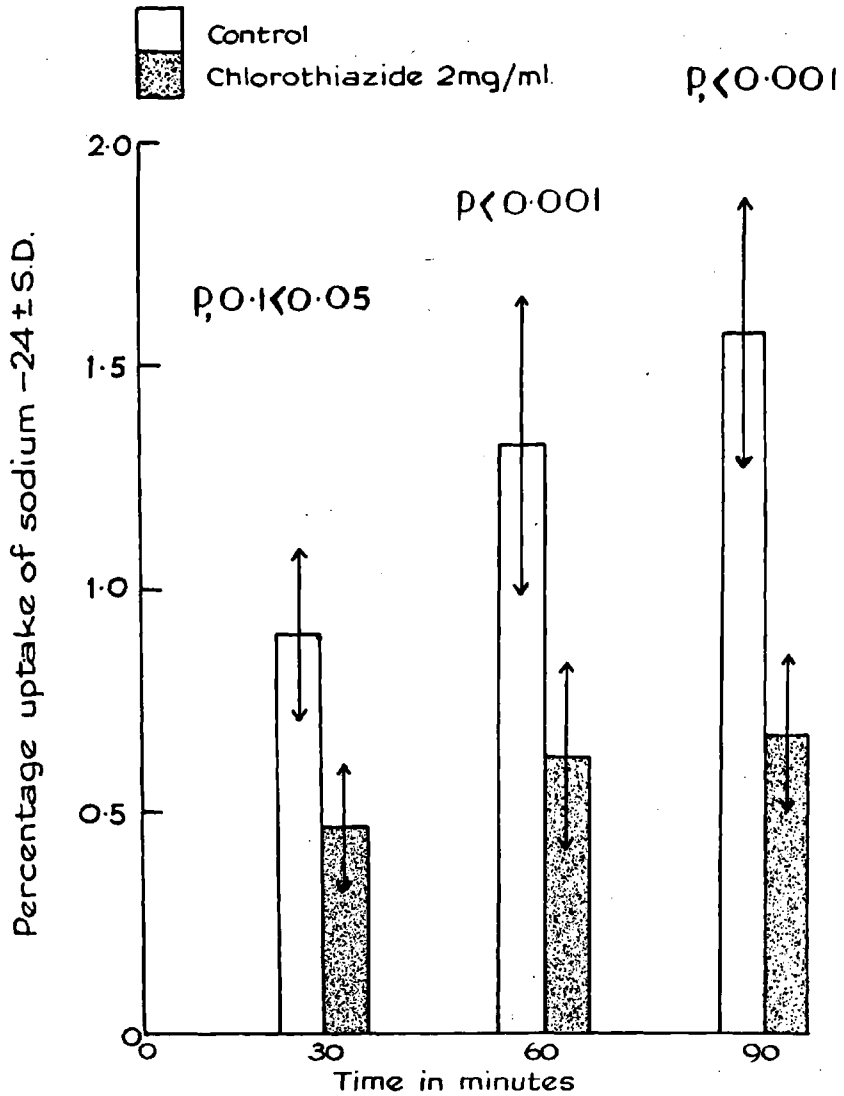

Fig. 104.

Uptake of sodium-24 by the isolated frog sartorius muscle. The vertical bars signify the standard deviation of the mean.

the isolated strips of rabbit aorta.

Efflux of sodium-24.

There was no apparent increase in the efflux of sodium-24 from the isolated strips of rabbit thoracic aorta (Fig. 105, page 292, Table 13, page 301) and from the isolated frog sartorius muscle.

Uptake of potassium-42.

There was no observable effect on the uptake of potassium-42 in the isolated strips of rabbit thoracic aorta (Fig. 106, page 293, Table 11, page 299) or in the isolated frog sartorius muscle (Fig. 107, page 294, Table 11, page 299).

Efflux of potassium-42.

Chlorothiazide in dose levels of 1 to 2 mg. per ml. did not cause release of potassium-42 from the isolated strips of rabbit aorta (Fig. 108, page 295, Table 12, page 300) and from the isolated frog sartorius muscle.

Experiments on the adenosinetriphosphatase activity of rat skeletal muscle.

Chlorothiazide in dose levels of 1 to 2 mg. per ml. did not modify the adenosinetriphosphatase activity of a suspension of rat hind leg muscles (Fig. 110, page 297, Table 14, page 302).

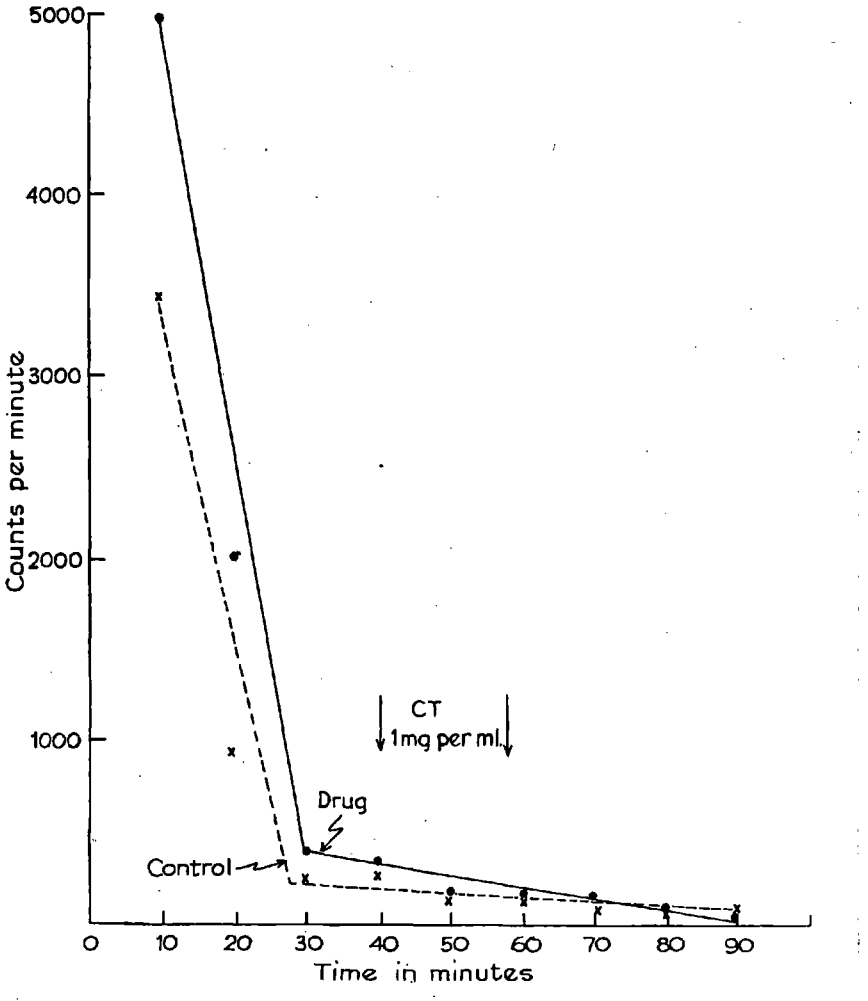

Fig. 105

Effect of chlorothiazide 1 mg. per ml. (CT) on the release of sodium-24 from isolated strips of rabbit aorta. The vertical arrows indicate the points of exposure of the test muscle to the drug.

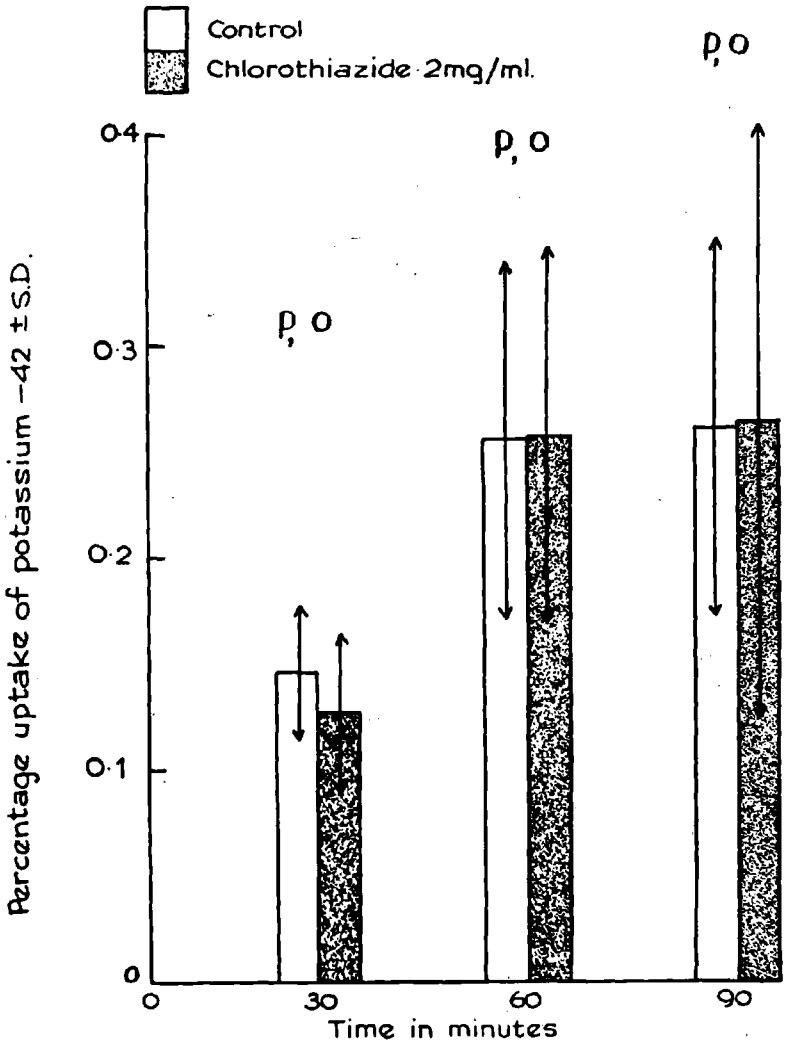

Fig. 106.

Uptake of potassium-42 by isolated strips of rabbit aorta.

The vertical bars signify the standard deviation of the mean.

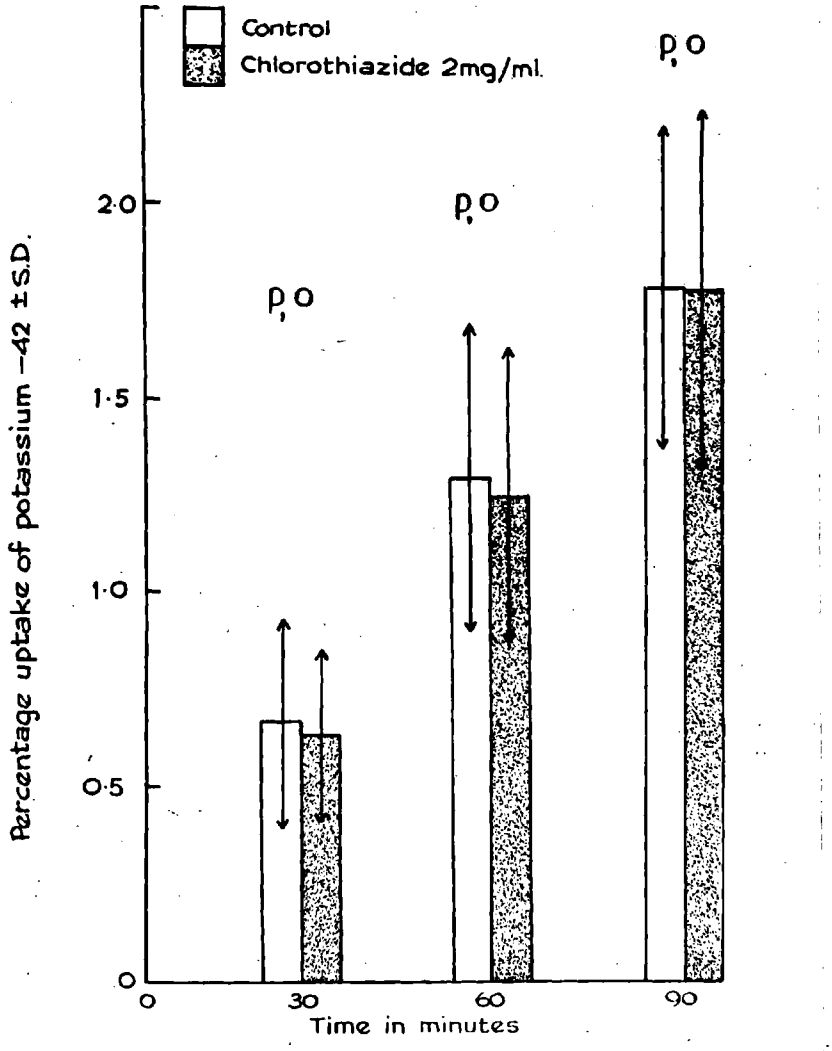

Fig. 107.

Uptake of potassium-42 by the isolated frog sartorius muscle. The vertical bars signify the standard deviation of the mean.

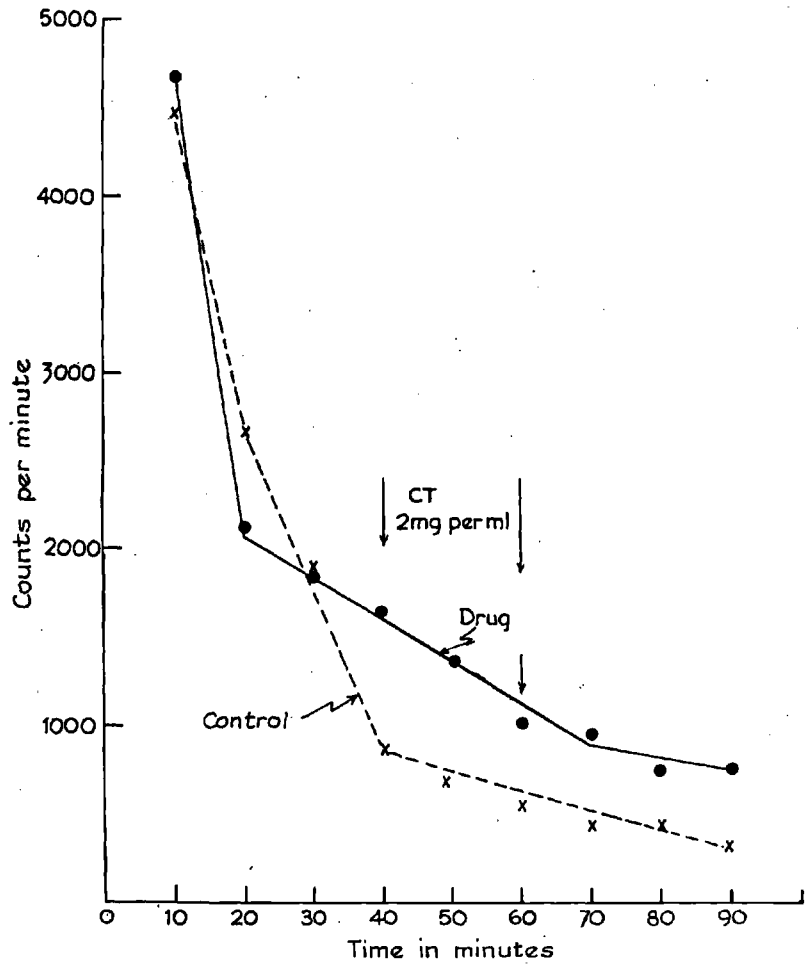

Fig. 108.

Effect of chlorothiazide 2 mg. per ml. (CT) on the release of potassium-42 from isolated strips of rabbit aorta. The vertical arrows indicate the points of exposure of the test muscle to the drug.

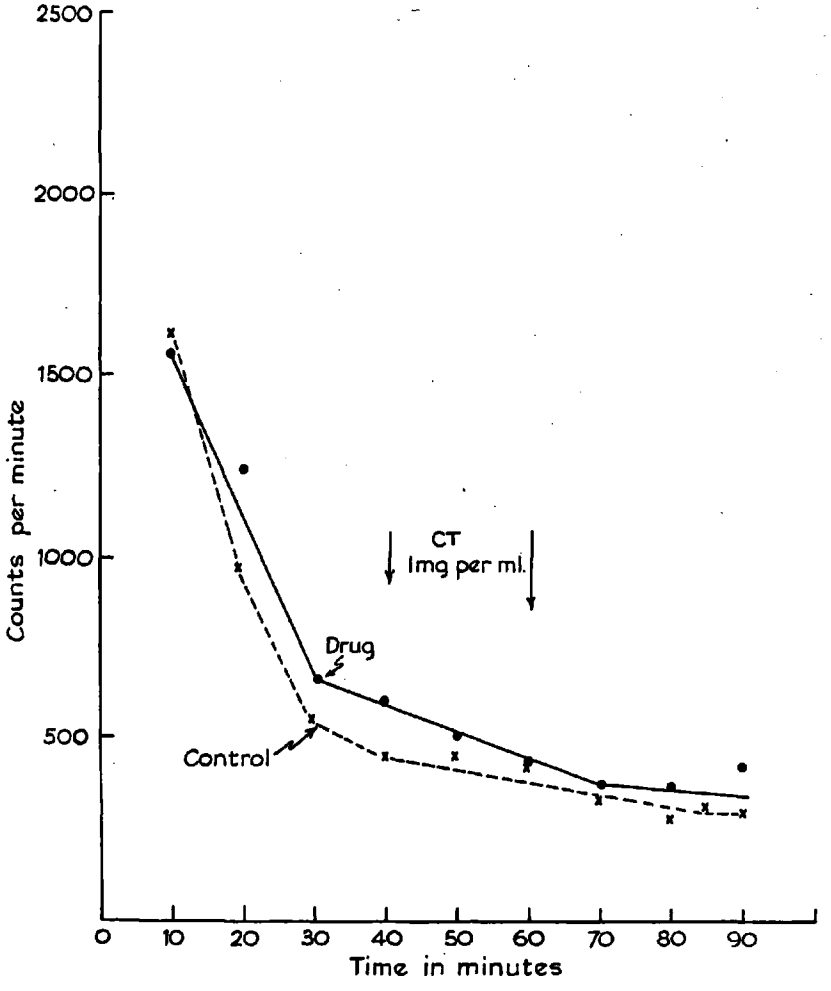

Fig. 109.

Effect of chlorothiazide 1 mg. per ml. (CT) on the release of potassium-42 from isolated frog sartorius muscle. The vertical arrows indicate the points of exposure of the test muscle to the drug.

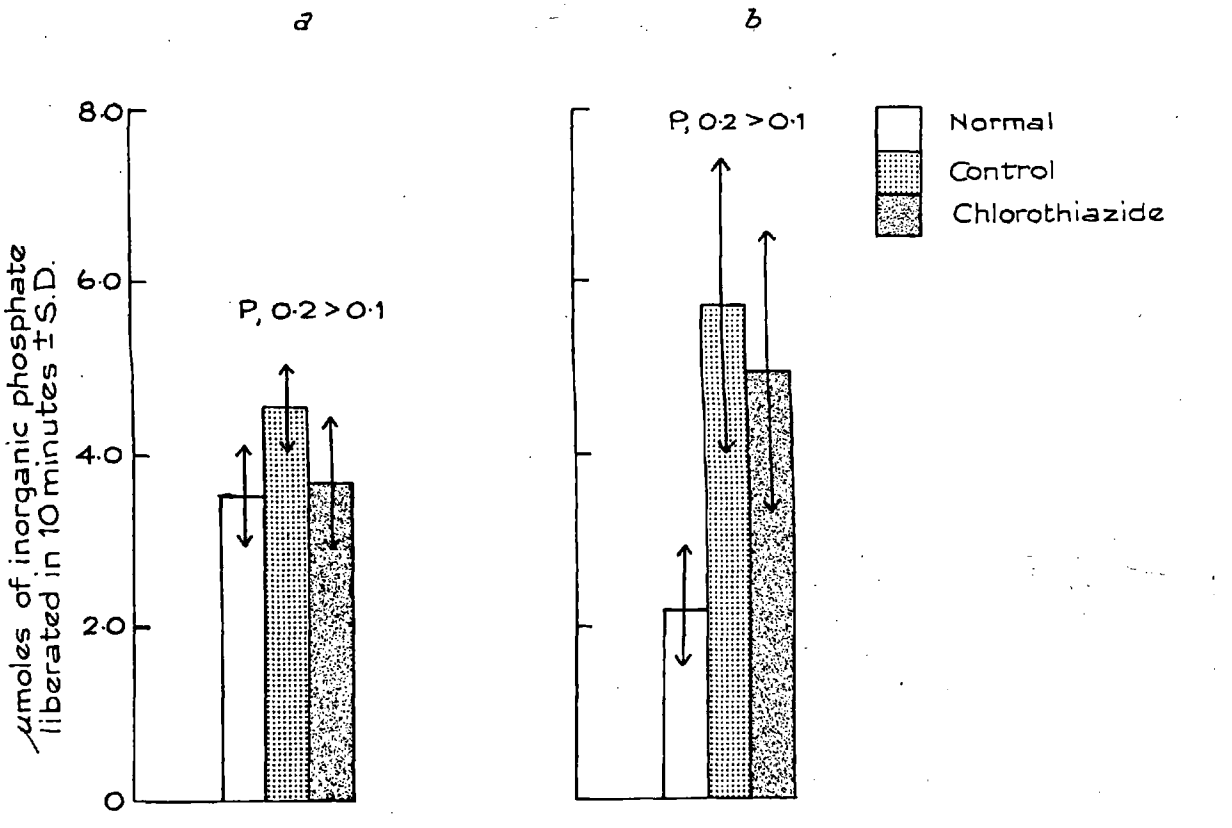

Fig. 110.

Effect of 1 mg. per ml. (at a) and 2 mg. per ml. (at b) of chlorothiazide on the <u>in vitro</u> adenosine triphosphatase activity of rat skeletal muscle.

Table 10. The effect of chlorothiazide upon the Uptake of Sodium-24

No. of experiments	Tissue used	Dose mg. per ml.	Mean percentage uptake of sodium-24 ± SD.					
			After 30 minutes		After 60 minutes		After 90 minutes	
			Control	Drug	Control	Drug	Control	Drug
10	Rabbit aorta strips	2	0.467 ± 0.082	0.344 ± 0.087	0.557 ± 0.10	0.379 ± 0.110	0.535 ± 0.100	0.345 ± 0.080
			P, 0.01 < 0.001		P, 0.01 < 0.001		P, > 0.001	
9	Frog sartorius muscle	2	0.89 ± 0.20	0.46 ± 0.14	1.32 ± 0.34	0.62 ± 0.21	1.57 ± 0.30	0.67 ± 0.18
			P, 0.1 < 0.5		P, < 0.001		P, < 0.001	

Table 11. Effect of chlorothiazide upon the Uptake of Potassium-42

No. of Experiments	Mean percentage uptake of potassium-42 ± SD							
	Tissue used	Dose mg. per ml.	After 30 minutes		After 60 minutes		After 90 minutes	
			Control	Drug	Control	Drug	Control	Drug
8	Rabbit aorta strips	2	0.663 ± 0.27 P,	0.633 ± 0.22 0	1.301 ± 0.41 P,	1.25 ± 0.37 0	1.785 ± 0.41 P,	1.783 ± 0.46 0
8	Frog sartorius muscle	2	1.45 ± 0.31 P,	1.62 ± 0.38 0	2.56 ± 0.85 P,	2.57 ± 0.90 0	2.62 ± 0.85 P,	2.64 ± 1.41 0

Table 12

Effect of chlorothiazide (2 mg. per ml.) on the release of potassium-42 from isolated strips of rabbit aorta

No. of Expt.	Muscle	Number of counts released during a period of 10 minutes									Total number of counts released by the muscle.	Ratio of T/C
		10	20	30	40	50	60	70	80	90		
1	Test	1824	821	389	321(T)	295(T)	230 (T)	192	181	178	4432	1.2
	Control	1527	638	264	245(C)	243(C)	198 (C)	163	152	108	3538	
2	Test	3535	1800	827	338(T)	399(T)	401 (T)	411	393	380	8484	0.9
	Control	4269	1829	880	397(C)	314(C)	374 (C)	318	303	244	8928	
3	Test	5519	1629	864	749(T)	671(T)	675 (T)	443	311	193	11054	1.06
	Control	5238	1328	892	637(C)	720 (C)	521 (C)	418	319	282	10355	
4	Test	9713	1295	306	146(T)	141(T)	98 (T)	95	70	89	11953	1.1
	Control	8769	844	240	121(C)	102(C)	93 (C)	154	123	98	10544	
5	Test	1338	1600	649	407(T)	332(T)	308 (T)	252	272	226	5384	1.1
	Control	1221	1160	557	418(C)	374(C)	359 (C)	303	210	76	4678	
6	Test	8076	960	390	240(T)	200(T)	180 (T)	150	120	60	10376	1.01
	Control	7826	896	430	226(C)	265(C)	191 (C)	166	148	90	10238	
7	Test	4679	2572	1848	1669(T)	1374(T)	1004 (T)	968	693	697	15504	1.2
	Control	4471	2700	1868	871(C)	686(C)	539 (C)	453	347	389	12324	
8	Test	2081	423	298	218(T)	156(T)	160 (T)	94	121	80	3631	0.9
	Control	2387	517	306	212(C)	152(C)	144(C)	122	95	76	4011	

(T) denotes the point of exposure of the muscle to drug solution
(C) denotes the point of exposure of the muscle to control solution

Table 13. Effect of chlorothiazide (1 mg. per ml.) on the release of sodium-24 from isolated strips of rabbit aorta

No. of Expt.	Muscle	Number of counts released during a period of 10 minutes									Total number of counts released by the muscle.	Ratio of T/C
		10	20	30	40	50	60	70	80	90		
1	Test	2119	795	484	287 (T)	227 (T)	160 (T)	120	116	69	4377	1.0
	Control	2153	855	410	253 (C)	193 (C)	161 (C)	108	95	82	4310	
2	Test	5896	1235	735	631 (T)	529 (T)	446 (T)	325	307	301	10405	0.96
	Control	6698	1130	748	512 (C)	405 (C)	358 (C)	381	343	224	10799	
3	Test	3480	729	631	388 (T)	295 (T)	282 (T)	210	192	180	6386	0.98
	Control	3960	660	482	300 (C)	289 (C)	261 (C)	182	193	182	6509	
4	Test	1675	1531	978	469 (T)	343 (T)	234 (T)	142	108	98	5570	1.06
	Control	1713	1333	852	454 (C)	318 (C)	216 (C)	128	115	102	5231	
5	Test	3489	1831	1011	868 (T)	492 (T)	367 (T)	302	218	198	8776	0.95
	Control	3448	1993	1255	935 (C)	483 (C)	382 (C)	291	211	163	9161	
6	Test	2903	1215	1131	813 (T)	499 (T)	307 (T)	381	290	152	7691	1.04
	Control	2819	1136	1031	832 (C)	450 (C)	397 (C)	301	219	188	7373	

(T) denotes the point of exposure of the muscle to drug solution.

(C) denotes the point of exposure of the muscle to control solution.

Table 14.

	The effect of chlorothiazide upon ATP-ase activity in rat skeletal muscle homogenate.				
No. of observations	Tissue used	Dose level mg. per ml.	Inorganic Phosphate (µmoles) released in 10 minutes (Mean ± SD).		
			Blank	Control	Drug
8	skeletal muscle	1	3.531 ± 0.582	4.353 ± 0.459 P = 0.2	3.545 ± 0.781 P = 0.1
9		2	2.216 ± 0.714	5.769 ± 1.614 P = 0.2	4.944 ± 1.552 P = 0.1

P = Significance of difference between drug and control treated tissue.

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C H A P T E R III

C H A P T E R III

Discussion

Among all the many synthetic and natural substances which produce a fall in the blood pressure level of normotensive animals there are few which induce hypotension of a sufficiently specific type to justify their therapeutic use as antihypertensive agents. Since the ability to produce a fall in blood pressure is an insufficient criterion in itself for the choice of an antihypertensive agent, the purpose of making a pharmacological analysis of the general, and especially of the circulatory properties, of hypotensive drugs is to bring out those characteristics which may indicate the mode of its antihypertensive action. To do this it is necessary to correlate the results obtained by different groups of workers.

Chlorothiazide does not produce a fall in the arterial blood pressure level of the normotensive anaesthetised cat. This has been confirmed by a number of workers using other animal species, including the dog and the rabbit (Preziosi, Bianchi, Loscalzo and Schaepdryver, 1959 and Preziosi, Schaepdryver, Marmo and Miele, 1961). It is also interesting to note that chlorothiazide does not reduce the arterial blood pressure level in normotensive human subjects, although it exhibits saluretic actions to the same extent as in hypertensive subjects (Freis, 1959). The absence of a hypotensive action in normotensive individuals may be due to the existence /

existence of a vascular compensatory mechanism which appears to be lacking in hypertension (Wilson and Freis, 1959).

The pharmacological studies of Preziosi, Marmo and Miele (1961) have revealed that chlorothiazide and hydrochlorothiazide potentiate the antihypertensive effects of reserpine in the anaesthetised cat. Clinically it is well established that chlorothiazide potentiates the antihypertensive actions of ganglion-blocking agents (Tapia, Dustan, Schneckloth, Corcoran and Page, 1957) and other antihypertensive agents including reserpine, hydrallazine and the Veratrum alkaloids (Moser and Macaulay, 1959 and Smirk, McQueen and Morrison, 1960). It has also been shown to potentiate the reduction of the blood pressure level produced by sympathectomy and adrenalectomy (Sellers, Barends, Goldman, Lindauer and Jeffers, 1958) and that due to a restricted intake of sodium.

Clinical data published in 1958 suggested that chlorothiazide could on its own reduce the blood pressure level in a proportion of hypertensive individuals (Wilkins, Hollander and Chobanian, 1958 and Freis, Wanko, Wilson and Parrish, 1958). These early observations were confirmed by Hall and Owen (1959) and Juel-Jensen and Pears (1960) who presented the results of a double blind trial of the effects of chlorothiazide used both alone and together with ganglion-blocking drugs in the treatment of hypertension. It was found that chlorothiazide was capable of producing a significant fall in the blood pressure level of 5 out of 15 hypertensive patients when given in doses of 250 mg. three

times /

times daily for a period of 6 weeks.

The mechanism by which chlorothiazide exerts its antihypertensive action has been disputed. As already mentioned (Chapter I), suggestions for possible mechanisms of action have been put forward by a number of groups of workers and these will be taken up at appropriate points in the discussion which follows.

A fall in the plasma volume following the administration of chlorothiazide was independently observed by Tapia, Dustan, Schneckloth, Corcoran and Page (1957) and Freis, Wanko, Wilson and Parrish (1958) and was confirmed by the investigations of Dustan, Cumming, Corcoran and Page (1959) and Macleod, Dustan and Page (1960). Nine patients were given 1 g. of chlorothiazide twice daily for a period of from 4 to 6 days. The cardiac output was decreased in 6 out of the 9 patients and there was a simultaneous fall in the blood pressure level. A consistent decrease in the plasma volume with an increase in the peripheral resistance was also observed. To explore the possibility that the decrease in cardiac output was due to a reduction in plasma volume one patient was given 500 ml. of dextran solution intravenously in the form of a slow infusion. This procedure increased the cardiac output and blood pressure levels, together with a reduction in the total peripheral resistance. An increased sensitivity to the depressor effects of tetra-ethylammonium chloride was also observed following chlorothiazide administration. On the basis of the above observations Dustan, Cumming, Corcoran and Page (1959) /

(1959) postulated that oligaemia with increased vasomotor activity was the primary mechanism by which chlorothiazide potentiated the action of antihypertensive agents such as reserpine and ganglion-blocking drugs. Crosley, Castillo, Freeman, White and Rowe (1958) also found that the hypotensive response to chlorothiazide was associated with a decrease in the pressure in the right heart and a fall in cardiac output. The normal arterial blood pressure is the product of the cardiac output and the peripheral resistance; a decrease in one without a compensatory increase in the other factor will result in a fall in the blood pressure level. It seems probable that the depletion of the plasma volume and shrinkage of the extracellular space, secondary to the loss of interstitial fluid, cause a fall in the filling pressure of the right heart.

Unlike Dustan, Cumming , Corcoran and Page (1959), Wilson and Freis (1959) and Lauwers and Conway (1960) did not find a reduction in the plasma volume during long term therapy with chlorothiazide. The period of their clinical investigations extended from 26 days to one year. A partial explanation for this difference resides in the fact that the studies of Dustan and her associates (1959) were performed on patients who were treated for a period of less than 8 days. This reduction of plasma volume in the short term studies was also confirmed in 1960 by Conway and Lauwers. A significant reduction in the plasma volume of non-oedematous hypertensive subjects following a period of from 3 to 8 days of chlorothiazide therapy was also observed by Wilson and Freis (1959), /

(1959), whereas the same was not true in patients treated for a period of one year.

If it is true that the reduction of the arterial blood pressure is associated with a fall in the cardiac output, which is dependent on the reduction of plasma volume, then restoration of the latter should return the blood pressure level to pre-treatment values. The effect of re-expansion of the plasma volume was determined by Wilson and Freis (1959) on patients who had been under continuous treatment with chlorothiazide for periods of two weeks to three months and who had shown a fall in the blood pressure. The administration to these patients of 500 ml. of 6 per cent dextran solution in isotonic saline resulted in an immediate rise in the blood pressure level. In order to determine whether the sodium ion was important in the reversal of the hypotensive effect of chlorothiazide, an infusion of dextran in 5 per cent glucose was given. A significant rise in blood pressure to the pre-treatment level was still observed. A further attempt to determine the factors responsible for the haemodynamic changes was made by Wilson and Freis (1959) and Conway and Lauwers (1960). These workers investigated the relationship between the body fluids and exchangeable sodium and other electrolytes following long term therapy with chlorothiazide. Following a period of control observations in patients, 500 mg. of chlorothiazide was given 3 times daily. During the first 48 hours a loss of sodium and chloride over and above the level of the intake occurred together with /

with a considerably less degree of potassium loss. The initial saluretic response gradually became less but the loss of the sodium and chloride was maintained. Serum concentrations of sodium and chloride did not change significantly and the potassium concentrations of the serum fell to a moderate extent. This reduction in serum potassium tended to increase when treatment was continued for a period of months. Since the serum concentration of sodium did not change significantly it was assumed that the extent of the sodium loss could be accounted for by depletion of the extracellular fluid volume. Wilson and Freis (1959) postulated that reductions in the plasma and extracellular fluid volumes in the early phase of chlorothiazide treatment were the factors causing the fall in blood pressure.

As already described Lauwers and Conway (1960) could find no significant changes in extracellular fluid or plasma volume but recorded a fall in body weight in almost every patient under treatment with chlorothiazide. They attributed this to a reduction in the total body water. On the basis of these observations it was concluded that the fluid loss was from the intracellular compartment of the body. Although the reduction in intracellular fluid following the administration of chlorothiazide may have been directly induced by the drug, it could also be a secondary effect due to vascular dehydration of another kind. Potassium depletion could be responsible for this change as it is known to occur during chlorothiazide therapy, (Wilkins, Hollander and Chobanian, /

Chobanian, 1958). It is also reported (Black and Milne, 1952) that a low potassium diet may reduce blood pressure levels in experimental animals and man (Perera, 1953).

Aleksandrow, Wysznacka and Gajewski (1959) suggested that the antihypertensive action of chlorothiazide was due mainly to its natriuretic effect. They observed a reduction in the peripheral resistance, without a significant fall in the cardiac output. The peripheral resistance was lowered soon after the onset of the natriuresis and disappeared when the stores of the body sodium were restored to normal after the withdrawal of the drug.

It has long been known that a low sodium intake or the increased elimination of sodium from the body enhances the effectiveness of various antihypertensive agents. In 1948, Stead, Reiser, Rapaport and Ferris observed that when the blood pressure level was reduced following the administration of a test dose of tetraethylammonium, a further decrease could be obtained by placing the patient on a low salt diet. Freis (1948) observed that adequate salt restriction augmented the hypotensive response to surgical sympathectomy or to antihypertensive agents such as the Veratrum alkaloids.

The role played by the sodium ion in hypertension is undoubtedly important, although it is not very clearly defined. Dahl and Love (1957) found that a close relationship existed between the incidence of hypertension and higher levels of sodium intake. It is also interesting

to /

to note that early observations made by a number of workers (Eichelberger, 1943; Laramore and Grollman, 1950 and Ledingham, 1953) have shown that there is a rise in the extracellular water and sodium levels in animals in which renal hypertension was produced experimentally. Laramore and Grollman (1950) observed that when experimental renal hypertensive rats were compared with normal animals the sodium and water content of all the tissues were greatly increased and the potassium content was decreased. On the other hand, Ledingham (1953) using inulin to estimate the volume of the extracellular fluid, has demonstrated an increase in the total extracellular fluid during the early stages of experimental renal hypertension. After a period of three months, despite the persistence of the hypertension, the extracellular fluid volume tended to return to a normal level. There is also evidence that the sodium and potassium content of aortic tissues was significantly increased in rats in which experimental renal and desoxycorticosterone hypertension was induced (Tobian and Binion, 1954). In similar experiments Tobian (1956) noticed that the smooth muscle cells in the wall of the rat aorta contained more water, sodium, potassium, magnesium and phosphorus per unit cell solids in the hypertensive than in the normotensive rat. He also observed that when the blood pressure level dropped to normal as a result of the low sodium diet, the sodium, potassium and phosphorus levels also returned to normal. These findings indicated that changes of this kind in the electrolyte content of arterial smooth muscle cells may be associated with renal hypertension.

Intracellular /

Intracellular concentrations of sodium and potassium ions in the rat aorta were found to be increased in desoxycorticosterone hypertension, adrenal regeneration hypertension and in the elevation of the blood pressure level which persisted after the excision of the ischaemic kidney (Tobian and Redleaf, 1958). Further, an elevation of the potassium content of the aorta in renal hypertensive rats was observed by Freed, George and Rosenman (1959), who noted a fall in the blood pressure level following potassium deprivation. This was followed by a proportional decrease in the potassium content of the aorta. Return of the blood pressure level to earlier values following administration of cortisone was associated with a return of the potassium content of the aorta to the elevated levels. These authors suggested that the arterial potassium content may also influence arterial tone and by altering peripheral resistance may be a significant factor in the regulation of the arterial blood pressure level.

It is possible to assume that in hypertension there may exist some derangement in the electrolyte metabolism of the cell but whether this is the cause or the result of the elevated blood pressure level is not, however, clear. Chlorothiazide, through its effects upon electrolyte excretion, may correct this deranged balance of the cellular electrolytes.

On the other hand, Heider, Dennis and Moyer (1958) and Wilkins, Hollander and Chobanian (1958) while agreeing that sodium loss may play an important role in the reduction of the blood pressure level by chlorothiazide, /

chlorothiazide, suggested that it might not be the only factor involved in the continued hypotensive response to chronic oral therapy with this drug. They based their conclusion on the following findings. Firstly, after the therapeutic use of chlorothiazide over a period of four weeks, they were unable to find significant changes in extracellular fluid volume or in total body sodium and potassium. Secondly, in splanchnic-ectomised patients or in those treated with ganglion-blocking drugs, the oral or intravenous administration of chlorothiazide frequently resulted in a hypotensive response within a few hours, i.e., before any considerable loss of sodium or body fluids had occurred. Thirdly, oral or intravenous administration of hydrochlorothiazide produced a marked reduction in the blood pressure level of hypertensive patients. Administration of the steroidal antagonist (3-(3-oxo-17-hydroxy-19-hor-4-androsten-17α-yl) to patients who had already responded to chlorothiazide, produced further losses of body sodium associated with a decrease in blood pressure levels.

Hollander, Chobanian and Wilkins (1959) postulated that chlorothiazide may reduce the blood pressure by inhibiting the formation of some pressor agent such as renin and also by interfering with the salt-retaining action of aldosterone. The observation that when chlorothiazide was given to sympathectomised patients and those receiving ganglion-blocking drugs, it produced a hypotensive response before any significant saluresis occurred could be explained by the fact that the blood pressure of the patients with impaired sympathetic vasoconstrictor reflexes /

reflexes responds even to small degrees of blood loss (Freis, Stanton, Finnerty, Schnaper, Johnson, Rath and Wilkins, 1951).

The arterial blood pressure of normotensive subjects does not change following the administration of chlorothiazide and yet the same reduction of plasma volume and extracellular fluid space is induced (Wanko and Freis, 1958). The vascular responsiveness to pressor and depressor stimuli is, however, considerably altered (Merrill, Guinand-Baldo and Giordano, 1958). Wanko and Freis observed that the hypertensive response to a given rate of infusion of noradrenaline was reduced when compared with that before the administration of chlorothiazide. An increase in the hypotensive response to trimetaphan camphor sulphonate following the use of chlorothiazide was also observed. They suggested that although the arterial blood pressure in normotensive subjects remains unaltered following the use of chlorothiazide, their cardio-vascular response becomes less reactive to pressor stimuli and more responsive to depressor agents.

As already indicated, chlorothiazide depresses the hypertensive response to injection of adrenaline in the cat and to both adrenaline and noradrenaline in the rat, but in the dog it has been reported to depress the pressor responses to adrenaline and noradrenaline (Beavers and Blackmore, 1958). Bock and Gross (1960) have demonstrated that when hydrochlorothiazide was given orally to dogs over a period of one week it depressed the pressor responses to adrenaline and noradrenaline. It is /

It is possible that chlorothiazide, by altering the electrolyte composition of arterial smooth muscle, depresses the vascular reactivity to circulating pressor agents. The fact that salt restriction has the same effect on these responses supports this view.

The pressor effect of desoxycorticosterone acetate is enhanced by sodium intake and reduced or abolished by sodium withdrawal (Knowlton, Loeb, Stoerkand Seegal, 1947, Gross, 1950 and Stamler, Pick and Katz, 1951). It is also known that the pressor effects of injected or infused noradrenaline in man are potentiated by the pre-administration of desoxycorticosterone. In adrenalectomised animals, however, the hypertensive responses to injections of adrenaline and noradrenaline were lowered when compared with those of normal dogs (Humphreys and Lepeschkin, 1950). These observations indicate that the activity of the adrenal cortex by virtue of its influence on electrolyte balance may be an important factor in determining the degree of cardiovascular responsiveness to adrenaline and noradrenaline. It was also pointed out that the pressor effect of desoxycorticosterone may be due to an inorease in the vascular reactivity to the actions of intrinsic catecholamines which resulted from intracellular deposition of sodium. When given to hypertensive patients the pressor effects of adrenaline and noradrenaline are significantly diminished or abolished by a sodium-free diet (Raab, Humphreys, Makous, De Grandpre and Gigee, 1952). It was observed by these authors that during sodium withdrawal, desoxycorticosterone failed to /

to increase the pressor effects of adrenaline and noradrenaline. These observations indicate that the presence of a certain concentration of sodium in the arteriolar smooth muscle cell is necessary to maintain the vascular responsiveness to circulating pressor agents. The relation which exists between vascular reactivity and exchange of sodium and potassium and other free ions in the vascular smooth muscle cell is not clear.

The mechanism by which the electrolytes pass through the living cell membrane is of extreme importance in the physiological function of these ions. There is no evidence for the assumption that the activity of vascular smooth muscle, like that of the cells of skeletal muscle or nerve cells, is accompanied by an exchange of potassium and sodium during depolarization and repolarization.

In order to estimate the importance of the sodium ion concentration in cellular activity, a short account of the role of sodium ion in the living cell will be given.

It is an established fact that nerve fibres become inexcitable when the sodium concentration of the medium is less than one tenth of that of Ringer's solution, isotonicity being maintained with glucose or sucrose. This fact has been demonstrated in the case of the Loligo giant axon (Webb and Young, 1940, and Hodgkin and Katz, 1949), Sepia giant axon (Keynes, 1951), Carcinus nerve (Katz, 1947), frog medullated nerve (Feng and Liu, 1949, and Huxley and Stämpfli, 1951). In most

cases /

cases conduction block occurs when the sodium in the outside medium is replaced by an inert, non-penetrating cation, for example, choline.

Overton in 1902 and 1904 demonstrated that musoles were paralysed in the absence of sodium ions even if the osmotic pressure of the medium was maintained by the addition of an indifferent non-electrolyte such as sucrose. This paralysis was fully reversible when sodium was added to the medium. The concentration of sodium at which paralysis is complete varies from one species to another. Frog muscle loses excitability at about 12 mM. of sodium per litre (Taugner, Siebert and Gottstein, 1953). According to Magee and Reid (1927), lowering of the sodium chloride concentration below 0.6 per cent reduces the size of contractions in the isolated intestine of the rabbit and ultimately almost abolishes them. Streeten (1950) found that guinea pig and human intestine were even more sensitive to a reduction in the sodium chloride level than was the rabbit gut. Clinical observations have indicated a close correlation between a low sodium chloride concentration in the plasma and paralytic ileus (Streeten, 1950, and Marriott, 1947). It was also observed that in salt-depleted subjects, water absorption was delayed due to gastrointestinal atonia. Streeten and Vaughan Williams (1952) studied the effects of sodium chloride depletion upon the rate of propulsion of intestinal fluid in the dog. Sodium chloride depletion was brought about by intraperitoneal infusions of 4.5 per cent dextrose solution containing appropriate amounts of potassium, calcium, magnesium /

magnesium and phosphate ions. This treatment caused an initial rise in the rate of intestinal propulsion but was soon followed by a marked reduction and ultimately by total paralysis of peristalsis. In some cases, the intestinal loop became completely toneless. It is of interest that the elasticity of the skin was also markedly impaired, indicating loss of tone in the plain muscle of the skin. The paralysis of intestinal peristalsis could be reversed within one minute by the injection of hypertonic sodium chloride solution. Streeten and Vaughan Williams suggested that the reduced level of sodium chloride in the blood induced a loss of potassium from the musole fibre, which was suggested to be the ultimate cause of the paralysis.

The hypothesis advanced by Hodgkin and Katz (1949) implies that a lowering of the membrane potential has two effects. One is a rapid and transient increase in sodium permeability and the other, a delayed but prolonged increase of potassium permeability. Both of these effects are assumed to be reversible in the sense that repolarisation restores the permeabilities to their original values. Fatt and Katz (1951) observed that the depolarising effect of the transmitter-acetyloholine led to a change of the potential across the muscle cell membrane. This was responsible for the initiation of the propagated muscle action potential which produced muscular contraction. Subsequent observations by Hodgkin (1951) revealed that in nerve, the action potential depended upon a rapid sequence of changes in the permeability to sodium and potassium /

potassium ions. The resting membrane was found to be moderately permeable to potassium ions and sparingly permeable to sodium ions. A large but transient increase in the permeability to sodium ions occurred when the fibre was depolarised. Sodium ions, therefore, entered the fibre at a high rate and reversed the potential difference across the cell membrane. This increase in permeability to sodium ions was followed by a similar rise in permeability to potassium ions. Entry of sodium ion was approximately balanced by the leakage of a corresponding quantity of potassium ions. It has been suggested that the sodium ion enters the fibre during the rising phase of the action potential and the potassium ion leaves during the falling phase. The entry of sodium ions and the exit of potassium ions provides an immediate source of energy for the propagation of the impulse.

The relation which exists between the action potential and the external sodium concentration has been studied by Hodgkin and Katz (1949) for the giant axon of the squid and they postulated that the rise in the action potential of the cell should be determined by the rate at which the membrane capacity was discharged by the entry of sodium into the cell. In their experiments Hodgkin and Katz (1949) found that the rate of rise of the action potential was roughly proportional to the external sodium concentration. It is of interest to note that the falling phase of the action potential, during which the fibre is supposed to be very slightly permeable to sodium, is much less affected by the external sodium concentration.

If /

If it is assumed that in hypertension a state of partial depolarization of the muscle cell membrane of the arteriolar smooth muscle is present, then it is possible that salt restriction or a lowering of the external sodium concentration may interfere with the activity of the vascular smooth muscle cells. This view is based on the fact that normal activity (action potential or initiation of impulse) is accompanied by the increased uptake of sodium by the cell.

A fall in the plasma concentration of sodium and a rise in the plasma potassium level was associated with a marked elevation of the blood pressure level, when a generalised vasoconstriction was produced by the administration of noradrenaline in the dog (Muirhead, Goth and Jones, 1954). Tobian and Fox (1956) have reported that noradrenaline infusions decreased the potassium content of the dog femoral artery and in some cases elevated its sodium content. On the other hand, the sodium concentration of the rat aorta was not altered, whereas its potassium content was markedly reduced under the influence of noradrenaline (Daniel, Dawkins and Hunt, 1957). It is possible that noradrenaline, by affecting the membrane of the smooth muscle cell, allows a loss of potassium and a gain of sodium ions. Since adrenaline and noradrenaline can induce a contraction of arterial smooth muscle, (Furchgott and Bhadrakom, 1953) it is possible that electrolyte shifts take place as a result of the change in the membrane potential. Chlorothiazide, by causing sodium depletion, may interfere in the normal function of the cell and depress the vascular reactivity /

reactivity to pressor agents.

It has been shown (Chapter 2, page 270) that the magnitude of the pressor response due to baroreceptor stimulation and that which follows bilateral occlusion of the common carotid arteries in the cat is also slightly reduced following the administration of large doses of chlorothiazide. This effect was not seen by Preziosi, Bianchi, Loscalzo and Schaepdryver (1959). It has been postulated (Heymans, 1954) that drugs which cause the muscular walls of the carotid sinuses to contract, increase their intrinsic tone and decrease their distensibility, thus stimulating the receptors at the endings of the carotid sinus nerves. This stimulation reflexly reduces the arterial blood pressure and decreases the hypertension normally produced by a decrease of the carotid sinus blood pressure. The pressor response which follows stimulation of the central end of the vagus is in part due to reflex adrenergic vasoconstriction and perhaps also due to stimulation of carotid body chemoreceptors following reflex hypoxia and the reflex is mediated through the higher centres of the brain. The fact that adrenergic blocking agents and ganglion blocking agents depress these reflex pressor responses supports this view. The observations that chlorothiazide does not modify the pressor responses to electrical stimulation of the cut central end of the vagus and that it has no ganglion blocking activity are in agreement with the observations of Preziosi, Bianchi, Loscalzo and Schaepdryver (1959) in the dog. There is no modification of the rhythmic activity of preparations of isolated /

isolated heart following the injection of chlorothiazide, except for a slight reversible depression seen when large doses are used. The responses of the isolated guinea pig auricles to adrenaline and noradrenaline are not modified by chlorothiazide. These observations confirm the findings of Preziosi and his associates (1959).

No direct vasodilator effect was observed on the isolated hindquarters of the rat, following perfusion of chlorothiazide even when the drug was used at high dose levels. A slight decrease in the constrictor responses to adrenaline and noradrenaline was noted. In the isolated hindquarters preparation of the rat, it is perhaps to be expected that little, if any, neurogenic vascular tone should remain so that in these circumstances, little or no vasodilatation would be expected.

In experiments performed on isolated strips of horse carotid artery, chlorothiazide had no direct relaxant effect, but markedly depressed the contractions of arterial strips elicited by adrenaline, noradrenaline, 5-hydroxytryptamine and acetylcholine. No direct depressant effect or antagonism to spasmogens was observed in other smooth muscle preparations, such as the guinea pig ileum or rabbit duodenum. These observations do not agree with the findings of Preziosi and his associates (1959) who found some depressant effect on the isolated guinea pig and rabbit colon following the use of chlorothiazide. Since chlorothiazide does not modify the normal tone of intestinal smooth muscle, it is quite probable that it may have some specific action on arterial smooth muscle. The

observation that chlorothiazide significantly depresses the uptake of sodium-24 in isolated strips of rabbit aorta may cast some light upon the link which may exist between the electrolyte metabolism of the cell and hypertension.

The mechanism by which chlorothiazide depresses the uptake of sodium by the muscle cell is not clear. Investigation of the influence of chlorothiazide on adenosine triphosphatase activity has not demonstrated any measurable effect upon the activity of this enzyme. It is possible that a common mechanism is involved in the movement of sodium ions across the tubular cell of the kidney and across the wall of the arteriolar smooth muscle cell. Chlorothiazide may depress the movements of the sodium ion by affecting this mechanism. The lesser effect on sodium-24 uptake observed on the sartorius muscle of the frog may indicate that the action of chlorothiazide on the arterial smooth muscle is relatively specific in nature.

From the data presented above it is not possible to suggest a single mechanism which can account for all the effects of chlorothiazide on the cardiovascular system of animals and in preparations of isolated tissues and organs.

The highly specific depression of sodium uptake by strips of aorta tends to confirm the views of Aleksandrow, Wysznacka and Gajewski (1959), that chlorothiazide reduces the sodium content of the artery wall by depressing sodium uptake from the plasma and extracellular fluid and that this /

this contributes to its antihypertensive activity. A fall of sodium content may also reduce the water content of the arterial wall (Lauwers and Conway, 1960) and reduce the swelling or oedema (Redleaf and Tobian, 1958), if this is present. This reduction in the thickness of the artery wall will lower the peripheral resistance which in turn leads to a fall in the arterial blood pressure.

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C H A P T E R IV.

CHAPTER IV

SUMMARY OF PART I (b).

In the introductory chapter, a review of the literature upon the pharmacology of chlorothiazide, hydrochlorothiazide and flumethiazide, together with a short historical account of the discovery and mode of diuretic action of sulphonamide derivatives are presented.

In Chapter II, the experimental techniques used in the investigation of the general pharmacological properties of chlorothiazide, hydrochlorothiazide and flumethiazide, and the results of this investigation are described. Chlorothiazide did not produce an observable fall in the blood pressure level of the normotensive anaesthetised cat or rat. A slight inhibition of the hypertensive response to adrenaline in the cat and to adrenaline and noradrenaline in the rat was observed following the administration of chlorothiazide. Chlorothiazide depressed the responses of isolated strips of horse carotid artery to adrenaline, noradrenaline, 5-hydroxytryptamine and acetylcholine.

Chlorothiazide produced a significant depression in the uptake of sodium-24 by isolated strips of rabbit thoracic aorta, but it did not produce any demonstrable effects on the uptake and release of potassium-42 by this tissue.

Chlorothiazide did not modify the adenosine triphosphatase activity of rat skeletal muscle homogenates tending, thereby to indicate /

indicate that the supply of energy yielded by adenosine triphosphate for the movements of sodium ions is not interfered with.

In Chapter III, the antihypertensive actions of chlorothiazide and their relationship to its saluretic effects are discussed.

The highly specific depression of sodium uptake by strips of rabbit aorta suggests that chlorothiazide may reduce the sodium content of the artery wall by depressing sodium uptake from the plasma and extracellular fluid and that this property may contribute to its antihypertensive activity. A fall in the sodium content may also reduce the water content of the artery wall and reduce the swelling or œdema if it is present. This reduction in the thickness of the arterial wall may lower the peripheral resistance which may, in turn, lead to a fall in the arterial blood pressure level.

PART II

STUDIES ON THE NATURE OF THE MUSCLE-RELAXANT ACTIONS OF ETHER AND SOME OTHER VOLATILE ANAESTHETICS.

C H A P T E R I

CHAPTER I.

INTRODUCTION

Since it was first shown by Auer and Meltzer (1914) that ether in anaesthetic concentrations was able to depress the magnitude of the response of the muscle to indirect stimulation, a number of workers have undertaken detailed studies upon the nature of this phenomenon. Thus it has been established that the muscle relaxant actions of non-depolarizing neuromuscular blocking agents such as tubocurarine are potentiated by ether (Cullen, 1944 and Watland, Long, Pittinger and Cullen, 1957). This action of ether is shared by chloroform and halothane. In their studies upon rabbits, Watland and his associates (1957) demonstrated that ether produced a depressant effect upon the response of the gastrocnemius muscle to indirect stimulation *via* the sciatic nerve. They also observed that when tubocurarine was given during the period of chloroform or halothane anaesthesia, there was a marked reduction in the magnitude of the muscular contractions when compared with the effect caused by tubocurarine alone. The tubocurarine was given to the unanaesthetised animal one hour after the termination of the anaesthesia. Earlier observations had revealed that ether exhibited /

exhibited curare-like actions on the motor end-plate (Auer and Meltzer, 1914). Githens and Meltzer (1914a) stated that during ether anaesthesia, even after respiration had ceased, the diaphragm muscle and phrenic nerve remained excitable. Forbes, McIntosh and Sefton (1916) recorded the action potentials of motor nerves during ether anaesthesia. They observed that the action potential remained unchanged, at concentrations of ether which were deep enough to cause cessation of respiration. Simonart and Simonart (1934) found that the muscular contractions of the cat gastrocnemius muscle-sciatic nerve preparation induced by intra-arterial injection of acetylcholine, could no longer be elicited during ether anaesthesia, and moreover, that eserine could antagonize this ether effect. Gross and Cullen (1943) and Poulsen and Secher (1949) using the gastrocnemius muscle-sciatic nerve preparations of the cat and the dog, found that ether was capable of inhibiting contractions due both to intra-arterial injection of acetylcholine, and to indirect stimulation of the muscle <u>via</u> the sciatic nerve. They also confirmed that neostigmine antagonized the effects of ether on the acetylcholine-induced contractions. Gross and Cullen (1943) and Poulsen and Secher (1949) concluded that the peripheral action of ether was due to an interference with the liberation of acetylcholine at

the /

the neuromuscular junction. In his efforts to differentiate the mechanisms of action of ether and of tubocurarine upon neuromuscular transmission and the muscle cell itself, Naess (1950a,b, and c) carried out a detailed study of the effects of tubocurarine and neostigmine upon ether induced neuromuscular block in the rabbit flexor digitorum longus muscle-sciatic nerve preparation. From his observations, Naess suggested that ether and tubocurarine decreased the sensitivity of the end-plate to depolarization but that the two drugs acted by different mechanisms. Secher (1950, 1951 a, b, c and d), using the rat phrenic nerve-diaphragm preparation, observed that ether could abolish muscular contractions induced by indirect stimulation and that the contractions recovered completely when addition of ether was discontinued. He also observed, that when given at a concentration which abolished contractions induced by indirect stimulation, ether did not inhibit contractions of the diaphragm due to direct stimulation. Muscle contractions caused by indirect stimulation ceased when the ether concentration in the medium was 0.22 per cent, whereas direct stimulation of the muscle was only affected when the ether concentration of the medium was increased to 0.57 per cent. From these observations, Secher (1951d) concluded that ether in the concentrations attained during general anaesthesia had very /

very little direct depressant effect upon the muscle cell but that its effects resulted primarily from an action on the motor end-plate. Naess (1950 a,b, and c) and Secher (1951 a,b,c, and d) have also indicated that, although the observable effects of ether were identical with those of tubocurarine in depressing neuromuscular transmission, the precise nature of the mechanism was different.

In contrast to the observations of Githens and Meltzer (1941b), chloroform in anaesthetic concentrations, has been reported by Blume (1925), Naess (1950a) and Secher (1951d), to possess a peripheral action on the neuromuscular junction. These authors reported that chloroform had a potentiating action on the tubocurarine-induced inhibition of muscular contractions elicited by indirect stimulation.

The precise nature of the mechanism by which ether produces relaxation of skeletal muscle is not known. The studies described in this thesis were carried out in the hope that the observations of the effects on the permeability of the cell membrane to sodium and potassium ions, under the influence of ether and tubocurarine, would shed further light on the problem.

For an understanding of the mode of action of volatile anaesthetics on the neuromuscular junction and its relationship

to /

to the permeability of the cell membrane to sodium and potassium ions, a brief account will be given of the mechanism of neuromuscular transmission and of the nature of the cell membrane.

Physiological processes at the neuromuscular junction during excitation are extremely complex and so are best considered from several viewpoints.

The role of acetylcholine

Transmission of impulses from the neurone to the effector cell is mediated by a specific chemical transmitter, acetylcholine, which is liberated from the nerve terminals (Katz, 1958a and Nachmansohn, 1959a and b). The specialised role of the motor end-plate in the mechanism of neuromuscular transmission has been discussed by Katz (1958b).

Depolarization occurs following the adsorption of acetylcholine molecules released by the nerve impulses upon the cholinergic receptors of the end-plate. Repolarization is effected following its enzymatic hydrolysis (Nachmansohn and Wilson, 1951 and Koelle, 1962). Depolarization of the end-plate produces a non-propagated end-plate potential (Eccles, Katz and Kuffler, 1941). A spontaneous and intermittent release of disorete quantal packets of acetylcholine also occurs at the neuromuscular junction during rest. This elicits minute potential changes in the post-synaptic membrane /

membrane which are insufficient to initiate an action potential and consequently a contraction of the muscle fibre (Fatt and Katz, 1952a and b, and Katz, 1956 and 1959). The spontaneous rupture of large numbers of quantal packets of acetylcholine in response to impulses traversing the motor nerve fibre (Katz, 1956) produces depolarization of the end-plate sufficient to generate a wave of excitation in the muscle fibre. The action potential propagates in both directions along the muscle fibre, inducing the muscle contraction. This process of depolarization is associated with rapid alterations in the permeability of the cell membrane to ions. During the passage of an impulse in a nerve or muscle fibre, sodium enters the fibre and potassium is subsequently released in equivalent amounts (Hodgkin, 1951). Under normal conditions, however, the cell membrane is moderately permeable to potassium ions and sparingly permeable to sodium ions. When an impulse travels along the motor axon, this exchange of cations proceeds until the impulse reaches the nerve terminals. Acetylcholine is liberated from the nerve terminals (Dale, Feldberg and Vogt, 1936) and by a specific action on the post-synaptic membrane, short-circuits the muscle cell membrane (Fatt and Katz, 1951). A propagated wave of excitation is initiated in the muscle fibre, involving an exchange of sodium and potassium ions similar to the one produced in the nerve axon (Nastuk and Hodgkin, 1950). The precise mechanism by which /

which acetylcholine is liberated and its relationship to the changes occurring in the permeability of the cell membrane to these ions is not clearly understood (Castillo and Katz, 1956 and Katz, 1958a and b). Fatt and Katz (1952a and b) suggested that acetylcholine was liberated from the nerve terminals by a specific exchange of sodium ions, which entered the terminal during the electrical activity. There is little evidence in favour of this hypothesis. A decrease in the release of acetylcholine would, therefore, be expected when the external sodium concentration was reduced.

The role of inorganic ions

Changes in the concentrations of cations in the environment of the neuromuscular junction may influence the release of transmitter (Nastuk, 1959). There is evidence that an excess of potassium ions when applied externally causes a liberation of acetylcholine from the nerve endings. This was observed in experiments on the perfused sympathetic ganglion (Brown and Feldberg, 1935). It is possible that an increase of potassium ion concentration may depolarize the cell membrane and therefore result in the liberation of acetylcholine. Hutter and Kostial (1954) observed that the potassium-induced release still occurred in a ganglion which had previously been made inexcitable by perfusing it with a sodium-free sucrose solution. It is believed that even

under /

under these conditions, potassium depolarizes the cell membrane and the release of transmitter may therefore be an indirect effect of the presence of potassium ions (Castillo and Katz, 1956).

The magnitude of the action potential of the nerve cell appears to depend upon the concentrations of the potassium ions in the outside medium. When this increases, the action potential is depressed. In the giant axon of the squid, the potential was reduced to zero, when the concentration of potassium ions in the bathing solution was raised by six times to that found in sea water (Curtis and Cole, 1942). When bathed in Ringer's solution containing four times the normal potassium concentration, isolated frog sciatic nerve showed a reduction in the height of the action potential (Rosenberg and Kitayama, 1930). Hertz (1945) observed an immediate reduction of the amplitude of the action potential, on immersing a single frog myelinated nerve fibre in Ringer's solution containing six to ten times the potassium concentration of normal Ringer's solution. According to Huxley and Stämpfli (1951), the depolarization of frog medullated nerve fibre by an increased potassium concentration takes place in less than one minute. Fenn (1940) noted that a moderate increase in the potassium concentration of the medium might enhance the mechanical response of skeletal muscle, while larger concentrations paralysed the muscle. Overton (1904) using an isolated nerve-muscle preparation /

preparation from the frog had already shown that about 10 mM of potassium per litre of Ringer's solution produced total muscular paralysis.

Nerve fibres become inexcitable when the sodium concentration of the medium is 10 per cent less than that of Ringer's solution, the isotonicity of the medium being maintained by suorose or glucose. This fact has been demonstrated by Webb and Young (1940) and Hodgkin and Katz (1949) using the Loligo giant axon. The same effeot was observed in the case of the Sepia giant axon (Keynes, 1951) and frog medullated nerve (Erlanger and Blair, 1938 and Huxley and Stämpfli, 1951).

The relationship between the aotion potential and the external sodium concentration has also been investigated by Hodgkin and Katz (1949) using the giant axon of the squid. The isotonicity of the medium was maintained by substituting dextrose for sodium ions. Hodgkin and Katz (1949) demonstrated that the rate of rise of the aotion potential was roughly proportional to the external sodium concentration. The falling phase of the action potential, during which the oell membrane was supposed to be very slightly permeable to sodium ions, was much less affected by the external sodium concentration than the rising phase. Hodgkin and Katz (1949) suggested that the rise in the aotion potential was determined by the rate at which the membrane capacity was discharged /

discharged by the entry of sodium. The detailed studies made by Fatt and Katz (1950, 1951, 1952a and 1952b) have done much to explain the role of sodium in the genesis of the end-plate potential. These authors used micro-electrodes which were introduced into the frog muscle fibre at the site of the end-plate. They found that the end-plate potential was reduced and nerve transmission blocked when the sodium concentration of the bathing Ringer's solution was lowered to from one half to one third of that in normal Ringer's solution. The reduction in the size of the end-plate potential was comparable to that obtained in a partially curarised muscle. The preparation, however, remained sensitive to externally applied acetylcholine. Nastuk (1954), using a frog nerve-muscle fibre preparation, found that although the magnitude of the resting potential of the end-plate was little affected by the complete removal of sodium from the bathing fluid, the magnitude of the end-plate potential produced by direct application of acetylcholine was significantly reduced. The changes produced by a deficiency of sodium ions were suggested to be due to a post-synaptic blocking action (Castillo and Katz, 1956).

It has been suggested by Katz (1958b) that the action of acetylcholine on membrane permeability persists even though the sodium on the outside of the cell membrane has been removed. Furukawa, Furukawa and Takagi (1957) observed that the substitution of /

of ammonium ions for sodium ions, while abolishing the spike potentials, did not reduce the spontaneous miniature potentials, nor did it interfere with the depolarizing action of externally applied acetylcholine.

The studies of Castillo and Engbaek (1954), using micro-electrodes, demonstrated that high concentrations of magnesium ions and low concentrations of calcium ions may reduce the amount of acetylcholine liberated. Large concentrations of calcium ions stimulate the release of acetylcholine (Castillo and Stark, 1952). Very high concentrations of both ions depress the direct excitability of the cell membrane.

The nature and structure of the cell membrane.

The muscle end-plate is a highly specialized development of the muscle cell membrane and may be expected to exhibit some of the general properties of membranes.

To interpret the results obtained from studies on the permeability of the cell membrane it is necessary to know something of its nature and its structure.

Unfortunately it has not been possible in the past to demonstrate the structure and chemical nature of the cell membrane by optical methods. Theories advanced by earlier workers are summarized /

summarized by Davson and Danielli (1952) and these are firstly, that the cell membrane is a thin layer of fatty material; secondly, that the cell membrane is a sieve with pores of molecular dimensions; thirdly, that the cell membrane consists of a mosaic made up of areas of more than molecular dimensions, each area possessing a different chemical nature; fourthly, that the cell membrane consists of a sieve-like structure superimposed upon a lipoid layer; and finally, that the cell membrane is a bimolecular leaflet of fatty molecules.

The view of Overton (1895 and 1900) that the cell membrane consisted of a thin layer of fatty material was based on the observation that substances penetrated the cells in the same relative order of effectiveness as their oil-water partition coefficients varied. Since then, it has been generally assumed that such a correlation indicates the presence of a larger proportion of lipoid material in the cell membrane, and that due to their higher lipid solubility, substances with high oil-water partition coefficients penetrate rapidly.

Overton (1901) and Meyer (1899-1901) believed that the potency of anaesthetic agents was directly related to their distributions between oil and water, i.e., to the oil-water partition coefficient. This was the first attempt in the field of pharmacology in which the physical properties of a group of drugs /

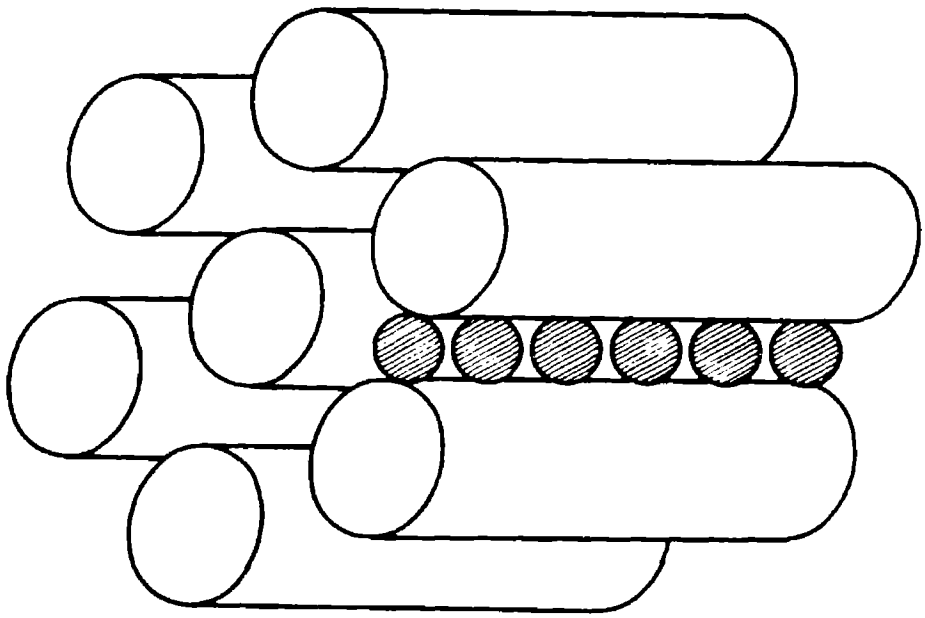

Fig. 135.

After Mullins (1956).

Diagram showing the arrangement of macromolecules shown in a regular hexagonal packing. The pores which exist between any three cylinders are characterised by the regularity of their size.

drugs were correlated with their pharmacological actions.

Danielli and Davson (1934) have put forward another theory according to which the lipoid layer is a few fatty molecules thick, with an adsorbed protein layer at each fat-water interface. Doubt was cast upon the stability of such a bimolecular lipoid layer sandwiched between two aqueous phases. This model of the cell membrane has been revised by Mullins (1956) who suggested that it was a macromolecular liquid membrane, in which pores were formed by the inability of macromolecules to fill all the spaces in the liquid. The measurement of the thermodynamic activity of anaesthetic agents gave values which implied that narcotic molecules would be orientated at the site of their action on the cell membrane as they would be in the pure liquid state. The small molecules of narcotics would become distributed almost uniformly throughout the membrane sites.

To explain the relationship between the distribution of narcotic molecules and increased permeability of the cell membrane to inorganic ions the following suggestion was put forward by Mullins (1956). It was said that pores formed by the cylindrical macromolecules were constantly undergoing thermal motion and so would show differences in size. The fact that the cell membrane is sparingly permeable to sodium ions and moderately permeable /

permeable to potassium ions was said to be due to the size of the pores existing in it. A resting cell membrane allowed the entry of ions of only a certain size. Sodium ions were larger than potassium ions, and their larger energy of hydration resulted in a slow mobility in aqueous solution and were not therefore capable of passing freely through the membrane pores in a resting cell. Organic molecules capable of passing through the membrane pores would distort the channels by accumulating in them. This effect was presumed to cause an increase in the size of neighbouring channels and pores on the surface of the cell membrane. Sodium rapidly entered and occupied the spaces so formed and a compensatory release of potassium ions occurred. Calcium ions were of approximately the same size as sodium ions and might compete with sodium for entry into the pores.

Mullins (1956) suggested that only molecules of a certain size could pass through the channels formed on the surface of the cell membrane. It is possible that the tubocurarine molecule, being larger and bulky, may not pass through these pores into the channels and may merely block their openings. The view presented by Mullins (1956) will be discussed in a later part of this chapter.

Recently Ahmed and Lewis (1961 and 1962) have indicated that the classification of a muscle relaxant as depolarizing or competitive /

competitive may be made, according to its ability or otherwise to increase the permeability of the cell membrane to sodium and potassium ions. Non-depolarizing agents such as tubocurarine and gallamine even at high dose levels had no observable effect upon the release or uptake of potassium and sodium ions. Depolarizing agents, for example decamethonium and suxamethonium, were shown to be capable of causing a significant increase in the uptake of sodium and in the release of potassium ions.

The literature available on the influence of volatile anaesthetics on the permeability of the cell membrane is both limited and controversial. The early observations of Alcock (1906) indicated that when frog nerve was treated with the vapour of chloroform or ether, a reversible decrease in the resting potential occurred. He interpreted his observations as being due to an increased permeability of the tissue to ions. Höber (1907) on the other hand, observed that narcotics such as chloroform and ether decreased the permeability of the cell membrane to ions and he attempted to relate the activity of narcotics to their activity on colloidal systems in the cell.

Lillie (1909) indicated that a narcotic substance exerted its actions by interfering with the permeability of the cell membrane during activity. He postulated that an anaesthetic agent decreased /

decreased the permeability of the cell membrane by adsorbing on to the cell's surface. This produced a narcotic effect by varying the rate of removal of carbon dioxide from the cell.

Winterstein (1916) measured the rate of increase of weight of frog sartorius muscles placed in a hypotonic salt solution. He concluded that the permeability of the cell to water was decreased when alcohol was added to the bath solution.

Davson (1940) and Davson and Reiner (1942), using cat erythrocytes, observed that amyl alcohol increased their permeability to potassium ions and decreased their permeability to sodium ions. A large variety of organic agents (chloroform, ether, benzene and butyl and amyl alcohols) had similar actions on the permeability to sodium ions.

The fact that in some cases narcotics cause a reduction in cellular permeability while in others they cause an increase in permeability to certain ions and to water, indicates that besides the chemical nature of the anaesthetic agent, the special nature of the membrane, and in addition, the experimental method used must also be considered.

Pharmacologically active agents exhibit their actions by either specific or non-specific mechanisms (Beckett, 1956, and Ing, 1959). Muscle relaxant drugs are presumed to act in a structurally specific manner and to combine with specific receptors, a typical example of this group being tubocurarine.

Some /

Some agents, however, exert a physical or physicochemical effect within the cell or on its surface, thereby causing a disturbance of cellular metabolic activity. Among compounds of this type are volatile anaesthetics such as ether, chloroform and cyclopropane and hypnotics (Albert, 1960).

Structural changes in the cellular membrane are known to play an essential role in the process of depolarization and in the propagation of excitation (Waser, 1960); it may therefore be assumed that this is a site of the muscle relaxant action of general anaesthetics. It has, for example, been shown that anaesthetics do not act as depressants when injected into the cell but do so when they are allowed to act from without. Therefore, Warburg (1921) suggested that depressant drugs exerted their effects by coating the outer surface of the cell membrane, and there inhibiting oxidative processes. Quastel and Wheatley (1932, 1934), using rat brain cortex in vitro, demonstrated that the oxidation of glucose, lactate and pyruvate, was inhibited by concentrations of narcotics approximately corresponding to those which were found during general anaesthesia. Quastel (1939, 1952) observed that only certain oxidative processes were inhibited by ether. He found that in rat brain slices in vitro, oxidation of pyruvate, glucose and lactate were inhibited but no effect was seen /

seen on succinate oxidation. Quastel (1939) considered that anaesthetic agents attacked the point which acted as the hydrogen carrier between pyruvic dehydrogenase and cytochrome oxidase. Buttler (1950), on the other hand, suggested that depression of respiration in cells caused by anaesthetic agents might have been the result and not the cause, of the reduoed nervous activity. It seems that mechanisms other than oxidative processes probably exist and are influenced by narcotics. This view is favoured by the fact that xenon, a physically and chemically inert gas, can produce narcosis when the total volume of the non aqueous phase of the nerve cell is occupied by its molecules. It was suggested by Albert (1960) that narcotics in general act by their physical accumulation in some part of the cell and not by adsorption at the surface as was suggested by Warburg (1921).

Goldacre (1952) has demonstrated that general anaesthetics can cause a reversible increase in the area of the cell membrane of the amoeba. He suggested that the membrane was an enzyme which acted as a non-diffuseable substrate in the cytoplasm and considered that a similar mechanism might be present in the nerve cell. It is very possible that vital enzyme systems which are located on the surface of the cell membrane are interfered with by the presence of narcotic agents.

The /

The nature of the cholinergic receptor.

The view that drugs exert their actions by interacting with certain receptors in the tissues has become generally accepted by workers in the fields of chemotherapy and pharmacology. Although this concept originated from the studies of Ehrlich (Ehrlich and Morgenroth, 1910), the original ideas have been considerably modified. Clark (1933) and Clark and Raventos (1937) used the concept to treat drug action mathematically and in more recent times, Ariëns, Van Rossum and Simonis (1956) and Van Rossum, Ariëns and Linssen (1958) have developed this approach to give an elegant theoretical interpretation of drug action. They visualize drug-receptor interaction in general terms as basically an interaction of fields of force originating in the drug molecule and the tissue. They then postulate that the general field determines the affinity of the drug for the receptor (i.e., its ability to enter into drug-receptor complex formation) while certain specific interactions within the general field determine the intrinsic activity or ability of the drug to evoke a biological response. Regions of high or low electron density in the drug molecule will contribute to the electrostatic components of the force field, while stereochemical factors will contribute to Van der Waal's bonding.

A more detailed picture of the acetylcholine receptor involved /

involved in neuromuscular transmission has been given by Waser (1960) who postulates that the receptor site might be a pore in the post-synaptic membrane which had an anionic wall and within the pore itself, an esteratic site, to which the ester groups of acetylcholine become attached. On these assumptions the neuromuscular blocking action of non-depolarizing relaxants such as tubocurarine could be visualized as due to a covering of the pores of the end-plate region by their large bulky molecules. That is, tubocurarine or gallamine may act by inhibiting the access of acetylcholine to the receptors and thus, by preventing the flow of ions through the membrane pores, prevent depolarization of the post-synaptic membrane. On the other hand, depolarizing drugs such as decamethonium and suxamethonium may attach themselves to the anionic sites and so bridge the pore at different chords. The molecules may not cover the whole circular area of the opening of the pore, but may increase depolarization by causing mechanical deformation. Ions are assumed to pass through the pore on both sides of the long thin molecules, especially when the circular shape of the pore is deformed by the strain exerted upon it.

Some years before Waser (1960) presented his theory of the nature of the cholinergic receptor and from this attempted to explain the mode of action of depolarizing and non-depolarizing muscle relaxants, /

relaxants, Mullins (1956) had suggested a structure for the cell membrane on the basis of which the action of narcotics could be explained. If Mullins' view is accepted, then it is possible that the thin elongated molecules of decamethonium, instead of bridging the pore, enter the channels directly through the pores on the cell membrane. These molecules by their presence may cause mechanical folding or dislocation of the channels which consequently causes an increase in the size of the pores on the cell's surface. Sodium, which can ordinarily only get into the few pores which are enlarged by the thermal agitation of the macromolecular cylinders, is now able to find a large number of pores and channels large enough for its entry. This process is presumed to cause an increase in the permeability of the cell membrane to sodium and potassium ions. If these assumptions are true, then molecules of intermediate stereochemical properties may by their partial entry into the channels, cause a disturbance of a similar nature in the normal distribution and permeability of the cell membrane to cations.

It is possible that the entry of narcotic molecules into the pores formed on the cell surface causes a physical derangement of these channels (similar in manner to those described for depolarizing agents) and so results in an increased influx of sodium together /

together with a correspondingly increased release of potassium ions.

Although ether is known to potentiate the muscle relaxant actions of tubocurarine, it has also been shown that ether causes an increase in the level of the potassium ions in the circulating blood (Kiersz, 1948). Potassium chloride on the other hand, is known to cause antagonism to the neuromuscular blocking actions of tubocurarine (Wilson and Wright, 1936; Altamirano and Huidobro, 1948; and Li, Jacobs, Aviado and Schmidt, 1952). The same has been found true for sodium (Fatt and Katz, 1952b). The role of sodium and potassium ions is vitally linked with the process of neuromuscular transmission and the normal excitation of the cell (Fatt and Katz, 1952b).

The present studies were undertaken with the object of approaching the nature of the muscle relaxant action of ether and other volatile anaesthetics.

Investigations have been carried out upon the movements of sodium-24 and potassium-42 ions under the influence of ether, chloroform, halothane and methyl-n-propyl ether (neothyl). The isolated frog sartorius and rectus abdominis muscles and isolated strips of rat diaphragm have been used.

The effects of ether, tubocurarine and neostigmine on the movements of sodium-24 and potassium-42 from the rat phrenic nerve-diaphragm /

diaphragm preparation during muscular activity were also studied.

The effects of ether on the *in vivo* serum potassium level of the cat were also investigated and compared with those of tubocurarine, adrenaline and neostigmine. A simultaneous study of the effect of these drugs on the responses of the gastrocnemius muscle of the cat to indirect stimulation was also made.

The results are described in the following chapters.

C H A P T E R II.

CHAPTER II

A. MATERIALS

The drugs used in the investigation which is described in this section of the thesis, together with their shortened names, are as follows:

(1) Acetylcholine chloride, is described as acetylcholine.

(2) Adrenaline bitartrate, is described as adrenaline.

(3) Decamethonium iodide, is described as decamethonium.

(4) Neostigmine methyl sulphate, is described as neostigmine.

(5) (+)-Tubocurarine chloride, is described as tubocurarine.

(6) Atropine sulphate, is described as atropine.

(7) Diethyl ether (Anaesthetic Ether, B.P.), is described as ether.

(8) Methyl-n-propyl ether (Neothyl, Macfarlan) is described as Neothyl.

(9) Chloroform (Chloroform, B.P.), is described as chloroform.

(10) Halothane (Fluothane, I.C.I.), is described as halothane.

Radioactive materials were obtained from the Radiochemical Centre, Amersham, England.

Potassium-42 was obtained as a sterilised isotonic solution of 42-KCl and had an activity of approximately 0.10 to 0.12 mc. per ml. at the time of dispensing.

Sodium-24 was obtained as a sterilised isotonic solution of 24-NaCl and had an activity of approximately 1.00 mc. per ml. at the time of dispensing.

Solutions of volatile anaesthetics were prepared by shaking 10 ml. of the anaesthetic liquid with 90 ml. of the appropriate saline solution in a separating funnel of 500 ml. capacity for a period of 15 minutes at room temperature. After shaking, the solutions were left for a period of 15 minutes so as to form two liquid layers. The anaesthetic-saturated saline solution was then drained from the lower outlet of the separating funnel for use in the experiment. These solutions were prepared freshly prior to each experiment.

When ether, neothyl, chloroform or halothane solution is referred to, this indicates the use of a solution of the anaesthetic in the appropriate saline solution.

The composition and methods of preparation of the physiological saline solutions used in this investigation are to be found in the Appendix I, page 452. The statistical method employed to calculate the experimental observations is described in Appendix I, page 451.

B. EXPERIMENTAL.

To investigate the effects of the volatile anaesthetics on ion fluxes, the following preparations were used:-

1. Isolated frog sartorius muscle.
2. Isolated strips of rat diaphragm.
3. The rat phrenic nerve-diaphragm preparation.
4. The cat gastrocnemius muscle-sciatic nerve preparation.
5. Isolated frog rectus abdominis muscle.

The /

The investigations carried out using these preparations are described below.

1. Isolated frog sartorius muscle.

Investigation of the effect of ether, neothyl, chloroform and halothane upon (a) the uptake of potassium-42, (b) the efflux of potassium-42, and (c) the uptake of sodium-24 by frog sartorius muscle.

2. Isolated strips of rat diaphragm.

Investigation of the effect of ether, neothyl, chloroform and halothane upon (a) the uptake of potassium-42, (b) the efflux of potassium-42, and (c) the uptake of sodium-24 by isolated strips of rat diaphragm.

3. The rat phrenic nerve-diaphragm preparation.

Investigation of the effect of ether, and tubocurarine upon the magnitude of the muscle twitch produced by direct or indirect stimulation of the rat diaphragm, and upon potassium-42 efflux.

4. The cat gastrocnemius muscle-sciatic nerve preparation.

Investigation of the effect of ether upon muscular contractions induced by indirect stimulation *via* the sciatic nerve and also upon the level of potassium-42 in the blood serum.

5. Isolated frog rectus abdominis musole.

Investigation of the effect of ether, neothyl, chloroform and halothane upon acetylcholine-induced contractions of the isolated frog rectus abdominis muscle.

1 (a) /

1. Isolated frog sartorius muscle.

(a) Uptake of potassium-42.

Method. The method used was based on that described by Lister and Lewis (1959). Spring frogs (Rana temporaria) unselected as to age or sex and weighing from 25 to 50 g. were stunned by a sharp blow on the base of the head, decapitated and pithed. The skin covering both thighs and the pelvic region was reflected to expose the attachments of both ends of the two sartorius muscles. Each muscle was carefully dissected free, extreme care being taken to prevent damage to the muscle fibres. The distal tendons were sectioned and the muscles freed by cutting through the pelvic attachments. The muscles were blotted dry and each rapidly weighed on a torsion balance. With careful dissection, paired muscles could be obtained differing in weight from one another by less than 1 mg. By means of two stainless steel hooks the muscles were then suspended in two centrifuge tubes each containing 10 ml. of frog Ringer's solution at room temperature.

In this solution, part of the stable potassium chloride was replaced by the radio-active salt. The control muscle was suspended in 10 ml. of radio-active frog Ringer's solution which had previously been gassed for a period of 30 minutes with a mixture of 95 per cent oxygen and 5 per cent carbon dioxide. The other member of the pair was suspended in an identical solution which contained the drug. The muscles were exposed to drug or control solution for 30 minutes, after which they /

they were removed from the radio-active solution, each side of the muscle washed for 5 seconds by means of a stream of non-radioactive frog Ringer's solution and blotted dry to remove adhering moisture. The muscle was then placed in a test tube as shown in Fig. 111 (page 368) and counted in a thallium-activated, sodium iodide, scintillation crystal (Ecko type N.597) connected through a photomultiplier unit to an automatic scaler (Ecko type N.530D).

The muscles were usually exposed to the radio-active Ringer's solution for a period of from one and one half to two hours, and the radio-activity of both the test and control muscles was measured at 30 minute intervals. Corrections for decay were not made as in every experiment a control muscle was compared with the test under identical conditions. After correcting for background, the counts were expressed as counts per minute.

(b) Potassium-42 efflux.

Method. Spring frogs (Rana temporaria) unselected as to age or sex and weighing from 25 to 50 g. were used. For the estimation of potassium-42 efflux, 0.5 ml. of isotonic 42-KCl solution which at the time of dispensing contained 0.10 to 0.12 mc. per ml. was injected into the dorsal lymph sac. After a period of two hours, which was allowed for equilibration to take place, the frog was pithed and the paired sartorius or rectus abdominis muscles moved, washed for five seconds with a stream of non-radioactive Ringer's solution, blotted dry and weighed. Two series, each containing nine 10 ml. centrifuge tubes were /

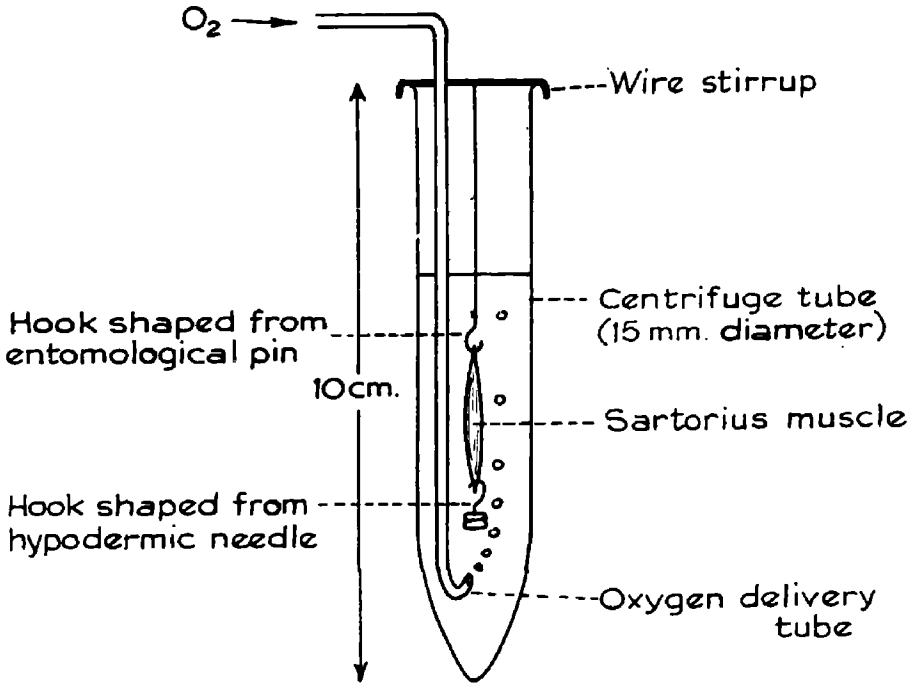

Fig. 111.

Diagram of the apparatus for suspending a muscle for the study of the uptake or release of radioactive ions.

were set up in parallel. Each tube contained 10 ml. of non-radioactive frog Ringer's solution at room temperature which had been previously well gassed with a mixture of 95 per cent oxygen and 5 per cent carbon dioxide; one series of tubes was used for the control muscle and the other for the test muscle. Each member of the pair of sartorius muscles was suspended from one end by means of a steel hook made from an entomological pin and stretched by a weight made out of a No. 20 hypodermic needle which was attached to the lower end (Fig. 111, page 368). The paired muscles were suspended, one in the first member of the control series of tubes and the other in the corresponding tube in the test series. After a period of 10 minutes in the first pair of tubes, the muscles were transferred to the second pair. The fluid which remained in the previous tube was counted, using a Geiger-Müller liquid counter (type M6) linked to an automatic scaler (Ecko type N.530D). In the test series, the drug was present in the fourth tube and in each case there was a ten minute period of contact between muscle and solution. After immersing it in the ninth tube of the series, the muscle was digested in 2 ml. of concentrated nitric acid, the volume adjusted with Ringer's solution to 10 ml. and the radioactivity counted in the manner described above.

(c) Uptake of sodium-24.

Method. The procedure adopted for the investigation of the effect of volatile anaesthetics upon the uptake of sodium-24 by isolated frog sartorius /

sartorius muscle was similar to that described on page 366. Muscles were suspended in centrifuge tubes containing 10 ml. of frog Ringer's solution in which part of the stable sodium chloride was replaced by 24-NaCl.

2. Isolated strips of rat diaphragm

(a) Uptake of potassium-42.

Method. Albino rats of either sex, weighing from 200 to 250 g., were stunned by a blow on the head. The throat was then cut and the animal allowed to bleed out. The diaphragm was dissected out and the tendinous parts cut away. Taking two strips from each side, four rectangular strips of about equal weight were prepared from each diaphragm. One member of each pair of strips was used as a control and the other for the test. The experiment was carried out in a manner similar to that described on page 366. Krebs'-Ringer's solution (Appendix I, page 452) was used as the bathing fluid.

(b) Potassium-42 efflux.

Method. Albino rats of either sex, weighing from 200 to 250 g., were injected intraperitoneally with 0.5 ml. of isotonic solution of 42-KCl. which at the time of dispensing contained 0.10 to 0.12 mc. per ml. After an equilibration period of 2 hours, the rats were killed and the diaphragm dissected out. The rest of the procedure was similar to that adopted for studying the release of potassium-42 from the isolated /

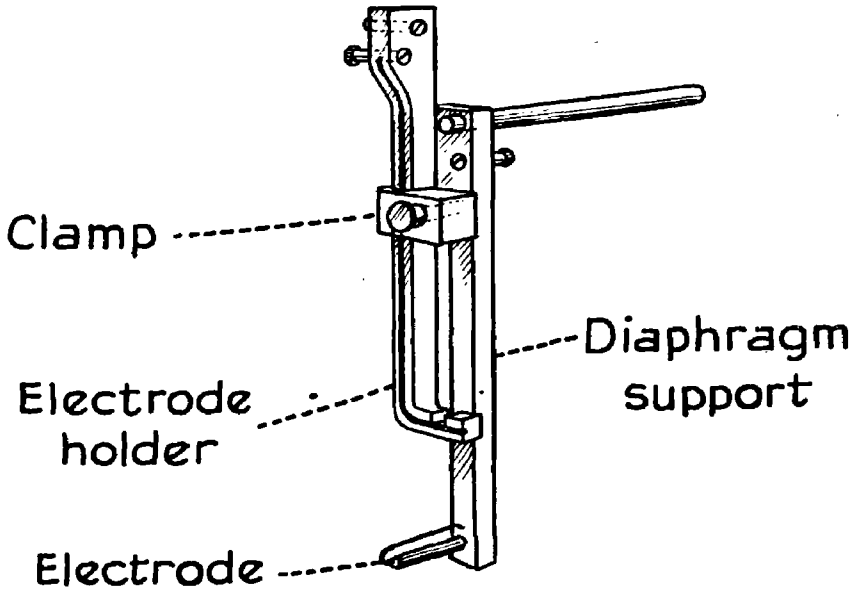

Fig. 112.

Diagram of Bell's electrode [Bell, G.H., (1952) Experimental physiology, 5th Edition, page 397 for direct or indirect stimulation of the rat phrenic nerve diaphragm preparation.

isolated sartorius muscle of the frog and described on page 367.

(c) Uptake of sodium-24.

Method. The procedure adopted for investigation of the effect of volatile anaesthetics upon the uptake of sodium-24 by isolated strips of rat diaphragm was similar to that described on page 366. Muscle strips were suspended in centrifuge tubes containing 10 ml. of Krebs'-Ringer's solution in which part of the stable sodium chloride was replaced by 24-NaCl.

3. The isolated rat phrenic nerve-diaphragm preparation

Method. The method adopted to record the contractions of the isolated rat phrenic nerve-diaphragm preparation was based on that of Bülbring (1946). The electrode described by Bell (1952) (Fig. 112, page 371) was used so that the muscle could be stimulated both directly and indirectly.

Adult rats of either sex were used. 0.25 ml. of 42-KCl was injected intraperitoneally and the animal left for a period of two hours for equilibration to take place. The rat was then killed by a blow on the head, the throat cut, and the blood allowed to drain out. The animal was now placed on its back and pinned upon a dissecting board. The skin over the thoracic wall was removed on both sides, the abdomen opened and a small incision made through the diaphragm from the abdominal aspect, at the point of its attachment to the ribs and close to one side of the sternum. One blade of a pair of scissors /

scissors was inserted through the incision and the ribs divided close to the sternum. This procedure was repeated on the other side of the sternum. The sternum was then dissected from the mediastinal tissue and removed. Starting between the fifth and sixth ribs, an incision was made across the ribs on the right side parallel to the diaphragmatic attachment. All the ribs above this incision were removed. A second transverse incision was made along a curved line just below the attachment of the diaphragm to the thoracic wall. The right phrenic nerve was identified as it entered the diaphragm. It was moved gently to the medial side and the lobes of the right lung cut away near the hilum and removed. The thoracic cage was washed with Tyrode's solution containing double the usual amount of glucose and the right phrenic nerve carefully dissected free from the mediastinal tissues. When the nerve had been freed as high in the neck as possible, a ligature was tied around it and the nerve severed above the ligature. A length of about 4 to 5 cm. of nerve was usually obtained. Two ligatures were now inserted through the costal segment which was attached to the diaphragm and lengths of threads were left with the ligatures. The ligatures were from 2 cm. to 2.5 cm. apart, with the point of entry of the phrenic nerve lying midway between them. They were pulled gently to stretch the diaphragm and to observe the direction in which the muscle fibres ran. Two cuts were made through the ribs and diaphragm, parallel to the muscle fibres and commencing just /

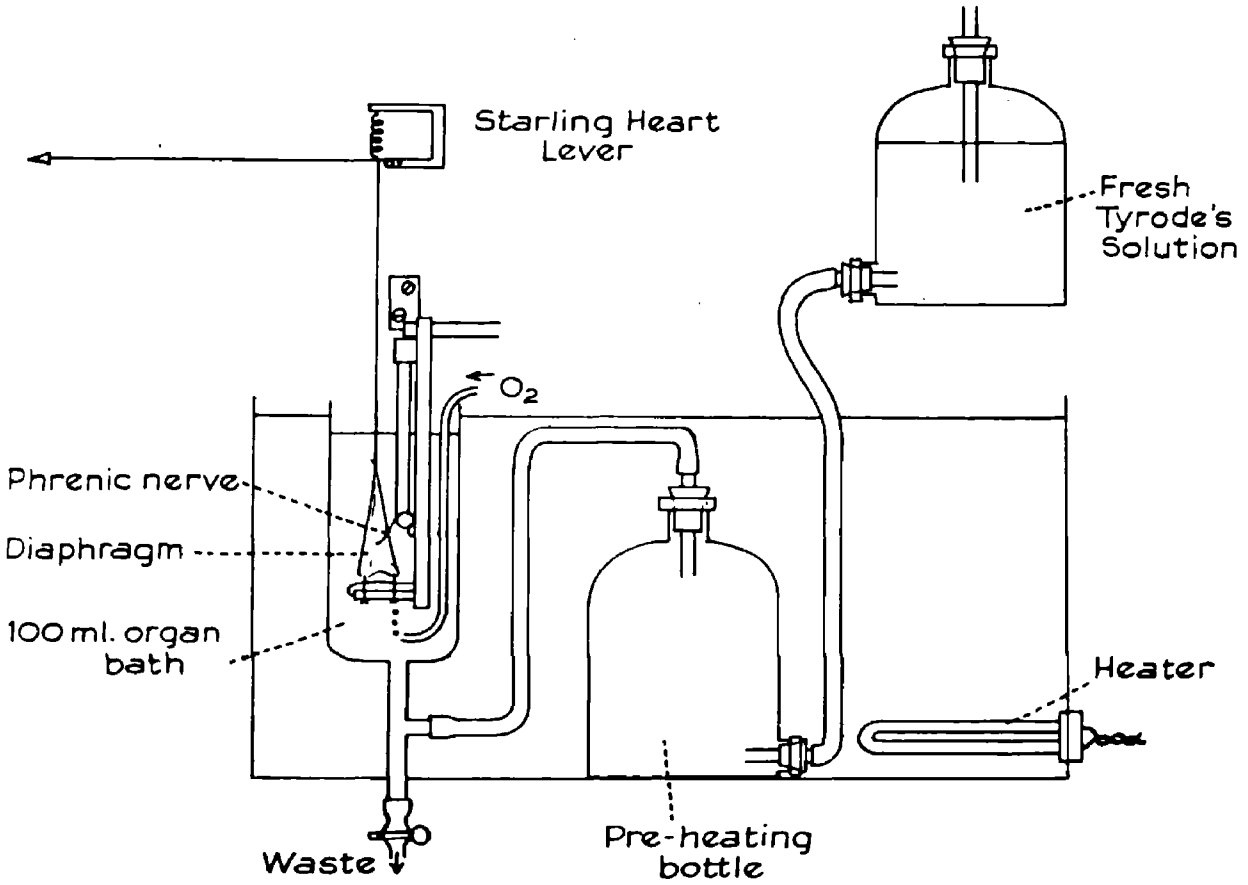

Fig. 113.

Diagram of the apparatus used for recording contractions of the rat diaphragm produced either by direct or indirect stimulation of the muscle using Bell's electrode.

just outside the costal ligatures, so as to converge on the central tendinous part of the diaphragm. The muscle-nerve preparation was then taken out of the animal and placed in a petri dish filled with Tyrode's solution. A similar preparation was also obtained from the left side of the animal. The right side preparation is, however, said to give better contractions, although the nerve is more difficult to dissect out than that on the left side where it is not so closely bound to the mediastinum.

The muscle-nerve preparation was then fixed on to the electrode assembly. The two ligatures which had been passed through the costal margin were tied firmly to the horizontal arm of the diaphragm support and a platinum wire tied to the tendinous part of the diaphragm. The other end of the wire was fixed to a light isotonic heart lever which recorded the contractions on a moving smoked paper surface. The phrenic nerve was passed into the groove described previously so that it lay across the two wires of the electrode. To keep the nerve firmly in place, the end of the phrenic nerve was fixed between the electrode holder and the diaphragm support. The whole assembly was immersed in a 100 ml. organ bath (Fig. 113, page 374) filled with Tyrode's solution containing double the usual amount of glucose. The solution in the organ bath was gassed with 95 per cent oxygen and 5 per cent carbon dioxide through a gas distributing tube fitted to the bottom of the organ bath. Due to the high volatility of the anaesthetic agents used, /

used, the experiments were carried out at room temperature. Anaesthetic agents, dissolved in Tyrode's solution, were added directly to the bath by means of a graduated pipette.

The preparation was stimulated from two Dobbie McInnes stimulators - one for indirect stimulation of the diaphragm via the phrenic nerve and the other for direct stimulation of the muscle. For indirect stimulation, the frequency was 6 to 8 stimuli per minute, the voltage 8 to 10, pulse width 0.5 to 1 m.sec. For direct stimulation, the frequency was 6 to 8, voltage 25 to 50 and pulse width 2 m.sec. In any one experiment, the frequency, voltage and pulse width both for direct and indirect stimulation were kept constant.

The effects of the solution of ether (2 to 10 ml.) upon the twitch height of the rat diaphragm preparation in response to both direct and indirect stimulation were observed. The effects of the solution of ether (2 to 10 ml.) and of tubocurarine (1 to 3 µg. per ml.) on the potassium-42 efflux from the rat diaphragm were also studied.

To estimate the levels of potassium-42 in the bath fluid before and after the addition of the solutions of ether and tubocurarine, two 2 ml. samples of fluid were taken at 10-minute intervals from the bath by means of a graduated pipette. The sample of bathing solution was then adjusted to 10 ml. with non-radioactive Tyrode's solution and the radioactivity of the sample measured by means of a Geiger Müller liquid /

liquid counter (type M.6) linked to an automatic scaler (Ekco type N 530D).

4. Experiments on the gastrocnemius muscle-sciatic nerve preparation of the anaesthetised cat.

Method. Cats of either sex, weighing from 1.75 to 2 kg. were used. Each animal was given an intraperitoneal injection of 4 ml. of 42-KCl solution which at the time of dispensing contained from 0.10 to 0.12 mc. per ml. A period of four hours was allowed for equilibration. An intraperitoneal injection of 1 mg. per kg. of atropine was given to the cat so as to reduce the bronchial secretions due to the irritant action of ether vapour. The cats were anaesthetised by means of an intraperitoneal injection of sodium pentobarbitone. A dose of 60 mg. per kg. was usually adequate for the induction of surgical anaesthesia.

The external jugular vein and the trachea of the anaesthetised cat were cannulated in a manner similar to that described on page 27 of this thesis. The left leg was now prepared for indirect stimulation of the gastrocnemius muscle via the sciatic nerve. The gastrocnemius muscle was partially dissected 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 then held with its long axis perpendicular to the operating table and fixed rigidly in position by means of two clamps - one /

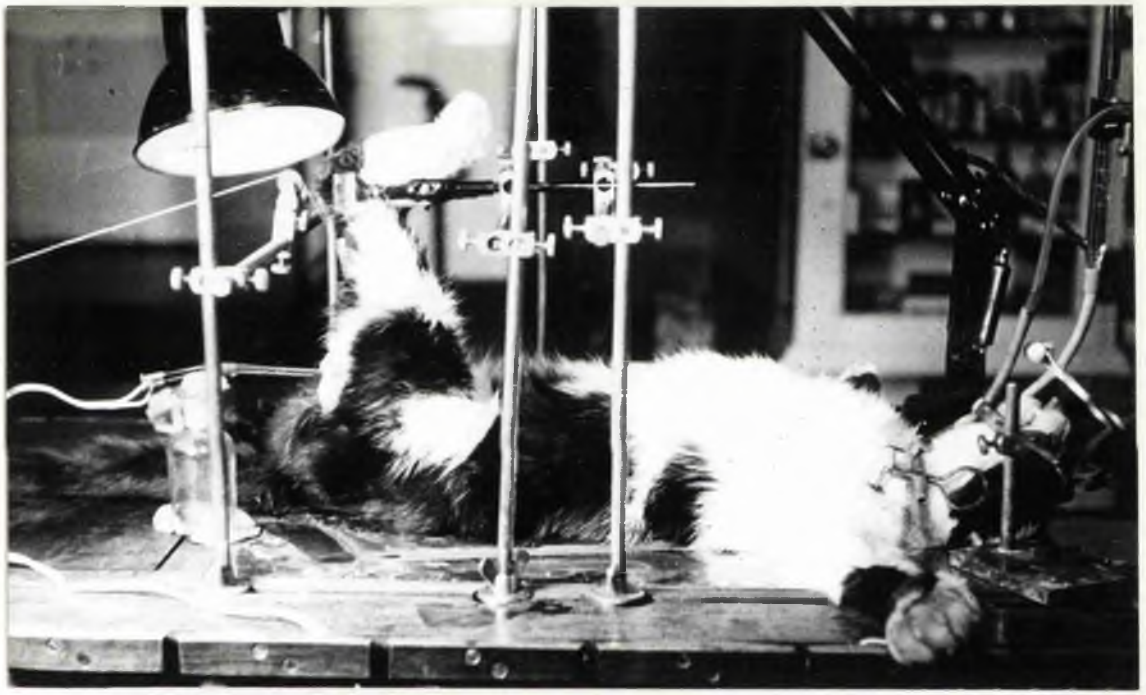

Fig. 114

Diagram of the cat gastrocnemius muscle-sciatic nerve preparation of the anaesthetized cat

one at the knee joint and the other at the ankle (Fig.114, page 378). 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 placed around the nerve distal to this point. When this dissection was completed, the iliac vein of the contralateral leg was cannulated through the femoral vein using a fine polythene cannula. This was connected to a 1 ml. graduated tuberculin syringe which was used for the collection of samples of venous blood. The cat was given an intravenous injection of 1,000 units of heparin through one of the external jugular veins. Two 1 ml. samples of blood were collected at 5 minute intervals into heparinised centrifuge tubes, shaken gently and then centrifuged at 1,500 revolutions per minute for a period of 10 minutes. The cat was allowed to inhale ether vapour through an ether bottle for a period of from 5 to 10 minutes. Duplicate blood samples were again collected at 5 minute intervals. Collection was continued in this way following the end of the period of ether inhalation until the potassium level in the serum returned to control levels or to values near these.

0.25 ml. of the plasma was adjusted to 10 ml. with normal saline and /

and the radioactivity measured using a Geiger Müller liquid counter (type M.6) linked to an automatic scaler (Ekco, type N 530D).

The effects of adrenaline (50 to 100 µg. per kg.), neostigmine (0.25 mg. per kg.) and tubocurarine (100 to 200 µg. per kg.) on ether-induced neuromuscular block and blood serum potassium-42 levels were studied.

The sciatic nerve was stimulated by means of a Dobbie McInnes square wave stimulator, using single shocks at a frequency of 4 to 8 per minute, at 10 to 20 volts, the pulse width being 2.0 to 3.0 m.sec. 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. The tension placed upon the muscle varied between 0.2 and 0.3 kg. In any one experiment the tension was kept constant. The tension produced by the contraction of the muscle in response to electrical stimulation varied from experiment to experiment but usually remained constant between 0.6 and 0.8 kg. The drugs administered were in the form of their solutions in normal saline. Injections were given into the rubber connection between the jugular vein cannula and the burette, and the drug solutions were then washed in by means of 2 ml. of saline.

5. Isolated frog rectus abdominis muscle.

Method. The method adopted was similar to that described previously

(page /

(page 47).

Contractions of the isolated rectus abdominis muscle of the frog in response to acetylcholine (0.1 to 0.5 µg. per ml.) were recorded in a conventional manner. Solutions of ether (2 to 10 ml.) in frog Ringer's solution were then added directly to the bath. The ether solution was allowed to remain in the bath for a period of from 2 to 5 minutes and after washing out, acetylcholine (0.1 to 0.5 µg. per ml.) added and the response recorded. This dose of acetylcholine was repeated at intervals of 3 minutes until the response returned to the control level. The effect of ether on the tubocurarine-induced (2 to 10 µg. per ml.) reduction of the response to acetylcholine was also investigated. A simultaneous estimation of potassium-42 levels in the bath solution was also made. The Ringer's solution in the bath was counted at the end of each reading and the effect of the stimulant drugs upon ion fluxes noted.

C H A P T E R III

CHAPTER III.

RESULTS.

The effects of volatile anaesthetics upon (a) the uptake of potassium-42, (b) the efflux of potassium-42 and (c) the uptake of sodium-24 by the isolated frog sartorius muscle and by isolated strips of rat diaphragm.

(a) Influence of ether upon the uptake of potassium-42 by the isolated frog sartorius muscle and by isolated strips of rat diaphragm.

Solution of ether (2 to 10 ml.) produced a significant decrease ($P < 0.001$) in the uptake of potassium-42 by the isolated frog sartorius muscle (Fig. 115b, page 383 and Table 15, page 412) and by isolated strips of rat diaphragm (Fig. 116b, page 384 and Table 15, page 412). The control muscle showed an increase in the uptake of potassium-42 when compared with the drug-treated muscle.

Neothyl

Solutions of neothyl (2 to 10 ml.) produced a significant decrease ($P < 0.001$) in the uptake of potassium-42 by the isolated frog sartorius muscle (Fig. 117a, page 385 and Table 19, page 416) and by isolated strips of rat diaphragm.

Halothane

Solution of halothane (2 to 10 ml.) produced a significant decrease ($P < 0.001$) /

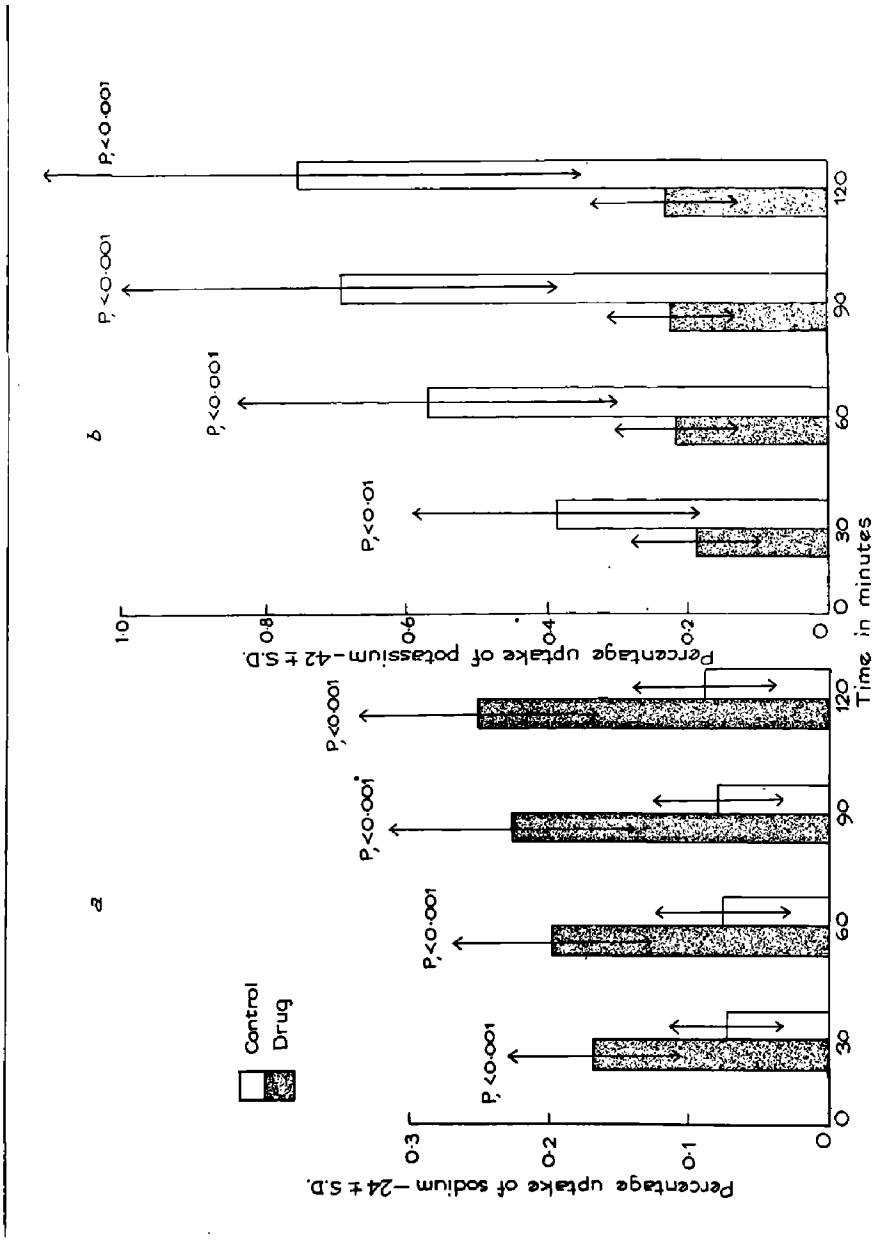

Fig. 115.

Uptake of sodium-24 (a) and potassium-42 (b) by isolated frog sartorius muscle in the presence of 10 ml. of ether solution.

The vertical bars signify the standard deviation of the mean.

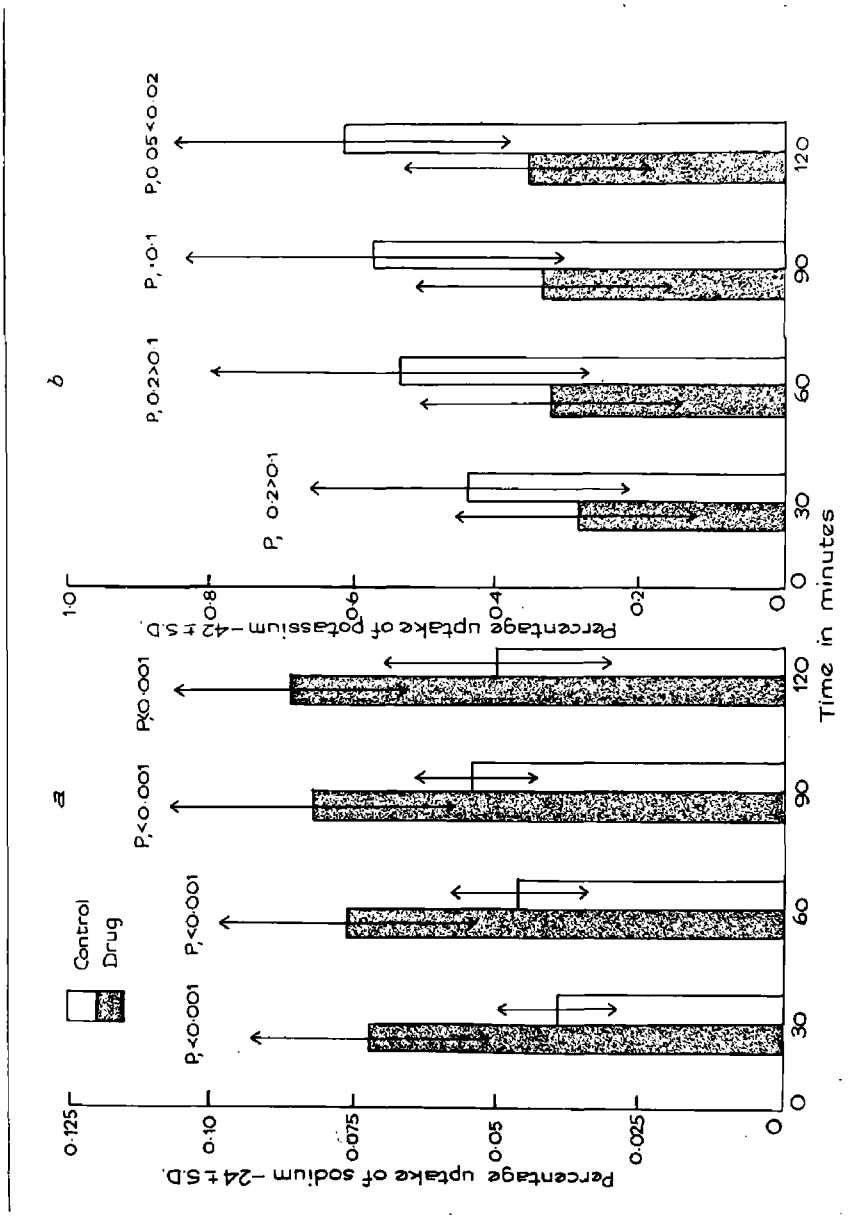

Fig. 116.

Uptake of sodium-24 (a) and potassium-42 (b) by the isolated strips of rat diaphragm in the presence of 5 ml. of ether solution.

The vertical bars signify the standard deviation of the mean.

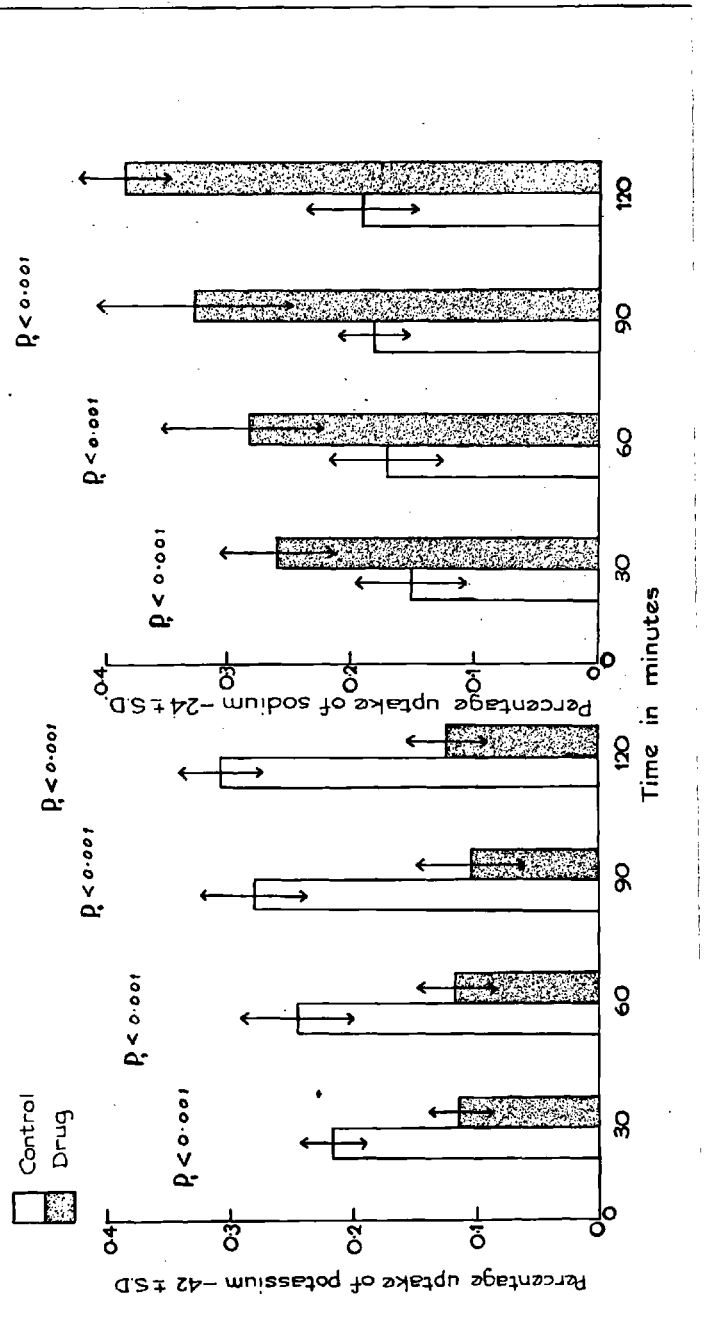

Fig. 117.

Uptake of potassium-42 (a) and sodium-24 (b) by isolated frog sartorius muscle in the presence of 10 ml. of neothyl solution.
The vertical bars signify the standard deviation of the mean.

($P < 0.001$) in the uptake of potassium-42 by the isolated frog sartorius muscle (Fig. 118, page 387 and Table 17, page 414) and by isolated strips of rat diaphragm.

Chloroform

Solution of chloroform (2 to 10 ml.) produced a significant decrease ($P < 0.001$) in the uptake of potassium-42 by the isolated frog sartorius muscle (Fig. 119a, page 388 and Table 19, page 416) and by isolated strips of rat diaphragm.

(b) Influence of ether upon the efflux of potassium-42 from isolated frog sartorius muscle and strips of rat diaphragm.

Solution of ether (5 to 10 ml.) produced a marked increase in the release of potassium-42 from the isolated frog sartorius muscle (Fig. 120, page 389 and Table 20, page 417) and from isolated strips of rat diaphragm (Table 21, page 418).

Neothyl

Solution of neothyl (2 to 5 ml.) produced a marked increase in the release of potassium-42 from the isolated frog sartorius musole (Fig. 121, page 390) and from isolated strips of rat diaphragm.

Halothane

Solution of halothane (2 to 5 ml.) produced a marked increase in the release of potassium-42 from the isolated frog sartorius musole (Fig. /

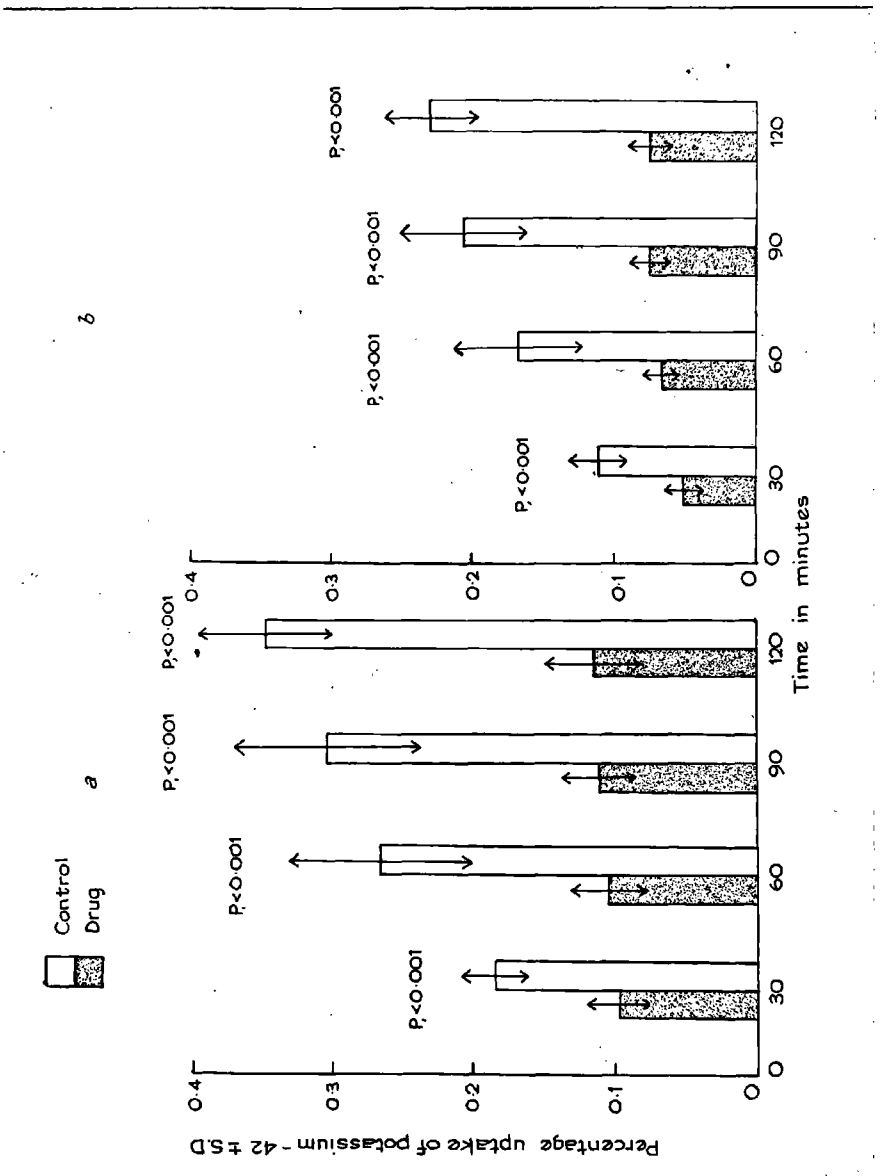

Fig. 118.

Uptake of potassium-42 by the isolated frog sartorius muscle in the presence of 5 ml. of halothane solution (at a) and 10 ml. of halothane solution (at b). The vertical bars signify the standard deviation of the mean.

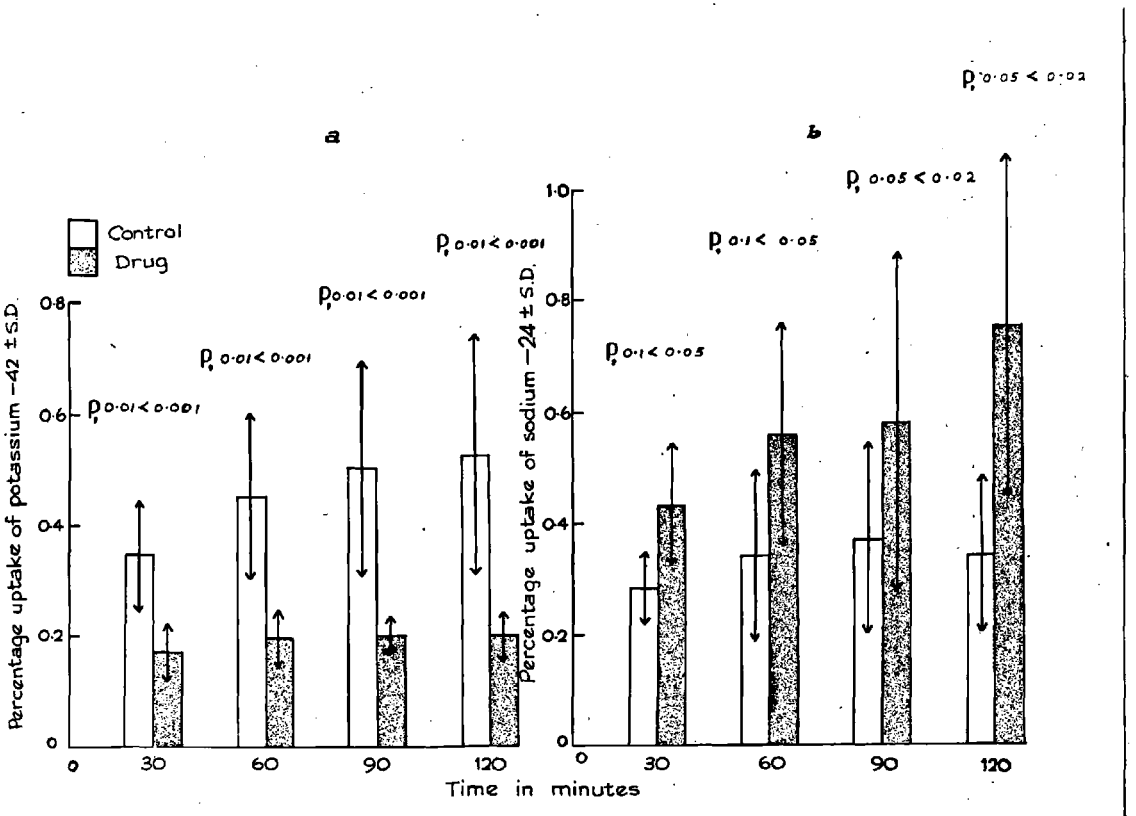

Fig. 119.

Uptake of potassium-42 (a) and sodium-24 (b) by isolated frog sartorius muscle in the presence of 5 ml. of chloroform solution.

The vertical bars signify the standard deviation of the mean.

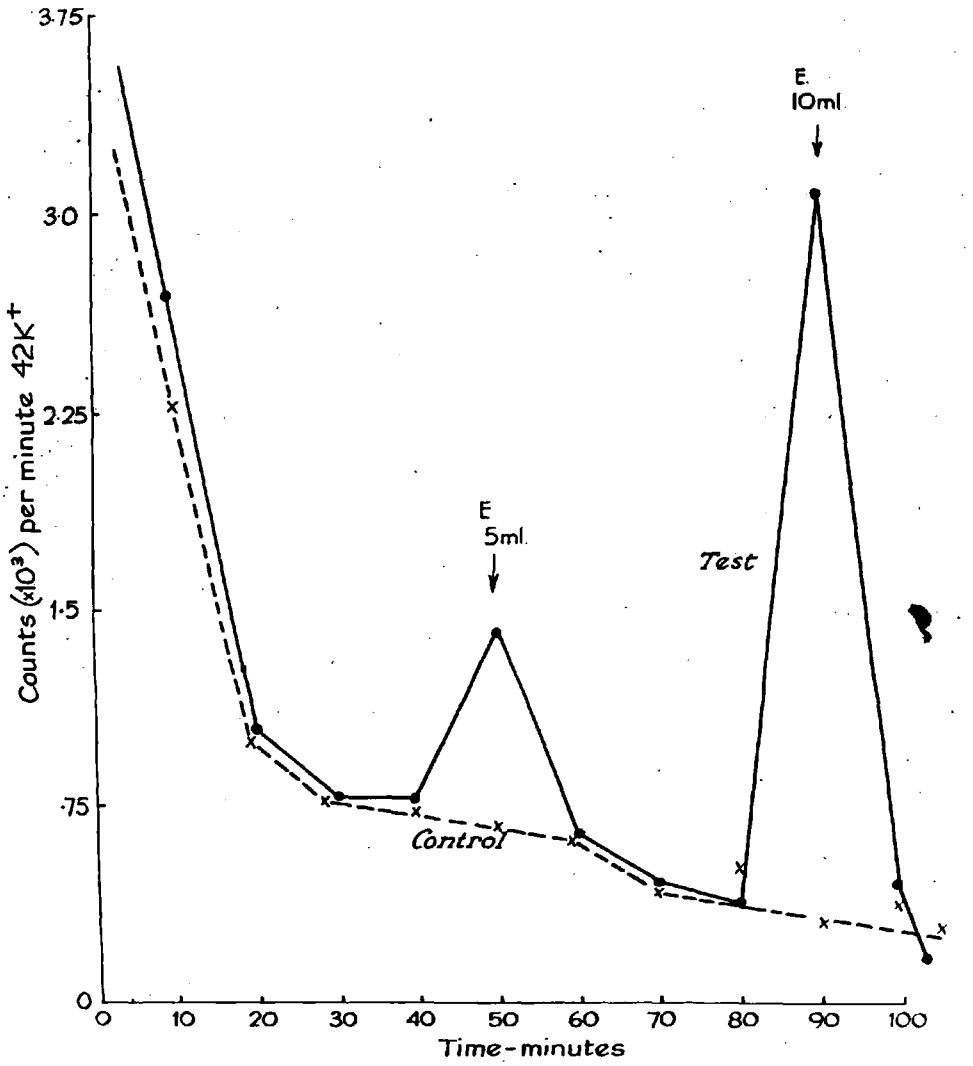

Fig. 120.

Effect of 5 ml. and of 10 ml. of ether solution on the release of potassium-42 from the isolated frog sartorius muscle. The vertical arrows indicate the point of exposure of the test muscle to the drug-containing solution.

The broken line indicates control muscle and the unbroken line the test muscle.

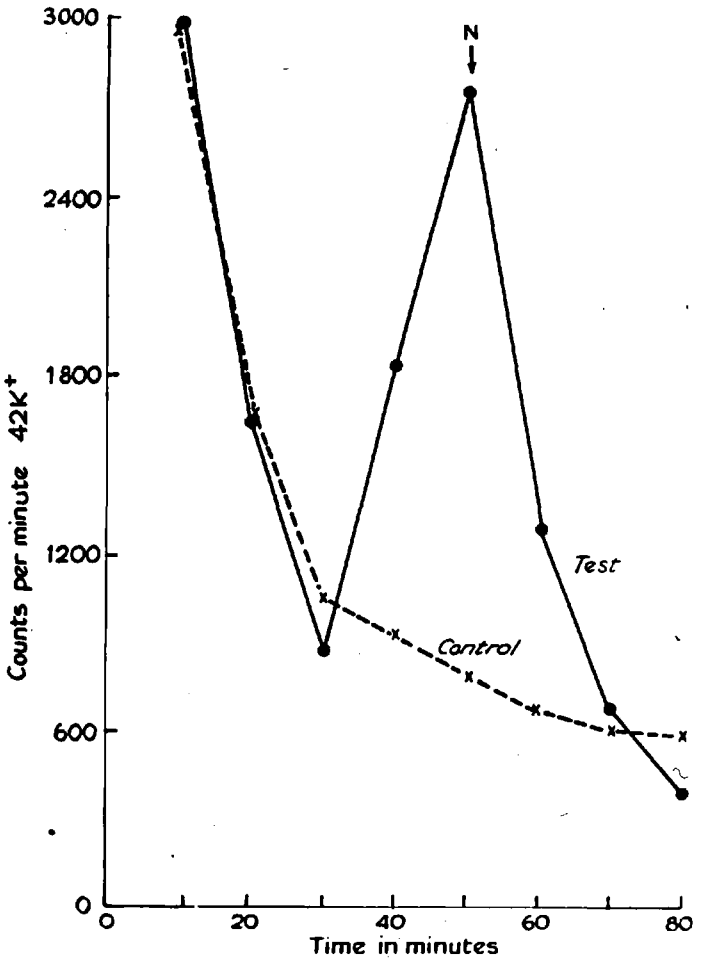

Fig. 121

Effect of 5 ml. of neothyl solution upon the release of potassium-42 from the isolated frog sartorius muscle. The vertical arrow indicates the point of exposure of the test muscle to the drug-containing solution.

The broken line indicates the control muscle and the unbroken line the test muscle.

(Fig. 122, page 392 and Table 22, page 419) and from isolated strips of rat diaphragm.

Chloroform

Solution of chloroform (2 to 10 ml.) produced a marked increase in the release of potassium-42 from the isolated frog sartorius muscle (Fig. 123, page 394) and from isolated strips of rat diaphragm.

(c) Influence of ether upon the uptake of sodium-24 by isolated frog sartorius muscle and by strips of rat diaphragm.

Solutions of ether (2 to 10 ml.) produced a significant increase ($P < 0.001$) in the uptake of sodium-24 by the isolated frog sartorius muscle (Fig. 115a, page 383 and Table 16, page 413) and by isolated strips of rat diaphragm (Fig. 116a, page 384 and Table 16, page 413). The control muscle did not show an increase in the uptake of sodium-24 when compared with the drug-treated muscle.

Neothyl

Solution of neothyl (2 to 10 ml.) produced a significant increase ($P < 0.001$) in the uptake of sodium-24 by the isolated frog sartorius muscle (Fig. 117b, page 385 and Table 18, page 415) and by isolated strips of rat diaphragm.

Halothane

Solutions of halothane (2 to 10 ml.) produced a significant increase ($P < 0.001$) in the uptake of sodium-24 by the isolated frog sartorius muscle (Table 23, page 420) and by isolated strips of rat diaphragm. /

diaphragm.

Chloroform

Solutions of chloroform (2 to 10 ml.) produced a significant increase ($P = 0.05 > 0.02$) in the uptake of sodium-24 by the isolated frog sartorius muscle (Fig. 119b, page 388 and Table 18, page 415) and by isolated strips of rat diaphragm.

The rat phrenic nerve-diaphragm preparation

The effect of ether and tubocurarine upon the magnitude of the muscle twitch produced by the direct and indirect stimulation of the rat diaphragm and upon potassium-42 efflux.

Solution of ether (5 to 10 ml.) when added to the bath, reduced the magnitude of the response to indirect stimulation via the phrenic nerve (Fig. 124, page 395). In the same concentration, ether increased the tubocurarine-induced (1 to 2 µg. per ml.) inhibition of the response to indirect stimulation (Fig. 124, page 395). A marked increase in the efflux of potassium-42 was also noted during the presence of ether solution in the bath. On the other hand, tubocurarine, although it caused a reduction in the magnitude of the contractions which was similar to that caused by ether, produced no demonstrable effect on the efflux of potassium-42 (Fig. 125, page 396).

Ether solution (10 to 30 ml.) produced complete inhibition of the muscular /

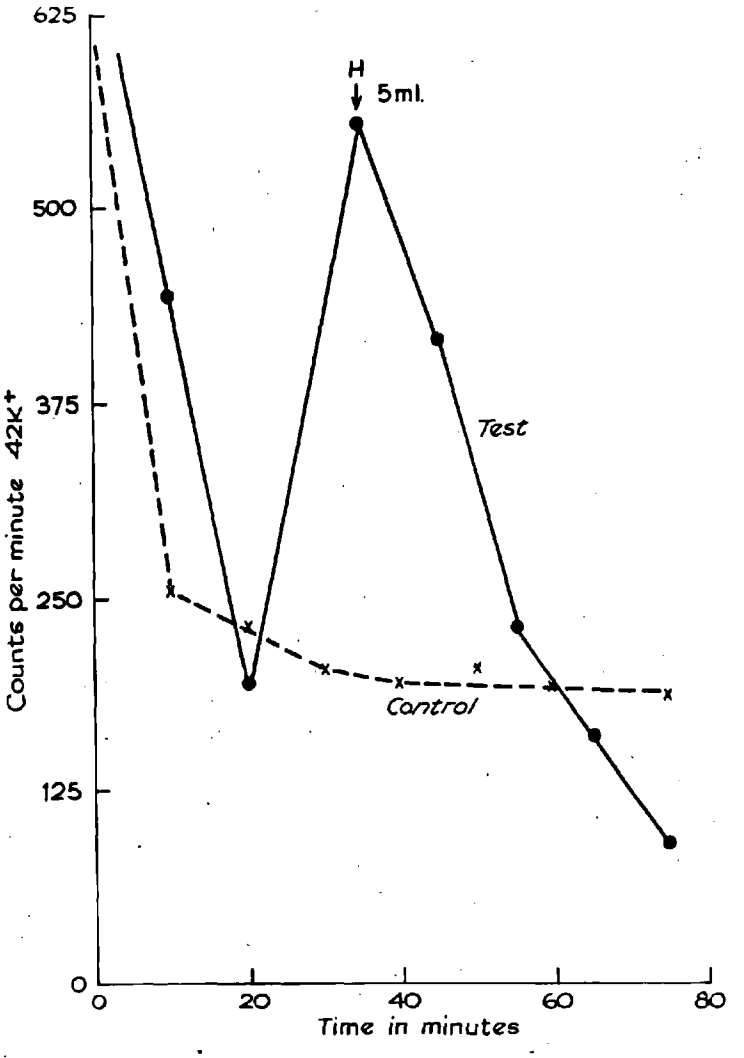

Fig. 122.

Effect of 5 ml. of halothane solution on the release of potassium-42 from the isolated frog sartorius muscle. The vertical arrow indicates the point of exposure of the test muscle to the drug-containing solution.

The broken line indicates the control muscle and the unbroken line the test muscle.

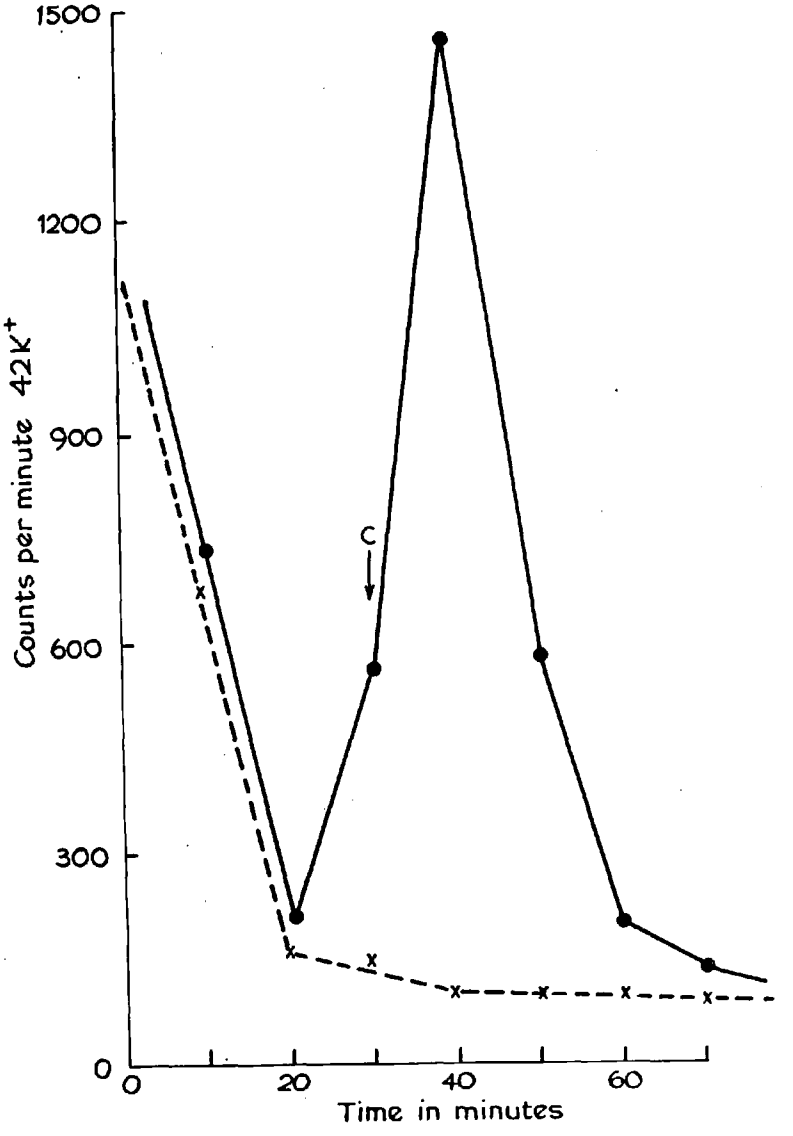

Fig. 123.

Effect of 5 ml. of chloroform solution on the release of potassium-42 from the isolated frog sartorius muscle. The vertical arrow indicates the point of exposure of the test muscle to the drug-containing solution.

The broken line indicates the control muscle and the unbroken line the test muscle.

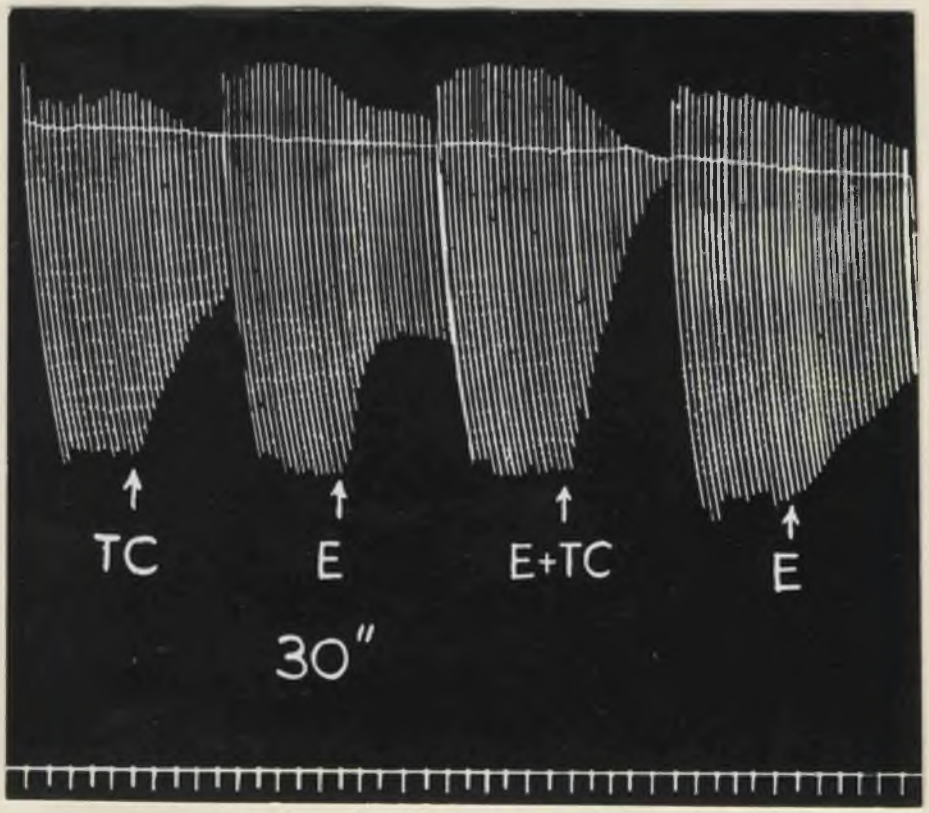

Fig. 124.

The isolated rat phrenic nerve diaphragm preparation.

Indirect stimulation via the phrenic nerve.

Contractions downwards.

At TC, 1 µg. per ml. of tubocurarine.
At E, 5 ml. of ether solution.
At E + TC, 5 ml. of ether solution and 1 µg. per ml. of tubocurarine.

Time interval (lower trace) = 30 seconds.

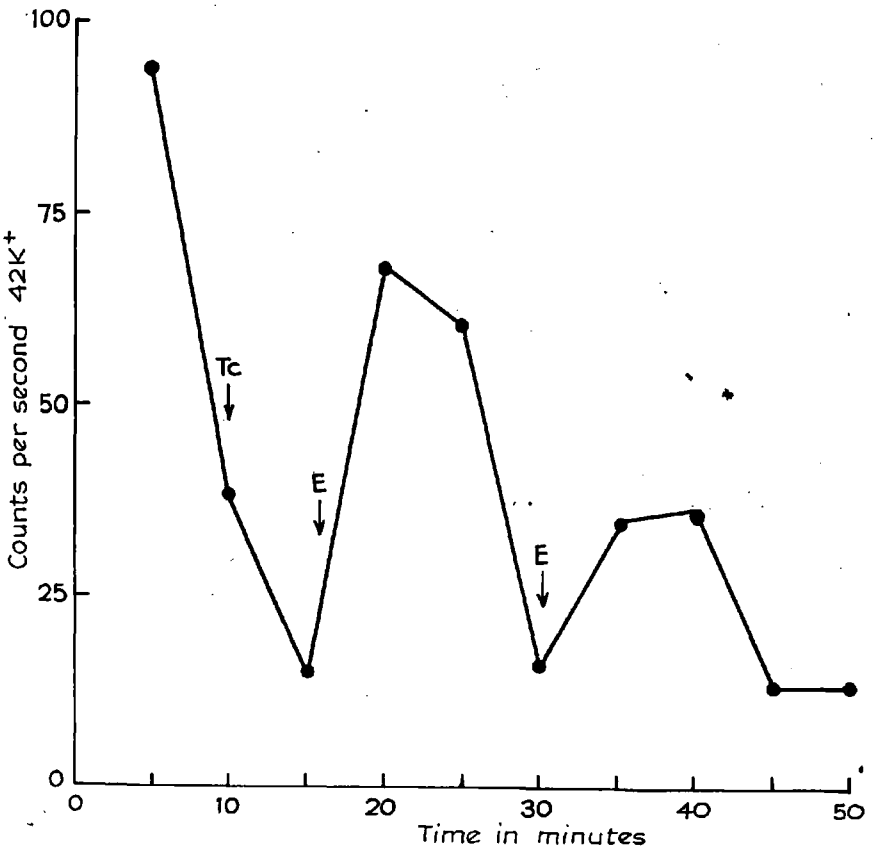

Fig. 125.

Effects of (2 µg. per ml.) tuboourarine (at Tc) and 5 ml. of ether solution (at E) in 50 ml. Tyrode's solution upon the efflux of potassium-42 from the rat phrenic nerve-diaphragm preparation. Vertical arrows indicate the point of exposure of the preparation to the drug-containing solution.

muscular contractions induced by indirect stimulation, whereas the responses of the diaphragm to direct stimulation were not affected (Fig. 126, page 398).

Decamethonium (10 to 20 µg. per ml.) produced a decrease in the magnitude of the contractions of the diaphragm to indirect stimulation and at the same dose level it potentiated the effect of ether upon the response of the muscle to indirect stimulation (Fig. 127a, page 399).

The depressant effects of ether solution (2 to 10 ml.) on the muscular contractions were also antagonized by the addition of neostigmine (2 to 10 µg. per ml.) (Fig. 127b, page 399).

The cat gastrocnemius muscle-sciatic nerve preparation

Effect of ether upon the muscular contractions induced by indirect stimulation *via* the sciatic nerve and also upon the level of potassium-42 in the blood serum of the cat.

Inhalation of ether for a period of 5 to 10 minutes by the pentobarbitone-anaesthetized cat, produced an inhibition of the contractions due to indirect stimulation (Fig. 128, page 400). A simultaneous increase in the level of potassium-42 in the blood serum was also observed. Tubocurarine (0.2 to 0.5 mg. per kg.) produced a similar decrease of the twitch height but did not alter the serum potassium-42 level (Fig. 129, page 401).

Adrenaline /

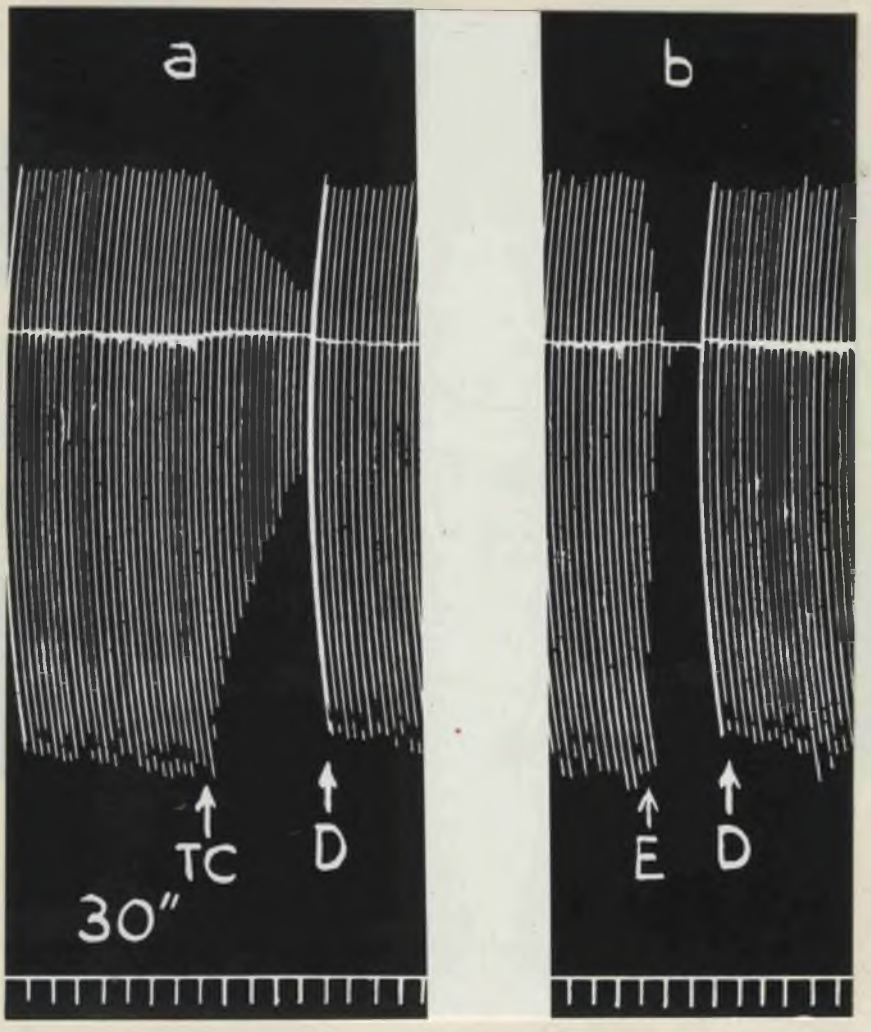

Fig. 126.

The isolated rat phrenic nerve diaphragm preparation. Contractions downwards.

Tracing a. Effect of 2 µg. per ml. tubocurarine upon the responses of the rat diaphragm to indirect stimulation (unlabelled contractions) and to direct stimulation at D.

Tracing b. Effect of 30 ml. of ether solution upon responses of the rat diaphragm to indirect stimulation (unlabelled contractions) and to direct stimulation at D.

Time interval (lower trace) = 30 seconds.

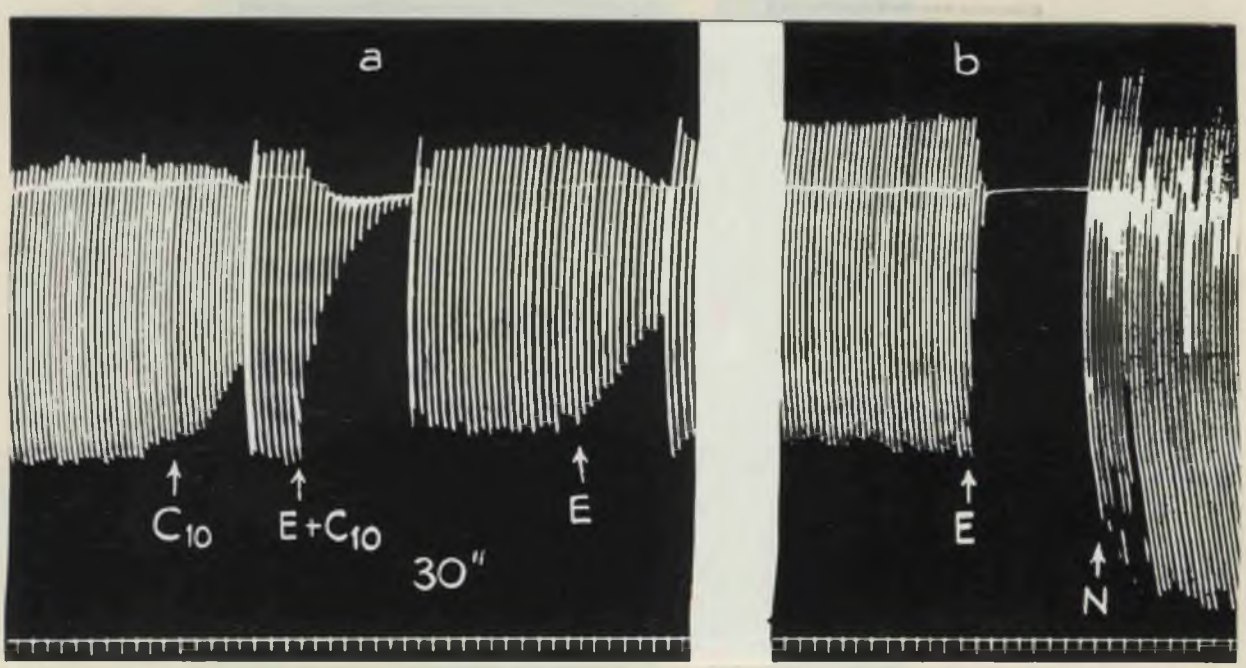

Fig. 127.

The isolated rat phrenic nerve-diaphragm preparation. Indirect stimulation via the phrenic nerve. Contractions downwards.

Tracing a. At C_{10}, 10 µg. per ml. of decamethonium.
At E + C_{10}, 5 ml. of ether solution and 10 µg. of decamethonium.
At E, 5 ml. of ether solution.

Tracing b. At E, 30 ml. of ether solution.
At N, 10 µg. per ml. neostigmine.

Time interval (lower trace) = 30 seconds.

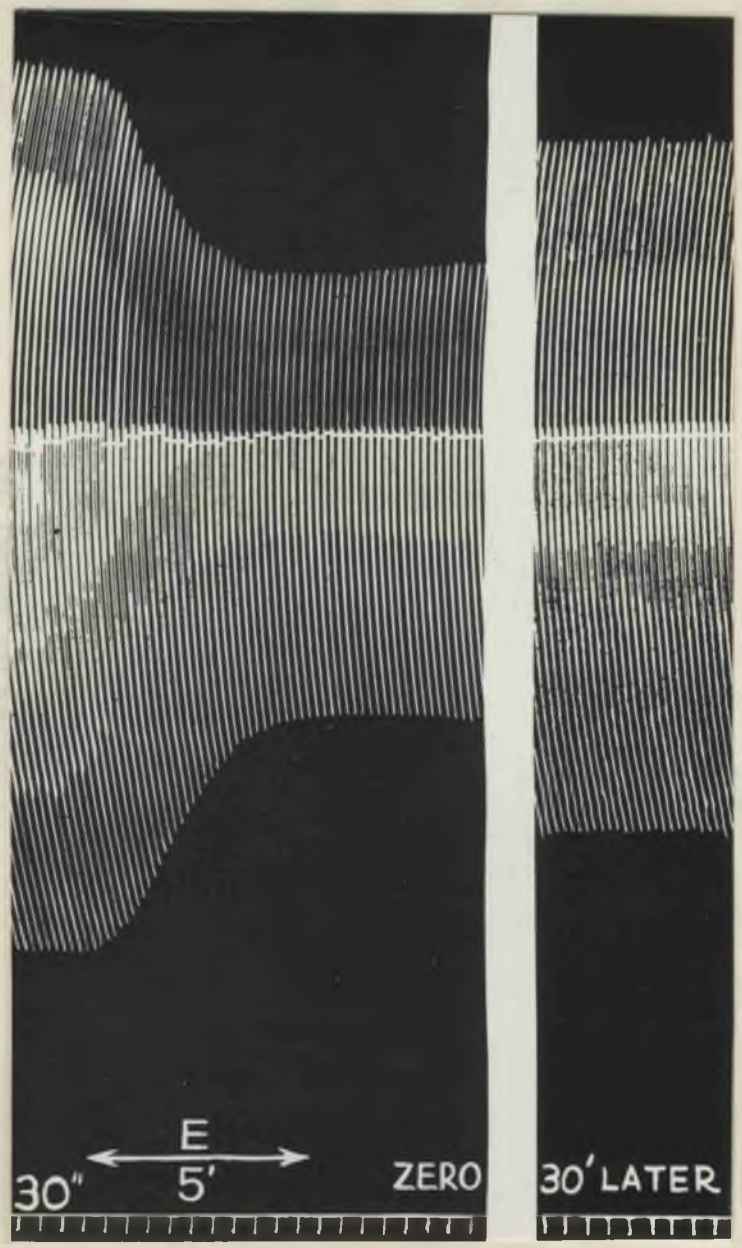

Fig. 128.

Cat gastroonemius muscle-sciatic nerve preparation.
Pentobarbitone anaesthesia (60 mg. per kg.).
Indirect stimulation via the sciatic nerve.
Contractions downwards.

At E, the cat was allowed to inhale ether for 5 minutes. Contractions recovered to about 70 per cent of control levels after 30 minutes.

Time interval (lower trace) = 30 seconds.

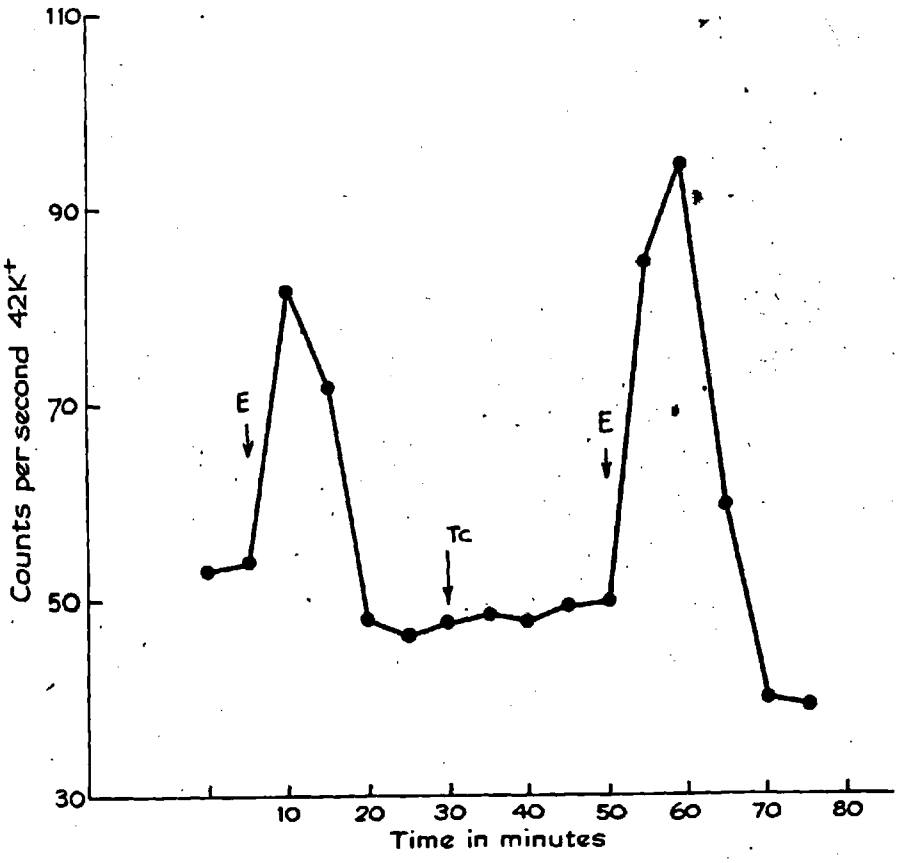

Fig. 129.

Effect of ether (E) and tuboourarine (Tc) upon the potassium-42 levels of the blood serum of the cat.

All drugs given intravenously.

At E, the cat was allowed to inhale ether for 5 minutes.

At To, 100 µg. per kg. of tubocurarine was given intravenously.

Vertical arrows indicate the points where ether and tubocurarine were given to the cat.

Adrenaline (20 to 50 µg. per kg.) when administered intravenously, antagonized the inhibition of the muscular contractions following inhalation of ether for from 5 to 10 minutes or the intravenous administration of tubocurarine (0.2 to 0.5 mg. per kg.) (Fig. 130, page 403). Administration of adrenaline (20 to 50 µg. per kg.) was always accompanied by a marked increase in the serum potassium-42 level as compared with the control. This effect is shown in Fig. 131, page 404.

Neostigmine (0.25 mg. per kg.) antagonized the depressant effects of ether upon the muscular contractions. This effect was also accompanied by a marked increase in the serum potassium-42 level of the cat (Fig. 132, page 405 and Fig. 133, page 406).

Isolated frog rectus abdominis muscle.

(a) The direct effect of ether, neothyl, chloroform and halothane upon the isolated frog rectus abdominis muscle.

(b) The effect of ether, neothyl, chloroform and halothane upon the acetylcholine-induced contractions of the muscle.

Solution of ether (2 to 10 ml.) produced a contractural response of the isolated frog rectus abdominis muscle which was accompanied by a marked increase in the release of potassium-42. The response of the muscle to ether solution was always followed by an inhibition of the responses to a subsequent dose of acetylcholine (0.1 to 1 µg. per ml. /

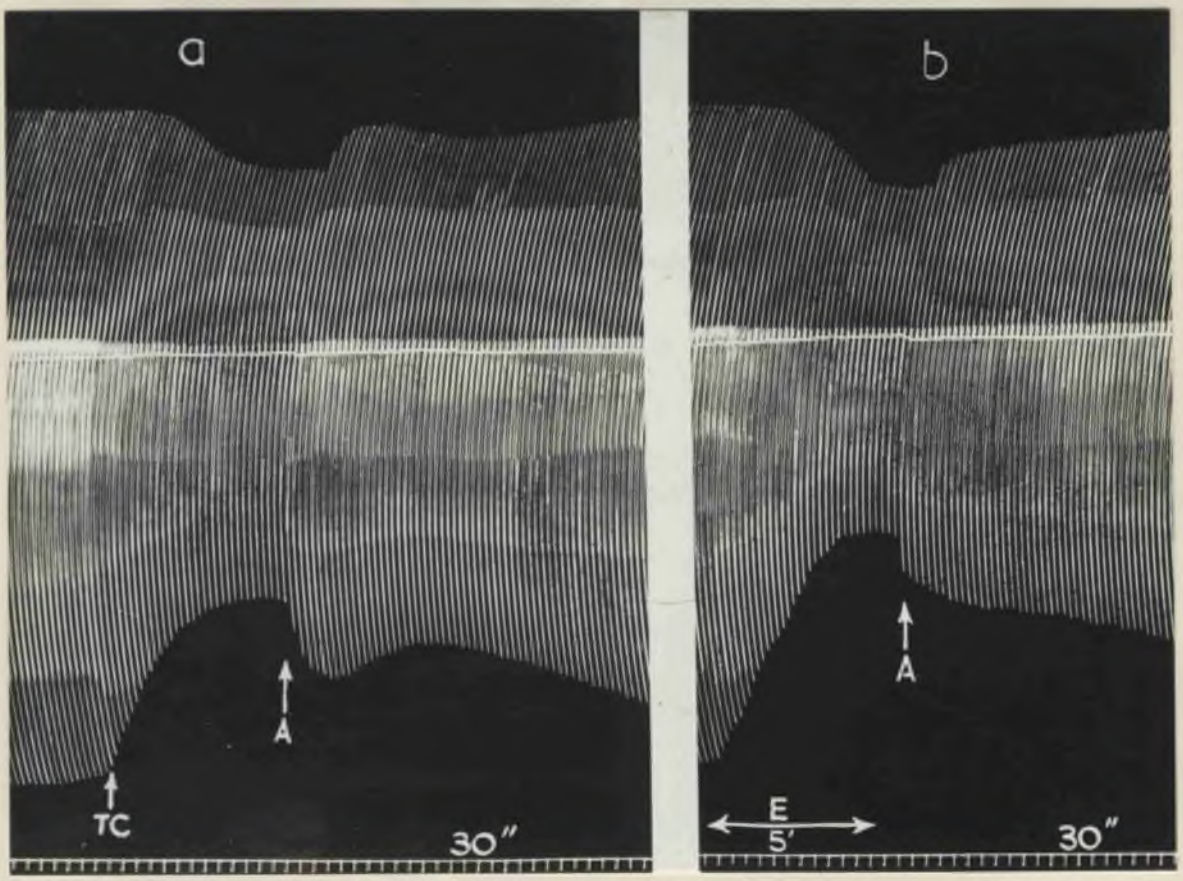

Fig. 130.

Cat gastrocnemius muscle-sciatic nerve preparation. Pentobarbitone anaesthesia (60 mg. per kg.). Indirect stimulation via sciatic nerve. Contractions downwards. Drugs given intravenously.

Tracing a. At Tc, 200 µg. per kg. of tubocurarine.
At A, 50 µg. per kg. of adrenaline.

Tracing b. At E, The cat was allowed to inhale ether for 5 minutes.
At A, 50 µg. per kg. of adrenaline.

Time interval (lower trace) = 30 seconds.

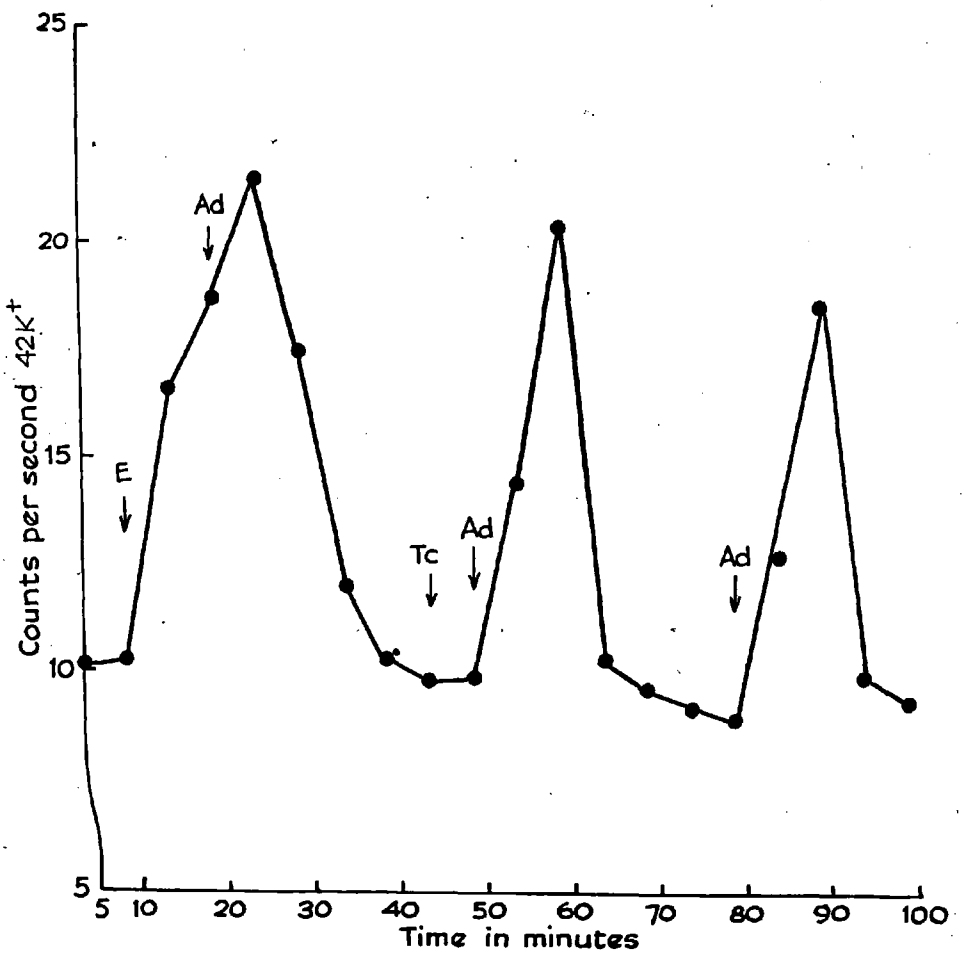

Fig. 131.

Effect of ether (E), tubocurarine (Tc) and adrenaline (Ad) upon the potassium-42 levels of the blood serum of the cat.

All drugs given intravenously.

At E, the cat was allowed to inhale ether for 5 minutes.

At Ad, 50 µg. per kg. of adrenaline.

At Tc, 0.2 mg. per kg. of tubocurarine.

Vertical arrows indicate the points where ether, adrenaline and tubocurarine were given to the cat.

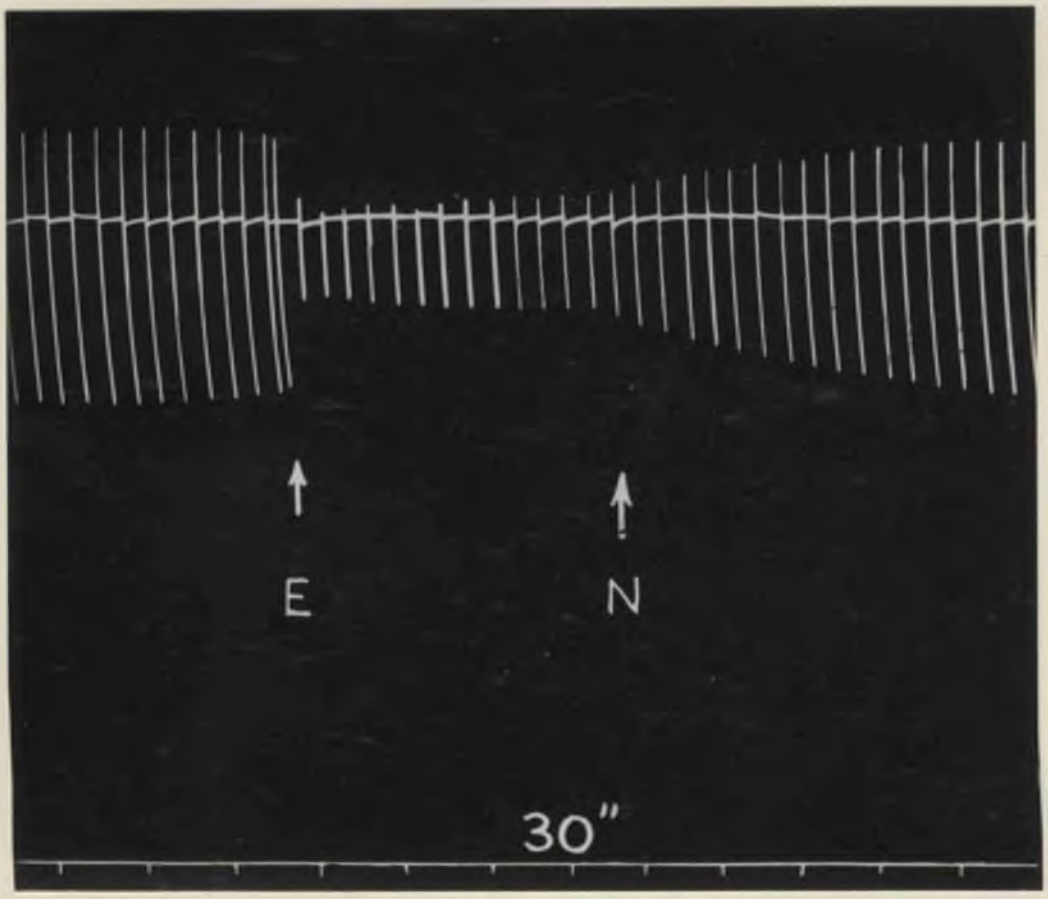

Fig. 132.

Cat gastrocnemius muscle-sciatic nerve preparation. 4 ml. of potassium-42 solution given 2 hours prior to the experiment.
Pentobarbitone anaesthesia (60 mg. per kg.).
Indirect stimulation via the sciatic nerve.
Contractions downwards.
All drugs given intravenously.

At E, the cat was allowed to inhale ether for 5 minutes.
At N, 0.25 mg. per kg. of neostigmine.

Time interval (lower trace) = 30 seconds.

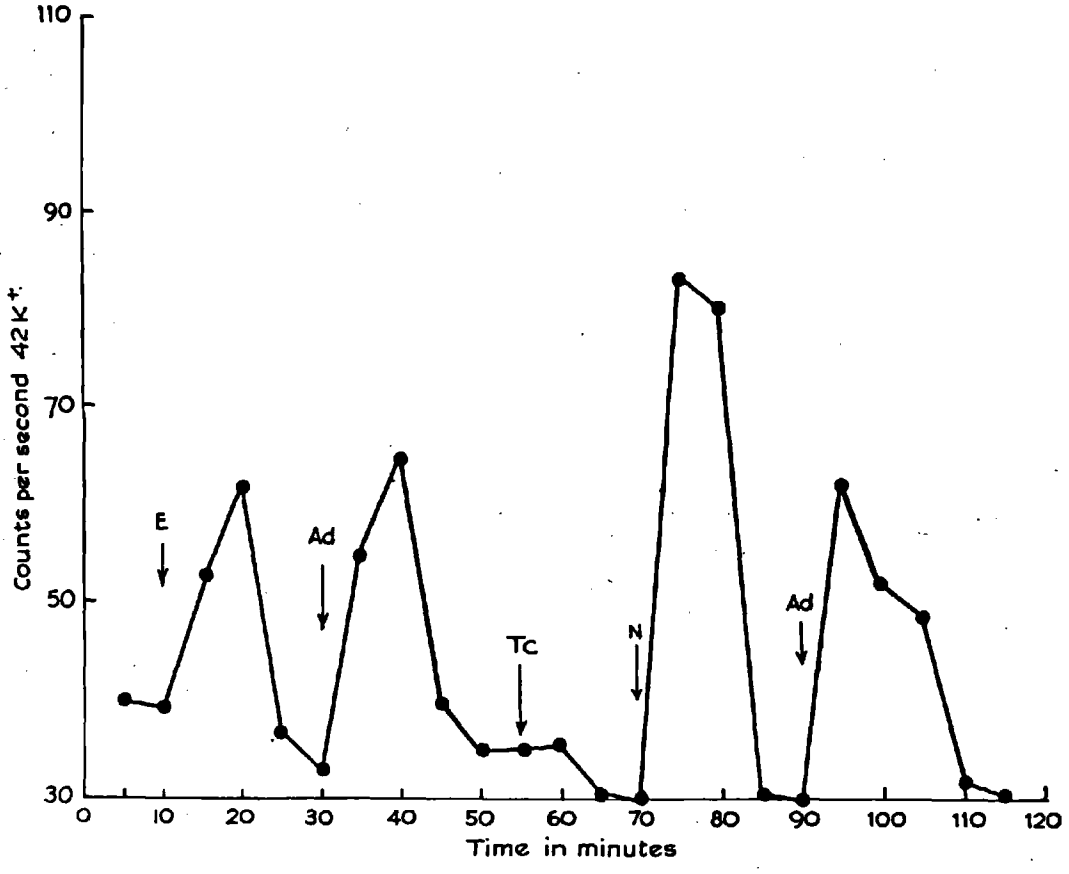

Fig. 133.

Effect of ether (E), adrenaline (Ad), tubocurarine (To) and neostigmine (N) upon the potassium levels of the blood serum of the cat.

All drugs given intravenously.

At E, the cat was allowed to inhale ether for 5 minutes.

At Ad, 50 µg. per kg. of adrenaline.

At Tc, 100 µg. per kg. of tubocurarine.

At N, 0.25 mg. per kg. of neostigmine.

Vertical arrows indicate the point where ether, tubocurarine, adrenaline and neostigmine were given.

ml.). When the muscle was exposed repeatedly to ether solution, the contractions failed to return to control levels (Fig. 134, page 408). Soaking the muscle in Ringer's solution containing three times as much potassium as normal Ringer's solution for a period of half an hour did not bring the acetylcholine-induced responses of the isolated muscle back to control levels. This may indicate that an irreversible change had taken place in the muscle cell. Ether solution (2 to 10 ml.) potentiated the action of tubocurarine (0.3 to 0.6 µg. per ml.) on the response of the frog rectus abdominis muscle (Fig. 136, page 409). At the same dose level tubocurarine had no effect upon potassium-42 efflux.

Neothyl solution (2 to 10 ml.) had similar depressant effects on the acetylcholine-induced contractions of the isolated frog rectus abdominis muscle (Fig. 137, page 410). It also had a direct stimulant effect on the muscle which was accompanied by an increase in the release of potassium-42.

Halothane (2 to 5 ml.) (Fig. 138, page 411) and chloroform (2 to 5 ml.) also had similar actions on the isolated frog rectus abdominis muscle but the doses required were less than those of ether and neothyl.

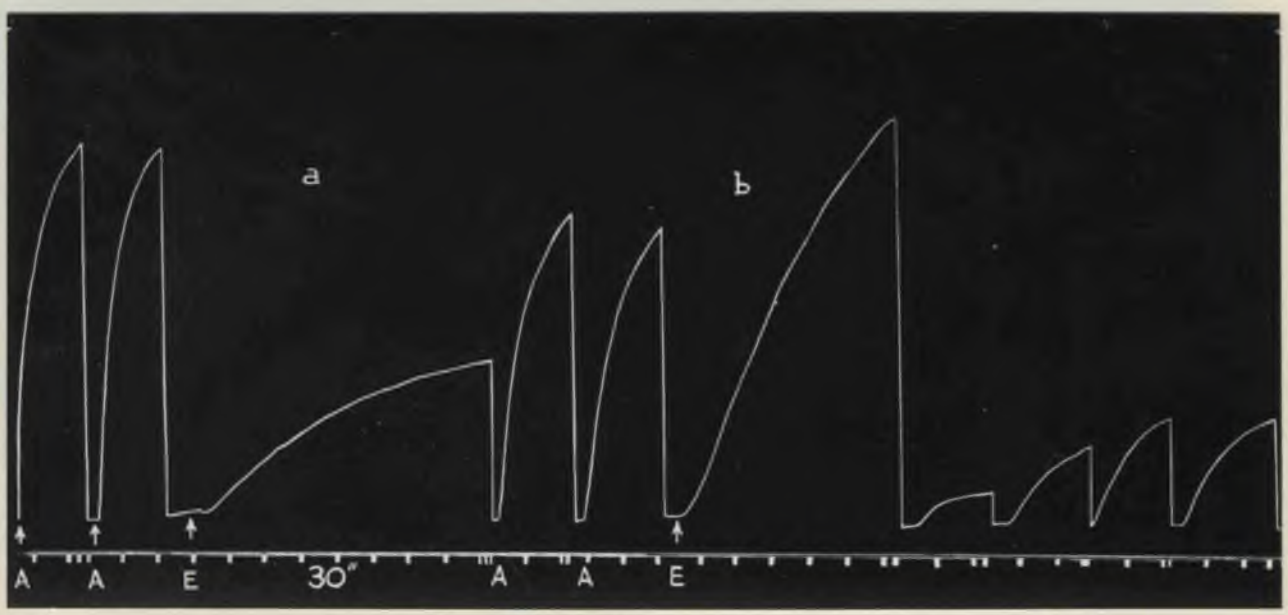

Fig. 134.

Effect of 5 ml. (at a) and 10 ml. (at b) of ether solution (E) on the isolated frog rectus abdominis muscle and on the acetylcholine-induced contractions.
All other contractions are due to 0.5 µg. per ml. of acetylcholine (A).
Time interval (lower trace) = 30 seconds.

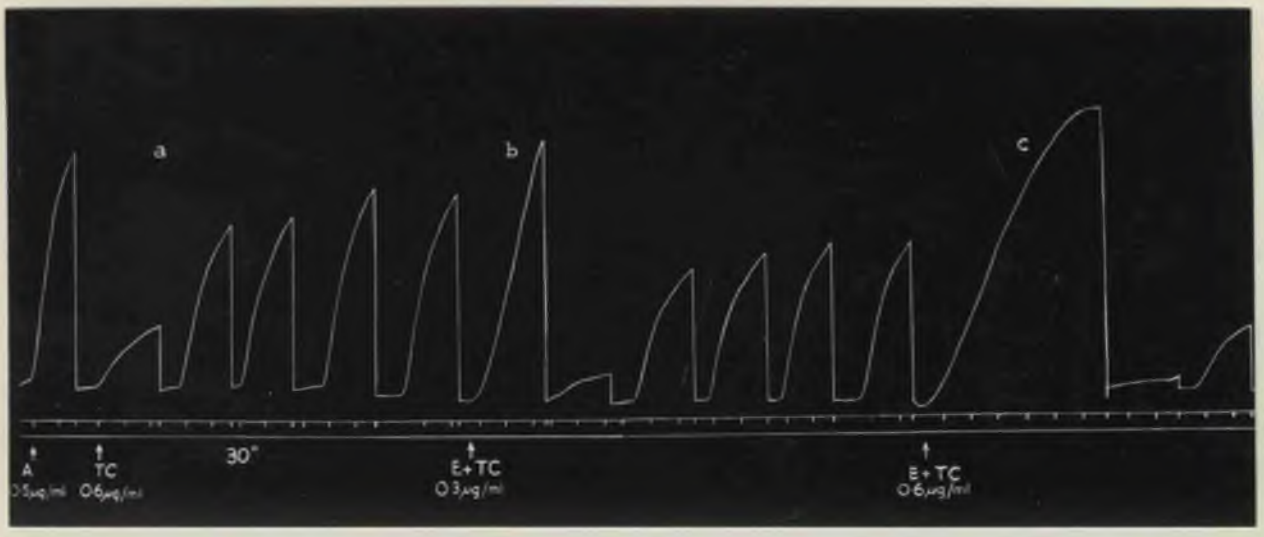

Fig. 136.

Effect of tubocurarine (TC) and ether solution (E) on the isolated frog rectus abdominis muscle and on the acetylcholine-induced contractions.

All other contractions are due to 0.5 ug. per ml. of acetylcholine.

Tracing a. At TC, 0.6 µg. per ml. of tubocurarine.

Tracing b. At E + TC, 5 ml. of ether solution and 0.3 µg. per ml. of tubocurarine.

Tracing c. At E + TC, 5 ml. of ether solution and 0.6 µg. per ml. of tubocurarine.

Time interval (lower trace) = 30 seconds.

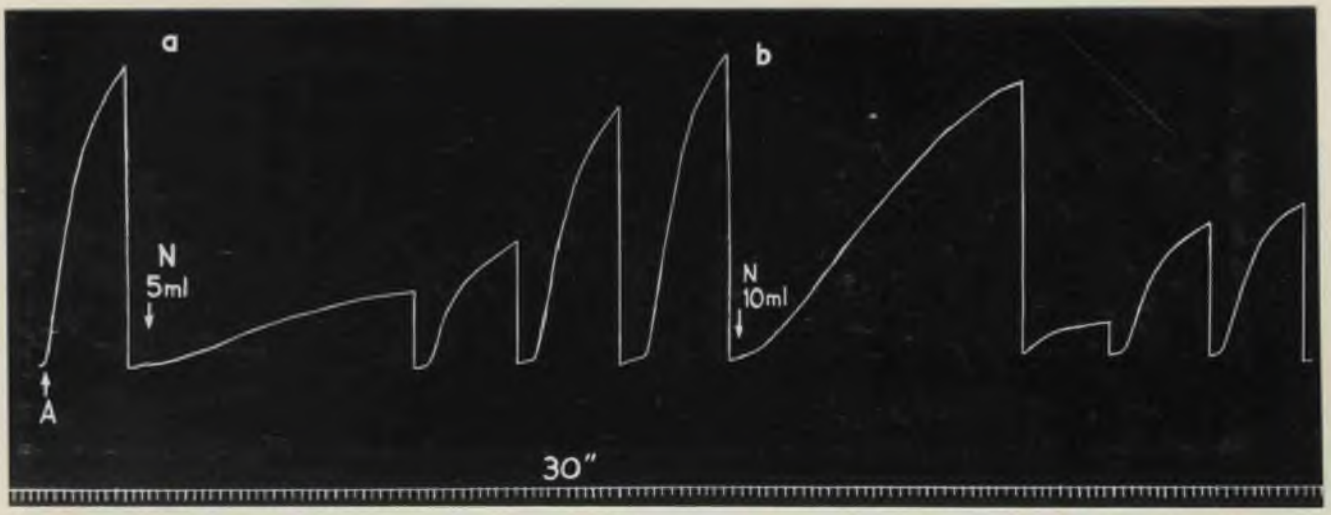

Fig. 137.

Effect of 5 ml. (at a) and 10 ml. (at b) of neothyl solution (N) on the isolated frog rectus abdominis muscle and on the acetylcholine-induced contractions. All other contractions are due to 0.5 µg. per ml. of acetylcholine (A).

Time interval (lower trace) = 30 seconds.

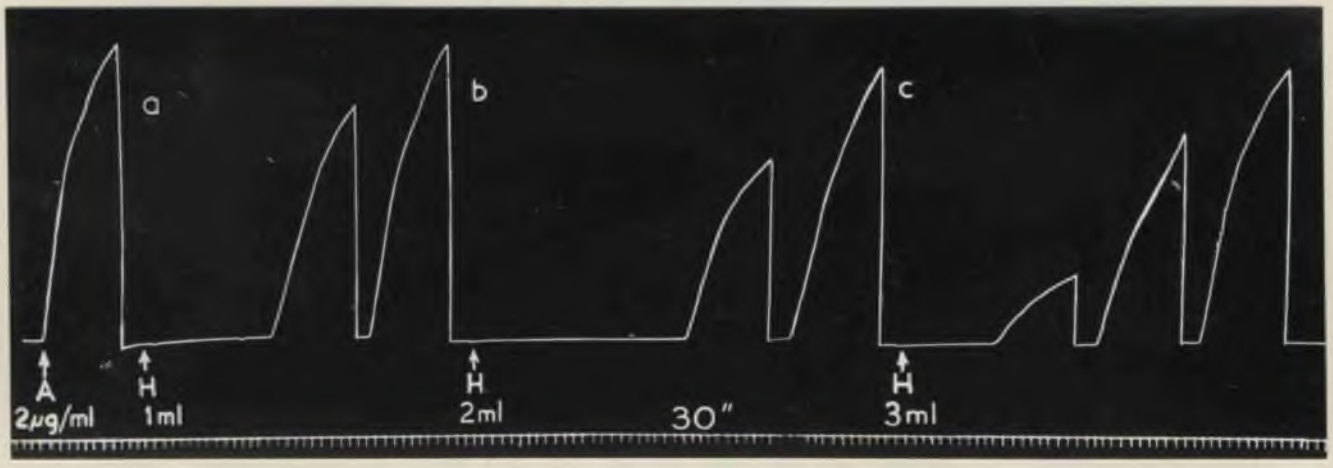

Fig. 138.

Effect of 1 ml. (at a), 2 ml. (at b) and 3 ml. (at c) of halothane solution (H) on the isolated frog rectus abdominis muscle and on the acetylcholine-induced contractions.

All contractions are due to 2 µg. per ml. of acetylcholine.

Time interval (lower trace) = 30 seconds.

Table 15.

Uptake of potassium-42 by isolated frog sartorius muscle and isolated strips of rat diaphragm in the presence of saturated solution of ether in Ringer's solution at 20°C.

No. of experiments	Tissue used	Dose level	Mean percentage uptake of potassium-42 ± SD.							
			After 30 minutes		After 60 Minutes		After 90 minutes		After 120 minutes	
			Control	Drug	Control	Drug	Control	Drug	Control	Drug
8	Rat diaphragm strips	10 ml.	0.045± 0.01	0.018± 0.004	0.052± 0.018	0.019± 0.004	0.0598± 0.015	0.020± 0.004	0.067± 0.02	0.023± 0.002
			P = 0.001		P = 0.001		P = 0.001		P = 0.001	
9	Frog sartorius muscle	10 ml.	0.386± 0.20	0.189± 0.09	0.573± 0.27	0.217± 0.08	0.682± 0.33	0.221± 0.089	0.754± 0.4	0.232± 0.1
			P, < 0.001		P, > 0.001		P, < 0.001		P, < 0.001	

Table 16.

Uptake of sodium-24 by isolated frog sartorius muscle and isolated strips of rat diaphragm in the presence of saturated solution of ether in Ringer's solution at 20°C.								
No. of experiments	Mean percentage uptake of sodium-24 ± SD.							
	Tissue used.	Dose level	After 30 minutes		After 60 minutes		After 90 minutes		After 120 minutes	
			Control	Drug	Control	Drug	Control	Drug	Control	Drug
8	Rat diaphragm strips	10 ml.	0.020± 0.001	0.053± 0.004	0.021± 0.001	0.060± 0.003	0.025± 0.005	0.066± 0.003	0.026± 0.005	0.063± 0.008
			P, < 0.001		P, < 0.001		P, < 0.001		P, < 0.001	
8	Frog sartorius muscle	10 ml.	0.067± 0.04	0.170± 0.054	0.076± 0.05	0.189± 0.067	0.079± 0.048	0.223± 0.081	0.084± 0.05	0.084± 0.05
			P, < 0.001		P, 0.01 < 0.001		P, < 0.001		P, < 0.001	

Table 17.

Uptake of potassium-42 by isolated frog sartorius muscle in the presence of saturated solution of halothane in frog Ringer's solution at 20°C.

No. of experiments	Tissue used	Dose Level	Mean percentage uptake of potassium-42 ± SD.							
			after 30 minutes		after 60 minutes		after 90 minutes		after 120 minutes	
			Control	Drug	Control	Drug	Control	Drug	Control	Drug
6	Frog sartorius muscle	10 ml.	0.111± 0.020	0.051± 0.01	0.165± 0.046	0.065± 0.01	0.201± 0.04	0.071± 0.009	0.227± 0.035	0.071± 0.009
			P, < 0.001		P, < 0.001		P, < 0.001		P, < 0.001	
6	Frog sartorius muscle	5 ml.	0.183± 0.028	0.096± 0.025	0.264± 0.063	0.106± 0.024	0.304± 0.064	0.11 ± 0.024	0.347-+ 0.045	0.114± 0.034
			P, < 0.001		P, < 0.001		P, < 0.001		P, < 0.001	

Table 18.

Uptake of sodium-24 by isolated frog sartorius muscle in the presence of saturated solution of neothyl or chloroform in Ringer's solution at 20°C.

No. of experiments	Tissue used	Dose Level	Mean percentage uptake of sodium-24 ± SD.							
			After 30 minutes		After 60 minutes		After 90 minutes		After 120 minutes	
			Control	Drug	Control	Drug	Control	Drug	Control	Drug
6	Frog sartorius muscle	Neothyl 10 ml.	0.015± 0.004	0.026± 0.005	0.017± 0.004	0.028± 0.007	0.018± 0.003	0.032± 0.008	0.019± 0.005	0.038± 0.009
			P, < 0.001		P, < 0.001		P, < 0.001		P, < 0.001	
6	Frog sartorius muscle	Chloroform 5 ml.	0.280± 0.090	0.431± 0.11	0.343± 0.16	0.564± 0.20	0.373± 0.18	0.583± 0.33	0.349± 0.14	0.762± 0.32
			P, 0.1 < 0.05		P, 0.1 < 0.05		P, 0.05 < 0.02		P, 0.05 < 0.02	

Table 19.

Uptake of potassium-42 by isolated frog sartorius muscle in the presence of saturated solution of neothyl or chloroform in Ringer's solution at 20°C.

No. of experiments	Tissue used	Dose level	Mean percentage uptake of potassium-42 ± SD.							
			After 30 minutes		After 60 minutes		After 90 minutes		After 120 minutes	
			Control	Drug	Control	Drug	Control	Drug	Control	Drug
6	Frog sartorius muscle	Neothyl 10 ml.	0.216± 0.028	0.112± 0.028	0.244± 0.049	0.116± 0.032	0.279± 0.045	0.101± 0.045	0.306± 0.035	0.123± 0.03
			P, < 0.001		P, < 0.001		P, < 0.001		P, < 0.001	
6	Frog sartorius muscle	Chloroform 5 ml.	0.387± 0.11	0.172± 0.03	0.452± 0.15	0.197± 0.04	0.504± 0.199	0.199± 0.033	0.541± 0.21	0.199± 0.028
			P, 0.01 < 0.001		P, 0.01 < 0.001		P, 0.01 < 0.001		P, 0.01 < 0.001	

Table 20. Effect of saturated solution of ether (10 ml.) in Ringer's solution at 20°C. on the release of potassium-42 from the isolated frog sartorius muscle.

No. of Expt.	Muscle	Number of counts released during a period of 10 minutes								Total number of counts released by the muscle.	Ratio of T/C
		10	20	30	40	50	60	70	80		
1	Drug	7252	1766	1545	9537 (T)	1193	832	333	120	22578	1.8
	Control	5516	1340	1245	1108 (C)	1155	842	706	458	12370	
2	Drug	6739	4656	2627	7966 (T)	3899	2262	860	520	29529	1.6
	Control	6250	5393	1684	1152 (C)	1120	1108	925	630	18262	
3	Drug	4392	2064	1569	2246 (T)	1634	670	4240	4480	21295	1.9
	Control	4042	1820	1450	1240 (C)	1028	520	482	428	11010	
4	Drug	1020	882	434	684 (T)	514	298	1026	984	5842	1.3
	Control	1298	1002	558	328 (C)	307	338	270	198	4299	
5	Drug	9023	2247	1480	1926 (T)	1137	492	481	392	17178	1.0
	Control	10467	2170	1172	987 (C)	933	630	511	306	17176	
6	Drug	2489	1852	1251	7813 (T)	3852	1022	634	322	19235	1.9
	Control	2951	1821	1352	1212 (C)	988	852	678	198	10052	
7	Drug	2895	2172	1852	6180 (T)	5205	3066	855	439	22664	1.8
	Control	2930	2450	1935	1321 (C)	1161	1202	969	431	12399	
8	Drug	2551	2821	1198	5839 (T)	3529	932	351	211	17432	1.6
	Control	3652	2298	1121	936 (C)	893	781	322	293	10296	

(T) denotes the point of exposure of the test muscle to the drug solution.

(C) denotes the point of exposure of the control muscle to the control solution.

Table 21.

Effect of saturated solution of ether (10 ml.) in Ringer's solution at 20°C. on the release of potassium-42 from isolated strips of rat diaphragm

No. of Expt.	Muscle	Number of counts released during a period of 10 minutes								Total number of counts released by the muscle.	Ratio of T/C
		10	20	30	40	50	60	70	80		
1	Drug	1541	728	579	2062 (T)	1441	223	209	168	6951	1.3
	Control	1968	755	634	493 (C)	421	369	309	283	5232	
2	Drug	7882	3870	2335	3788 (T)	442	469	341	293	19420	1.2
	Control	7825	3372	2297	921 (C)	583	484	407	288	16177	
3	Drug	2508	1584	1112	2174 (T)	505	423	375	280	8961	1.2
	Control	2468	1070	1022	958 (C)	704	428	419	252	7321	
4	Drug	7722	6424	2047	3438 (T)	3008	658	320	219	23836	1.09
	Control	8967	6824	1711	1540 (C)	1353	633	334	371	21733	
5	Drug	4632	3855	1227	2064 (T)	1803	335	169	108	14193	1.2
	Control	4379	4092	1026	813 (C)	633	339	208	101	11591	
6	Drug	9350	6212	1179	2603 (T)	1482	682	503	423	22434	1.1
	Control	8921	6208	1225	925 (C)	878	739	756	451	20103	
7	Drug	7751	3689	1728	2947 (T)	1158	423	299	289	18284	1.1
	Control	7623	3796	1866	1002 (C)	899	389	321	203	16099	

(T) denotes the point of exposure of the test muscle to the drug solution.

(C) denotes the point of exposure of the control muscle to the control solution.

Table 22.

Effect of saturated solution of halothane (10 ml.) in Ringer's solution at 20°C. on the release of potassium-42 from the isolated frog sartorius muscle

No. of Expt.	Muscle	Number of counts released during a period of 10 minutes									Total number of counts released by the muscle.	Ratio of T/C
		10	20	30	40	50	60	70	80	90		
1	Test	1706	800	500	1709 (T)	463	437	545	428	315	6903	1.38
	Control	1625	797	509	459 (C)	403	302	351	288	311	4985	
2	Test	3092	1286	882	1546 (T)	1518	867	698	483	267	10639	1.33
	Control	2807	1329	982	688 (C)	593	483	452	369	275	7978	
3	Test	2524	1588	982	2370 (T)	518	563	375	288	210	9418	1.15
	Control	2367	1831	1102	651 (C)	631	582	463	322	208	8157	
4	Test	1406	919	903	2207 (T)	872	672	448	391	288	8036	1.23
	Control	1521	699	803	755 (C)	731	651	582	421	328	6491	
5	Test	1359	1272	923	2486 (T)	343	211	240	203	197	7234	1.38
	Control	1288	1160	932	459 (C)	440	221	319	218	201	5238	
6	Test	3718	1821	4061	3948 (T)	1000	417	306	289	232	15792	1.45
	Control	3851	1950	1262	1151 (C)	928	631	500	322	292	10887	
7	Test	2739	1169	968	3077 (T)	1149	648	380	289	192	10611	1.19
	Control	2821	1452	1008	982 (C)	881	731	480	326	229	8910	

(T) denotes the point of exposure of the test muscle to the drug solution.

(C) denotes the point of exposure of the control muscle to the control solution.

TABLE 23.

No. of experiments	Uptake of sodium-24 by isolated frog sartorius muscle in the presence of saturated solution of halothane in frog Ringer's solution at 20° C.							
	Mean percentage uptake of sodium-24 ± SD.							
	Tissue used	Dose level	After 30 minutes		After 60 minutes		After 90 minutes		After 120 minutes	
			Control	Drug	Control	Drug	Control	Drug	Control	Drug
6	Frog sartorius muscle	5 ml.	0.006±	0.010±	0.0063±	0.012±	0.0065±	0.015±	0.0066±	0.0175±
			0.002	0.005	0.002	0.005	0.002	0.004	0.002	0.004
			P, 0.01 < 0.001		P, < 0.001		P, < 0.001		P, < 0.001	
6	Frog sartorius muscle	10 ml.	0.0072±	0.015±	0.0075±	0.018±	0.0077±	0.020±	0.0078±	0.0216±
			0.0035	0.0038	0.0038	0.004	0.0038	0.004	0.0037	0.0071
			P, 0.01 > 0.001		P, < 0.001		P, < 0.001		P, 0.001	

C H A P T E R IV.

C H A P T E R IV.

DISCUSSION

The experimental evidence suggests, that in various concentrations, ether is capable of producing an increase in the permeability of the cell membrane to inorganic ions.

There was observed a marked increase in the release of potassium ions, associated with a significant increase in the uptake of sodium ions and a corresponding decrease in the uptake of potassium ions, by the isolated frog sartorius muscle and isolated strips of rat diaphragm.

In similar concentrations, ether was shown in separate experiments to potentiate the effect of tubocurarine upon the acetylcholine-induced contractions of the isolated frog rectus abdominis muscle. In the same preparation, ether, chloroform, halothane and neothyl were found to be capable of producing a muscle contracture - followed by a reversible depression of the muscular activity. This was associated with an increased release of potassium ions. This effect is identical to the action of depolarizing agents observed by Ahmed and Lewis (1962) using decamethonium and suxamethonium.

It was also observed, that concentrations of ether which were sufficient to cause inhibition of neuromuscular transmission in the rat phrenic nerve-diaphragm preparation and in the cat gastrocnemius muscle- /

muscle-sciatic nerve preparation were capable of causing an increase in the release of potassium ions. When used upon the same preparation tubocurarine, although it caused a potentiation of the neuromuscular block induced by ether, had no observable effect on the flux of ions.

Adrenaline and neostigmine were found to antagonize the effects of ether on the response to indirect stimulation of the cat gastrocnemius muscle-sciatic nerve preparation. Adrenaline produced an increase in the serum potassium-42 levels of the cat. This observation was in agreement with the earlier findings of D'Silva (1933) and Cicardo and Ashman (1958). A marked increase in the serum potassium-42 level of the cat was also observed with neostigmine.

When the results obtained from the present investigations are considered from the point of view of Overton's lipoid cell membrane theory it seems possible that ether and other volatile anaesthetics (by virtue of their high solubilizing action on fats) are acting on the cell membrane in such a manner as to alter its permeability to inorganic ions. This physical action may depress the activity of the cell which is reflected in the appearance of an injury potential. It was observed by Galeotti and Di Cristina (1910) that the local application of ether to an isolated muscle surface produced a spot which became electrically negative when compared to the rest of the muscle and this effect was not completely reversible. He suggested that /

that this phenomenon was similar to irreversibly damaging the muscle by cutting it. It may also be assumed, that it is associated with an increased release of potassium and an increased uptake of sodium ions. If this assumption is true, then the question arises as to the manner in which the ability of the muscle cell to respond to electrical or chemical stimuli returns to normal. According to Mullins (1956) a local transformation of one part of the cell membrane cannot readily be compensated for without creating further disturbances of the cell membrane. This may indicate that some other mechanism of action is involved which brings about a momentary change in the uptake and release of sodium and potassium ions, i.e. the type of effect produced by depolarizing drugs. This fact is supported by the observations of Ahmed and Lewis (1962) that depolarizing agents such as suxamethonium and decamethonium caused an increase in the uptake of sodium and calcium ions and increased the release of potassium ions. In contrast to this observation, competitive neuromuscular blocking agents, i.e. tubocurarine and gallamine, were found to exert no influence on ion fluxes.

The experimental evidence presented in Chapter III, indicates that the mechanisms of the muscle relaxant actions of the volatile anaesthetics and tubocurarine differ. It seems that ether may cause depolarization of the motor end-plate in the first phase of its action, possibly due to the presence of a high local concentration /

concentration of potassium and that this effect may be followed by a non-depolarizing action (Foldes, Wnuck, Hodges, Thesleff and de Beer, 1957).

The question arises as to why an increase in the concentration of potassium ions in the medium, which ordinarily causes antagonism to the effects of tubocurarine, (Paton, 1956) does not antagonize the effect of tubocurarine in this preparation. It is possible that this is due to the very large concentration of potassium ions in the bathing solution which produces a secondary inhibitory effect on the excitability of the cell (Fenn, 1940).

It was also observed that the concentrations of ether in Tyrode's solution which were capable of producing complete inhibition of the responses to indirect stimulation, did not alter the response of the diaphragm muscle to direct stimulation. This confirms the observations of Secher (1951d) that although ether in concentrations which are capable of depressing neuromuscular transmission, does not alter the activity of the muscle cell itself, yet it can cause an increase in the permeability of the cell membrane to ions. It is well known that neuromuscular transmission is affected more rapidly than the muscle cell itself (Nachmansohn, 1959 a and b).

The action of ether clearly differs from that of tubocurarine and is more like that of the depolarizing agents such as suxamethonium and decamethonium. This fact is further supported by the observations of

Watland /

Watland, Long, Pittinger and Cullen (1957) who suggested that the initial stimulant effect of chloroform on the responses of the gastrocnemius muscle to indirect stimulation was due to a depolarizing action.

Jenden, Kamijo and Taylor (1951 and 1954) suggested that depolarizing agents can produce a two phase block of neuromuscular transmission. From in vitro studies using the isolated rabbit lumbrical muscle preparation, they indicated that phase one block set in rapidly, was of short duration, and had the characteristics described by Paton and Zaimis (1949), Burns, Paton and Vianna Dias (1949) and Paton and Zaimis (1952) for decamethonium-induced block in the cat muscle-nerve preparation. The neuromuscular block in the phase one could be antagonized by tubocurarine but was not affected by potassium or anticholinesterases. This phase one block was followed by an increase in the neuromuscular block which now resembled that of a non-depolarizing block and this was antagonized by anticholinesterases and potassium ions. This effect persisted as long as the preparation was kept in contact with the depolarizing relaxant. It was also observed by Brennan (1956) that, although depolarizing and non-depolarizing drugs should antagonize the neuromuscular actions of one another, the neuromuscular block induced by small doses of gallamine was intensified by prior use of suxamethonium. Similar effects were reported in man by Hodges and Foldes (1956), who observed that when administered for

a /

a prolonged period, suxamethonium potentiated the neuromuscular block induced by tubocurarine.

In the present study using the rat phrenic nerve-muscle preparation, it was observed that ether potentiated the reduction in the magnitude of the responses to indirect stimulation induced by decamethonium. It was also observed, that the inhibition of responses to indirect stimulation by ether was antagonized by neostigmine. It has previously been reported, that tubocurarine is capable of antagonizing a depolarizing block produced by decamethonium both in laboratory animals (Foldes, 1954; Burns and Paton, 1951; Jenden, Kamijo and Taylor, 1951 and 1954; and Thesleff, 1955) and in man (Macfarlane, Unna, Pelikan, Cazort, Sadove and Nelson, 1950).

Although Paton and Zaimis (1952) have suggested that non-depolarizing agents are antagonized by depolarizing agents, this does not seem invariably to be true. When, for example, a depolarizing relaxant is administered after a non-depolarizing agent (Hutter and Pascoe, 1951), the administration of decamethonium does not antagonize the effect of a subsequent dose of tubocurarine (Paton and Zaimis, 1949). On the other hand, there is evidence (Brennan, 1956; Hodges and Foldes, 1956; Foldes, Wnuck, Hodges, Thesleff and de Beer, 1957) that prolonged administration of depolarizing drugs such as decamethonium, instead of antagonizing the effect of tubocurarine, may potentiate it. Zaimis (1953), Jenden and his associates /

associates (1951 and 1954) and Foldes and his co-workers (1957), using the sciatic nerve-muscle preparation of the dog, cat or rabbit, have demonstrated that the end-plate became less sensitive to the effects of decamethonium and suxamethonium when these were administered over a prolonged period. Neostigmine was found to be capable of antagonizing their effects. Foldes and his associates (1957) observed that when the isolated sartorius muscle-nerve preparation of the frog was kept in prolonged contact (15 minutes) with depolarizing agents, there was potentiation of the tubocurarine-induced inhibition. These authors postulated that when the end-plate was exposed to depolarizing drugs for a certain length of time, it developed a resistance to them. The same observation was made by Thesleff (1955). When the isolated nerve-muscle preparation of the frog was kept in a bath containing acetylcholine, the initially depolarized end-plate became repolarized after a certain period. The same was found true when suxamethonium and decamethonium were used.

The release of potassium-42 in the circulating blood of the cat observed following the use of ether is in agreement with results of Kiersz (1948). It has been said to be due to the reflex release of adrenaline from the suprarenal glands (Elliott, 1912) or sympathetic stimulation (Bhatia and Burn, 1933). The fact that adrenaline causes an increase in the potassium ion concentration of the cat blood /

blood was first observed by D'Silva (1933). Adrenaline, on the other hand, antagonizes the effect of the neuromuscular block produced both by tubocurarine and by ether.

During a neuromuscular block due to inhalation of ether vapour, there was an associated increase in the blood potassium level. When, however, adrenaline was given, the blood potassium rose further but the block diminished. This may mean that the release of potassium ions seen during neuromuscular block is not due to the release of endogenous adrenaline but some other mechanism. It is possible that the source of blood potassium is merely from the erythrocytes and not from the muscle cells (Davson, 1940). It may be due to a mechanism of depolarization resembling that of decamethonium. This fact is supported by the evidence that in the isolated frog sartorius muscle and isolated strips of rat diaphragm an increased release of potassium ions was observed, when these tissues were exposed to ether.

Although ether exhibits muscle relaxant actions which resemble those of tubocurarine, it seems to have a mechanism of action different from that of tubocurarine. It is tempting to accept the theory of Foldes and his associates (1957) that basically there is no difference between the mechanism of action of depolarizing and non-depolarizing agents. Both compete with acetylcholine for the cholinergic receptors of the end-plate membrane. After their successful competition with acetylcholine, the sequence of events seems to be first determined by the /

the chemical characteristics of the relaxant and second by the properties of the end-plate (as a marked variation is seen in different species). Those relaxants which chemically resemble acetylcholine, especially in relation to the size of the cationic head (Bovet, 1951) have a tendency to produce initial depolarization. The degree and duration of depolarization depends upon the sensitivity of the end-plate and the type of depolarizing agent used. If the sensitivity of the end-plate is low as seen in the monkey, depolarization block can not be produced (Zaimis, 1953).

From a consideration of the results of experiments recorded in this part of the thesis it seems that ether and other volatile anaesthetics tested, increase the permeability of the cell membrane to sodium and potassium ions in a manner similar to that of depolarizing agents.

This assumption is favoured by the evidence presented by Mullins (1956) and Waser (1960). It is likely that ether depresses neuromuscular transmission by depolarizing the motor end-plate membrane. It is felt that the peripheral actions of ether in increasing a non-depolarizing block, are due to the mechanism suggested above.

CHAPTER V.

SUMMARY OF PART 2.

In the introduction, a survey has been made upon the work of earlier investigators upon the muscle relaxant actions of volatile anaesthetics. A discussion of the role of inorganic ions in the mechanism of neuromuscular transmission is included together with a consideration on theories of the nature of the cell membrane and the cholinergic receptor.

In Chapter II, the experimental techniques used to investigate the mode of muscle relaxant action of ether and some other volatile anaesthetics are described.

In Chapter III, the results of the investigations described in Chapter II, are presented. Ether, halothane, chloroform and neothyl in various concentrations were found significantly to increase the uptake of sodium-24 and the efflux of potassium-42 by the isolated frog sartorius muscle and by isolated strips of rat diaphragm. In other series of experiments ether, halothane, chloroform and neothyl were found to inhibit the acetylcholine-induced contractions of the isolated frog rectus abdominis muscle. This inhibitor effect was preceded by a reversible contracture and an increase in the efflux of potassium ions. Ether was capable of potentiating the effects

of /

of tubocurarine on acetylcholine-induced contractions of the isolated frog rectus abdominis muscle.

In lower concentrations, ether was found to be capable of depressing the response of the isolated rat phrenic nerve-diaphragm preparation to indirect stimulation, whereas larger concentrations were required to cause a similar effect on the response of the rat diaphragm to direct stimulation. The inhibition of neuromuscular transmission in this preparation was associated with an increase in the efflux of potassium-42.

When pentobarbitone-anaesthetised cats were allowed to inhale ether for 5 to 10 minutes, an increase was observed in the level of potassium-42 in the venous blood. This effect was associated with a simultaneous inhibition of the response of the gastrocnemius muscle to indirect stimulation. Tubocurarine, although it caused an inhibition of the response of the gastrocnemius muscle to indirect stimulation, did not cause any observable change in the serum potassium-42 levels.

Neostigmine and adrenaline were found to cause an increase in the serum potassium level and also to antagonize the effects of ether on the responses of the isolated rat diaphragm and cat gastrocnemius muscle to indirect stimulation.

In Chapter IV, the significance of the increased permeability of the cell membrane to sodium and potassium ions in the muscle relaxant action /

action of ether is discussed.

It is concluded that the muscle relaxant actions of ether and other volatile anaesthetics are probably due to a mechanism which resembles that of depolarizing agents.

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