THE MODE OF ACTION OF SOME DRUGS AND CHEMICALS USED TO TREAT HYPERTENSION, OR AS MUSCLE RELAXANTS

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KAMALUDDIN AHMAD, M.B.B.S. (Dacca)

Experimental Pharmacology Division,
Institute of Physiology, and
Department of Materia Medica
and Therapeutics,
The University of Glasgow.

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LIST OF PUBLICATIONS

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

1. On the pharmacology of petaline chloride, a convulsant alkaloid from Leontice leontopetalum Linn.
   J. Pharm. Pharmacol. (1960), 12, 163-174.

2. The effects of tubocurarine, decamethonium, suxamethonium, edrophonium and neostigmine upon flux of calcium-47 in frog skeletal muscle.
   J. Pharm. Pharmacol. (1961), 13, 123-127.

3. The effects of gallamine, carbachol, nicotine, ryanodine and protoveratrine A and B upon flux of calcium-47 in frog skeletal muscle.
   J. Pharm. Pharmacol. (1961), 13, 383-384.

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

British Pharmacological Society in London in January, 1960 - Pharmacology of petaline chloride.

P A R T I

STUDIES ON THE EFFECT OF CERTAIN DRUGS UPON THE FLUX OF CALCIUM, POTASSIUM AND SODIUM IONS IN SKELETAL MUSCLE.

CHAPTER I

INTRODUCTION

The influence of monovalent and divalent cations on excitable tissues has been of interest to physiologists and pharmacologists for many years and the properties of pharmacological agents and physiological mechanisms are in some cases most readily understood in terms of ion distribution and ion movements. The most extensively studied cations are calcium, potassium, sodium and magnesium. Their importance, if not their precise roles, in maintaining the normal functioning of excitable tissues has been clearly established.

CALCIUM

The effects of calcium ions upon the isolated "intact" heart and cardiac tissue preparations.

Ringer (1, 2, 3) in 1883 was successful in preparing a solution containing sodium chloride, potassium chloride, sodium bicarbonate and calcium chloride or calcium phosphate in quantities which made it suitable as a substitute for blood, and which kept frog and eel hearts contracting in vitro for several hours. Later on, Mines (4) in 1913 made simultaneous records of the mechanical responses and electrical changes in the perfused frog heart and observed that alterations/

alterations in the concentrations of hydrogen ions and calcium ions in the perfusion fluid could abolish mechanical movements, but did not influence the regular electrical variations which remained in a normal form. In 1920, De Burgh Daly and Clark (5) observed that alterations in the concentrations of the ions normally present in Ringer's solution would, if sufficiently extensive, impair all functions of all parts of the frog heart, but a moderate degree of change influenced the different regions of the heart to an unequal extent. Alterations in the potassium ion content produced a greater impairment of the conduction of electrical variations, whereas reduction of the calcium ion content had little effect. Lack of potassium or sodium ions and excess of calcium ions all produced an increase of systolic tone and caused somewhat similar variations in the electrocardiogram.

Green and his colleagues (6) while using cat papillary muscle to study the contractility and excitability of the mammalian myocardium, noted that contractility depended upon the calcium ion concentration in the bathing medium.

Solomon (7) studied the influence of altered calcium ion concentration in the perfusing fluid, upon the contractile force and electrical potentials of the cat papillary muscle. He observed that increasing the calcium ion content of the perfusing fluid increased the force of the contraction and inverted the T wave. Decreasing the calcium ion content reduced the force of contraction, shortened the R-T/

R-T interval and raised the R-T segment.

Wilbrandt and Koller (8) while experimenting with isolated frog hearts, observed that dilution of the Ringer's solution with isotonic sucrose produced the same result as an increase in the calcium ion content of the solution. They suggested that the site of action of calcium ions in the heart was probably the cell membrane. In this case, they suggested that the action depended quantitatively on the calcium ion concentration at the membrane, which was presumably not identical with the concentration in the surrounding solution, but related to it by an equilibrium constant. In general they thought it would be higher. From the calculation of the equilibrium constant Wilbrandt and Koller (8) found that dilution of the external solution increased the calcium ion concentration at the membrane itself.

Recently Weidmann (9) studied the effect of increasing the calcium ion concentration during a single heart beat. He raised the calcium ion concentration rapidly after the beginning of an action potential by applying a calcium-rich solution (100 mM calcium chloride in Ringer's solution) through a cannula inserted into the coronary artery of the isolated turtle ventricle. His observations made it clear that an increment of contractile strength could be obtained if the calcium ion concentration in the perfusate started to rise at the beginning of systole, i.e. after the onset of membrane depolarization. On the basis of this result Weidmann/

Weidmann (9) concluded that throughout the period of membrane depolarization calcium ions mediate between electrical and mechanical activity.

Niedergerke (10) studied the staircase phenomenon of cardiac tissue using combined methods of electrical and mechanical recording. His experiments suggested that the staircase effect was due to a facilitation of a process by which the action potential gave rise to a muscular contraction. He observed that a similar facilitation could be produced without repetitive stimulation by certain changes in the ionic environment, notably by an increase in the calcium ion concentration, and to a less marked extent, by a reduction of the potassium ion concentration. Niedergerke (11) also studied the potassium chloride contracture of cardiac tissue and its modification by calcium ions. Tension and surface depolarization of strips of frog's ventricle were recorded during potassium chloride contracture. High calcium ion concentrations increased the amplitude and rate of rise of the contracture, and slowed relaxation. When calcium ions were added to or withdrawn from a depolarized muscle, rapid changes occurred in the strength of the contracture. He concluded that the calcium ion alters the relation between depolarization and tension. It enhances tension at all levels of depolarization, but particularly strongly at small depolarizations. These effects, produced by a change in the external calcium ion concentration, develop and subside rapidly and may, therefore, be presumed to take place at the cell surface. Niedergerke (12) also studied the rate of/

of action of calcium ions on the contraction of the heart. He observed that changes in tension due to an altered calcium ion concentration were faster than the corresponding net movements of calcium ions between the tissue and the surrounding medium. An appreciable fraction of the net movement of calcium ion (more than 50 per cent) however, takes place while the tension rises or decays.

Niedergerke and Lüttgau (13) observed that every known feature of the action of calcium ion on the cardiac tissue could be simulated by a diminution of the sodium ion concentration. They suggested that calcium and sodium ions competed for a compound which controlled contraction. Niedergerke and Harris (14) found that replacement of sodium ions by sucrose or lithium ions, or the omission of potassium ions from Ringer's solution, caused the cardiac tissue to accumulate calcium ions. In both of these cases they observed an increased uptake of calcium ion, whereas the rate of release of calcium ions was diminished.

Lüttgau and Niedergerke (15) studied the effects of sodium and calcium ions on the contraction of strips of frog heart by recording the tension of isometric twitches and potassium chloride contractures. They measured the corresponding action potentials and the depolarizations and using microelectrodes also determined the cellular membrane potentials. It was observed that calcium ions and sodium ions influenced contractility in an antagonistic way and that twitch and contracture tensions depended approximately upon the ratio of calcium ions to sodium ions in the bathing fluid./

fluid. They suggested that calcium ions and sodium ions competed at the cell surface or in some other cellular region readily accessible to the external solution, for a negatively charged substance to form either a calcium compound activating contraction, or a sodium compound which is inactive in this respect.

Niedergerke (16) investigated the influence of calcium ions on the activation of the contraction in cardiac tissue by studying the uptake of calcium ions in frog ventricles during rest and contracture. He perfused the ventricles with sodium ion-depleted Ringer's solution, replacing sodium ions with potassium ions and observed an increased uptake of calcium ions. When the ventricles were transferred (after a five minute perfusion in potassium Ringer's solution) to normal Ringer's solution there was a loss of calcium. This suggested a reversible competition between sodium ions and calcium ions for a cellular site. When the ventricles were depolarized by raising the external potassium ion concentration to 100 mM (which was done by adding solid potassium chloride to the perfusion fluid) it was observed that the depolarization caused by added potassium increased the calcium ion uptake. On the basis of these results Niedergerke (16) suggested that the inward movement of calcium ions activated the contraction of cardiac tissue.

Sekul and Holland (17) studied the exchange of calcium ions in atrial fibrillation using radioactive calcium-45 as a tracer. Freshly dissected rabbit atria were attached to/

to platinum-iridium electrodes and suspended in Ringer's solution. Fibrillation was induced by stimulating the atria at 1,200 cycles per minute for one minute. It was found that fibrillation caused an increased influx, but had no influence on the efflux of calcium ions.

Thomas (18) studied the uptake of calcium-45 by heart muscle during potassium ion-lack contracture using frog ventricle for his work. He observed that when the ventricles were perfused with potassium ion-free Ringer's solution there was an increase in the uptake of calcium. This increased uptake of calcium coincided with the gradual development of the contracture and was associated with a loss of potassium ion and a gain of sodium ion.

It appears from a study of the observations cited above, that the calcium ion plays a vital role in the normal functioning of the intact heart and of isolated cardiac tissue. Contractile strength seems to depend on the amount of calcium ion available and the observation (16-18) that contracture or fibrillation were associated with an increased calcium ion content of cardiac tissue indicates that depolarization of the membrane may be associated with a net entry of calcium ions into the myofibril. There seems to be an antagonistic relationship between calcium ions and sodium ions on the cardiac cell surface, while there is strong evidence that in the heart the calcium ion may supply the link between the electrical and mechanical events.

The/

The influence of calcium ions on the heart in situ.

Rubin and his colleagues (19) made simultaneous records of the cortical electrogram and the electrocardiogram during intravenous injection of salts of potassium, calcium and magnesium in the cat. They observed that salts of potassium and calcium produced no changes in the cortical electrogram until the development of intraventricular block or cardiac arrest. There was subsequently a slowing of the cortical electrogram.

Clarke (20) studied the action of calcium ions on the human electrocardiogram. He observed that the changes produced were progressive and depended upon the calcium ion concentration of the blood. The earliest changes were bradycardia, sinus arrhythmia, shifting of the pacemaker and various degrees of heart block. Calcium ion was found to act directly upon the ventricular muscle, increasing its excitability, and producing foci of idiopathic ventricular rhythmsand ventricular extrasystoles of large and unusual form. There were also alterations in blood pressure, the systolic level was raised and the diastolic pressure usually depressed.

The influence of drugs upon the flux of calcium ions in the isolated "intact" heart and on cardiac tissue preparations.

Loewi (21) in 1918 suggested that the action of strophanthin on the heart was due to an increased sensitivity to calcium ions and Thomas (22) in 1957 suggested that digitoxin altered the affinity of binding sites in heart muscle for/

for calcium ions.

Harvey and Daniel (23) using calcium-45 as a tracer investigated in the isolated perfused guinea-pig heart, the possibility that digitalis acted by altering the influx and/or efflux of calcium ions in the heart muscle. Perfusion with a normal medium was sustained for 30 minutes, after which a calcium-45 labelled medium was introduced for periods of from 5 minutes to 1 hour along with different concentrations of digitoxin. At the end of this time the hearts were analysed for calcium-45 content. It was observed that even in intoxicated hearts the calcium ion content did not vary significantly with regard to either the time of perfusion or digitoxin treatment.

Thomas and his co-workers (24) studied the effects of potassium ion-free perfusion and certain contracture producing agents, e.g. ouabain, adrenaline and iodoacetate on calcium-45 uptake by isolated frog hearts. Electrically stimulated ventricles mounted on Straub cannulae were perfused with calcium-45 labelled Ringer's solution. It was found that ventricles perfused with potassium ion-free Ringer's solution to the point of final contracture, accumulated 100 per cent more calcium-45 than the controls. Ventricles put into contracture by toxic doses of ouabain took up 20 per cent more calcium-45 than the controls, but non-toxic doses of ouabain had no effect. Ventricles put into contracture by adrenaline or iodoacetate took up no more calcium-45 than the controls.

Holland/

Holland and Sekul (25) studied the effect of ouabain on calcium-45 exchange in isolated rabbit atria. Freshly dissected atria weighing between 300 and 400 mg. obtained from young rabbits were employed. Influx was determined by incubating the atria for periods of 2 hours in Ringer's solution containing calcium-45 in the presence or absence of ouabain. After the incubation period, the atria were washed with non-radioactive Ringer's solution for a period of 5 minutes, weighed and homogenized in distilled water and the counts were made upon evaporated samples of the homogenate. Efflux was determined by incubating the atria in Ringer's solution containing calcium-45 for a period of 2 hours. At the end of this time the preparations were washed three times with non-radioactive Ringer's solution. The atria were then incubated in a number of samples of non-radioactive Ringer's solution with and without ouabain for a period of 1 hour, after which they were blotted dry, weighed and homogenized in distilled water. The number of counts remaining in the tissue was determined by counting the evaporated samples of the homogenate.

It was observed that ouabain increased calcium ion influx but did not alter calcium ion efflux. The action on the influx was enhanced by an increase of calcium ion, and blocked by an increase of potassium ion in the medium. Thomas (26) also found that in frog ventricles when sufficient ouabain was given to cause contracture, it increased the rate of influx but not of the efflux of calcium-45.

Sekul/

Sekul and Holland (17) using rabbit atria found that acetylcholine had no demonstrable action on the calcium ion exchange, but in acetylcholine-induced fibrillation the rate of entry of calcium ionswas greater than in the rapidly stimulated atria.

The effect of calcium ions upon skeletal muscle.

Calcium is known to exist in biological materials in both the ionic and "bound" states. The influence of calcium ions in the process of muscle contraction has received the attention of many workers. There is no evidence that bound calcium plays a part in the activity of nerve or muscle.

Ringer (2, 3) in 1887 observed that calcium salts added to a saline solution sustained contractility in frog skeletal muscle and Mines (27) in 1908 using the method of submaximal stimulation, observed that addition of a small amount of calcium chloride to a sodium chloride solution which was bathing isolated frog sartorius muscle, caused an immediate diminution in the muscular excitability towards electrical currents of long duration.

Brown and Harvey (28) in 1940 observed that a kid kept on a diet deficient in calcium showed a defect in neuromuscular transmission, such that a single maximal motor nerve volley failed to elicit a maximal response from the muscle. With repeated stimulation, at a sufficiently high frequency, each successive response of the muscle became greater until transmission was fully restored at the fifth or sixth response.

Heilbrunn/

Heilbrunn and Wiercinski (29) studied the action of various cations on the muscle protoplasm by injecting solutions of various salts into the interior of isolated frog muscle fibres. The effects of these solutions on the lengths of the muscle fibres were then determined by measurements made under the microscope. Calcium ions even in rather high dilutions caused an immediate and pronounced shortening of the muscle fibres. This effect was not shared by any one of the other cations normally present in any quantity in muscle.

Merlis (30) studied the effect of changes in the calcium ion content of a balanced salt solution (Na, 0.141; K, 0.0033; Ca, 0.00125; Mg, 0.0012; Cl, 0.152; HPO_4, 0.00048; HCO_3, 0.021; glucose, 0.0034; urea, 0.0022 moles per litre) perfused through the lower spinal sub-arachnoid space at constant pressure and temperature in dogs with spinal cord transectioned at T10. He observed that calcium ion-free solutions produced an augmentation of the spinal flexion reflex, an increase in muscle tone and spontaneous twitching of the muscles of the lower half of the body.

Bülbring and her colleagues (31) studied the behaviour of isolated frog skeletal muscle in a calcium ion-deficient medium and observed that lowering the calcium ion content of the bathing medium led to a fall in the membrane potential.

Del Castillo and Stark (32) studied the relation between the size of the motor end plate potential and the calcium/

calcium ion concentration using frog sartorius muscle for their experiments. It was observed that over a wide range of change of calcium ion concentration there was an approximately linear proportionality between the calcium ion concentration and the amplitude of the end plate potential. It was noted that by increasing the calcium ion concentration from 0.45 to 7.2 mM the size of the end plate potential increased from 0.15 to 2.26 times that obtained in Ringer's solution (1.8 mM calcium ion concentration).

Harris (33) studied the exchange of calcium-45 in frog sartorius muscle. He soaked the muscles for a period of from 4 to 12 hours in a bicarbonate saline solution containing 0.5 mM calcium chloride which included a portion of calcium-45 and found that the muscles took up 10 to 25 per cent of the calcium-45. He also followed the loss of calcium-45 into Ringer's solution from muscles loaded with calcium-45. He observed a rapid initial loss which became slow after 1 to 2 hours. The rate of loss was increased when the muscles were placed in calcium ion-free solutions, but returned to the previous slow rate after replacement of the calcium ion.

Harris (34) extended his work further and studied the uptake and loss of calcium-45 in the sartorius muscles of Rana temporaria. He observed an initial rapid uptake which diminished with time. The radioactivity of muscle measured at 16 hours and 20 hours showed no change. In some experiments calcium-45 content became constant after a 1 hour period of exposure. He noted that calcium-45 uptake reaches/

reaches a limit, after which there is no further increase. In experiments in which the muscles were exposed to solutions containing calcium-45 for about 16 hours, it was observed that the exchange between the calcium ions in the solutions and the muscle calcium ions was far from complete. Even exposure to labelled solutions for a period of 60 hours did not bring about any noticeably increased exchange. Observations on the loss of calcium-45 were made upon muscle which had been labelled by exposure for 16 hours at 4°C. The output of calcium-45 was initially rapid but slowed down after about 1 hour. In experiments lasting from 4 to 5 hours there was from 10 to 20 per cent of the original calcium-45 content remaining in the muscle and most of it could not be removed by treatment for 16 hours with ethylenediamine tetra acetate. The output was reduced if the temperature of the system was reduced from the usual 20°C to 0°C. When the muscles were immersed in calcium ion-free solutions there was sometimes a definite acceleration of the efflux but this was variable. In some experiments no acceleration of calcium ion efflux could be detected at all. Acceleration of calcium-45 efflux in calcium ion-free solution was stopped by the addition of 5 mM magnesium ion. Addition of ethylenediamine tetra acetate always caused an increased output. Stimulation at either 15 or 75 impulses per minute did not cause any change in the rate of efflux of calcium-45 from the muscle. From the results of these experiments it was concluded that the muscle calcium was held principally in a bound form, but the strength of the binding forces was variable./

variable.

Gilbert and Fenn (35, 36) studied the uptake of calcium-45 by isolated frog sartorius muscle. Before use the muscles were kept overnight in Ringer's solution at 5°C, followed by standing for 2½ to 3 hours at room temperature. They were then soaked for various periods of time in Ringer's solution containing calcium-45 and then weighed and ashed. The ash was dissolved in water, transferred to planchets and dried. The radioactivity was measured by using a thin mica end window Geiger Müller tube. The radioactivity of the sample was expressed as a percentage of the radioactivity in Ringer's solution. Although the biological variability was fairly large, they observed that the surface phase took up calcium-45 in about 1 minute, the extracellular space and connective tissue in about 30 minutes and the intracellular space in about 300 minutes. The distribution of the total calcium ion in the whole muscle immersed in Ringer's solution was calculated to be 10 per cent in the surface phase, 12 per cent in the extracellular water space, 17 per cent in the dry connective tissue, 24 per cent in the intracellular space and 37 per cent as non-exchangeable calcium ion.

Shanes and Bianchi (37) studied the distribution and kinetics of the release of calcium-45 in tendon and skeletal muscle, using the sartorius muscle and Achilles tendon of the frog (Rana pipiens). The muscles and tendons were mounted on glass rods and soaked for 4 hours in oxygenated Ringer's solution containing 2 microcuries per ml. of calcium-45./

calcium-45. After this period the muscles and tendons were placed at regular intervals in a volume of 2 or 3 ml. of non-radioactive Ringer's solution. The radioactivity of each collection and that of the acid extract of the ashed tissues obtained at the end of the wash out series, were measured with a low background gas flow counter. In some of the experiments Shanes and Bianchi introduced two modifications. In the first of these one member of a pair of muscles which had been soaked in Ringer's solution containing calcium-45, was allowed to lose its calcium-45 into non-radioactive, calcium ion-free Ringer's solution while its partner was kept in normal Ringer's solution. It was observed that an additional fraction of the calcium-45 was retained by the tissue in calcium ion-free Ringer's solution. In the second modification it was noted that in the case of the labelled tissue exposed for a period to calcium ion-free, non-radioactive Ringer's solution, addition of calcium at the normal level caused a rapid additional release of calcium-45. This showed that the retained calcium ion actually consisted of easily accessible, exchangeable bound calcium. From the results of these experiments Shanes and Bianchi (37) concluded that in muscle the exchangeable bound calcium ion is in the connective tissue, on superficial fibre sites and in the myoplasm. About 80 per cent of the superficial sites on the fibres can lose calcium ionsin exchange for other physiological cations when calcium ion is lacking in the medium, the remaining 20 per cent can only exchange with calcium ion.

Cosmos/

Cosmos (38) studied the movement of calcium ions in frog and rat muscles in vivo using calcium-45 as a tracer. Both frogs and rats were injected intraperitoneally with 15.6 microcuries per 100 g. body weight calcium-45 in a 0.7 per cent sodium chloride solution. The injected frogs were stimulated using a Grass stimulator. The duration and voltage employed for each stimulus were adjusted so that each shock elicited a complete extension of the hind legs, followed by a return to the normal sitting position. The duration of the experiment was from 40 to 520 minutes. Control animals were run in a parallel fashion. The animals were weighed before and after stimulation. After the end of the experiment the frogs were pithed, blood was taken from the aortic arches, allowed to clot and then centrifuged. The serum was separated, an aliquot of serum was ashed in a furnace and the calcium estimated. Muscles were removed from the hind legs immediately following the blood sampling. The rats were killed with pentobarbitone at various time intervals from 20 to 1,080 minutes after the time of injection. Blood samples were drawn from heart punctures, following which leg muscle samples were removed. The results show that approximately 400 minutes of periodic stimulation was required for all the muscle calcium ion in the frog to exchange with calcium-45, whereas the rat exchanged all its muscle calcium within 100 to 150 minutes without any artificial stimulation. From the results of these experiments Cosmos (38) concluded that with muscular activity there is increased movement of calcium ion, implying/

implying the conversion of bound calcium ion of the resting muscle to an ionized form, free to exchange with available calcium-45 during the active state of the muscle. Frank (39) also observed that there was an inward movement of the calcium ion when the muscle fibre was depolarized and he suggested that this calcium ion acted as a link between the electrical and mechanical events in muscle contraction.

Woodward (40) studied the release of calcium-45 from muscle during electrical stimulation. He used frog sartorius muscles for his work and immersed them for a period of 3 to 4 hours at 5°C. in Ringer's solution containing 5 microcuries of calcium-45 per ml. The muscles were then removed, rinsed and washed in 5 to 7 successive baths, each containing the same volume of Ringer's solution, the time spent in each bath being the same. After washing, the muscles were stimulated electrically while immersed for the same length of time in a bath of Ringer's solution. It was found that electrical stimulation caused an increase of from 30 per cent to 200 per cent in the amount of calcium-45 released into the bathing medium.

Bianchi and Shanes (41) using the sartorius muscle of the frog (Rana pipiens) and calcium-45 as a tracer studied the calcium ion influx in skeletal muscle at rest, during activity and during potassium ion contracture. They observed that on stimulation of the muscle the influx of calcium ions was almost double the influx in the resting condition. It was also noted that potassium ion contracture markedly augmented the entry of calcium ion into the muscle.

Shanes/

Shanes and Bianchi (42) also studied the calcium ion release by stimulated and potassium ion-treated frog sartorius muscle using calcium-45 as a tracer. They observed that the muscles exhibited an increased release of calcium-45 during the period of stimulation. No difference was seen when the stimulation was at a rate of 20 per sec. or at 0.5 per sec. Prior stimulation, however, appreciably increased the loss per impulse. The release of calcium-45 during potassium ion depolarization depended upon the nature of the contracture. When the contracture was isometric the rate of release was doubled and when it was isotonic the rate of release was four times greater than normal. Shanes and Bianchi pointed out that the close equality of calcium ion entry and exit during stimulation and potassium ion depolarization was not inconsistent with a net entry of calcium ion.

It seems likely that a part at least of the muscle calcium may exchange with the available radioactive calcium and this exchange is enhanced by mechanical activity of the muscle. Both the inward and outward movements of calcium ion during stimulation and potassium ion contracture indicate that calcium ion movement is intimately connected with the process of depolarization.

<u>The effects of calcium ions on nerves, nervous tissue and the functioning of nerves</u>

Brink (43) has reviewed the role of the calcium ion in neural processes and Shanes (44) has reviewed extensively the/

the electrochemical aspects of its physiological and pharmacological action in excitable cells.

Libet and Gerard (45) studied the physico-chemical and nervous factors controlling spontaneous potentials in the olfactory bulb of the isolated frog brain which continued to show its in vivo electrical activity. They observed that moderately increasing the calcium ion content of the Ringer's solution produced slow electrical waves, whereas reducing the calcium ion content produced fast ones - an indication that calcium ions were concerned in the maintenance of normal electrical activity in the olfactory bulb.

Sidney Saloway and his colleagues (46) studied the transport of calcium-45 in chela axons of the crayfish Cambarus virilis. These axons are thinly myelinated and essentially free from connective tissue. The nerves were incubated for a period of 1 hour in a volume of 6 ml. of van Harreveld's solution (47) in which stable calcium was replaced by calcium-45. The nerves were then washed for 10 minutes in a large excess of non-radioactive van Harreveld's solution, after which they were transferred to an aluminium planchet containing 1 ml. of the same solution. The nerves were allowed to remain in the planchet for a length of time sufficient to yield to the surrounding medium an amount of calcium-45 which could be easily measured. They were then transferred to the second planchet and the process repeated. This procedure was continued until a total of about 8 planchets had been employed. The nerves were then coiled at the bottom of the 9th planchet and dried under a lamp along with/

with the entire set of planchets. The radioactivity of each planchet was then measured. The results indicated that the loss of calcium-45 from crayfish nerve could be described by a curve consisting of two exponential terms. Lowering the calcium ion concentration of the bathing medium did not affect the rate of calcium-45 loss. When the temperature of the system was raised to 25°C from the usual 15°C there was either very little or no change in the process of calcium-45 loss.

Hodgkin and Keynes (48) studied the movements of labelled calcium ions in squid giant axons using calcium-45 as a tracer. They observed that depolarization of the membrane by raising the external potassium ion concentration caused a five-fold increase and stimulation at 156 impulses per second a twenty-fold increase in the calcium ion influx.

Frankenhaeuser (49) studied the influence of different external calcium ion concentrations on the membrane action potentials in myelinated frog nerve fibres and observed that the action potential amplitude was smaller when the calcium ion concentration was low.

Dalton (50) while studying the transmembrane potentials in the crayfish giant axons observed that a decrease in the external calcium ion concentration resulted in a decrease in both the resting potential and the action potential.

Adelman and Adams (51) studied the action potential in motor axons from the lobster limbs and observed that upon exposure to a calcium ion-free solution the spike amplitude was/

was rapidly decreased and this was followed within a few minutes by a slow linear decline.

Frankenhaeuser and Hodgkin (52) while studying the action of calcium ions on the electrical properties of squid axons, observed that changes in the calcium ion concentration and changes in the membrane potential had similar effects on the system which allowed sodium ion and potassium ion to move through the membrane during the spike.

Adelman (53) pointed out that if in calcium ion lack a reduction in membrane resistance was assumed, it might be concluded that this means an increased membrane permeability to both sodium ions and potassium ions. Stämpfli and Nishie (54) observed that calcium ion lack enhanced the membrane permeability, particularly for sodium ions but also for potassium ions.

Shanes and his co-workers (55) from experiments on the voltage clamped squid axon suggested that multivalent ions reacted with the cell membrane to reduce the number of sites available for the passage of monovalent ions at lower levels of depolarization - yet at higher levels of depolarization more sites were available. This, they suggested, could be accounted for if calcium ions occupied sites normally available to monovalent ions with activity. Calcium ions interact with the membrane to cause more of such sites to appear and occupy them prior to activity. When depolarization takes place calcium ions leave these sites, which are then free to function in the transfer of monovalent ions.

Dalton/

Dalton and Adelman (56) stated that the reduction in the external concentration of calcium ions produced changes in the lobster axon which could not be accounted for solely on the basis of alterations in the potassium potential. Their results indicated that in low calcium ion solution there was a direct action on the action potential and low external calcium ion affects to some extent the resting potential and the action potential independently, i.e. calcium ions may have a dual role in the production and maintenance of transmembrane potentials. Adelman and Dalton (57) have also concluded that the calcium ion has a direct effect upon the spike-producing mechanism which is mediated through sodium ion, and that there is also direct interaction between calcium ion and potassium ion on the spike generating mechanism.

It seems likely that calcium ion plays a distinct role in maintaining the transmembrane potentials. When the equilibrium is altered by depolarization, permeability to the calcium ion and other cations at the membrane is increased, leading to a change in the ionic environment.

The effects of calcium ions on smooth muscle.

Bacq and Rosenblueth (58) observed that intravenous injection of calcium and potassium ions caused contractions of the non-pregnant uterus of the cat. Dumont and his colleagues (59) found that calcium ion was necessary for the active transport of sodium ion in the rat small intestine. Dowdle and his co-workers (60) and Schachter and his co-workers (61) have shown that transport of calcium ions across the wall of/

of the everted rat gut sac depended on the presence of vitamin D, as did calcium ion accumulation in respiring slices of rat small intestine.

Robertson (62) studied the influence of calcium ion on contractility in depolarized smooth muscle. In a thin preparation of rabbit ileum exposed to calcium ion-free potassium-Ringer's solution, he observed a gradual reduction in the response to acetylcholine, eventually leading to an almost negligible response. There was a quick recovery which was significant at 15 seconds and complete 2 minutes after calcium ion was added to the bathing fluid. This result showed that the presence of calcium ions in the bathing fluid were necessary for isolated smooth muscle, already depolarized by potassium ion, to contract with acetylcholine.

<u>The effects of calcium ions on ganglion cells and the adrenal medulla</u>.

Bronk and his colleagues (64) studied the influence of an altered chemical environment on the activity of ganglion cells. They perfused the stellate ganglion and observed that an increase of calcium ion concentration in the perfusion fluid blocked transmission. When calcium ion concentration was reduced by perfusion with sodium citrate, the cells discharged spontaneously at a time when synaptic transmission was blocked. Harvey and MacIntosh (65) investigated the role of the calcium ion on synaptic transmission in a sympathetic ganglion. They perfused the superior cervical ganglion/

ganglion of the cat and observed that when calcium was omitted from the perfusion fluid the ganglion cells discharged spontaneously and, owing to the failure of the preganglionic impulses to liberate acetylcholine, synaptic transmission failed.

Bacq and Rosenblueth (58) studied the effects of intravenous injection of calcium and potassium ions on the adrenal medulla and nictitating membrane of the cat. Both the ions had a stimulant action on adrenal medullary secretion and a "sympathomimetic" action on the nictitating membrane evoking a contraction.

Katz and Katz (66) studied the effect of potassium and calcium ions on the adrenal medulla of the spinal cat and observed that both caused a discharge of adrenaline.

The influence of drugs upon the flux of calcium ions in skeletal and smooth muscle.

Lister and Lewis (63) found that protoveratrine A caused an increased release of calcium ion from frog sartorius muscle labelled with calcium-45. Robertson (62) observed that acetylcholine markedly increased the uptake of calcium-45 in depolarized smooth muscle.

POTASSIUM

The effects of potassium ions on skeletal muscle.

Potassium is generally acknowledged to be the primary intracellular cation. Skeletal muscle requires a definite concentration/

concentration of potassium ion both intracellularly and extracellularly for optimum physiological functioning. Normally the concentration of intracellular potassium ion is maintained in skeletal muscle at a higher level than in the body fluids. Many investigations (67, 68) have shown that muscular activity leads to a loss of potassium ion from the cell and an entry of sodium ion from the extracellular fluid.

Mitchell and Wilson (69) perfused both legs of pithed frogs through a cannula inserted into the dorsal aorta. The muscles of one leg were subjected to prolonged tetanizing stimuli, firstly by means of the nerve, until they failed to respond, and then by direct stimulation of the muscle until no further response was obtained. The potassium ion content of the muscles of the stimulated leg was considerably less than the potassium ion content of the rested leg.

Hirschfelder and Haury (70) investigated the plasma potassium and magnesium ion levels in clinical "grand mal" epilepsy. In epileptics whose plasma was collected during or just before convulsions, the following deviations from normal values were observed. In five cases of status epilepticus, high potassium was found in five and low magnesium in four. In thirteen epileptics with very severe convulsions high potassium was observed in six and low magnesium in nine. In twelve moderately severe epileptics, high potassium was noted in eight and low magnesium in three. In five epileptics with mild convulsions a high potassium/

potassium level was found in three and low magnesium in two. In seventeen epileptics who were not having convulsions, the levels of plasma potassium and magnesium were normal. McQuarrie (71) also found that the potassium balance was strongly negative during convulsive seizures in epileptic patients. Since most of the potassium ion in the body is within the cells, this marked increase in potassium excretion indicated a leakage through the cell membrane.

Baetjar (72) while studying the diffusion of potassium ionsfrom resting cat skeletal muscle following a reduction in the blood supply, carried out some experiments in which blood samples were collected whilst the muscles were contracting rhythmically as a result of stimulation of the 6th and 7th anterior spinal nerve roots. The potassium ion content of the blood samples collected during the period when the muscles were completely exhausted showed an increase of from 22 to 50 per cent above normal values.

Fenn and Cobb (73) studied the electrolyte changes in muscle during muscular activity in the rat and frog. They stimulated muscle both directly and indirectly and found that in rat muscle stimulation caused an exchange of about 15 per cent of the potassium ion content of the muscle for sodium ion from the extracellular fluid. This change was largely reversible during a recovery period of from 1 to 3 hours. In frog muscles they observed a loss of potassium ion with certainty only when the muscle was stimulated directly.

Fenn (74) studied the loss of potassium ion during voluntary/

voluntary muscular contractions in the rat. One sciatic nerve of the animal was cut and the rat made to swim. Immediately afterwards the muscles were analysed for their potassium ion content. It was observed that the muscular contractions produced a decrease in potassium ion content of the muscles of the healthy limb.

Drahota (75) studied the changes in the potassium ion content of various muscles of the rat immediately after denervation. He observed that after denervation there was a rise in the potassium ion content in the tibialis anterior, extensor digitorum longus, soleus and diaphragmatic muscles of the rat.

Fenn and his colleagues (76) studied the electrolyte changes in cat skeletal muscles during stimulation. After stimulating one leg of a cat for 30 minutes, they analysed the muscles of both legs for potassium, sodium, calcium and magnesium content. The results calculated per 100 g. dry weight of muscle showed a loss of potassium ion which was balanced by a gain in sodium ion. After a recovery period of a few hours, there was a more or less complete reversal of the changes in the electrolytes from that which occurred during stimulation.

Tipton (77) observed that during stimulation, cat muscle lost about 20 per cent of the total potassium ion from the muscle fibres (potassium ion exchanging with sodium ion from the plasma), lost magnesium and gained water, along with sodium, chloride and small amounts of calcium. He further noted/

noted that after 2½ hours of stimulation muscles showed a much greater loss of potassium ion (with an approximately equivalent gain of sodium ion) than did muscles stimulated for 30 minutes or less. During recovery these changes were almost completely reversed. Injection of potassium chloride intravenously increased the loss of potassium ion during activity.

Heppel (78) using young rats (about 5 to 6 weeks old), studied the electrolyte changes in muscle during stimulation. He found that the loss of potassium ion on muscular stimulation increased progressively as the duration of the contraction was lengthened. After 30 minutes there was no further change for at least another half hour. The changes in chloride and water were almost maximal after 5 minutes of stimulation. He noted further that young rats (about 6 weeks old) lost more potassium ion from their muscles on stimulation than adult rats. Feeding with diets low in potassium ion content and poisoning with monoiodoacetic acid had little or no effect on the potassium ion exchanges during stimulation.

Wood and his colleagues (79) studied the exchange of potassium ion during rest, stimulation and recovery by analysing the arterial and venous blood of the isolated dog gastrocnemius muscle perfused by means of a heart-lung preparation. The muscle was stimulated both indirectly and directly. They observed a rapid loss of potassium ion (about 2 mg. per 100 g. muscle) during indirect stimulation for a period of from 100 to 250 seconds. Similar results were/

were obtained by direct stimulation of a normal muscle, a curarized muscle or a muscle to which the motor nerve had been transected 13 days earlier.

Fenn (80) studied the factors which affected the loss of potassium ion from stimulated muscles. He observed that the loss of potassium ion from stimulated muscle was somewhat less when the tendon was cut than when the muscle pulled against an isometric lever at a high initial tension. Reducing the circulation through a stimulated muscle and thus partially asphyxiating it, decreased the loss of potassium ion. It was also decreased if the muscle was tetanized continuously. He concluded that the loss of potassium ion was in general proportional to the magnitude of the contraction but was particularly favoured by rhythmic contractions.

Joseph and his colleagues (81) studied the absorption, distribution and excretion of potassium ions using potassium-42 as a tracer. Rats of either sex weighing between 200 and 300 g. were given potassium-42 by stomach tube or by intraperitoneal injection. After intervals varying from 10 minutes to 22 hours the animals were anaesthetized with chloroform, bled by heart puncture and certain tissues and organs (stomach, small intestine, liver and skeletal muscle) were dissected out for analysis. The radioactivity was extracted by covering the tissue with from 10 to 20 ml. of 20 per cent trichloroacetic acid and grinding it up with clean sand. The filtered extract was transferred to an ashing capsule and evaporated to dryness. The radioactivity of the sample was measured on the electroscope. Extraction/

Extraction was completed by ashing the tissue residue and testing it for any residual activity. Joseph and his colleagues (81) observed that the uptake of potassium-42 by skeletal muscle was slow, but increased continuously for at least 4 hours and remained at a constant level for about 22 hours. The constant rate of increase of potassium-42 in skeletal muscle indicated the participation of the potassium ion in muscle metabolism. Similarly Noonan and his colleagues (82) observed that after subcutaneous or intraperitoneal injection into rats, potassium-42 was absorbed rapidly into most of the tissues of the body. The rate of absorption was high in liver, heart, kidney, lung and gastrointestinal tract, intermediate in muscle and skin and low in the testes, erythrocytes and brain. After equilibrium was established, the bulk of the potassium-42 was found in the muscles. Fenn and his colleagues (83) carried out similar investigations using rabbit, cat and frog for their experiments. In these animals it was also observed that the visceral organs took up potassium-42 more rapidly than skin, muscle, testes or brain. These experiments indicated that the potassium-42 was probably taken up quickly by the visceral organs from the plasma and then slowly transferred to the muscles.

Ginsburg and Wilde (84) studied the time course of the specific activity of potassium-42 in a number of organs in rats. They found that kidney, lung and intestine equilibriated very rapidly with plasma in about 4 to 10 minutes; liver, skin and spleen exchanged at an intermediate rate reaching equilibrium with plasma in about 100 minutes; muscle and testes/

testes exchanged slowly reaching equilibrium in about 600 minutes and brain and erythrocytes exchanged very slowly. Since the specific activities of all the organs studied either equilibriated with or continued to approach equilibrium with plasma, it indicated that all the body potassium ion was exchangeable.

Abbott (85) investigated the potassium exchange using frog muscles. He used sartorius, semimembranosus, extensor digitorum longus and other foot muscles for his work. The muscles were soaked for a few hours in a physiological saline of known potassium ion content with a trace of potassium-42. The muscles were then washed for one minute in a similar but non-radioactive solution. Finally the muscles were dissolved in acid. The radioactivity of the dissolved muscles was compared with that of a known dilution of the soaking medium. It was observed that the degree of potassium ion exchange attained by the different muscles was the same when similar experimental conditions were applied.

Harris (86) also studied the exchange of frog muscle potassium ion using potassium-42 as a tracer. He used the sartorius, semimembranosus and semitendinosus muscles for his experiments. A muscle was exposed for about 4 hours to a solution containing a portion of potassium-42 and a number of readings of radioactivity, giving a measure of the potassium ion exchanged, were made during the period. The muscle was then put into a solution chemically identical and maintained at the same temperature but free from potassium-42. The loss/

loss of radioactivity was measured for a further period of 4 hours. It was observed that most of the potassium ion in frog muscle was exchangeable.

Keynes (87) studied the uptake of potassium ion using potassium-42 as a tracer. He used the extensor digitorum longus and sartorius muscles of the frog for his work. It was observed that the uptake of potassium-42 followed an exponential course, the time constant of which was close to that for the subsequent release of potassium-42. A final steady state appeared which corresponded to a virtually complete exchange of the internal potassium ions.

Creese (88) studied the cation fluxes in resting rat diaphragm strips using potassium-42 as a tracer. He observed that the potassium ion content of the diaphragm strip was entirely exchangeable.

Creese and his colleagues (89) studied the movements of potassium ions in the contracting rat diaphragm. Diaphragm muscle was soaked for from 2 to 4 hours in saline containing potassium-42. It was then transferred to a holder which was suitably wired for direct stimulation of the muscle. The radioactivity of the saline coming from the muscle after stimulation was measured. It was observed that when the muscle was stimulated at a frequency of 2 impulses per second, there was an increase of about 23 per cent in the rate of outward movement of potassium-42.

It appears from the findings of various workers that almost all the potassium ion content of the muscle is exchangeable/

exchangeable with radioactive potassium. Contraction of muscle seem to be associated with a distinct loss of potassium ion and this indicates that extrusion of potassium ion is a factor in the process of depolarization.

The influence of drugs upon the flux of potassium ions in skeletal muscle.

Hardt and Fleckenstein (90) carried out experiments to find whether potassium ion was liberated when there was a muscle contraction induced by substances such as caffeine, chloroform, tribromethanol and nicotine, or by heat and to see whether local anaesthetics, which under certain conditions prevented contraction, exerted any influence upon the liberation of potassium ions. Hardt and Fleckenstein used frog gastrocnemius muscle for their work. The muscles after being removed from the frogs were immersed for 1 hour in saline, after which an isotonic lever was attached to the muscle and the latter immersed in saline for a further period of 20 minutes. Finally the muscle was immersed in saline containing the drug for a further period of 20 minutes. The resulting contraction was recorded on a slowly moving surface. The amount of potassium ion which diffused outwards in the second and third immersions was measured. It was observed that the drugs tested caused a release of potassium ion from isolated frog muscles. When the muscles were previously treated with local anaesthetics to the point when no contraction could be produced, there was no release of potassium ion. When the muscles were suddenly transferred from saline at $18^{o}C$/

18°C to saline at a higher temperature, contracture occurred from about 30°C upwards. The muscles also released potassium in this process. Hardt and Fleckenstein concluded that during muscular contraction, the mechanical process of shortening was closely associated with liberation of the potassium ion. Potassium ion liberation began with the onset of shortening and increased with the increase in the amplitude of contraction. Local anaesthetics which inhibited contraction had reinforcing and stabilising actions on the muscle membrane which were opposite to the drugs tested. By stabilising the superficial layers the local anesthetics suppressed the process which underlies every stimulus - membrane depolarisation.

Klupp and Kraupp (91) investigated whether depolarisation of the end plate region induced by substances similar to decamethonium was also associated with release of potassium ions from muscle. They used suxamethonium, decamethonium and octamethylbiscarbaminoylcholine for their work. Both conscious and chloralose-anaesthetised dogs were used for the experiments. Blood samples were collected by means of a needle inserted into the saphenous vein. After collecting two control specimens of blood the drug was injected intravenously. Starting 3 minutes after injection, 5 ml. of blood was collected on each occasion at intervals of from 30 to 40 seconds. Further blood samples were collected at longer intervals (3 to 5 minutes) for 1 hour after injection of the drug. The anaesthetised animal had a trachea cannula inserted half an hour before the injection of the drug and was/

was on artificial respiration throughout the experiment. Immediately after the experiment all blood samples were centrifuged and the potassium ion content of the blood plasma estimated by flame photometry. They observed an average increase of from 3 to 4 mg. per cent of blood potassium ion in the first few minutes after injection of the drug. During the course of the next few minutes the blood potassium ion values were diminished, but there was a second increase from the 5th or 6th minutes. This rise in blood potassium ion content persisted for some time and returned to normal in about 30 to 50 minutes. Klupp and Kraupp could not exclude the possibility that the rise in the blood potassium content in the first few minutes was not due to restlessness or temporary asphyxia after injection of the drug. To avoid this possibility they carried out further experiments in which they used only chloralose-anaesthetised dogs. The animals were anaesthetised 2 hours earlier and put on artificial respiration 30 minutes before the beginning of the experiment. It was found that injection of the drugs caused a rise in the blood potassium ion level. In these experiments they also observed a biphasic increase in the blood potassium ion level.

To measure a possible increase in the excretion of potassium ion after administration of the drug, a constant osmotic diuresis was produced in dogs by slow intravenous injection of 25 per cent mannitol solution. The speed of infusion was so regulated that a constant flow of urine occurred per minute. It was observed in these experiments that/

that shortly after administration of the drug there was an increase in the excretion of potassium ions, which returned to normal in an exponential manner.

To estimate the amount of potassium ion liberated, both hind limbs of cats under chloralose anaesthesia were perfused through the aorta with a blood-Tyrode's mixture, or with unmodified Tyrode's solution. The upper half of the animal was separated from the perfused preparation by a crush clamp and drugs were injected into the aortic cannula. The potassium ion content of the perfusate was estimated at intervals of 30 seconds or 1 minute. After injection of the drug there was a considerable increase in the potassium ion content of the perfusate. From these experiments Klupp and Kraupp concluded that the depolarising neuromuscular blocking agents caused a release of potassium ions from the musculature.

Kraupp and his colleagues (92) also studied the liberation of potassium ions from innervated and denervated skeletal muscle following intra-arterial injection of acetylcholine. One extremity of a cat was aseptically denervated 8 to 12 days before the experiment by cutting the sciatic and femoral nerves. The hindquarters of the animal were perfused with a blood-Tyrode's mixture through a cannula inserted into the aorta. The perfusate was collected separately from the two iliac veins every 2 to 4 minutes and centrifuged immediately. The potassium ion content of the supernatant fluid was estimated by means of the flame photometer. Acetylcholine was injected into the aortic cannula. After intra-arterial injection of acetylcholine an increased release/

release of potassium ion was found both in the innervated and in the denervated extremities.

Kraupp and his colleagues (93) studied the influence of tubocurarine and eserine on the acetylcholine-induced release of potassium ion from the skeletal musculature of the cat using a similar experimental preparation, and observed that eserine caused a lowering of the threshold dose of acetylcholine for liberation of potassium ion. Tubocurarine either caused suppression of potassium ion liberation or increased the threshold dose of acetylcholine for the liberation of potassium ion.

Werner and his colleagues (94) studied the influence of tetramethylammonium bromide on the liberation of potassium ion from skeletal muscle in a similar experimental preparation. They administered tetramethylammonium bromide either as a single dose into the aortic cannula or by dissolving it in the perfusing fluid in varying concentrations. They observed a demonstrable release of potassium ion from the musculature after a single injection of tetramethylammonium bromide. When perfusion was carried on using a solution containing different concentrations of tetramethylammonium bromide, there was an increase in the release of potassium ionswhich reached a maximum in about 10 to 15 minutes.

Kraupp and his colleagues (95) also studied the resting potential of the gracilis muscle and the arteriovenous concentration difference for potassium ion in the isolated perfused/

perfused hind limbs of cats. After a control perfusion with Tyrode's solution, decamethonium bromide was added to the perfusion fluid in various concentrations. It was observed that decamethonium caused a significant depolarization of the end plate-rich parts of the gracilis muscle (from 91 mV. before to 74 mV. 10 to 40 minutes after the start of the decamethonium perfusion). In all the experiments, depolarization was accompanied by a release of potassium ion from the hind limbs. Both potassium ion release and depolarization followed the same time course.

Fenn and his colleagues (96) investigated the influence of the neuromuscular blocking agents - tubocurarine and dihydro- β -erythroidine, along with mephenesin and acetylcholine - on the release of potassium ion from perfused and isolated frog muscles. In these perfusion experiments a cannula was inserted into the abdominal aorta and perfusion carried out using either 3 per cent acacia (dialysed) in Ringer's solution also containing 10 per cent of washed beef red blood cells, or with unmodified Ringer's solution. The renal portal vein was tied off and all the perfusate from the legs collected by means of a cannula in samples of about 2 to 3 ml. These were centrifuged and analysed for potassium ion content by the flame photometer. Drugs were added to the perfusing fluid. Fenn and his colleagues used the sartorius, semitendinosus or similar small muscles for their work. The muscles were immersed for varying periods of time in 200 ml. of Ringer's solution with or without the addition of drugs. Finally the muscles were/

removed, blotted gently, weighed, ashed and analysed for potassium ion content. It was observed that tubocurarine had no influence on the release of potassium ion from muscle tissue. Similar results were obtained with dihydro-β-erythroidine and mephenesin. They noted, however, a small release of potassium ion from muscle tissue after treatment with acetylcholine.

There are also references in the literature indicating that the Veratrum alkaloids cause a release of potassium ion from skeletal muscle (97, 98).

SODIUM

The effects of sodium ions on skeletal muscle

Overton (99) in 1902 found that frog muscles became inexcitable when they were immersed in solutions containing less than 10 per cent of the amount of sodium ion normally present in Ringer's solution. He also showed that chloride ions were not an essential constituent of Ringer's solution since excitability was maintained in solutions of sodium nitrate, benzoate, sulphate or phosphate. Hagiwara and Watanabe (100) observed that in the absence of sodium ions, choline and tetramethylammonium were not completely effective in restoring transmission in frog sartorius muscle fibres. The experiments of Koketsu and Nishi (101, 102) on neuromuscular transmission in frog muscle-nerve preparations in sodium ion-free media, suggested that the hydrazinium ion might act as a substitute for sodium ion, but the presence of calcium ionsin the medium was essential for the effective restoration/

restoration of neuromuscular transmission.

Nastuk and Hodgkin (103) studied the electrical activity of single muscle fibres taken from the frog sartorius muscle (Rana temporaria). They recorded the resting potential and the action potential of the muscle fibres. The muscle was first immersed in Ringer's solution and electrical changes recorded from 6 to 12 fibres, after which a modified Ringer's solution in which 80 per cent of the sodium ion was replaced by choline was run into the bath. The bath was washed thoroughly with the new solution and a period of 5 minutes allowed for equilibration. At the end of this time, records of action potential and resting potential were taken from 6 to 12 muscle fibres at intervals of from 1 to 2 minutes. Unmodified Ringer's solution was re-introduced after the muscle had been immersed in the test solution for about 20 minutes. A period of 5 minutes was again allowed for equilibration and a further set of records obtained. It was observed that the resting potential increased slightly in the sodium-free solution, but the action potential was greatly reduced and was consistently less than the resting potential. The values obtained during the final period in unmodified Ringer's solution showed that the effect was reversible. The action potential recovered promptly. From the results of this experiment Nastuk and Hodgkin concluded that the action potential was due to a specific increase in the permeability to sodium ion.

The effects of potassium and sodium ions on the functioning of nerves

Keynes/

Keynes and Lewis (104) studied the exchange of potassium-42 in resting crab nerve. They made parallel determinations of the potassium-42 exchanged and the total potassium ion content of nerves soaked for long periods in Ringer's solution containing a proportion of potassium-42. It was observed that about 97 per cent of the intracellular potassium ion exchanged readily with potassium-42.

Cowan (105) observed that when Maia nerves were stimulated in a small volume of sea water there was a definite loss of potassium ion from the nerve. The loss continued at a slower rate after the stimulation had been stopped.

Young (106) studied the effect of stimulation on the potassium ion content of Limulus leg nerves. He found a loss of 6 to 7 per cent of potassium ion during a tetanus of 15 minutes duration.

Arnett and Wilde (107) showed that stimulation of frog nerve for 60 to 210 minutes caused an appreciable leakage of potassium ion.

Asano and Hurlbut (108) observed that stimulation of intact or desheathed frog sciatic nerves produced an increase in the sodium ion content and a decrease in the potassium ion content of this tissue.

Keynes (109) studied the loss of potassium-42 from stimulated crab nerve. He observed that when nerves previously loaded with potassium-42 were stimulated in non-radioactive Ringer's solution there was a marked increase in/

in the rate at which potassium-42 was lost. When stimulation was carried on at 50 impulses per second there was an approximately tenfold increase in the loss of potassium-42 over the resting value.

Keynes (110) also studied the movements of potassium-42 and sodium-24 in resting and stimulated nervous tissue, using Carcinus and Sepia axons for his work. He observed an influx of sodium-24 and an efflux of potassium-42 from nerve during stimulation and concluded that during the rising phase of the action potential the membrane becomes highly and specifically permeable to sodium-ions.

Keynes and Lewis (111) studied the sodium ion and potassium ion content of cephalopod nerves. They applied the technique of activation analysis to make simultaneous determinations of the sodium ion and potassium ion content of single axons. The nerves were irradiated in a neutron pile and the amount of sodium-24 and potassium-42 which had been formed were estimated by taking beta and gamma counts of the irradiated samples using the appropriate filters. Comparison of the ionic contents of the resting and stimulated Sepia axons showed that during activity there was a net entry of sodium ion and a net loss of potassium ion. Similar results were obtained by analysis of samples of extruded squid axoplasm.

Keynes (112) further studied the ionic movements during nervous activity in Sepia axons using sodium-24 and potassium-42 as tracer substances. He obtained the average values/

values for inward and outward movements of sodium-24 and potassium-42 under resting conditions and compared these values with those obtained during stimulation. It was observed that during stimulation the inward sodium ion flux increased 18 times and the outward potassium ion flux increased 9.1 times.

Erlanger and Blair (113) studied the influence of isotonic, salt-free solutions on conduction in medullated nerve fibres. They used the spinal roots of the bullfrog for their experiments and substituted isotonic glucose solution for a salt solution. They recorded the action potential in the spinal root and observed that conduction in the spinal roots of the bullfrog was blocked in a few seconds by treatment with isotonic glucose solution. The block, when it had not been maintained for too long, was reasonably well reversed following treatment with Ringer's solution. By recording the action potential of single fibres in the spinal root it was found that topical application of glucose solution to short stretches of the spinal root 2 to 3 mm. apart, caused block at a definite locus within a few seconds. It was concluded that the block by glucose solution was due to reduced excitability. Block from excessive treatment with glucose solution could not be reversed with Ringer's solution but could be effectively reversed by treatment with isotonic sodium chloride solution.

Huxley and Stämpfli (114) studied the saltatory conduction in peripheral myelinated nerve fibres. They used single myelinated fibres isolated from the sciatic nerves/

nerves of large specimens of Rana temporaria and Rana esculenta for their experimental work. It was observed that in freshly dissected fibres conduction was blocked within one second if the Ringer's solution surrounding the fibre was replaced by an isotonic sugar solution.

Hodgkin and Katz (115) studied the influence of sodium ion concentration on the form and size of the action potential recorded with an internal electrode in the giant axons of the squid. They observed that the action potential was abolished in sodium-free solutions, but returned to its former value when sea water was replaced. When sea water was diluted with isotonic dextrose solution there was a large and reversible decrease in the height of the action potential. The height of the action potential was increased by a hypertonic solution containing additional sodium chloride, but was not increased by addition of dextrose to the sea water. They noted that the changes produced by dilution of the sea water with isotonic dextrose solution were caused by reduction of the sodium ion concentration and not by alterations in the concentration of other ions. It was concluded that the reversal of the membrane potential during the action potential could be explained if it was assumed that the permeability conditions of the membrane in the active state were the reverse of those in the resting state. The resting membrane was taken to be more permeable to potassium ions than to sodium ions and the active membrane to be more permeable to sodium ions than to potassium ions.

Huxley/

Huxley and Stämpfli (116) studied the effect of potassium ions and sodium ions on the resting potential and action potential of single myelinated frog nerve fibres. At the beginning of the experiment they measured the values for the resting and action potentials in normal Ringer's solution. After these measurements had been made the Ringer's solution was replaced by a series of solutions containing different concentrations of potassium ions and sodium ions. It was observed that lowering the potassium ion concentration caused an increase and raising the potassium ion concentration a decrease in both the resting potential and the "overshoot" (excess of action potential over resting potential). When part of the sodium chloride in the Ringer's solution was replaced by choline chloride it was found that the "overshoot" was markedly decreased while the resting potential was slightly raised.

Hodgkin (117) suggested that the active depolarization of a nerve fibre was determined by an entry of sodium ion. Hodgkin and his colleagues (118) further suggested that depolarization increased the permeability to potassium ions as well as the permeability to sodium ions. There was, however, an important difference in the time scale. When the membrane was suddenly depolarized the permeability to sodium ions increased rapidly and reached its maximum in a fraction of a millisecond. On the other hand the permeability to potassium ions did not increase instantaneously, but required 1 or 2 milliseconds to reach its maximum. The two processes might be sufficient to account for the rise and fall/

fall of the action potential. During the rising phase the inward sodium current exceeded the outward potassium current with the result that the membrane was depolarized. Later the potassium ion permeability rose to a high value and the outward potassium current became greater than the inward sodium current. The membrane potential was, therefore, restored to its resting value and the sodium permeability thus reduced to its normal level.

The effects of potassium and sodium ions on the functioning of other organs.

Kehar and Hooker (119) studied the potassium ion content of the perfusate of normally beating and artificially fibrillating dog hearts. It was observed that while the potassium ion content of the perfusate was unchanged by passage through the normally beating heart, immediately after fibrillation was induced by tetanic stimulation of the ventricles there was an appreciable increase in the potassium ion content of the outflowing perfusate.

Conn and Wood (120) studied the sodium ion exchange in the isolated heart of the normal dog using sodium-24 as a tracer, and observed that sodium ion entry into the cell occurred almost entirely during the spike potential and was negligible during the resting potential and the remainder of the action potential.

Durbin and Jenkinson (121) studied the effect of carbachol upon potassium ion flux in depolarized smooth muscle using the taenia coli of the guinea-pig for their work./

work. They observed that after a brief initial period the loss of potassium-42 from the tissue followed a simple exponential curve. When carbachol was applied there was a marked increase in the rate of efflux. Atropine was found to abolish reversibly this effect as well as the carbachol contracture.

In recent years there has been a great deal of progress in the understanding of the physical and biochemical changes that occur during muscular activity. Dale and his colleagues (122) showed that stimulation of motor nerve fibres to perfused voluntary muscles caused the appearance of acetylcholine in the venous fluid. Direct stimulation of a normal muscle, or of one deprived only of its autonomic nerve supply had a similar result, but when the muscle was completely denervated no acetylcholine appeared in response to stimulation. When transmission of excitation from nerve to the perfused muscle was prevented by curarine, stimulation of the motor nerve caused the usual release of acetylcholine. When conduction from the motor nerve fibres to the perfused muscle failed from exhaustion, following prolonged stimulation, acetylcholine was no longer released by stimulation of either motor nerve or muscle. They concluded that acetylcholine acted as the direct stimulant of the nerve or of the muscle end plate, releasing essentially new propagated waves of excitation in nerve or muscle fibres.

Cowan (123) suggested that in the nerve-muscle preparation acetylcholine liberated at the nerve ending initiated/

initiated a propagated response in the muscle fibres by producing a local depolarization. Similarly Eccles and his co-workers (124) also suggested that the chemical transmitter - acetylcholine - caused a transient depolarization of the muscle membrane.

Fatt and Katz (125) observed that the depolarizing effect of the transmitter - acetylcholine - led to a change of potential across the muscle membrane. This was responsible for the initiation of the propagated muscle action potential which produced the muscular contraction.

Hodgkin (126) stated that the action potential depended on a rapid sequence of changes in the permeability to sodium and potassium ions. The resting membrane behaved as though it were 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 depolarized. Sodium ions, therefore, entered the fibre at a high rate and reversed the potential difference across the cell membrane. This increase in the permeability to sodium ions was followed by a similar rise in the permeability to potassium ions. Entry of sodium ions 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 the immediate source of energy for the propagation of the impulse./

impulse.

Katz (127) summarized the complex mechanism of muscle contraction. He stated that the stimulus from the nerve was received by the muscle membrane and transmitted to the contractile substance. A special situation existed at the myoneural junction where a large post-synaptic cell was triggered into activity by an impulse arriving in the small pre-synaptic axon branches. Direct local circuit transmission came to a halt at this point and was replaced by chemical transmitter action. Acetylcholine was released, in discreet quantal parcels, from the nerve endings and acting on receptors upon the external surface of the end plate membrane, caused a large increase of permeability to sodium, potassium and possibly other free ions. This gave rise to local currents of sufficient intensity to depolarize and excite the surrounding area of the muscle fibre and so initiated a new propagated impulse.

Much work on the physiology of neuromuscular transmission has been carried out using neuromuscular blocking agents. A neuromuscular blocking agent may be defined as a drug which interferes with the transmission of the nerve impulse through the neuromuscular junction to the muscle fibres, without modifying conduction in the nerve, and without affecting the twitch tension in response to direct stimulation of the muscle. Clinically useful neuromuscular blocking agents are all quaternary bases. Attempts have been made by various workers (128, 129, 130, 131) to classify the neuromuscular/

neuromuscular blocking agents. The method of classification proposed by Paton and Zaimis (128) distinguishes three types of neuromuscular block; (i) competitive block, (ii) depolarization block and (iii) mixed or intermediate block.

Competitive neuromuscular block is caused by drugs which produce their effects by competing with acetylcholine for receptors at the end plate region. The end plate potential is gradually diminished in amplitude and shortened in duration. Eventually the amplitude of the end plate potential is insufficient to initiate a propagated muscle action potential and thus contraction does not occur. A large number of naturally occurring and synthetic substances of this type are known. Tubocurarine is generally considered to be the typical representative of this group, but compounds which have a more purely non-depolarizing action are known.

Depolarization block may be contrasted with that caused by competition by virtue of the fact that depolarizing drugs produce a persisting partial depolarization of the motor end plate similar to that caused by acetylcholine when it is protected from hydrolysis by an anticholinesterase. The amplitude of the end plate potential is thereby reduced to below that necessary for the initiation of a propagated electrical response in the muscle fibre, and thus contraction does not occur. On the basis of its action on mammalian (cat), avian (chick) and amphibian (frog) muscle, decamethonium may be taken as being representative of the group of drugs which produce neuromuscular block by depolarization, although suxamethonium is thought to be more typical/

typical by some. End plate depolarization caused by a depolarizing drug is associated with an initial phase of excitation. During this phase there is seen transient muscular fasciculations. As depolarization persists the phase of increased excitability soon passes over into one of inexcitability.

The properties of drugs producing mixed or intermediate types of block are different from those described. The block produced by this type of drug is not typical of either competitive block or of depolarizing block. As an example of this group the properties of tridecamethonium may be considered. Tridecamethonium is a higher member of the methonium series. When injected into chicks it produces at first a contractural response followed slowly by a type of paralysis in which the legs are contracted but the head and neck are flaccid. Finally there appears a typical generalized paralysis.

Foldes (129) in 1954 suggested that neuromuscular blocking agents could be divided into two instead of three groups; (i) anti-depolarizing agents corresponding to the competitive agents in the classification of Paton and Zaimis (128) and exemplified by tubocurarine and (ii) depolarizing agents, e.g. decamethonium. Foldes observed that the anti-depolarizing agents produced an anti-depolarization block at the neuromuscular junction of all the species investigated but the type of neuromuscular block caused by the depolarizing agents depended on the properties of the receptor/

receptor protein of the species in question. In avians, amphibians and some mammals (e.g. cat) where the configuration of the receptor protein could be changed relatively easily, depolarizing agents (e.g. decamethonium) caused depolarization block. In other mammals (e.g. monkey) where the receptor protein was less inclined to configuration changes, decamethonium caused an anti-depolarization block.

Bovet and his co-workers (130) proposed a method of classification of neuromuscular blocking agents on the basis of their molecular shape. He called molecules such as decamethonium leptocurares, long, thin molecules and those bigger, bulkier molecules such as tubocurarine pachycurares, i.e. thick molecules.

Van Rossum, Ariëns and Linssen (131) noted that both the depolarizing and competitive neuromuscular blocking agents had affinity towards specific acetylcholine receptors, but there was a difference in intrinsic activity. The depolarizers, including acetylcholine had a high and the competitors a very low intrinsic activity. Non-depolarizing drugs such as dodecyltrimethylammonium were not antagonized by neostigmine while their action was synergistic with the depolarizers. The latter finding was interpreted as a non-competitive inhibition of acetylcholine.

On the basis of this concept three types of curariform drugs with shades of activity in between have been suggested:-
Type I. The depolarizers, which may also be called cholinomimetics./

cholinomimetics. This group induced a flaccid paralysis in most mammalian muscles which was not reversed by neostigmine. In avian muscles they induced a spastic paralysis and in the isolated frog rectus abdominis muscle a contracture.

Type II. The curarimimetics, cholinolytics or competitors. This group induced a flaccid paralysis of both mammalian and avian muscles which was easily reversed by neostigmine.

Type III. The non-competitive antagonists of acetylcholine which may also be called non-competitors. This group induced a flaccid paralysis of both mammalian and avian muscle which was not reversed by neostigmine.

Neuromuscular blocking agents have been used to study the flux of potassium ions in mammalian and in frog skeletal muscle. Depolarizing neuromuscular blocking agents, e.g., suxamethonium and decamethonium were found to cause an increased release of potassium ion from the skeletal muscles of the dog and the cat (91, 95). Similar results were obtained using other depolarizing agents, e.g., acetylcholine (92, 96) and tetramethylammonium bromide (94). Competitive neuromuscular blocking agents, e.g, tubocurarine and dihydro-β-erythroidine did not on the other hand show any such effect (93, 96). Tubocurarine either caused a suppression of potassium ion liberation or increased the threshold dose of acetylcholine required for the liberation of potassium ions (93).

Wilson and Wright (132) have demonstrated in 1936 that potassium ions antagonized the neuromuscular block induced by

by curare, but Paton and Zaimis (133) did not observe such an effect in the neuromuscular block produced by decamethonium.

Since potassium ions are capable of causing depolarization of the end plate membrane (116) it is not surprising that they should show antagonism to a competitive neuromuscular blocking agent and be ineffective against (or even enhance) a depolarization block. There is no doubt that the processes of depolarization and contraction imply a flux of potassium ions as an integral part of the physiological mechanism but we do not yet understand fully the mechanisms which control this flow and other ionic fluxes. The study to be described is partly directed towards this end.

It is a very old observation that the potassium ion of living organisms is largely confined to the interior of the cells (134), very little being present in the extracellular spaces, whereas the opposite is true with respect to sodium. This rule for the distribution of the two ion species holds true in the case of nearly all living cells, microorganisms, plant cells and animal cells alike.

The nature of the forces maintaining the intracellular potassium at a high and relatively constant level has been the subject of much speculation in the past, and even now no general agreement has been achieved. Ussing (135) has summarised the hypotheses advanced to explain the phenomenon:-

(i) The cell membrane is impermeable to potassium.

(ii) The potassium ions are free in the cell interior but are kept from leaving the cell by the electric potential/

potential difference between the cell and its surroundings (Donnan equilibrium).

(iii) The potassium ions are chemically bound by, or adsorbed on to non-diffusible cell constituents.

(iv) The potassium ions are supposed to be free in the cell interior but are transported actively inward through the cell membrane.

The low concentrations of sodium ion inside most cells is assumed to be due to active transport of this ion in the outward direction ("sodium pump").

The greatest amount of calcium in the body is present in the skeleton. Under conditions of inadequate calcium intake, increased calcium requirements or abnormal calcium metabolism, the bones give up calcium to the blood. There are references in the literature (35, 36) which suggest that at least part of the calcium in the muscle is intracellular.

Muscle fibres share with most other cells the property of having a continuous exchange of sodium as well as of potassium with the surrounding medium. But besides these resting exchanges, muscle, like other excitable tissues, including nerve and electric organs, exhibits grossly increased potassium and sodium shifts during activity. The electrical manifestations of activity are associated with a net loss of potassium and a net gain of sodium. During recovery the ionic composition of the fibres is reconstituted.

It is generally assumed that the active state and the associated action potentials of nerve and muscle arise as the/

the consequence of a sudden breakdown of the potassium selectivity of the membrane so that the other ions can move through freely. In recent years the experiments of various workers (38-42, 136) have indicated that activity in muscle is associated with increased movements of calcium ions both in the inward and the outward directions.

Considering these views it was thought that it would be fruitful to carry out some investigations on the uptake and release of calcium ion in a simple preparation such as the frog sartorius muscle, using drugs with known pharmacological properties and comparing calcium with potassium and sodium ion movements.

Drugs having depolarizing properties, e.g., suxamethonium, decamethonium and nicotine, drugs having mainly muscarinic properties, e.g., carbachol, drugs having anticholinesterase activity such as neostigmine and edrophonium and drugs producing contracture in skeletal muscle, including ryanodine, were selected for the study. Ryanodine is an alkaloid obtained from Ryania speciosa Vahl. The most remarkable property of this compound is its ability to induce an irreversible contracture in mammalian skeletal muscle and smooth muscle and in frog muscle (137-140). It is highly toxic and used as an insecticide.

An attempt was made to compare the effects of these drugs with that of the non-depolarizers (competitors) tubocurarine and gallamine.

Two Veratrum alkaloid esters, protoveratrine A and protoveratrine B/

protoveratrine B were also included in the investigation because of the characteristic veratrinic response (production of a secondary tetanus, following the normal twitch induced by single direct or indirect shocks) to Veratrum alkaloids by skeletal muscle.

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CHAPTER II

A. MATERIALS

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

(i) Succinylcholine chloride dihydrate is described as suxamethonium.

(ii) Decamethonium iodide is described as decamethonium.

(iii) Neostigmine methylsulphate is described as neostigmine.

(iv) Tubocurarine chloride is described as tubocurarine.

(v) Gallamine triethiodide is described as gallamine.

(vi) Nicotine hydrogen tartrate is described as nicotine.

Radioactive materials for these investigations were obtained from the Radiochemical Centre, Amersham. Calcium-47 was obtained as calcium-47 chloride in sterilised isotonic saline and had an activity of approximately 0.02 mc. per ml. at the time of despatch from Amersham. Potassium-42 was obtained as sterilised isotonic solution of potassium-42 chloride and had an activity of approximately 0.10 mc. per ml. at the time of despatch from Amersham. Sodium-24 was obtained as sodium-24 chloride in serilised isotonic saline and had an activity of approximately 1.0 mc. per ml. at the time of despatch from Amersham.

The/

The following drugs and their controls were obtained as commercial solutions:-

(i) Suxamethonium,
(Scoline, Allen & Hanburys Ltd.).

(ii) Decamethonium,
(Eulissin A, Allen & Hanburys Ltd.).

(iii) Neostigmine,
(Prostigmin, Roche Products Ltd.).

(iv) Edrophonium,
(Tensilon, Roche Products Ltd.).

(v) Tubocurarine,
(Tubarine, Burroughs. Wellcome & Co.).

(vi) Gallamine,
(Flaxedil, May & Baker, Ltd.).

The following drugs were obtained in crystalline form:-

(i) Nicotine
(ii) Carbachol
(iii) Ryanodine
(iv) Protoveratrine A
(v) Protoveratrine B

Nicotine, carbachol and ryanodine were soluble in water. In Appendix II, page 395 , are detailed the methods used in preparing solutions of protoveratrine A and protoveratrine B.

The composition and methods of preparation of physiological saline solutions used in this investigation are to be found in Appendix II , page 393 .

B./

B. EXPERIMENTAL

Frog sartorius muscle was used in the investigation. Experiments were designed to study the uptake of calcium-47, potassium-42 and sodium-24 by isolated muscle, and the release of calcium-47 and potassium-42 from similar muscle under the influence of drugs. The experiments fall into the following groups:-

(1) Investigation of the effect of drugs on calcium-47 uptake by isolated frog sartorius muscle.
(2) Investigation of the effect of drugs on potassium-42 uptake by isolated frog sartorius muscle.
(3) Investigation of the effect of drugs on sodium-24 uptake by isolated frog sartorius muscle.
(4) Investigation of the effect of drugs on calcium-47 release from isolated frog sartorius muscle.
(5) Investigation of the effect of drugs on potassium-42 release from isolated frog sartorius muscle.

1. Investigation of the effect of drugs on calcium-47 uptake by isolated frog sartorius muscle.

METHOD

The method used was based on that described by Lister and Lewis (1). Common frogs (Rana temporaria) unselected as to age or sex and weighing from 25 to 70 g. were stunned by a sharp blow on the base of the head and decapitated with scissors. A pithing needle was passed down the spinal canal to destroy this and to prevent reflex movements. The skin covering/

covering both the thighs and 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 as close to the pelvis as possible. The muscles were blotted with absorbant tissues and then rapidly weighed on a torsion balance. With very careful dissection it was found that paired muscles could be obtained differing in weight from one another by less than 1 mg. The muscles were then suspended by means of two stainless steel hooks in centrifuge tubes containing 10 ml. of frog Ringer's solution at room temperature, in which part of the stable calcium chloride was replaced by calcium-47 chloride. One hook was fastened by a thread to a wire stirrup fitted across the top of the centrifuge tube. The other hook consisted of a No. 20 hypodermic needle which was bent into the required shape (Fig. 1 , page 84). The control muscle was placed in the radioactive frog Ringer's solution and the test muscle was placed in the radioactive frog Ringer's solution to which the drug had been added. The radioactive bathing solutions were continuously agitated and oxygenated by a stream of oxygen. The muscles were left immersed for 30 minute periods, they were then removed from the radioactive bathing solution and each side of the muscle washed by a stream of non-radioactive frog Ringer's solution and lightly blotted with absorbant tissues to remove adhering moisture. The hooks used for suspending the muscle in the radioactive/

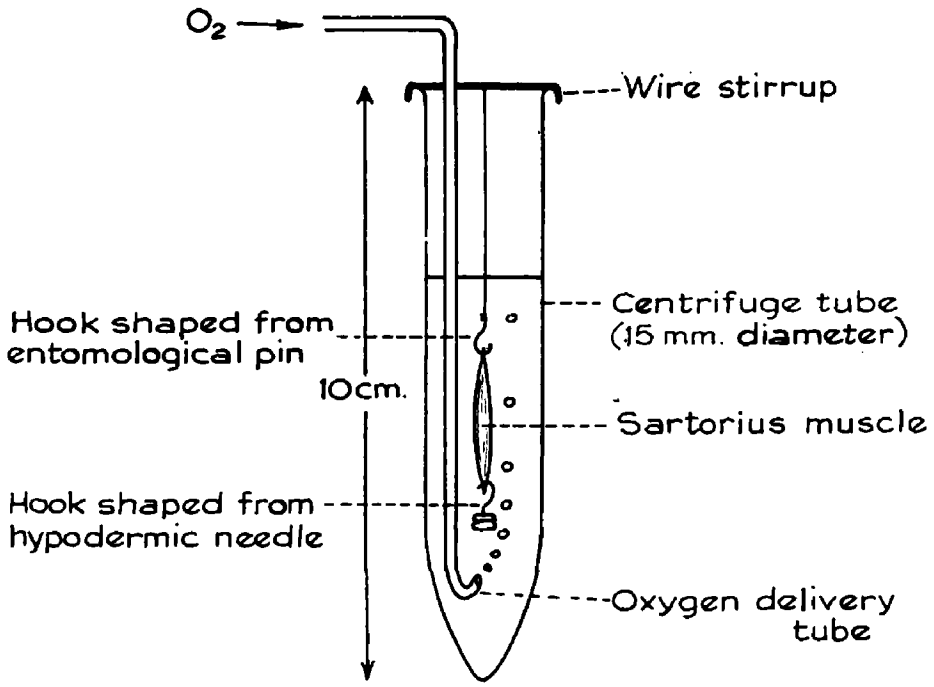

Fig. 1.

Diagram of the apparatus for suspending a muscle for the study of the uptake or release of radioactive ions.

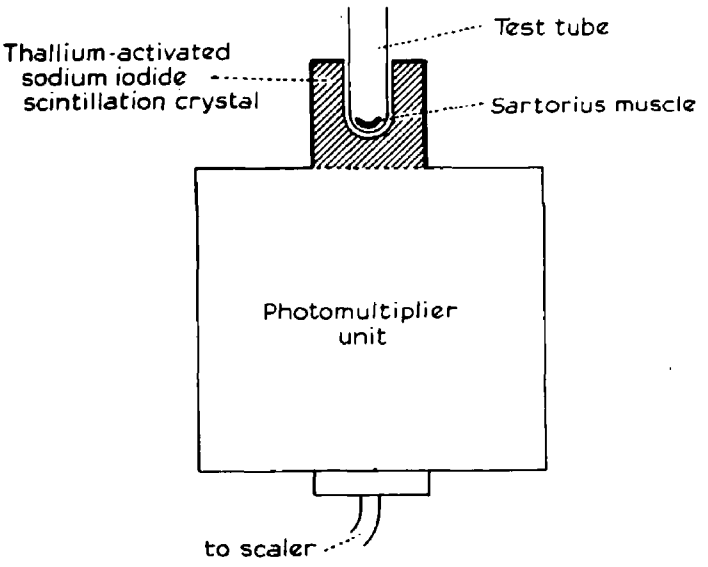

Fig. 2

Diagram of the apparatus used for measuring radioactivity in muscle.

radioactive bathing solution were removed and the muscle put into a test tube. The radioactivity in the muscle was then counted in a thallium-activated, sodium iodide, scintillation crystal (Ekco type N 597) connected through a photomultiplier unit to an automatic scaler (Ekco type N 530 D), (Fig. 2, page 85). After counting, hooks were again attached to the muscle and it was returned to the radioactive bathing fluid.

The muscles were usually exposed to the radioactive frog Ringer's solution for a period of from 2 to 3 hours, the radioactivity of both the control and the test muscle being measured at 30 minute intervals. The total radioactivity in the muscle at the end of the experiment was measured by dissolving it in 1 ml. of concentrated nitric acid, putting it in a test tube and counting the digest in the scintillation counter. Corrections for decay were not made as a control muscle was compared under identical conditions in every experiment. After correcting for background the counts were expressed as counts per minute. A specimen protocol of experiment is shown in Appendix I (page 388).

An appropriate dilution of the radioactive bathing solution was made and its radioactivity measured in the scintillation counter. From this measurement and the amount of radioactivity taken up by the muscle at different time intervals, the percentage of calcium-47 uptake by the muscle was worked out. Finally the results were plotted as percentage uptake of calcium-47 by the muscle against time.

2. Investigation of the effect of drugs on potassium-42 uptake by isolated frog sartorius muscle.

METHOD/

METHOD

Paired sartorius muscles of common frogs (Rana temporaria) unselected as to age or sex and weighing from 25 to 70 g. were used in these experiments. The dissection was carried out as described in section 1, pages 82-83 . The muscles were suspended in centrifuge tubes containing 10 ml. of frog Ringer's solution in which part of the stable potassium chloride was replaced by potassium-42 chloride. The procedure for suspending the muscle was similar to that described in the previous section and shown in Fig.1 (page 84). The control muscle was placed in the radioactive frog Ringer's solution and the test muscle was placed in the radioactive frog Ringer's solution to which the drug had been added. The radioactive bathing solution was continuously agitated and oxygenated by a stream of oxygen. At intervals of 30 minutes the muscles were removed from the bathing solution, each side washed by a stream of non-radioactive frog Ringer's solution and lightly blotted with absorbant tissues to remove the adhering moisture. The radioactivity in the muscle was then counted in a thallium-activated, sodium iodide scintillation crystal (Ekco type N 597) connected through a photomultiplier unit to an automatic scaler (Ekco type N 530 D), (Fig. 2, page 85). The muscle was returned to the bathing fluid after counting.

The muscles were usually exposed to the radioactive frog Ringer's solution for a period of from 1½ to 2½ hours, the radioactivity of both control and test muscles being measured/

measured at intervals of 30 minutes. At the end of the experiment the total radioactivity in the muscle was measured by dissolving it in 1 ml. of concentrated nitric acid and counting the digest in the scintillation counter. Corrections for decay were not made as a control muscle was compared under identical conditions in every experiment. After correcting for background the counts were expressed as counts per minute. A specimen protocol of experiment is shown in Appendix I (page 389).

A suitable dilution of the radioactive bathing solution was made and its radioactivity measured in the scintillation counter. From this measurement and the amount of radioactivity taken up by the muscle at different time intervals, the percentage of potassium-42 uptake by the muscle was worked out. Finally the results were plotted as percentage uptake of potassium-42 by the muscle against time.

3. Investigation of the effect of drugs on sodium-24 uptake by isolated frog sartorius muscle.

METHOD

Paired sartorius muscles from common frogs (Rana temporaria), unselected as to age or sex and weighing from 25 to 70 g. were used in these experiments. Dissection was carried out as described in section 1, (pages 82-83). The 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 sodium-24 chloride. The procedure for suspending the muscle was similar to that described for uptake/

uptake of calcium-47 and is shown in Fig. 1, page 84. The control muscle was placed in the radioactive frog Ringer's solution and the test muscle was placed in the radioactive frog Ringer's solution to which the drug had been added. The radioactive bathing solution was continuously agitated and oxygenated by a stream of oxygen. The muscles were removed at intervals of 30 minutes from the bathing solution, each side washed by a stream of non-radioactive frog Ringer's solution and blotted dry with absorbant tissues. The radioactivity in the muscle was then counted in a thallium-activated, sodium iodide scintillation crystal (Ekco type N 597) connected through a photomultiplier unit to an automatic scaler (Ekco type N 530 D), (Fig. 2, page 85). The muscle was returned to the bathing fluid after counting.

The muscles were usually exposed for a period of 1½ to 2 hours in the radioactive frog Ringer's solution and the radioactivity of both the control and the test muscle measured at intervals of 30 minutes. At the end of the experiment the total radioactivity in the muscle was measured by dissolving it in 1 ml. of concentrated nitric acid and counting the digest in the scintillation counter. Corrections for decay were not made as a control muscle was compared under identical conditions in every experiment. After correcting for background the counts were expressed as counts per minute. A specimen protocol of experiment is shown in Appendix I (page 390).

An appropriate dilution of the radioactive bathing solution was made and its radioactivity measured in the scintillation counter. From this measurement and the amount of radioactivity taken up by the muscle at different time intervals/

intervals, the percentage of sodium-24 uptake by the muscle was worked out. Finally the results were plotted as percentage uptake of sodium-24 by the muscle against time.

4. Investigation of the effect of drugs on calcium-47 release from isolated frog sartorius muscle.

METHOD

Common frogs (Rana temporaria) unselected as to age or sex and weighing from 25 to 70 g. were used in these experiments. Dissection was carried out as described in Section 1, pages 82-83 . The muscles were blotted dry with absorbant tissues and rapidly weighed on a torsion balance. They were then soaked in 10 ml. of frog Ringer's solution at room temperature in which part of the stable calcium chloride was replaced by calcium-47 chloride. The muscle was suspended in a manner similar to that described for uptake of calcium-47 and shown in Fig. 1, page 84 . The radioactive bathing solution was continuously agitated and oxygenated by a stream of oxygen. The soaking was continued for a period of from 3 to 4 hours.

After soaking the muscles were removed from the radioactive frog Ringer's solution, each side washed by means of a stream of non-radioactive frog Ringer's solution, and using hooks attached to the two ends of the muscles, suspended in the first pair of two parallel series of centrifuge tubes each containing 10 ml. of non-radioactive, calcium chloride-free oxygenated frog Ringer's solution at room temperature, one series being for control purposes only. After a 10 minute/

minute period of immersion in the first tube the muscle was transferred to the next tube in the series. After removing the muscle from the tube the calcium-47 content of the bathing fluid was measured by means of a thallium-activated, sodium iodide scintillation crystal (Ekco type N 597) connected through a photomultiplier unit to an automatic scaler (Ekco type N 530 D). Corrections for decay were not made as a control muscle was compared under similar conditions in every experiment. After correcting for background the counts were expressed as counts per minute.

Each test or control series contained 7 centrifuge tubes. In the control series each tube contained 10 ml. of calcium chloride-free, oxygenated frog Ringer's solution. In the test series the fourth tube contained 10 ml. of oxygenated, calcium chloride-free frog Ringer's solution together with the drug, the remaining tubes in this series contained 10 ml. of calcium chloride-free oxygenated frog Ringer's solution. A specimen protocol of experiment is shown in Appendix I (page 391).

Any change in the nature of the release of calcium-47 when the muscle was in contact with the drug was shown by a change in the slope of the curve obtained by plotting the number of counts released against time. The total activity remaining in the muscle was measured by dissolving it in 1 ml. of concentrated nitric acid and counting the digest in the scintillation counter. The release of calcium-47 by the test muscle and the control muscle was compared in both cases by plotting the output of calcium-47 against time.

5. Investigation of the effect of drugs on potassium-42 release from isolated frog sartorius muscle.

METHOD/

METHOD

The method used was based on that described by Lister and Lewis (1). Common frogs (Rana temporaria), unselected as to age or sex, weighing from 25 to 70 g. were injected with 0.5 to 1.0 ml. of radioactive potassium-42 chloride into the dorsal lymph sac. After a 2 hour equilibration period the frogs were stunned, decapitated and pithed. Dissection was carried out as described in section 1, pages 82 - 83 , and the sartorius muscles removed from both sides. One muscle served as the test object and the other was used as the control.

The muscles were washed on each side by a stream of non-radioactive frog Ringer's solution and suspended successively by means of stainless steel hooks attached to each end of the muscle in two parallel series of centrifuge tubes, each containing 10 ml. of oxygenated potassium chloride-free frog Ringer's solution at room temperature. After a 10 minute period of immersion in the first tube the muscle was transferred to the next tube in the series. After removing the muscle the potassium-42 content of the bathing fluid was measured by means of a Geiger-Müller liquid counter (type M6). The counter tube was connected through a probe unit to an automatic scaler (Ekco type N 530 D). Corrections for decay were not made as in every experiment a control muscle was compared under similar conditions. After correcting for background the counts were expressed as counts per minute.

Each/

Each test or control series contained 7 centrifuge tubes. In the control series each tube contained 10 ml. of potassium chloride-free oxygenated frog Ringer's solution. In the test series the fourth tube contained 10 ml. of potassium chloride-free oxygenated frog Ringer's solution, together with the drug. The remaining tubes in this series contained 10 ml. of potassium chloride-free oxygenated frog Ringer's solution. A specimen protocol of experiment is shown in Appendix I (page 392).

Any change in the nature of the release of potassium-42 taking place when the muscle was in contact with the drug was shown by a change in the slope of the curve obtained by plotting the number of counts released against time. The total activity remaining in the muscle was measured by dissolving it in 1 ml. of concentrated nitric acid and counting the digest in a Geiger-Müller liquid counter (type M6). The release of potassium-42 by the test muscle and the control muscle were compared in both cases by plotting the output of potassium-42 against time.

C. Statistical Analysis

The standard Students' t test was used to determine the significance of the difference of the means ($\bar{x}_1$ and $\bar{x}_2$) of the test and control uptake experiments. Calculations were made using the standard equations modified to facilitate machine calculation (2, 3).

The variance (S^2) from the mean ($\bar{x}$) for each set of variates was calculated by the equation,

S^2/

$$S^2 = \frac{\Sigma x^2 - \frac{(\Sigma x)^2}{n}}{n - 1}$$

where n is the number of variates.

The standard deviation from the mean ($S_{\bar{d}}$) for two sets of variates was calculated by the equation,

$$S_{\bar{d}}^2 = \frac{S_1^2}{n_1} + \frac{S_2^2}{n_2}$$

The value of t was then obtained by applying the equation,

$$t = \frac{\bar{x}_1 - \bar{x}_2}{S_{\bar{d}}}$$

In entering the tables of t, to obtain the values of P, the degrees of freedom (D.F.) were calculated from the expression,

$$D.F. = n_1 + n_2 - 2$$

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CHAPTER III

RESULTS

Uptake of calcium-47, potassium-42 and sodium-24 by isolated frog sartorius muscle in the presence of suxamethonium.

Suxamethonium blocks neuromuscular transmission in skeletal muscle by a persistent depolarization of the end plate region. The paralysis which it produces resembles that caused by decamethonium and large doses of acetylcholine in the presence of an anticholinesterase. Neuromuscular depression is preceded by transient stimulation.

Calcium-47

Suxamethonium in doses of from 1 mg. to 10 mg. per ml. caused a significant ($P, > 0.001$) increase in the uptake of calcium-47 by isolated frog sartorius muscle. The control muscle showed a lower percentage uptake than the drug-treated muscle. Table 1 (page 152) and Fig. 3(A)(page 97) show the results obtained in a group of ten experiments using 5 mg. per ml. of suxamethonium.

Potassium-42

Suxamethonium in doses of from 1 mg. to 10 mg. per ml. caused a significant ($P, > 0.001$) decrease in the uptake of potassium-42 by isolated frog sartorius muscle. The control muscle showed an increased percentage uptake when compared with/

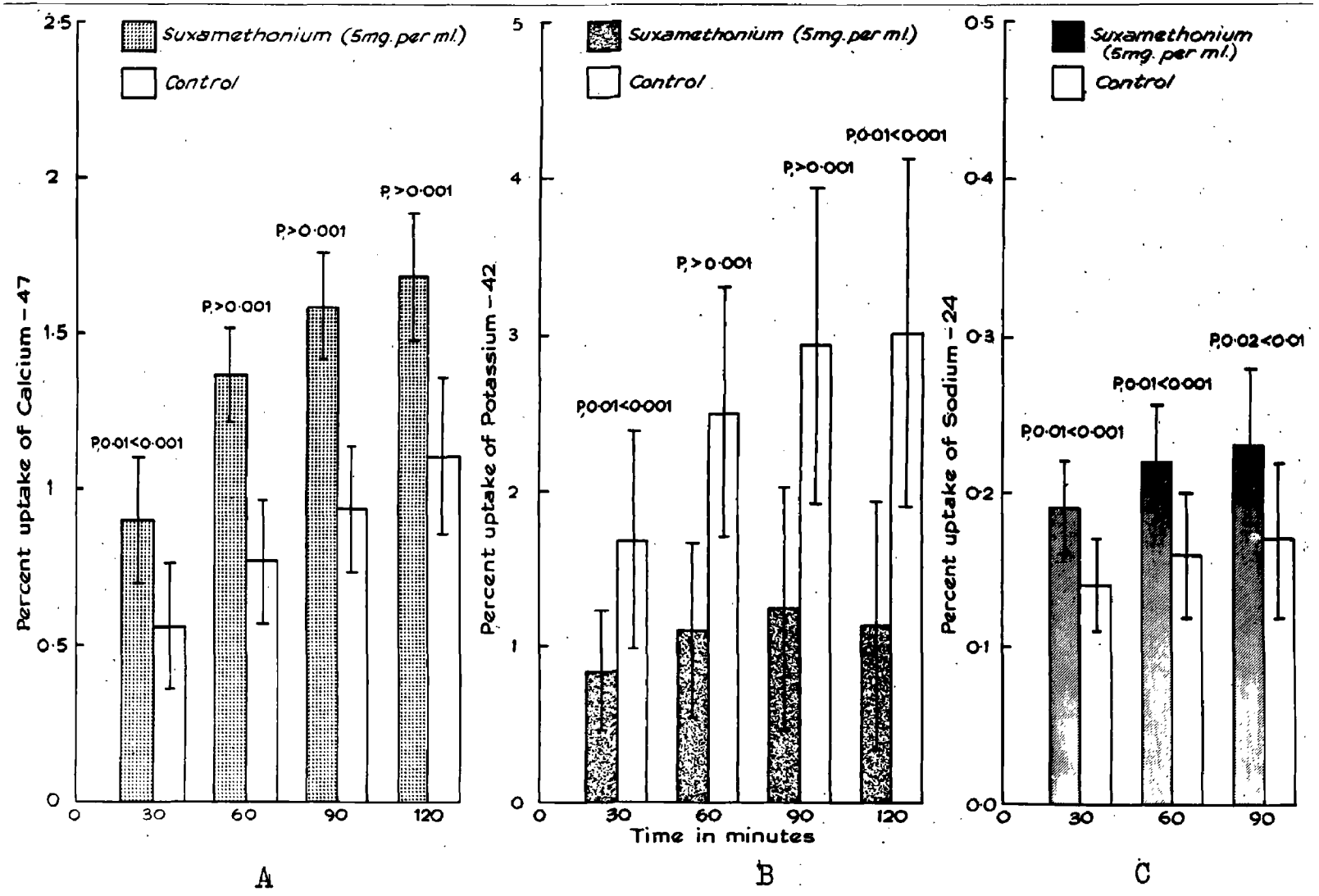


Fig. 3.

Uptake of calcium-47 (A), potassium-42 (B) and sodium-24 (C) by isolated frog sartorius muscle in the presence of suxamethonium (5 mg. per ml.).

compared with the drug-treated muscle. Table 1 (page 152) and Fig. 3(B) (page 97) show the results obtained in a group of ten experiments using 5 mg. per ml. of suxamethonium.

Sodium-24

Suxamethonium in doses of from 1 mg. to 10 mg. per ml. caused a significant (P, $0.01 < 0.001$) increase in the uptake of sodium-24 by isolated frog sartorius muscle. The control muscle showed a lower percentage uptake when compared with the drug-treated muscle. Table 1 (page 152) and Fig.3(C) (page 97) show the results obtained in a group of ten experiments using 5 mg. per ml. of suxamethonium.

Uptake of calcium-47, potassium-42 and sodium-24 by isolated frog sartorius muscle in the presence of decamethonium.

The main action of decamethonium on skeletal muscle is to depolarize persistently the motor end plate region and thus prevent muscular contraction in response to acetylcholine liberated at the neuromuscular synapse. Prior to causing paralysis, decamethonium evokes transient muscular fasciculations and causes a contracture.in avian muscle.

Calcium-47

Decamethonium in doses of from 50 µg. to 0.5 mg. per ml. caused a significant (P, $0.05 < 0.02$) increase in the uptake of calcium-47 by isolated frog sartorius muscle. The control muscle showed a lower percentage uptake when compared with the drug-treated muscle. Table 2 (page 153) and Fig./

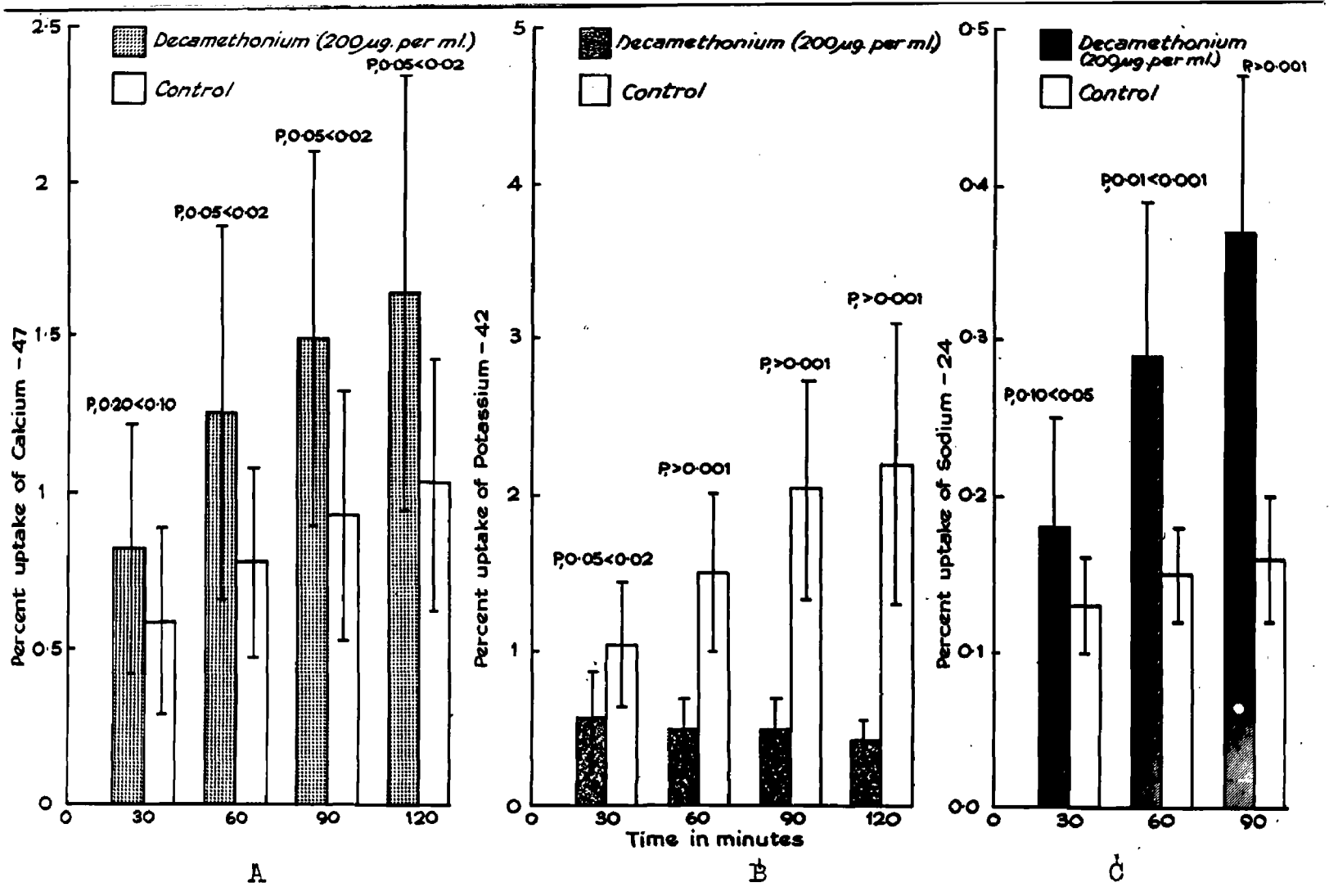


Fig. 4.

Uptake of calcium-47 (A), potassium-42 (B) and sodium-24 (C) by isolated frog sartorius muscle in the presence of decamethonium (200 µg. per ml.)

Fig. 4(A) (page 99) show the results obtained in a group of eleven experiments using 200 μg. per ml. of decamethonium.

Potassium-42

Decamethonium in doses of from 50 μg. to 0.5 mg. per ml. reduced significantly (P, > 0.001) the uptake of potassium-42 by isolated frog sartorius muscle. The control muscle showed an increased percentage uptake when compared with the drug-treated muscle. Table 2 (page 153) and Fig. 4(B) (page 99) show the results obtained in a group of ten experiments using 200 μg. per ml. of decamethonium.

Sodium-24

Decamethonium in doses of from 50 μg. to 0.5 mg. per ml. caused an increased (P, $0.01 < 0.001$) uptake of sodium-24 by isolated frog sartorius muscle. The control muscle showed a lower percentage uptake when compared with the drug-treated muscle. Table 2 (page 153) and Fig.4 (C) (page 99) show the results obtained in a group of ten experiments using 200 μg. per ml. of decamethonium.

Uptake of calcium-47, potassium-42 and sodium-24 by isolated frog sartorius muscle in the presence of nicotine.

Sollmann (1) has summarised the actions of nicotine in the frog as follows, "The injection of nicotine into frogs produces fibrillary twitching merging into tonic contracture... The minor grades may cease temporarily on section of the nerve, but return, especially on stimulation. They occur also in excised muscle and after degeneration of the nerve/

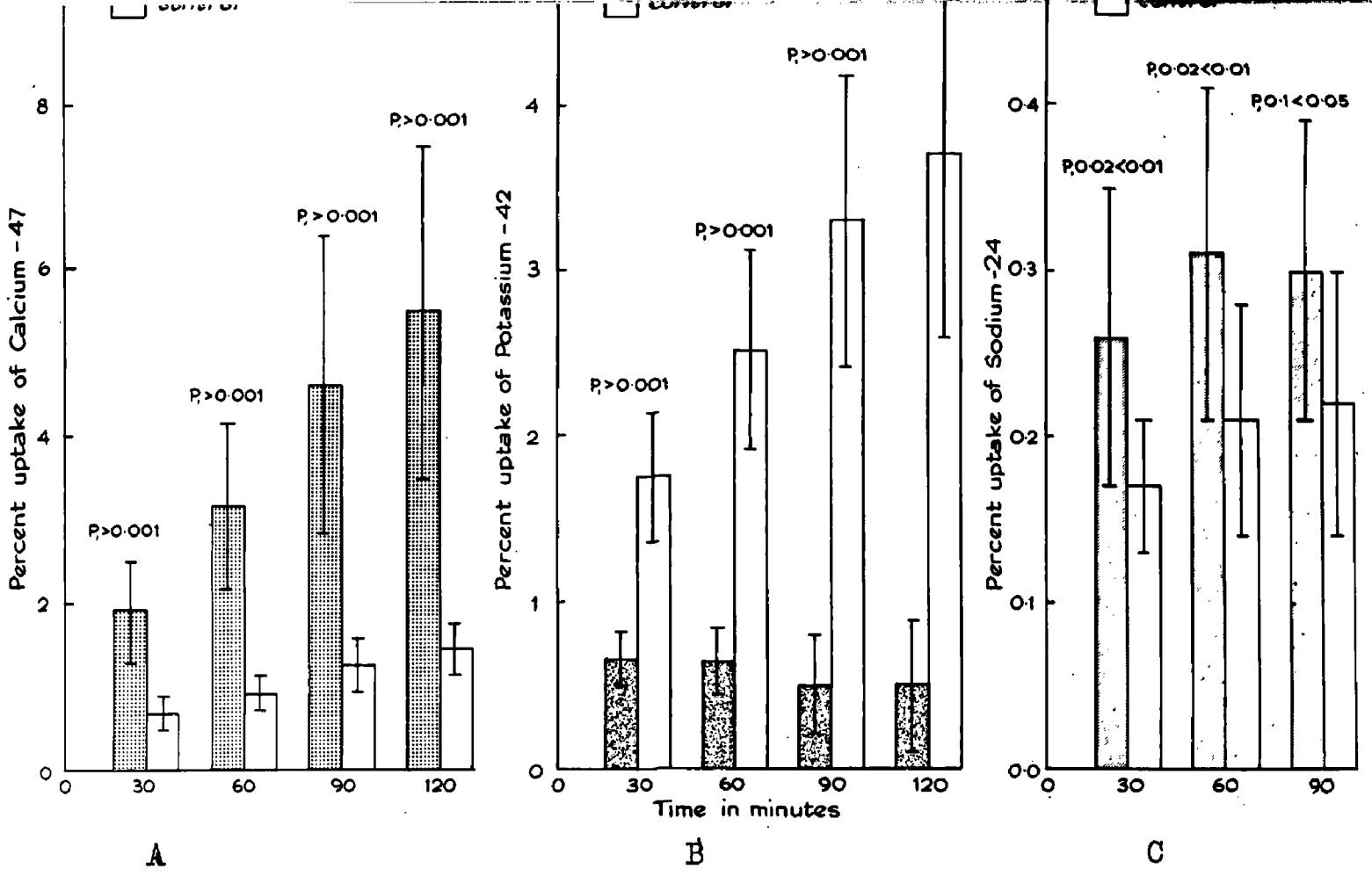


Fig. 5.

Uptake of calcium-47 (A), potassium-42 (B) and sodium-24 (C) by isolated frog sartorius muscle in the presence of nicotine (1 mg. per ml.).

nerve endings."

Calcium-47

Nicotine in doses of from 200 μg. to 2 mg. per ml. caused a significant ($P, > 0.001$) increase in the uptake of calcium-47 by isolated frog sartorius muscle. The control muscle showed a lower percentage uptake when compared with the drug-treated muscle. Table 3 (page 154) and Fig.5(A) (page 101) show the results obtained in a group of ten experiments using 1 mg. per ml. of nicotine.

Potassium-42

Nicotine in doses of from 200 μg. to 2 mg. per ml. significantly ($P, > 0.001$) reduced the uptake of potassium-42 by isolated frog sartorius muscle. The control muscle showed an increased percentage uptake when compared with the drug-treated muscle. Table 3 (page 154) and Fig.5 (B) (page 101) show the results obtained in a group of ten experiments using 1 mg. per ml. of nicotine.

Sodium-24

Nicotine in doses of from 200 μg. to 2 mg. per ml. caused a significant ($P, 0.02 < 0.01$) increase in the uptake of sodium-24 by isolated frog sartorius muscle. The control muscle showed a lower percentage uptake when compared with the drug-treated muscle. Table 3 (page 154) and Fig.5(C) (page 101) show the results obtained in a group of ten experiments using 1 mg. per ml. of nicotine.

Uptake/

Uptake of calcium-47, potassium-42 and sodium-24 by isolated frog sartorius muscle in the presence of ryanodine.

On skeletal muscle of the frog and of mammals, ryanodine causes an irreversible contracture. This action occurs without the resting membrane potential or the adenosine-triphosphatase activity being affected and it is independent of the innervation (2-4).

Calcium-47

Ryanodine in doses of from 10 μg. to 100 μg. per ml. caused a significant (P, $0.01 < 0.001$) increase in the uptake of calcium-47 by isolated frog sartorius muscle. The control muscle showed a lower percentage uptake when compared with the drug-treated muscle. Table 4 (page 155) and Fig. 6(A) (page 104) show the results obtained in a group of nine experiments using 50 μg. per ml. of ryanodine.

Potassium-42

Ryanodine in doses of from 10 μg. to 100 μg. per ml. significantly (P, > 0.001) reduced the uptake of potassium-42 by isolated frog sartorius muscle. The control muscle showed an increased percentage uptake when compared with the drug-treated muscle. Table 4 (page 155) and Fig. 6 (B) (page 104) show the results obtained in a group of nine experiments using 50 μg. per ml. of ryanodine.

Sodium-24

Ryanodine in doses of from 10 μg. to 100 μg. per ml. significantly (P, > 0.001) increased the uptake of sodium-24 by/

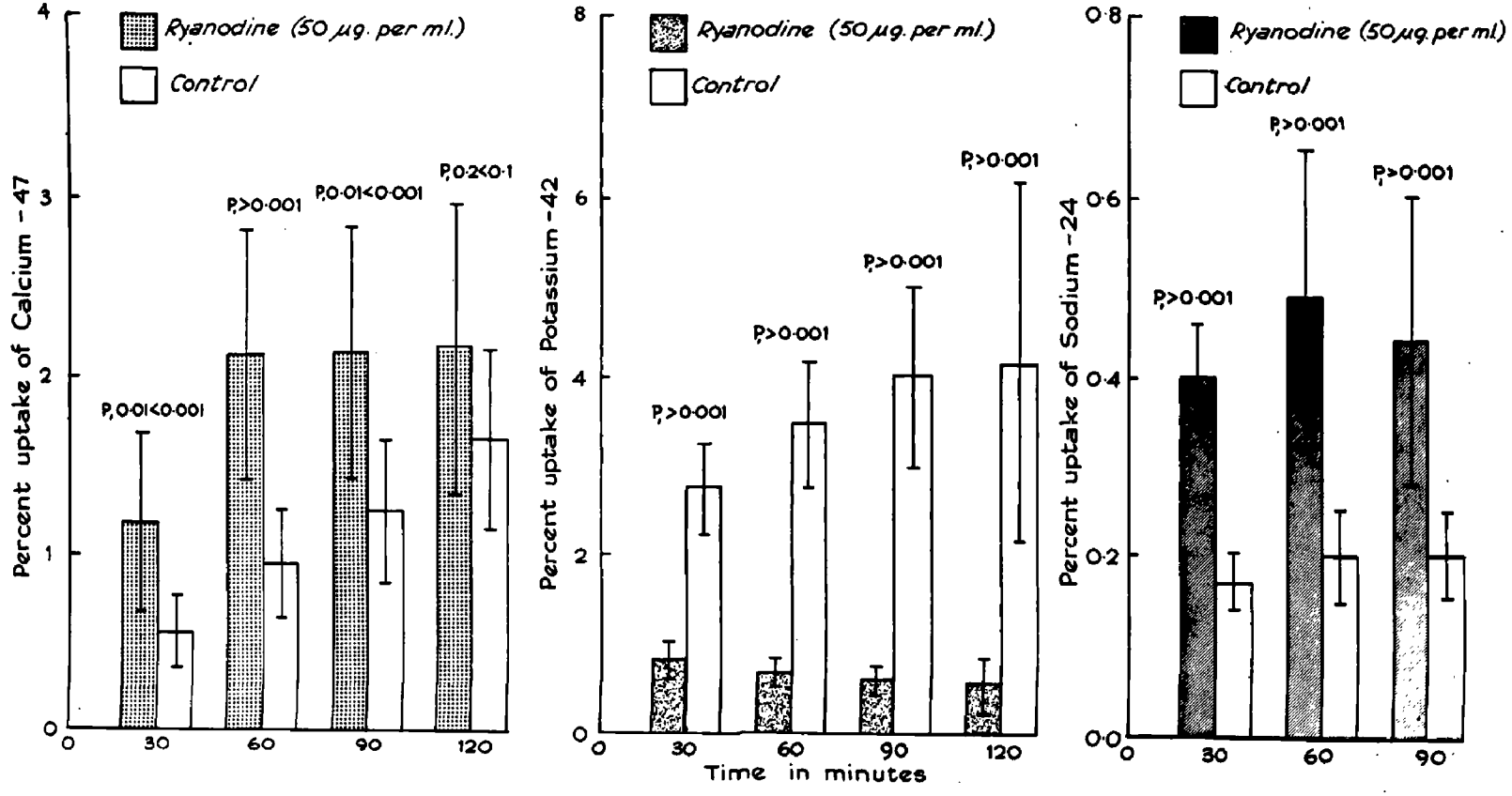


Fig. 6.

Uptake of calcium-47 (A), potassium-42 (B) and sodium-24 (C) by isolated frog sartorius muscle in the presence of ryanodine (50 µg. per ml.).

by isolated frog sartorius muscle. The control muscle showed a lower percentage uptake when compared with the drug-treated muscle. Table 4 (page 155) and Fig. 6 (C) (page 104) show the results obtained in a group of ten experiments using 50 μg. per ml. of ryanodine.

Uptake of calcium-47, potassium-42 and sodium-24 by isolated frog sartorius muscle in the presence of carbachol.

Carbachol is one of the numerous synthetic acetylcholine analogues but it is more stable and is not susceptible to destruction by cholinesterase. In this compound the muscarinic actions of acetylcholine are retained but nicotinic activity is reduced. Although selective affinities of carbachol for particular organs are not readily demonstrable in intact animals, it has been suggested that it has a greater action on the gastrointestinal and urinary tracts than upon the cardiovascular system or respiration.

Calcium-47

Carbachol in doses of from 1 mg. to 5 mg. per ml. did not significantly (P, $0.3 < 0.2$) alter the uptake of calcium-47 by isolated frog sartorius muscle. There was no significant difference between the percentage uptake by the control and that of the drug-treated muscle. Table 5 (page 156) and Fig. 7(A)(page 106) show the results obtained in a group of ten experiments using 5 mg. per ml. of carbachol.

Potassium-42

Carbachol in doses of from 1 mg. to 5 mg. per ml. did not/

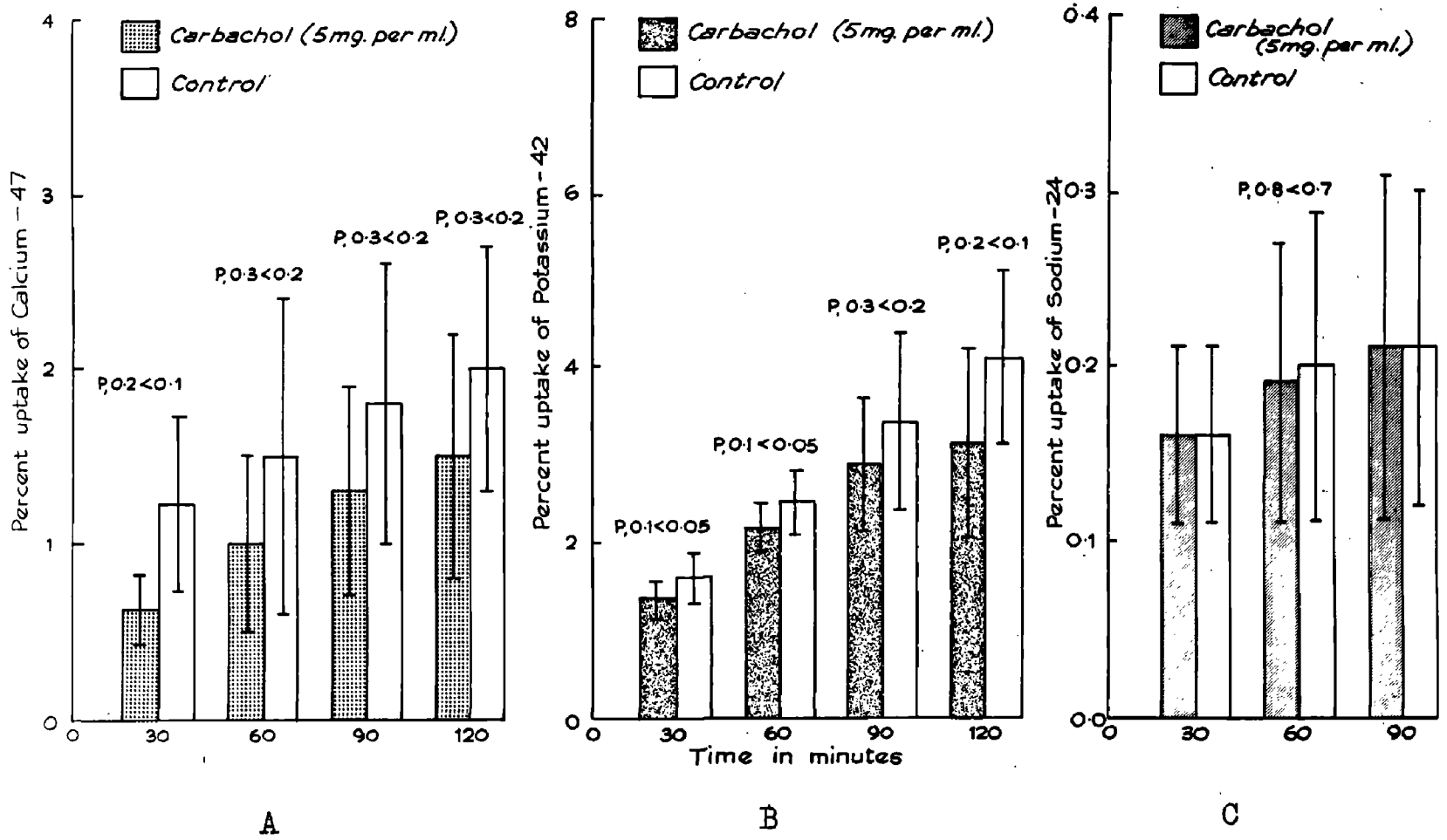


Fig. 7.

Uptake of calcium-47 (A), potassium-42 (B) and sodium-24 (C) by isolated frog sartorius muscle in the presence of carbachol (5 mg. per ml.).

not significantly (P, $0.1 < 0.05$) alter the uptake of potassium-42 by isolated frog sartorius muscle. There was no significant difference between the percentage uptake by the control and that of the drug-treated muscle. Table 5 (page 156) and Fig. 7(B) (page106) show the results obtained in a group of ten experiments using 5 mg. per ml. of carbachol.

Sodium-24

Carbachol in doses of from 1 mg. to 5 mg. per ml. did not cause any significant (P, $0.8 < 0.7$) change in the uptake of sodium-24 by isolated frog sartorius muscle. There was no significant difference between the percentage uptake by the control and that of the drug-treated muscle. Table 5 (page 156) and Fig. 7(C) (page 106) show the results obtained in a group of ten experiments using 5 mg. per ml. of carbachol.

Uptake of calcium-47, potassium-42 and sodium-24 by isolated frog sartorius muscle in the presence of neostigmine

Neostigmine is a potent anticholinesterase. In addition to this it has a direct depolarizing action on the muscle fibres. Denervated skeletal muscle contracts when treated with neostigmine and close intraarterial injection of this compound elicits a contractural response in skeletal muscle.

Calcium-47

Neostigmine in doses of from 25 µg. to 150 µg. per ml. did not influence significantly (P, $0.2 < 0.1$) the uptake of/

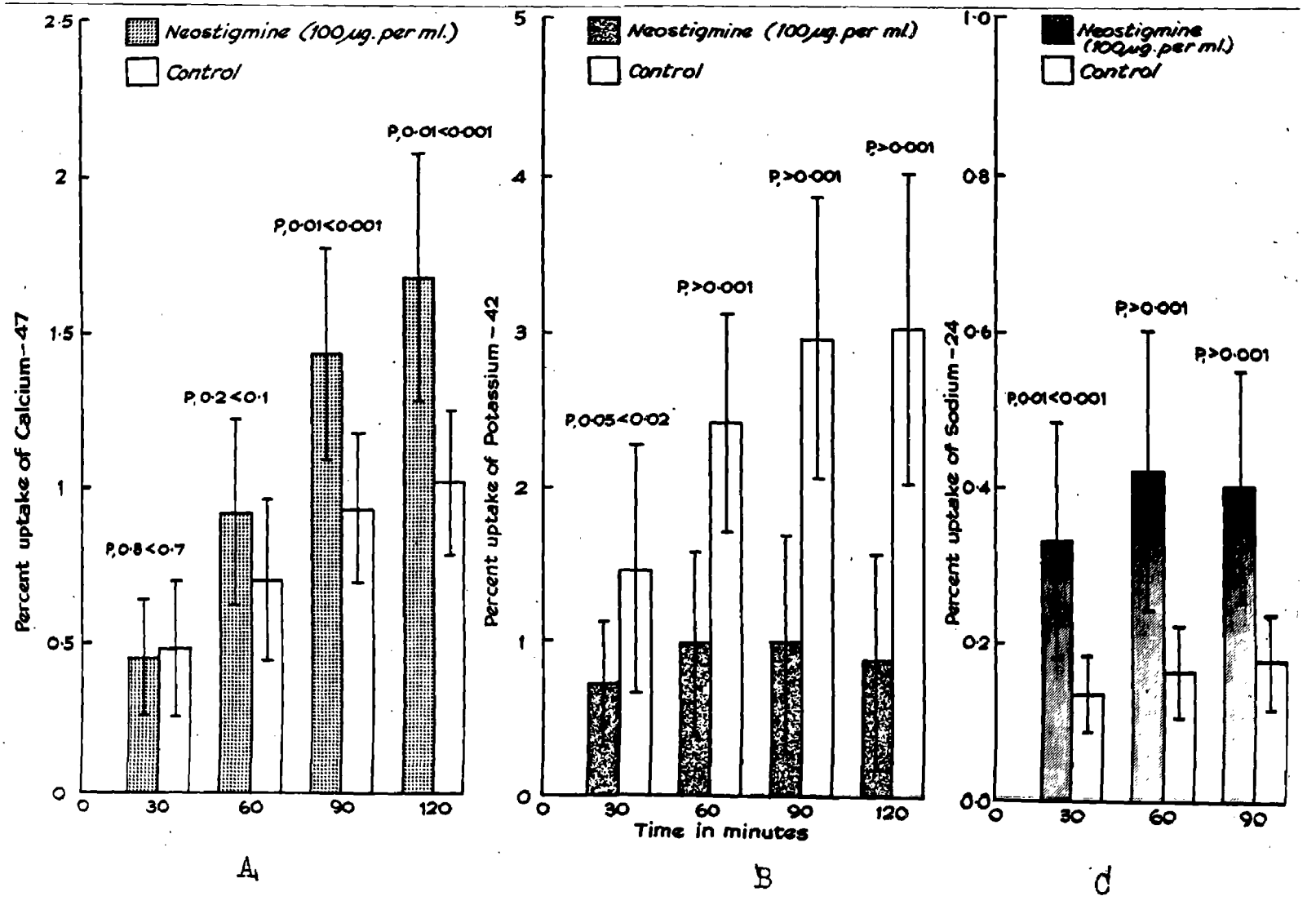


Fig. 8.

Uptake of calcium-47 (A), potassium-42 (B) and sodium-24 (C) by isolated frog sartorius muscle in the presence of neostigmine (100 µg. per ml.).

of calcium-47 by isolated frog sartorius muscle at the beginning of the experiment, but after a period of about one hour's exposure to the drug there was a significant (P, $0.01 < 0.001$) increase in the percentage uptake by the drug-treated muscle. Table 6 (page 157) and Fig. 8 (A) (page 108) show the results obtained in a group of nine experiments using 100 μg. per ml. of neostigmine.

Potassium-42

Neostigmine in doses of from 25 μg. to 150 μg. per ml. caused a significant (P, > 0.001) decrease in the uptake of potassium-42 by isolated frog sartorius muscle. The control muscle showed a higher percentage uptake when compared with the drug-treated muscle. Table 6 (page 157) and Fig. 8 (B) (page 108) show the results obtained in a group of nine experiments using 100 μg. per ml. of neostigmine.

Sodium-24

Neostigmine in doses of from 25 μg. to 150 μg. per ml. caused a significant (P, > 0.001) increase in the uptake of sodium-24 by isolated frog sartorius muscle. The control muscle showed a lower percentage uptake when compared with the drug-treated muscle. Table 6 (page 157) and Fig. 8 (C) (page 108) show the results obtained in a group of ten experiments using 100 μg. per ml. of neostigmine.

Uptake of calcium-47, potassium-42 and sodium-24 by isolated frog sartorius muscle in the presence of edrophonium.

Edrophonium is a compound with properties similar to those/

those of neostigmine with a rapid and brief action. It is a competitive antagonist of drugs which act like tubocurarine and acts by displacing them from receptors on the post-synaptic membrane of the neuromuscular synapse, so permitting the acetylcholine to depolarize the end plate region.

Calcium-47

Edrophonium in doses of from 0.5 mg. to 2 mg. per ml. caused a significant (P, $0.01 < 0.001$) decrease in the uptake of calcium-47 by isolated frog sartorius muscle. The control muscle showed a higher percentage uptake when compared with the drug-treated muscle. Table 7 (page 158) and Fig.9 (A) (page 111) show the results obtained in a group of ten experiments using 2 mg. per ml. of edrophonium.

Potassium-42

Edrophonium in doses of from 0.5 mg. to 2 mg. per ml. significantly ($0.05 < 0.02$) reduced the uptake of potassium-42 by isolated frog sartorius muscle. The control muscle showed a higher percentage uptake when compared with the drug-treated muscle. Table 7 (page 158) and Fig. 9 (B) (page 111) show the results obtained in a group of ten experiments using 2 mg. per ml. of edrophonium.

Sodium-24

Edrophonium in doses of from 0.5 mg. to 2 mg. per ml. did not influence significantly (P, $0.4 < 0.3$) the uptake of sodium-24 by isolated frog sartorius muscle. There was no significant difference between the percentage uptake by the control/

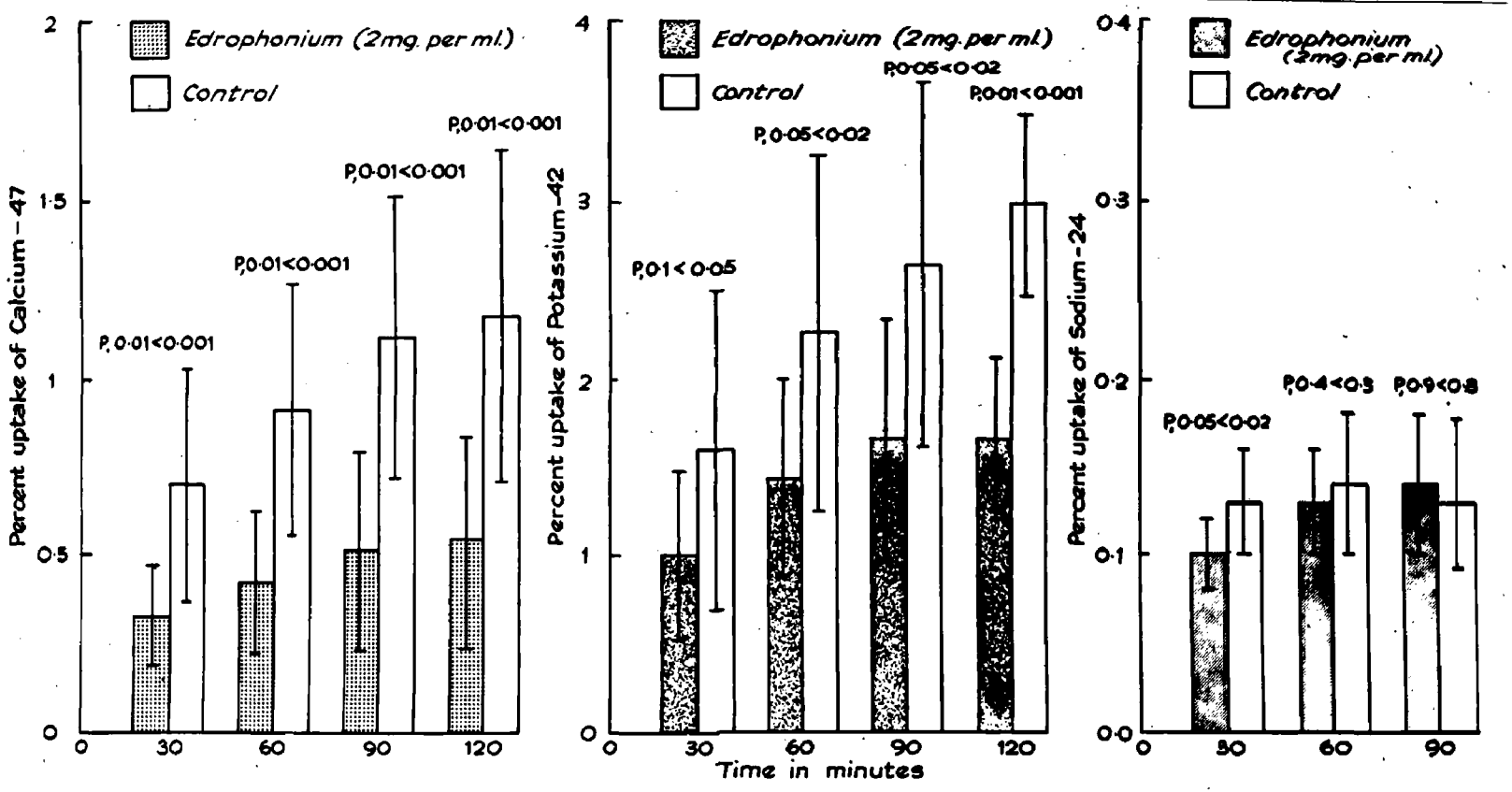


Fig. 9.

Uptake of calcium-47 (A), potassium-42 (B) and sodium-24 (C) by isolated frog sartorius muscle in the presence of edrophonium (2 mg. per ml.).

control muscle and that of the drug-treated muscle. Table 7 (page 158) and Fig. 9 (C) (page 111) show the results obtained in a group of ten experiments using 2 mg. per ml. of edrophonium.

Uptake of calcium-47, potassium-42 and sodium-24 by isolated frog sartorius muscle in the presence of tubocurarine.

Tubocurarine causes a paralysis of skeletal muscle by preventing the response to motor nerve impulses, i.e. to acetylcholine liberated. The muscle can still be made to contract by direct stimulation. There is no initial phase of excitation as is seen with depolarizing agents such as suxamethonium and decamethonium.

Calcium-47

Tubocurarine in doses of from 50 μg. to 0.5 mg. per ml. possibly significantly (P, $0.1 < 0.05$) altered the uptake of calcium-47 by isolated frog sartorius muscle. There was no clearly significant difference between the percentage uptake by the control muscle and that of the drug-treated muscle. Table 8 (page 159) and Fig. 10 (A) (page 113) show the results obtained in a group of ten experiments using 200 μg. per ml. of tubocurarine.

Potassium-42

Tubocurarine in doses of from 50 μg. to 0.5 mg. per ml. did not cause a significant (P, $0.8 < 0.7$) change in the uptake of potassium-42 by isolated frog sartorius muscle. There was no significant difference between the percentage uptake by/

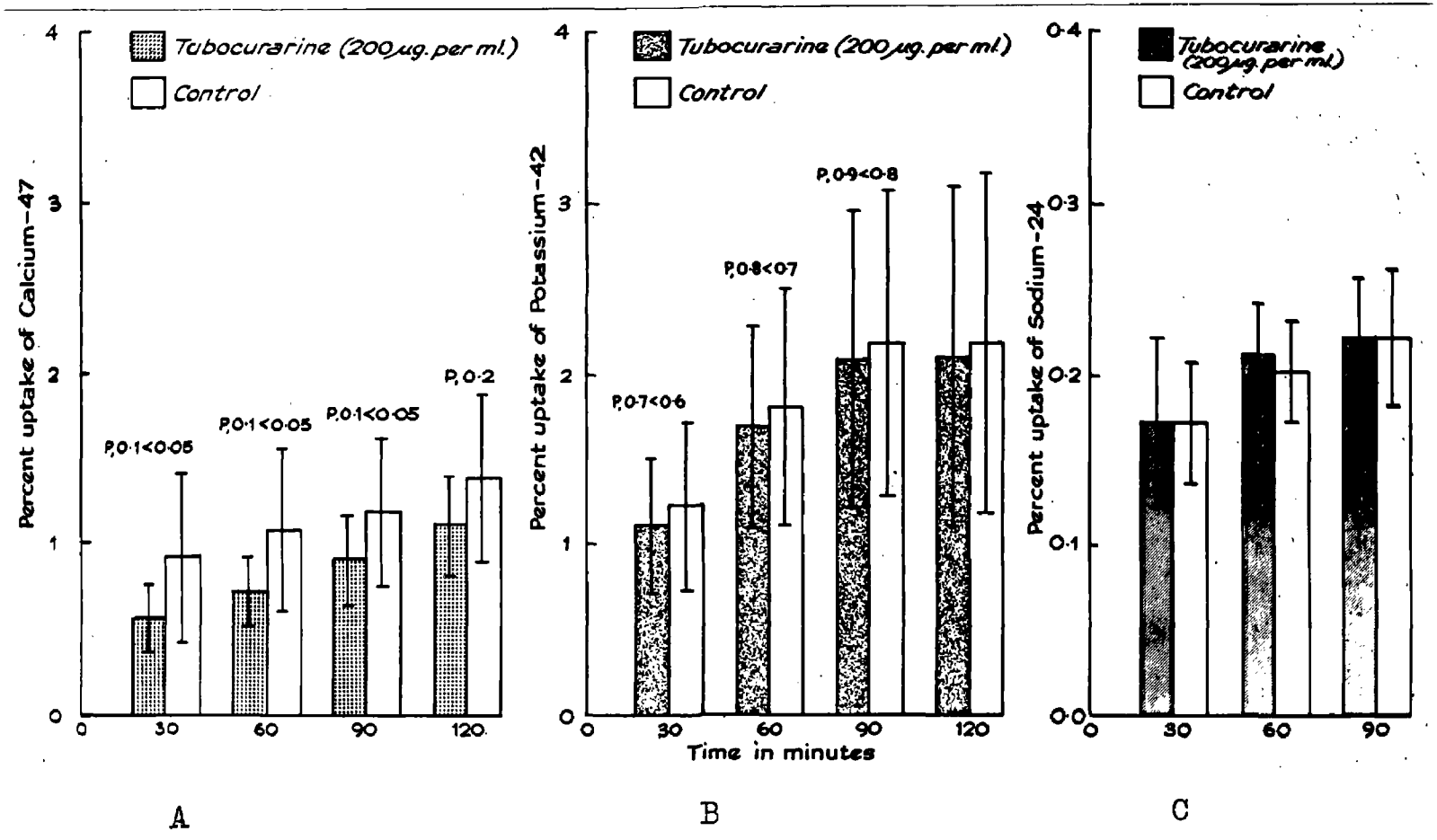


Fig. 10.

Uptake of calcium-47 (A), potassium-42 (B) and sodium-24 (C) by isolated frog sartorius muscle in the presence of tubocurarine (200 µg. per ml.).

by the control muscle and that of the drug-treated muscle. Table 8 (page 159) and Fig.10 (B) (page 113) show the results obtained in a group of nine experiments using 200 μg. per ml. of tubocurarine.

Sodium-24

Tubocurarine in doses of from 50 μg. to 0.5 mg. per ml. did not cause a significant change in the uptake of sodium-24 by isolated frog sartorius muscle. There was no significant difference between the percentage uptake by the control muscle and that of the drug-treated muscle. Table 8 (page 159) and Fig. 10 (C)(page 113) show the results obtained in a group of nine experiments using 200 μg. per ml. of tubocurarine.

Uptake of calcium-47, potassium-42 and sodium-24 by isolated frog sartorius muscle in the presence of gallamine.

Gallamine is one of a series of synthetic curare substitutes prepared by Bovet and his co-workers (5). The major action of gallamine is a curare-like paralysis of skeletal muscle. Both neuromuscular transmission and the effects of acetylcholine on the neuromyal junction are blocked. Its duration of action is shorter than that of tubocurarine.

Calcium-47

Gallamine in doses of from 1 mg. to 8 mg. per ml. did not cause a significant (F, $0.4 < 0.3$) change in the uptake of calcium-47 by isolated frog sartorius muscle. There was no/

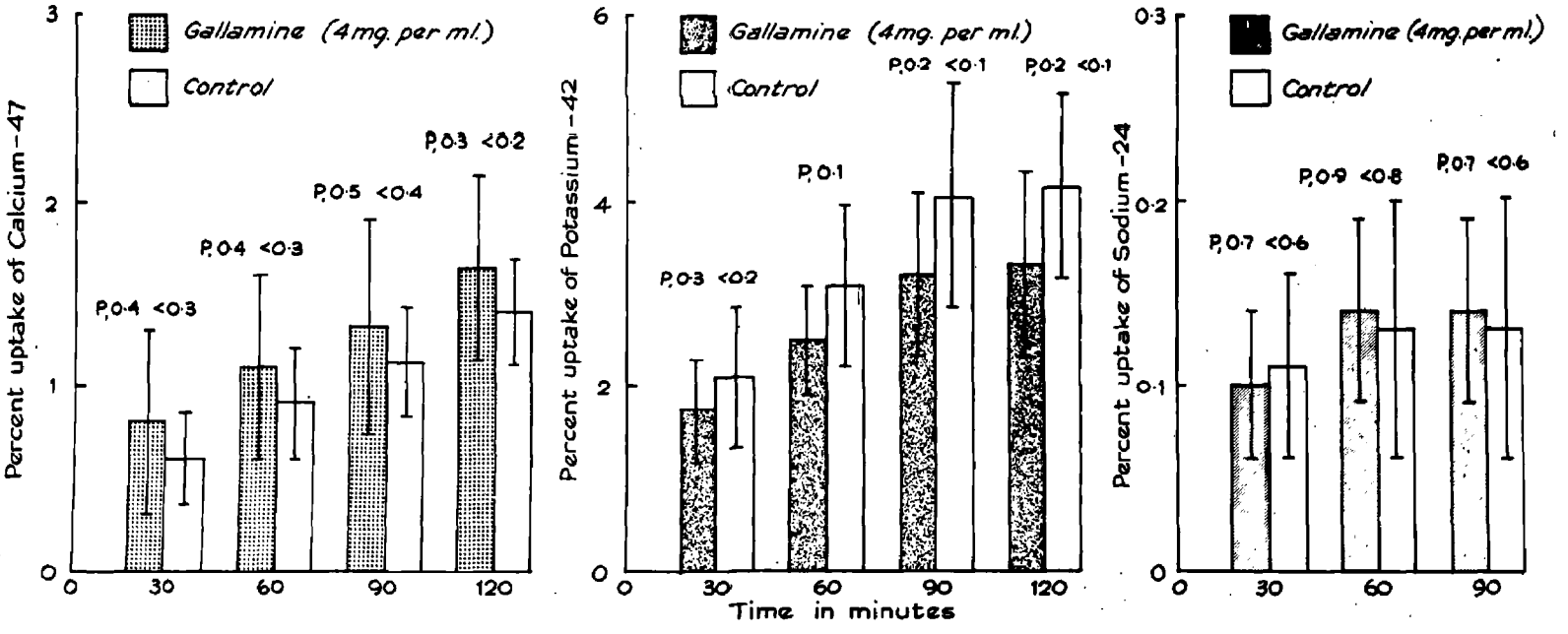


Fig. 11.

Uptake of calcium-47 (A), potassium-42 (B) and sodium-24 (C) by isolated frog sartorius muscle in the presence of gallamine (4 mg. per ml.).

no significant difference between the percentage uptake by the control muscle and that of the drug-treated muscle. Table 9 (page 160) and Fig. 11 (A) (page 115) show the results obtained in a group of nine experiments using 4 mg. per ml. of gallamine.

Potassium-42

Gallamine in doses of from 1 mg. to 8 mg. per ml. did not cause a significant (P, $0.2 < 0.1$) change in the uptake of potassium-42 by isolated frog sartorius muscle. There was no significant difference between the percentage uptake by the control muscle and that of the drug-treated muscle. Table 9 (page 160) and Fig. 11(B) (page 115) show the results obtained in a group of ten experiments using 4 mg. per ml. of gallamine.

Sodium-24

Gallamine in doses of from 1 mg. to 8 mg. per ml. did not cause a significant (P, $0.7 < 0.6$) change in the uptake of sodium-24 by isolated frog sartorius muscle. There was no significant difference between the percentage uptake by the control muscle and that of the drug-treated muscle. Table 9 (page 160) and Fig. 11(C) (page 115) show the results obtained in a group of ten experiments using 4 mg. per ml. of gallamine.

Uptake of calcium-47, potassium-42 and sodium-24 by isolated frog sartorius muscle in the presence of protoveratrine A.

Resting amphibian skeletal muscle treated with certain Veratrum/

Veratrum alkaloids especially veratrine, shows no obvious abnormality. If a stimulus is delivered to the muscle, either directly, or indirectly via the nerve, the magnitude of the twitch is as a rule higher than normal and is followed by a phase of slow relaxation. This is the typical veratrinic response.

Protoveratrine A is an ester alkaloid obtained from Veratrum album and it has typical veratrinic actions.

Calcium-47

Protoveratrine A in doses of from 50 μg. to 0.5 mg. per ml. caused a significant (P, $0.05 < 0.02$) depression in the uptake of calcium-47 by isolated frog sartorius muscle at the beginning of the experiment, but after a period of about 1 hour's exposure to the drug there was no significant (P, $0.4 < 0.3$) change in the percentage uptake by the control muscle and that of the drug-treated muscle. Table 10 (page 161) and Fig.12(A) (page118) show the results obtained in a group of nine experiments using 100 μg. per ml. of protoveratrine A.

Potassium-42

Protoveratrine A in doses of from 50 μg. to 0.5 mg. per ml. did not cause a significant (P, $0.6 < 0.5$) change in the uptake of potassium-42 by isolated frog sartorius muscle. There was no significant difference between the percentage uptake by the control muscle and that of the drug-treated muscle. Table10(page 161) and Fig. 12(B) (page 118) show the/

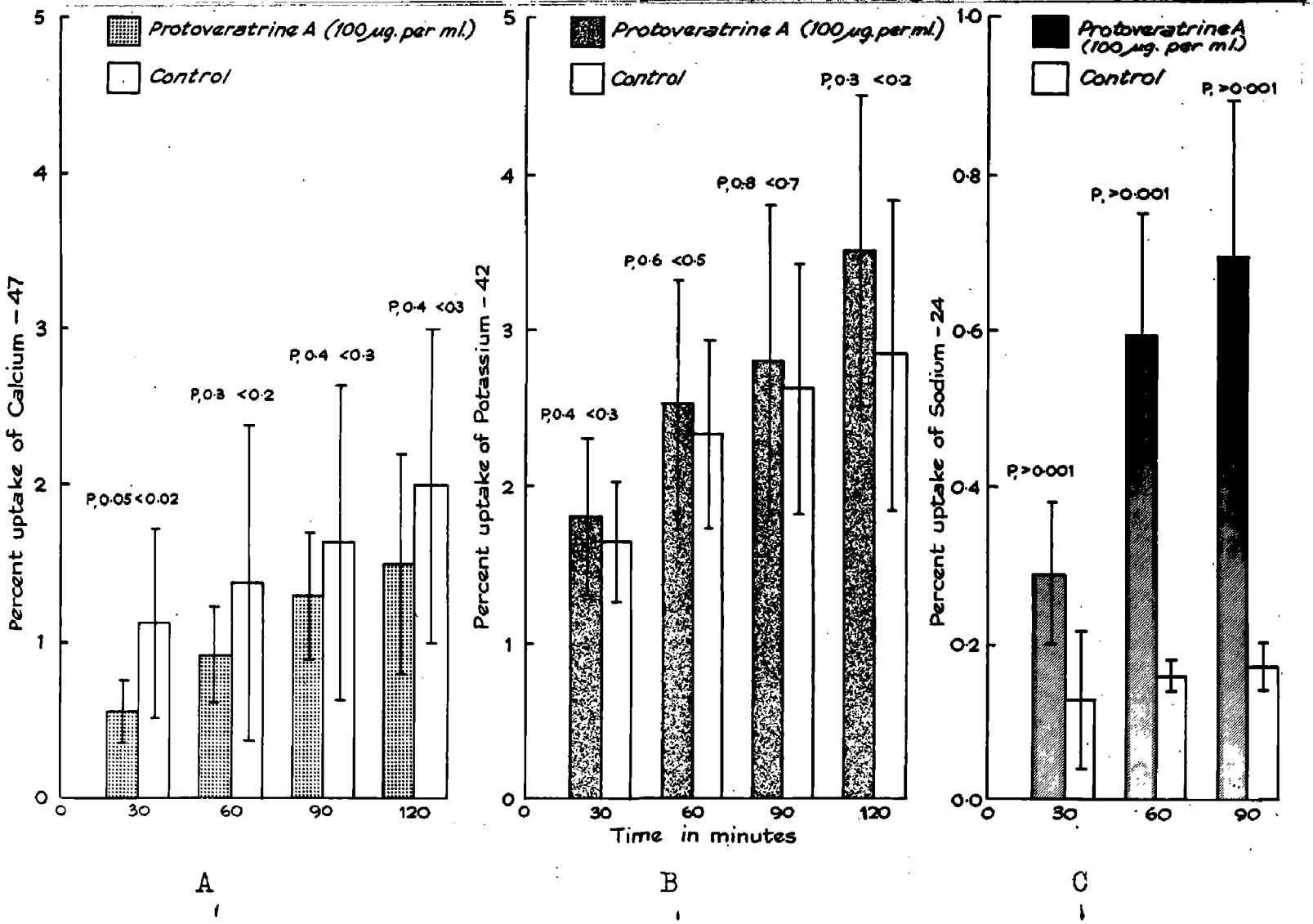


Fig. 12.

Uptake of calcium-47 (A), potassium-42 (B) and sodium-24 (C) by isolated sartorius muscle in the presence of protoveratrine A (100 µg. per ml.).

the results obtained in a group of ten experiments using 100 μg. per ml. of protoveratrine A.

Sodium-24

Protoveratrine A in doses of from 50 μg. to 0.5 mg. per ml. caused a significant ($P, > 0.001$) increase in the uptake of sodium-24 by isolated frog sartorius muscle. The control muscle showed a lower percentage uptake than the drug-treated muscle. Table 10 (page 161) and Fig. 12 (C) (page 118) show the results obtained in a group of ten experiments using 100 μg. per ml. of protoveratrine A.

Uptake of calcium-47, potassium-42 and sodium-24 by isolated frog sartorius muscle in the presence of protoveratrine B.

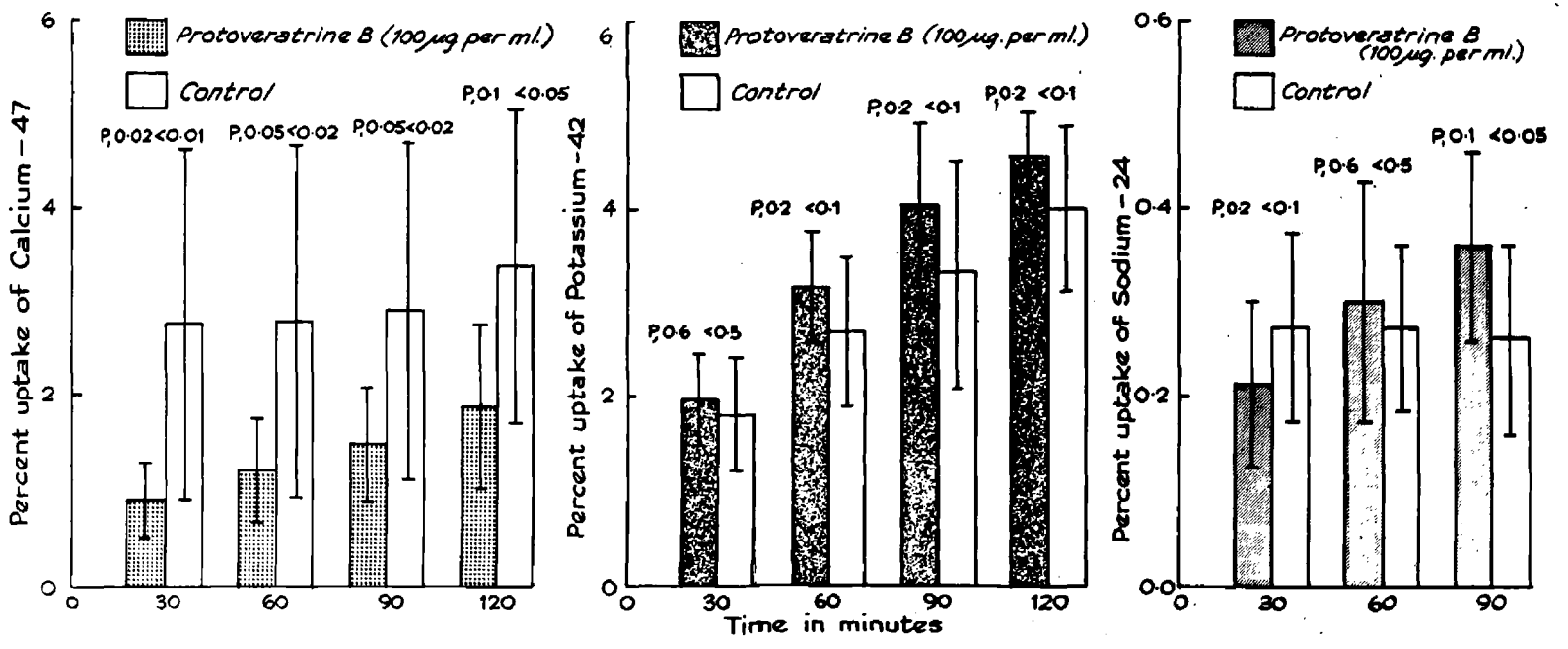
Protoveratrine B is an ester alkaloid obtained from Veratrum album, which like protoveratrine A has typical veratrinic actions.

Calcium-47

Protoveratrine B in doses of from 50 μg. to 0.5 mg. per ml. caused a significant ($P, 0.05 < 0.02$) decrease in the uptake of calcium-47 by isolated frog sartorius muscle. The control muscle showed a higher percentage uptake when compared with the drug-treated muscle. Table 11 (page 162) and Fig. 13(A) (page 120) show the results obtained in a group of nine experiments using 100 μg. per ml. of protoveratrine B.

Potassium-42

Protoveratrine B in doses of from 50 μg. to 0.5 mg. per/



A B C

Fig. 13.

Uptake of calcium-47 (A), potassium-42 (B) and sodium-24 (C) by isolated frog sartorius muscle in the presence of protoveratrine B (100 µg. per ml.).

per ml. did not cause significant (P, 0.2< 0.1) change in the uptake of potassium-42 by isolated frog sartorius muscle. There was no significant difference between the percentage uptake by the control muscle and that of the drug-treated muscle. Table 11 (page 162) and Fig. 13 (B) (page 120) show the results obtained in a group of nine experiments using 100 μg. per ml. of protoveratrine B.

Sodium-24

Protoveratrine B in doses of from 50 μg. to 0.5 mg. per ml. did not cause a significant (P, 0.2< 0.1) change in the uptake of sodium-24 by isolated frog sartorius muscle. There was no significant difference between the percentage uptake by the control muscle and that of the drug-treated muscle. Table 11 (page 162) and Fig. 13 (C) (Page 120) show the results obtained in a group of ten experiments using 100 μg. per ml. of protoveratrine B.

Effect of suxamethonium on the release of calcium-47 and potassium-42 from isolated frog sartorius muscle.

Calcium-47

Suxamethonium in doses of from 1 mg. to 10 mg. per ml. caused an increase in the release of calcium-47 from isolated frog sartorius muscle. The increase in the release of calcium-47 was more pronounced with the increase in the dose of suxamethonium. Fig. 14 (page 122) shows the results of a typical experiment and Table 12 (page 163) shows the results of a group of nine experiments using 5 mg. per ml of suxamethonium.

Potassium-42/

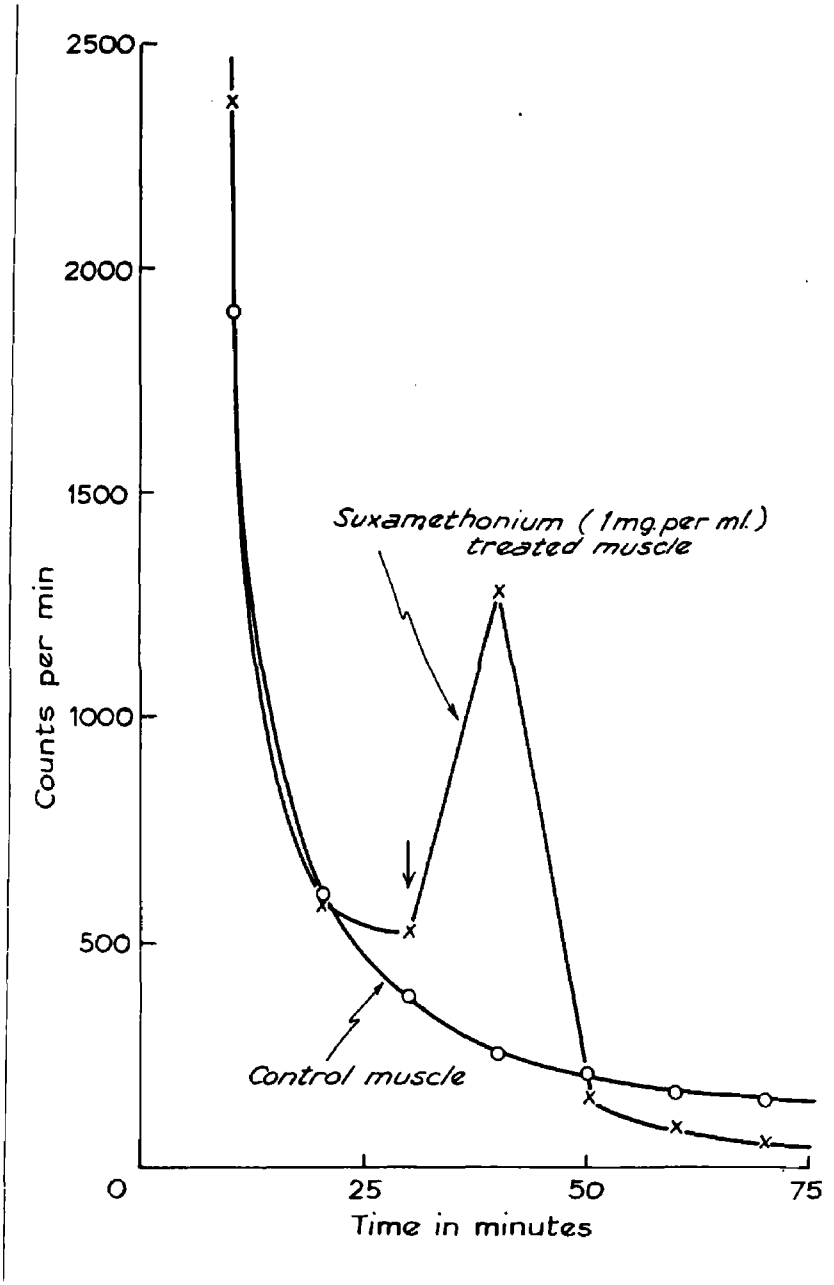


Fig. 14

Effect of suxamethonium (1 mg. per ml.) on the release of calcium-47 from isolated frog sartorius muscle. The vertical arrow indicates the point of exposure of the test muscle to the drug.

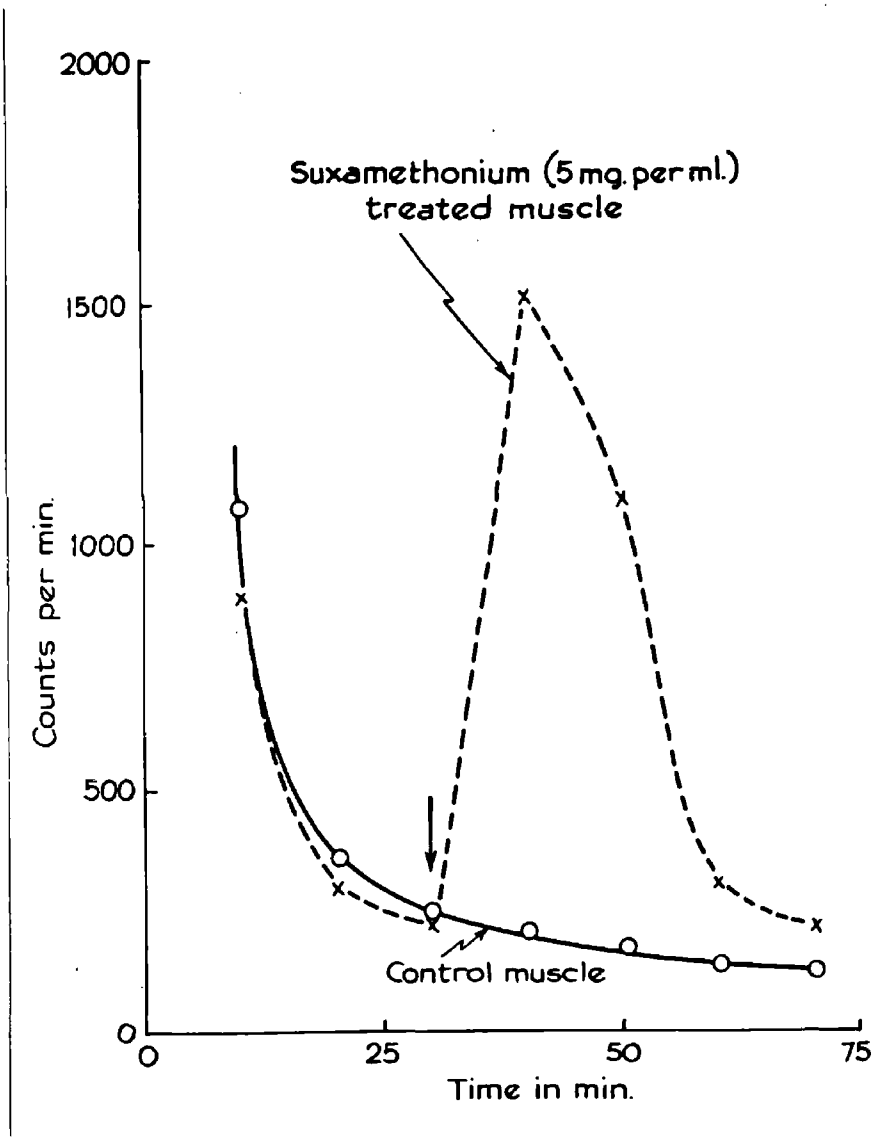


Fig. 15

Effect of suxamethonium (5 mg. per ml.) on the release of potassium-42 from isolated frog sartorius muscle. The vertical arrow indicates the point of exposure of the test muscle to the drug.

Potassium-42

Suxamethonium in doses of from 1 mg. to 10 mg. per ml. caused an increase in the release of potassium-42 from isolated frog sartorius muscle. The increase in the release of potassium-42 was more pronounced with the increase in the dose of suxamethonium. Fig. 15 (page 123) shows the results of a typical experiment and Table 23 (page 174) shows the results of a group of nine experiments using 5 mg. per ml. of suxamethonium.

Effect of decamethonium on the release of calcium-47 and potassium-42 from isolated frog sartorius muscle.

Calcium-47

Decamethonium in doses of from 50 μg. to 0.5 mg. per ml. had no significant effect on the release of calcium-47 from isolated frog sartorius muscle, although in a few experiments there was apparently a very slight increase in the release of calcium-47. Fig. 16 (page 125) shows the results of an experiment showing no increased release of calcium-47 and Table 13 (page 164) shows the results of a group of ten experiments using 200 μg. per ml. of decamethonium.

Potassium-42

Decamethonium in doses of from 50 μg. to 0.5 mg. per ml. caused an increase in the release of potassium-42 from isolated frog sartorius muscle. The increase in the release of potassium-42 was more pronounced with the increase in the dose of decamethonium. Fig. 17 (page 126) shows the results of a typical experiment and Table 24 (page 175) shows the results of a group/

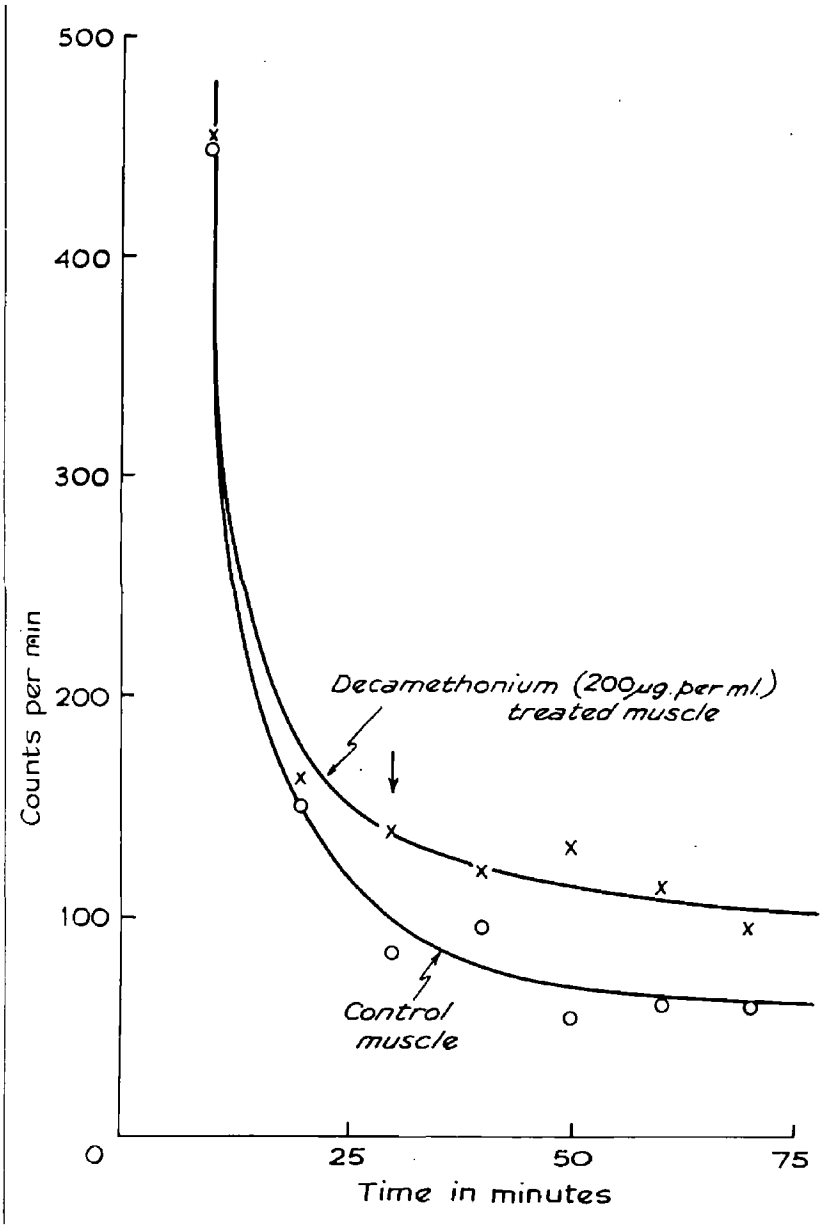


Fig. 16

Effect of decamethonium (200 µg. per ml.) on the release of calcium-47 from isolated frog sartorius muscle. The vertical arrow indicates the point of exposure of the test muscle to the drug.

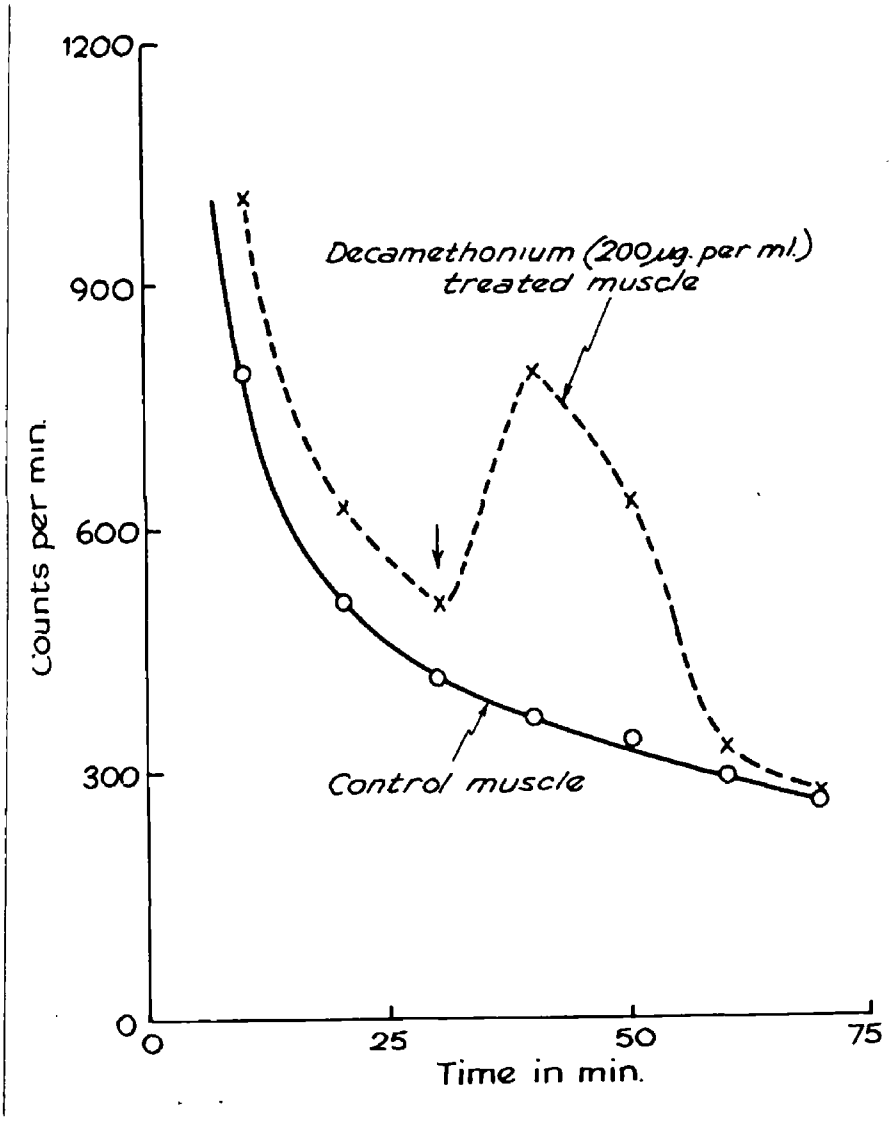


Fig. 17

Effect of decamethonium (200 µg. per ml.) on the release of potassium-42 from isolated frog sartorius muscle. The vertical arrow indicates the point of exposure of the test muscle to the drug.

group of nine experiments.using 200 µg. per ml. of decamethonium.

Effect of nicotine on the release of calcium-47 and potassium-42 by isolated frog sartorius muscle.

Calcium-47

Nicotine in doses of from 200 µg. to 2 mg. per ml. caused an increase in the release of calcium-47 from isolated frog sartorius muscle. The increase in the release of calcium-47 was more pronounced with the increase in the dose of nicotine. Fig. 18 (page 128) shows the results of a typical experiment and Table 14 (page 165) shows the results of a group of eight experiments.using 1 mg. per ml. of nicotine.

Potassium-42

Nicotine in doses of from 200 µg. to 2 mg. per ml. caused an increase in the release of potassium-42 from isolated frog sartorius muscle. The increase in the release of potassium-42 was more pronounced with the increase in the dose of nicotine. Fig. 19 (page129) shows the results of a typical experiment and Table 25 (page 176) shows the results of a group of ten experiments.using 1 mg. per ml. of nicotine.

Effect of ryanodine on the release of calcium-47 and potassium-42 from isolated frog sartorius muscle.

Calcium-47

Ryanodine in doses of from 10 µg. to 100 µg. per ml. caused an increase in the release of calcium-47 from isolated frog sartorius muscle. The increase in the release of calcium-47/

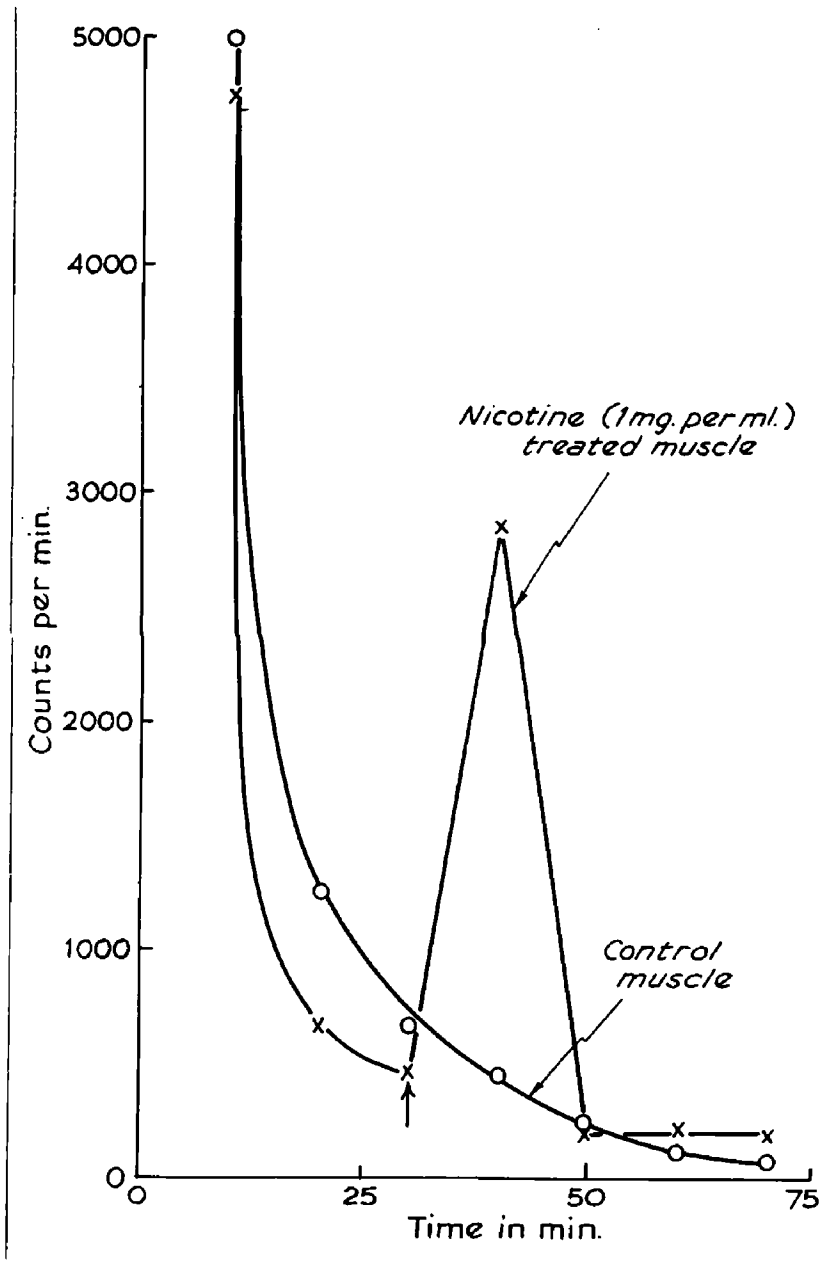


Fig. 18

Effect of nicotine (1 mg. per ml.) on the release of calcium-47 from isolated frog sartorius muscle. The vertical arrow indicates the point of exposure of the test muscle to the drug.

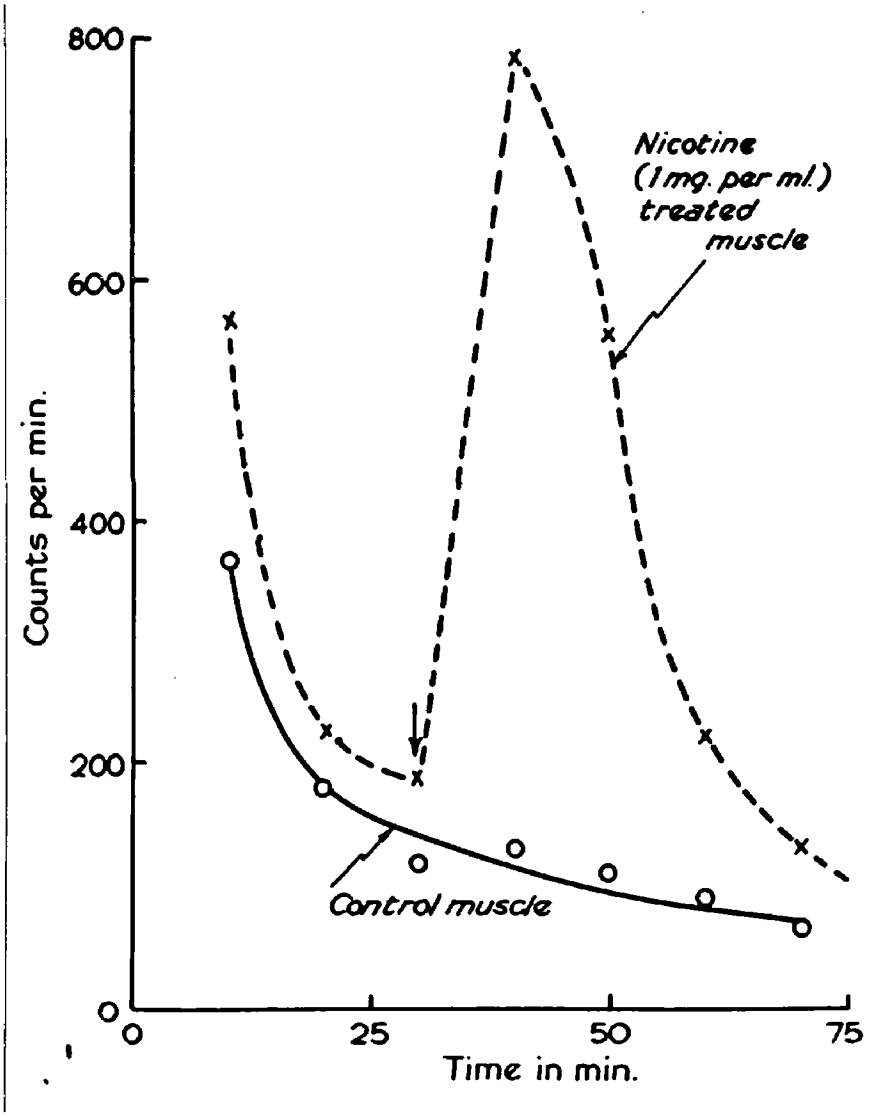


Fig. 19

Effect of nicotine (1 mg. per ml.) on the release of potassium-42 from isolated frog sartorius muscle. The vertical arrow indicates the point of exposure of the test muscle to the drug.

calcium-47 was more pronounced with the increase in the dose of ryanodine. In some experiments the release of calcium-47 was delayed for 10 to 20 minutes after exposure of the test muscle to the drug and in a few experiments the release was not very marked. Fig. 20 (page 131) shows the results of an experiment showing increased release of calcium-47 and Table 15 (page 166) shows the results of a group of ten experiments using 50 μg. per ml. of ryanodine.

Potassium-42

Ryanodine in doses of from 10 μg. to 100 μg. per ml. caused an increase in the release of potassium-42 from isolated frog sartorius muscle. The increase in the release of potassium-42 was more pronounced with the increase in the dose of ryanodine. In some experiments the release of potassium-42 was delayed for 10 to 20 minutes after exposure of the test muscle to the drug. Fig.21 (page 132) shows the results of one such experiment and Table 26 (page 177) shows the results of a group of nine experiments using 50 μg. per ml. of ryanodine.

Effect of carbachol on the release of calcium-47 and potassium-42 from isolated frog sartorius muscle.

Calcium-47

Carbachol in doses of from 1 mg. to 5 mg. per ml. did not apparently cause either a marked or a consistent effect on the release of calcium-47 from isolated frog sartorius muscle. In a few experiments there was apparently a very slight increase in the release of calcium-47 but in others no such/

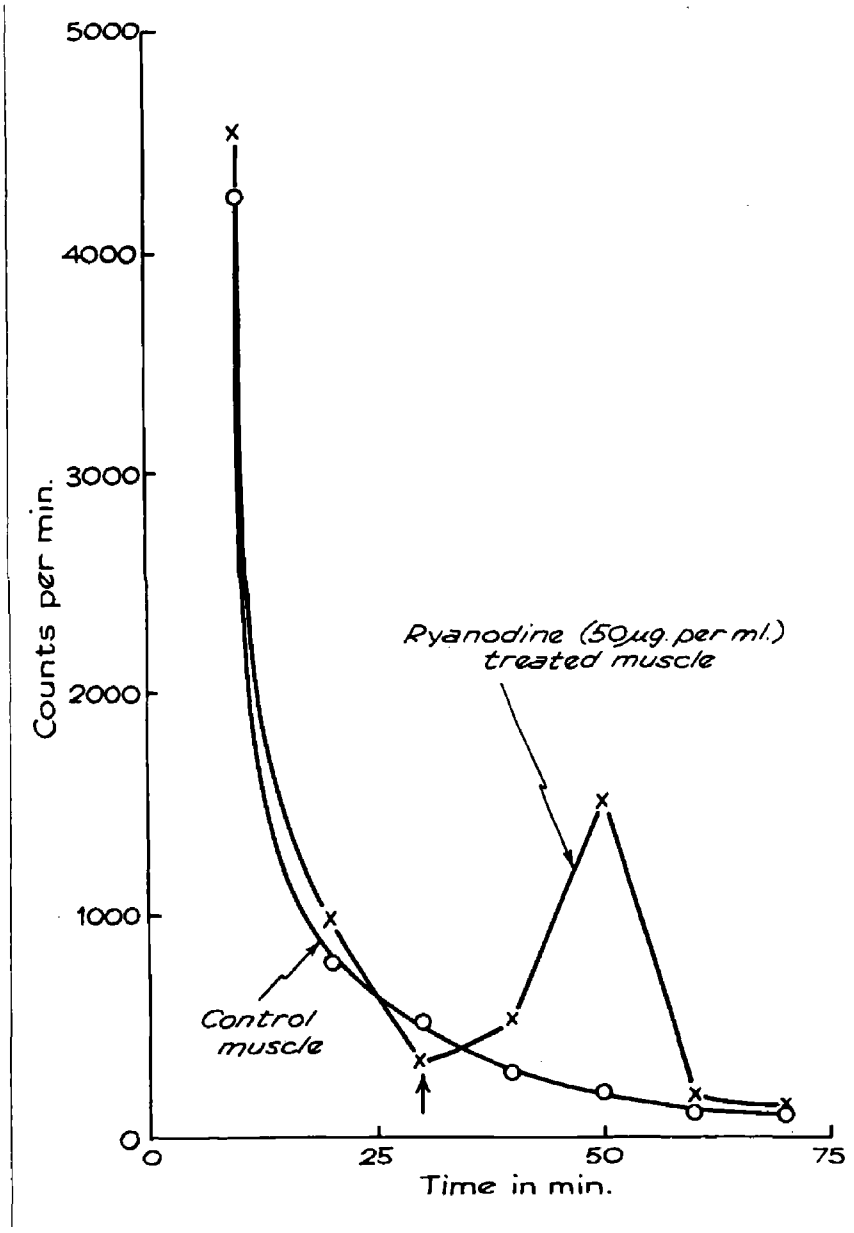


Fig. 20

Effect of ryanodine (50 µg. per ml.) on the release of calcium-47 from isolated frog sartorius muscle. The vertical arrow indicates the point of exposure of the test muscle to the drug.

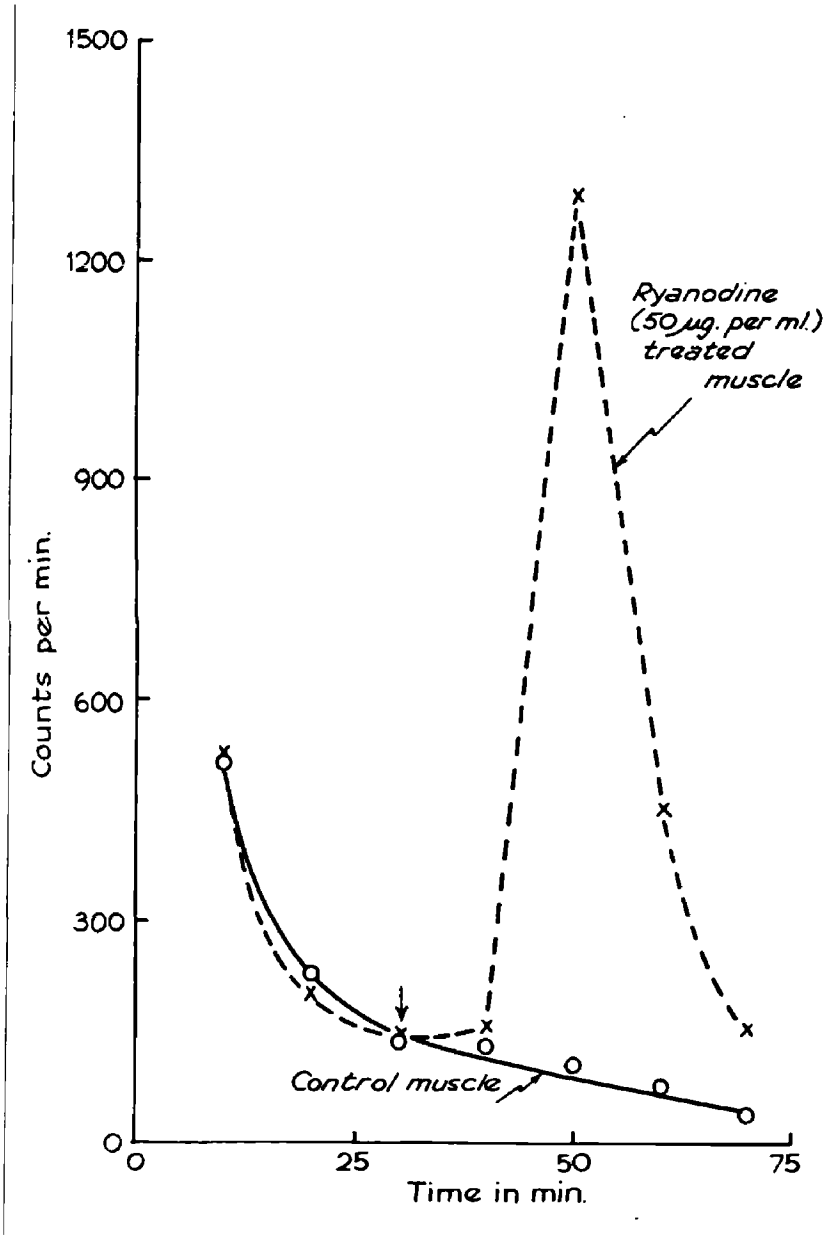


Fig. 21

Effect of ryanodine (50 µg. per ml.) on the release of potassium-42 from isolated frog sartorius muscle. The vertical arrow indicates the point of exposure of the test muscle to the drug.

such effect was observed. Fig. 22 (page 134) shows the result of an experiment showing no release of calcium-47 and Table 16 shows the results of a group of eight experiments using 5 mg. per ml. of carbachol.

Potassium-42

Carbachol in doses of from 1 mg. to 5 mg. per ml. caused an increase in the release of potassium-42 from isolated frog sartorius muscle. The increase in the release of potassium-42 was more pronounced with the increase in the dose of carbachol. Fig. 23 (page 135) shows the results of a typical experiment and Table 27 (page 178) shows the results of a group of ten experiments using 5 mg. per ml. of carbachol.

Effect of neostigmine on the release of calcium-47 and potassium-42 from isolated frog sartorius muscle.

Calcium-47

Neostigmine in doses of from 25 µg. to 150 µg. per ml. caused an increase in the release of calcium-47 from isolated frog sartorius muscle. The increase in the release of calcium-47 was more pronounced with the increase in the dose of neostigmine. Fig. 24 (page 137) shows the results of a typical experiment and Table 17 (page 168) shows the results of a group of eight experiments using 100 µg. per ml. of neostigmine.

Potassium-42

Neostigmine in doses of from 25 µg. to 150 µg. per ml. caused an increase in the release of potassium-42 from isolated frog sartorius muscle. The increase in the release of/

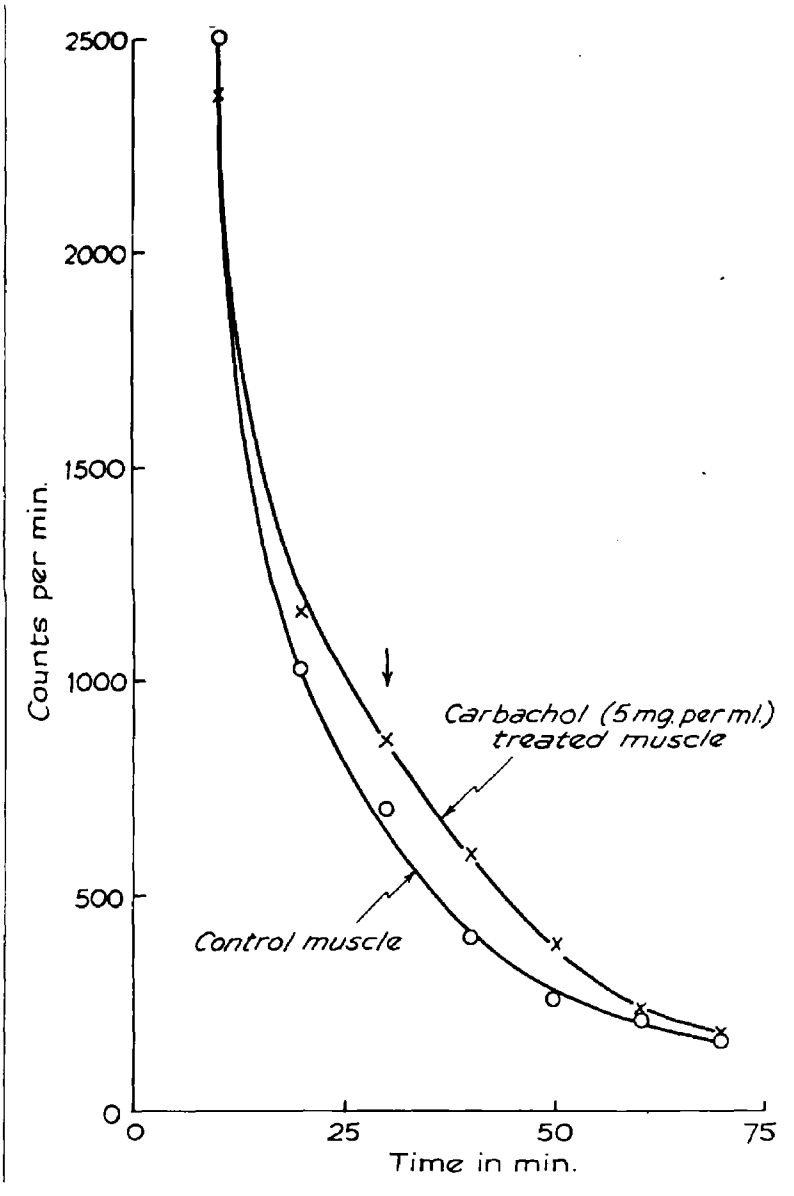


Fig. 22

Effect of carbachol (5 mg. per ml.) on the release of calcium-47 from isolated frog sartorius muscle. The vertical arrow indicates the point of exposure of the test muscle to the drug.

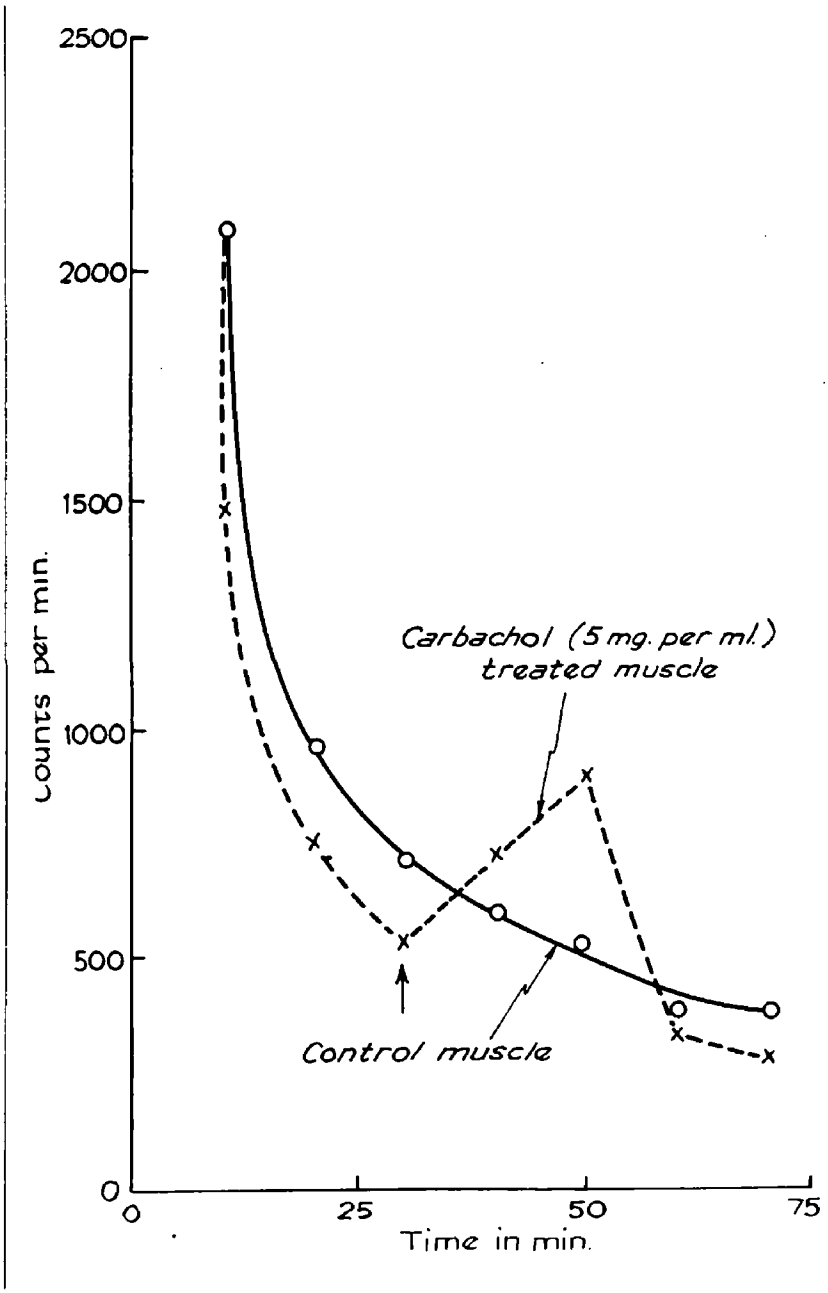


Fig. 23

Effect of carbachol (5 mg. per ml.) on the release of potassium-42 from isolated frog sartorius muscle. The vertical arrow indicates the point of exposure of the test muscle to the drug.

of potassium-42 was more pronounced with the increase in the dose of neostigmine. Fig. 25 (page 138) shows the results of a typical experiment and Table 28 (page 179) shows the results of a group of nine experiments.using 75 μg. per ml. of neostigmine.

Effect of edrophonium on the release of calcium-47 and potassium-42 from isolated frog sartorius muscle.

Calcium-47

Edrophonium in doses of from 0.5 mg. to 2 mg. per ml. caused an increase in the release of calcium-47 from isolated frog sartorius muscle. The increase in the release of calcium-47 was more pronounced with the increase in the dose of edrophonium. Fig. 26 (page 139) shows the results of a typical experiment and Table 18 (page 169) shows the results of a group of nine experiments.using 2 mg. per ml. of edrophonium.

Potassium-42

Edrophonium in doses of from 0.5 mg. to 2 mg. per ml. did not apparently cause change in the release of potassium-42 from isolated frog sartorius muscle. Fig. 27 (page 140) shows the results of a typical experiment and Table 29 (page 180) shows the results of a group of ten experiments.using 2 mg. per ml. of edrophonium.

Effect of tubocurarine on the release of calcium-47 and potassium-42 from isolated frog sartorius muscle.

Calcium-47

Tubocurarine in doses of from 50 μg. to 200 μg. per ml. did not apparently affect the release of calcium-47 from isolated/

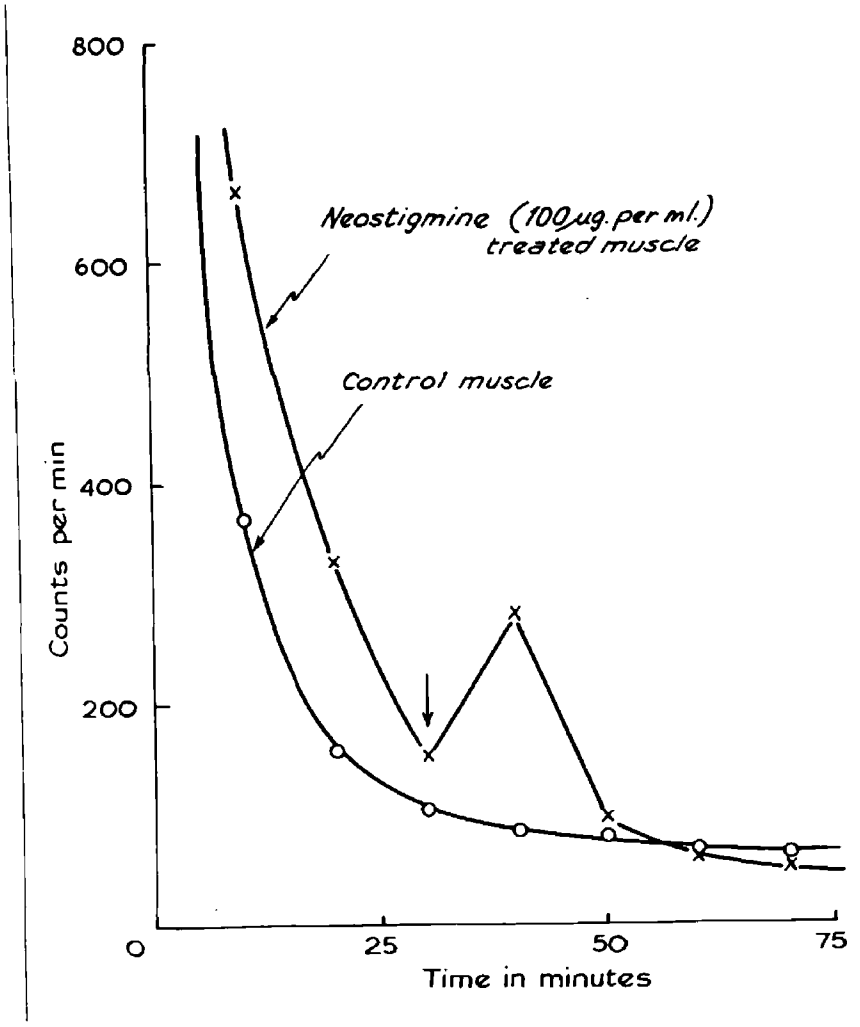


Fig. 24

Effect of neostigmine (100 µg. per ml.) on the release of calcium-47 from isolated frog sartorius muscle. The vertical arrow indicates the point of exposure of the test muscle to the drug.

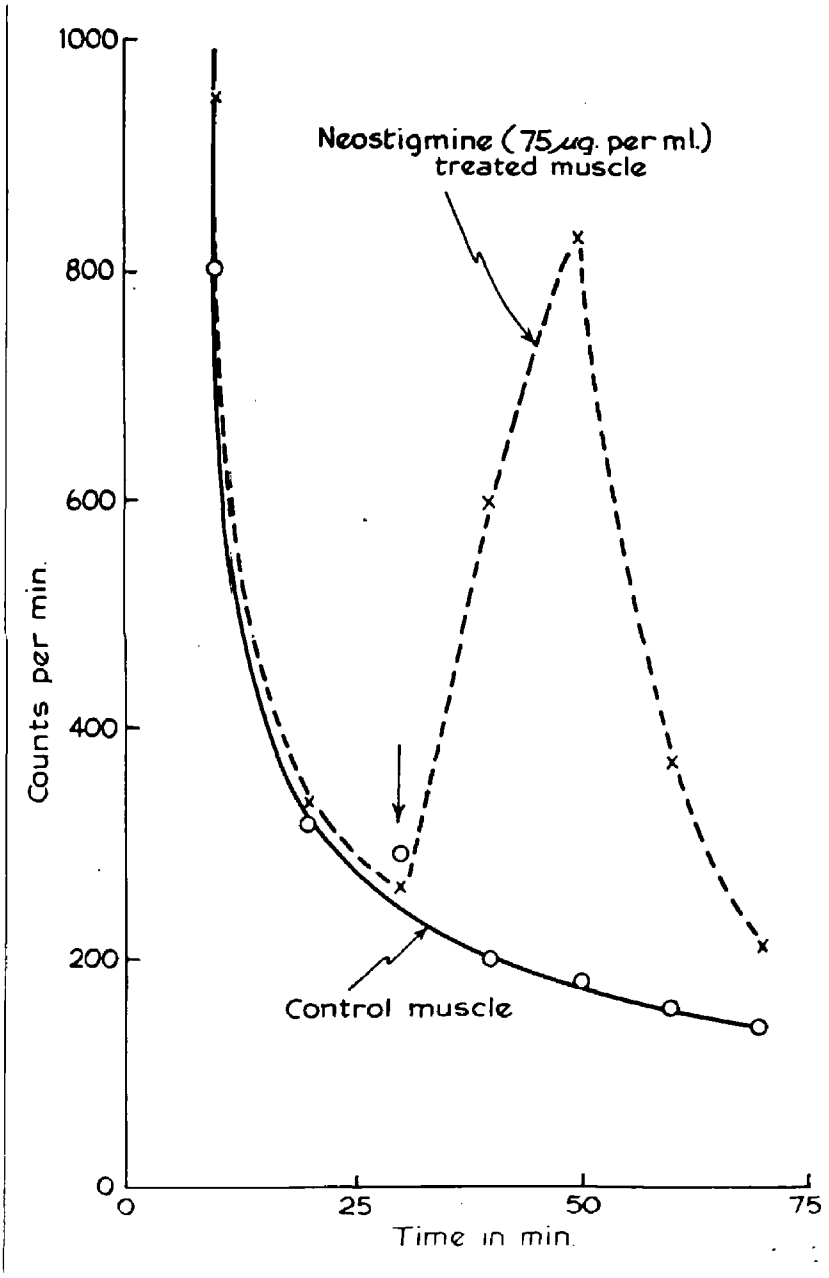


Fig. 25

Effect of neostigmine (75 µg. per ml.) on the release of potassium-42 from isolated frog sartorius muscle. The vertical arrow indicates the point of exposure of the test muscle to the drug.

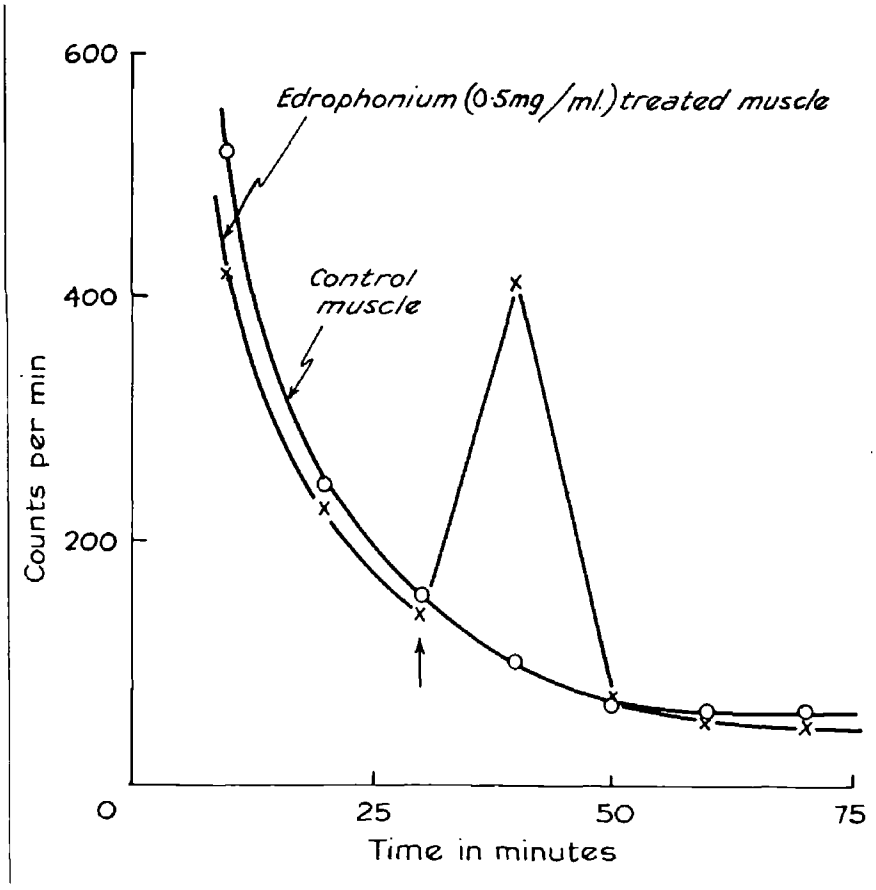


Fig. 26

Effect of edrophonium (0.5 mg. per ml.) on the release of calcium-47 from isolated frog sartorius muscle. The vertical arrow indicates the point of exposure of the test muscle to the drug.

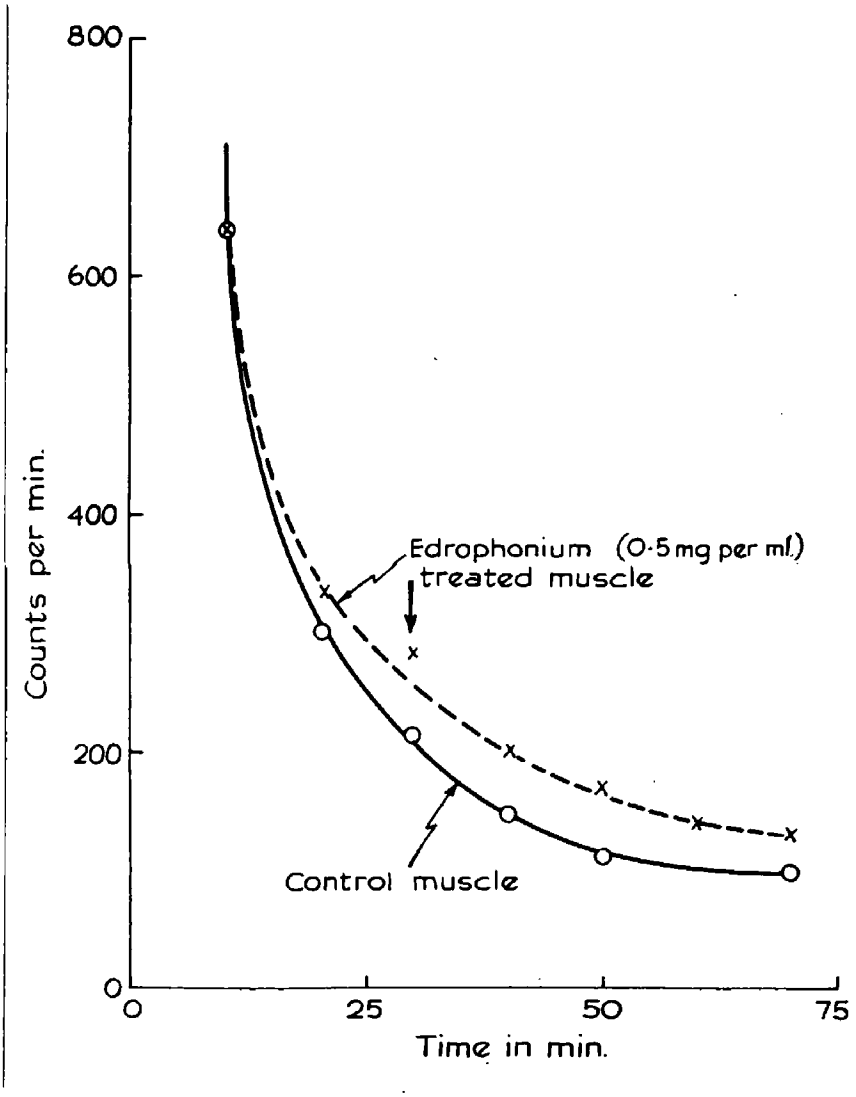


Fig. 27

Effect of edrophonium (0.5 mg. per ml.) on the release of potassium-42 from isolated frog sartorius muscle. The vertical arrow indicates the point of exposure of the test muscle to the drug.

isolated frog sartorius muscle, but larger doses (0.5 mg. to 1 mg. per ml.) caused an increase in the release of calcium-47 in some experiments. The result of one such experiment is shown in Fig. 28 (page 142). Table 19 (page 170) shows the results of a group of nine experiments.using 1 mg. per ml. of tubocurarine.

Potassium-42

Tubocurarine in doses of from 50 µg. to 1 mg. per ml. did not apparently cause any effect on the release of potassium-42 from isolated frog sartorius muscle. Fig. 29 (page 143) shows the results obtained during a typical experiment and Table 30 (page 181) shows the results from a group of nine experiments.using 1 mg. per ml. of tubocurarine.

Effect of gallamine on the release of calcium-47 and potassium-42 from isolated frog sartorius muscle.

Calcium-47

Gallamine in doses of from 1 mg. to 8 mg. per ml. did not cause any apparent effect on the release of calcium-47 from isolated frog sartorius muscle. Fig. 30 (page 144) shows the results of a typical experiment and Table 20 (page 171) shows the results of a group of eight experiments.using 4 mg. per ml. of gallamine.

Potassium-42

Gallamine in doses of from 1 mg. to 8 mg. per ml. did not cause any apparent effect on the release of potassium-42 from isolated frog sartorius muscle. Fig. 31 (page 145) shows the results of a typical experiment and Table 31 (page 182) shows/

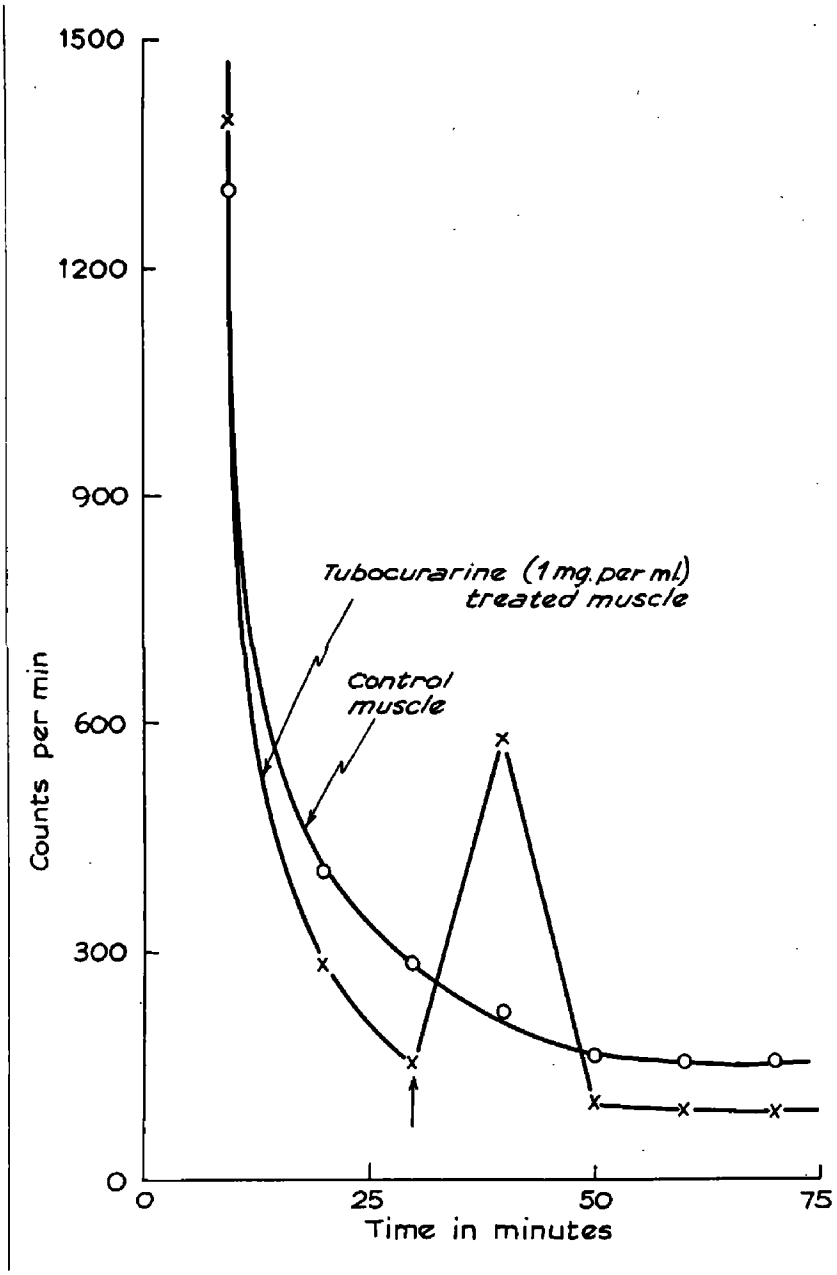


Fig. 28

Effect of tubocurarine (1 mg. per ml.) on the release of calcium-47 from isolated frog sartorius muscle. The vertical arrow indicates the point of exposure of the test muscle to the drug.

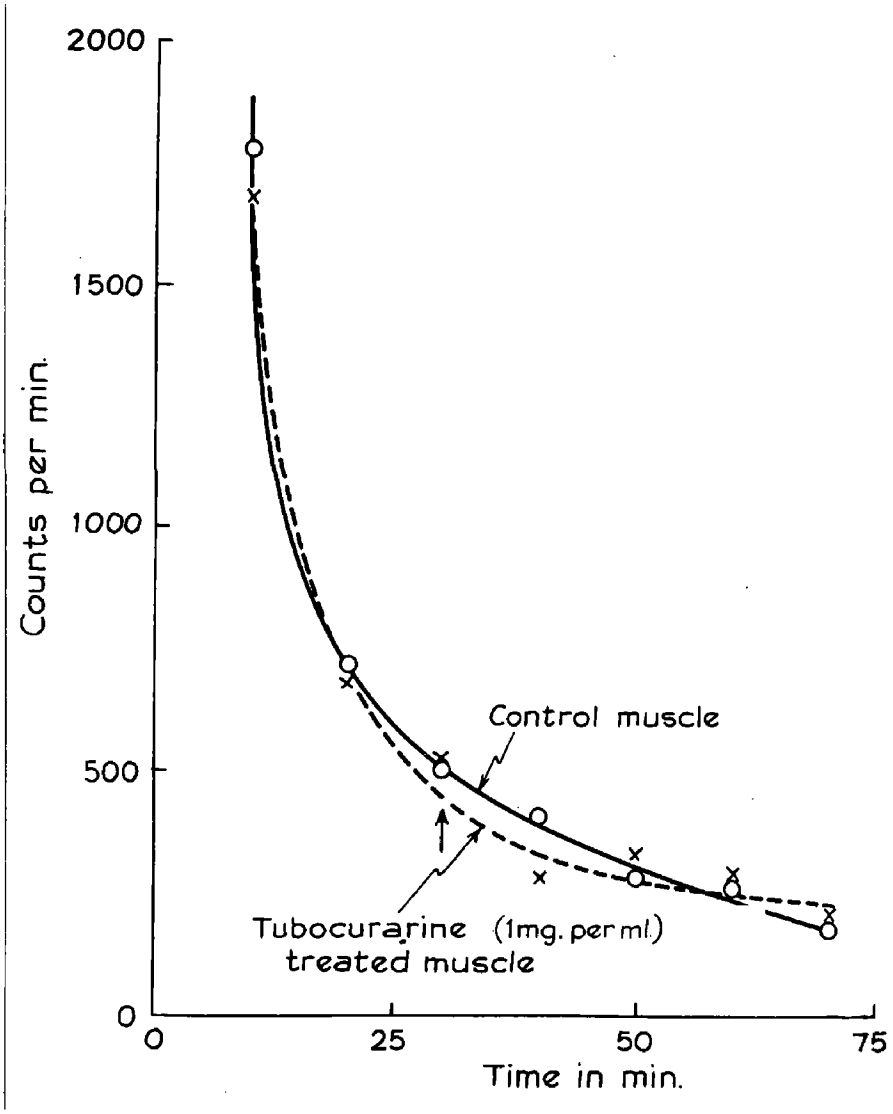


Fig. 29

Effect of tubocurarine (1 mg. per ml.) on the release of potassium-42 from isolated frog sartorius muscle. The vertical arrow indicates the point of exposure of the test muscle to the drug.

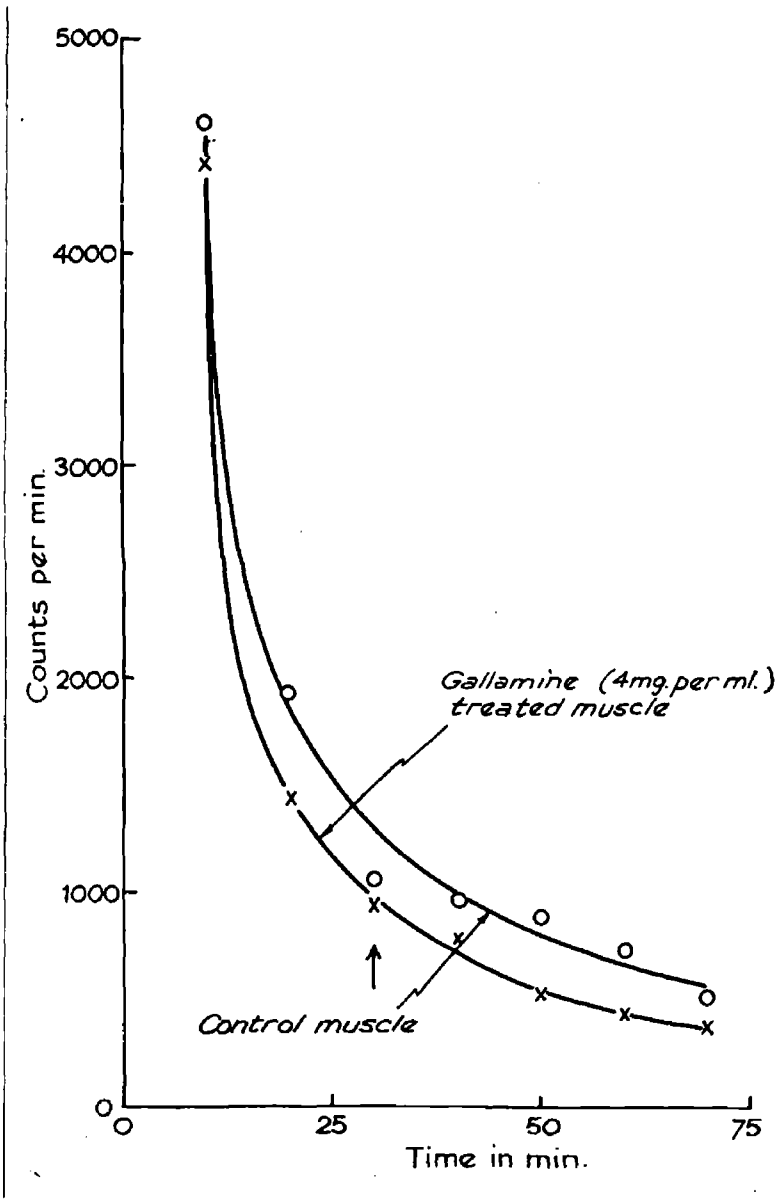


Fig. 30

Effect of gallamine (4 mg. per ml.) on the release of calcium-47 from isolated frog sartorius muscle. The vertical arrow indicates the point of exposure of the test muscle to the drug.

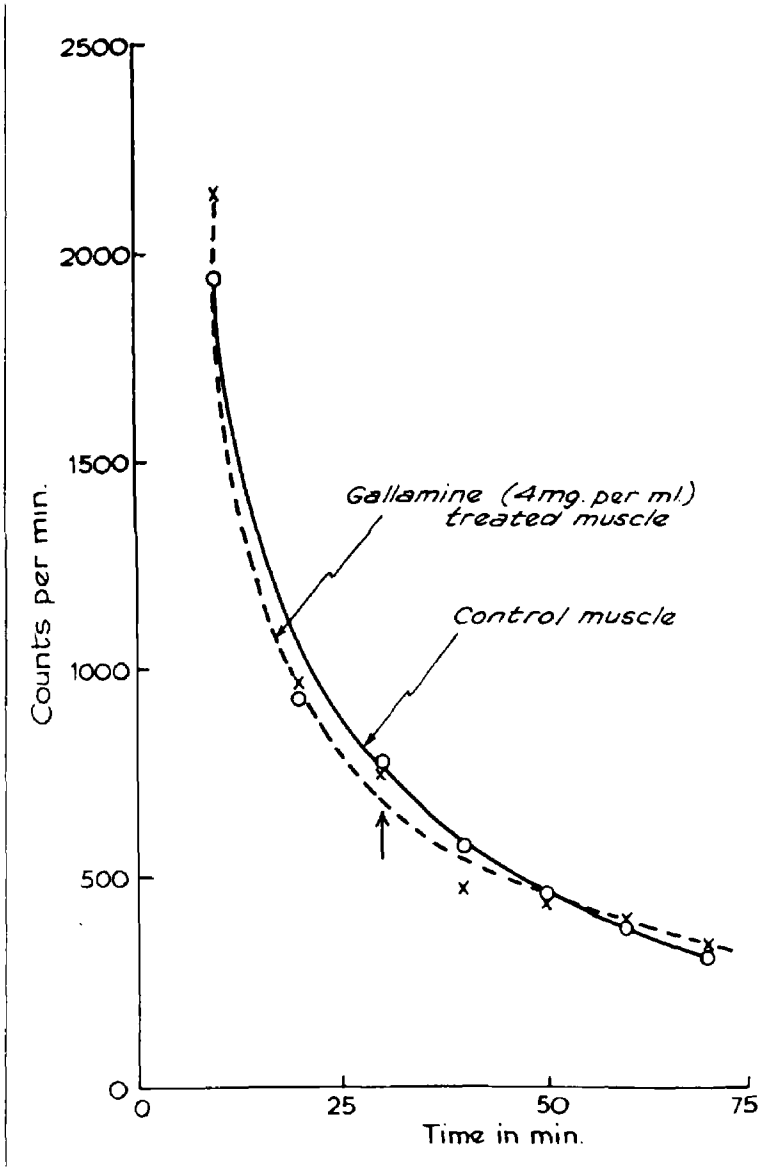


Fig. 31

Effect of gallamine (4 mg. per ml.) on the release of potassium-42 from isolated frog sartorius muscle. The vertical arrow indicates the point of exposure of the test muscle to the drug.

shows the results of a group of eight experiments using 4 mg. per ml. of gallamine.

Effect of protoveratrine A on the release of calcium-47 and potassium-42 from isolated frog sartorius muscle.

Calcium-47

Protoveratrine A in doses of from 50 µg. to 0.5 mg. per ml. had variable effects upon the release of calcium-47 from isolated frog sartorius muscle. In some experiments there was an increased release of calcium-47 whereas in others no distinct release was observed. In a few experiments the release of calcium-47 was delayed by from 10 to 20 minutes after initial exposure of the test muscle to the drug. The result of one such experiment is shown in Fig. 32 (page 147). Table 21 (page 172)shows the results of a group of nine experiments.using 100 µg. per ml. of protoveratrine A.

Potassium-42

Protoveratrine A in doses of from 50 µg. to 0.5 mg. per ml. caused an increased release of potassium-42 from isolated frog sartorius muscle in most of the experiments, whereas in a few experiments no increase in release could be seen. Fig. 33 (page 148) shows the results of an experiment showing increased release of potassium-42 and Table 32 (page 183) shows the results of a group of ten experiments.using 100 µg. per ml. of protoveratrine A.

Effect of protoveratrine B on the release of calcium-47 and potassium-42 from isolated frog sartorius muscle.

Calcium-47

Protoveratrine B/

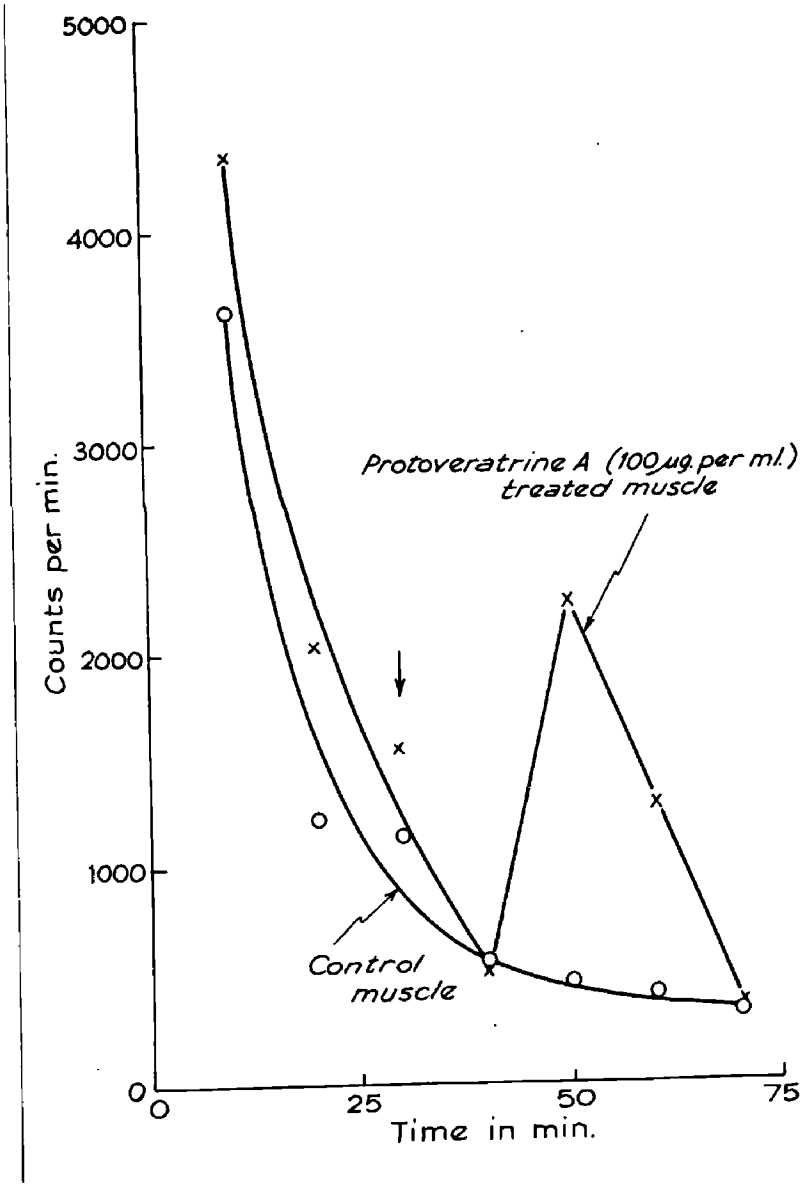


Fig. 32

Effect of protoveratrine A (100 µg. per ml.) on the release of calcium-47 from isolated frog sartorius muscle. The vertical arrow indicates the point of exposure of the test muscle to the drug.

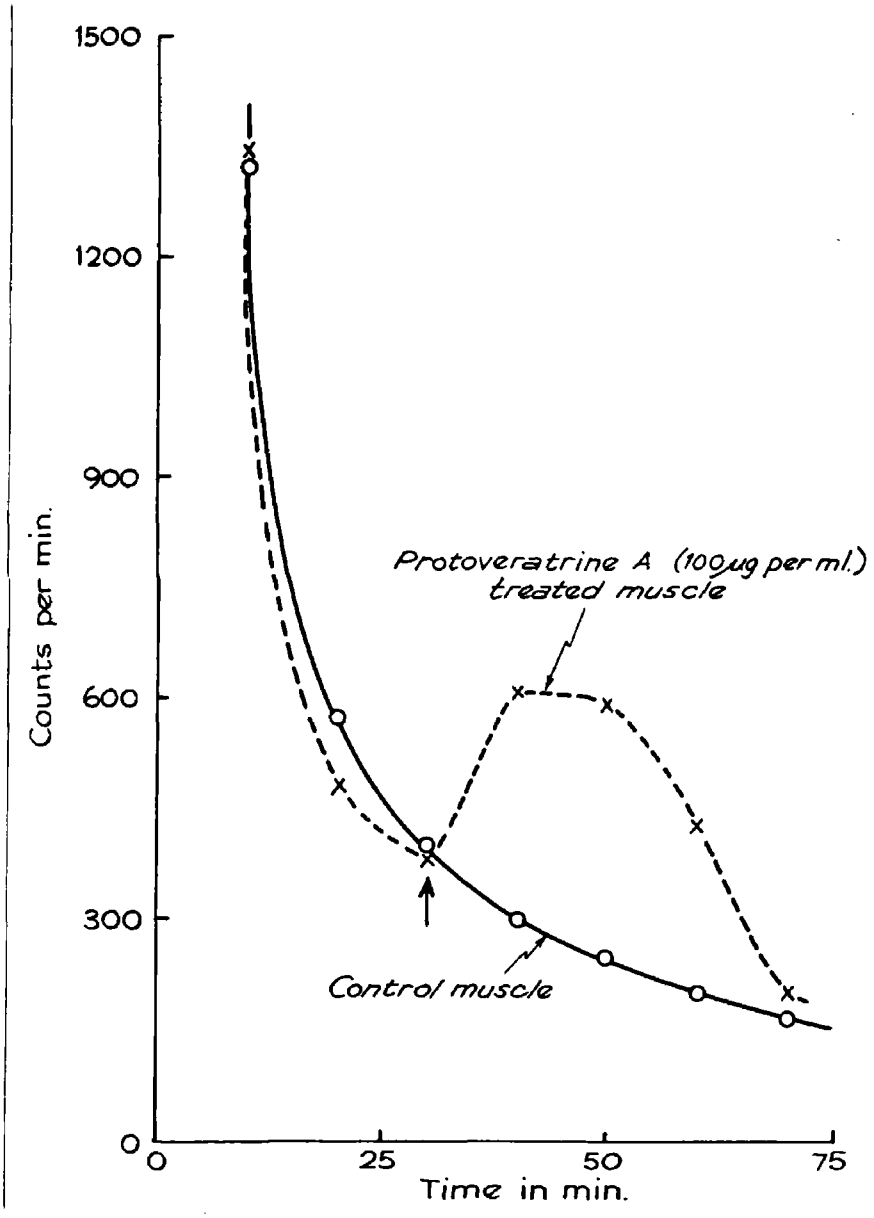


Fig. 33

Effect of protoveratrine A (100 µg. per ml.) on the release of potassium-42 from isolated frog sartorius muscle. The vertical arrow indicates the point of exposure of the test muscle to the drug.

Protoveratrine B in doses of from 50 μg. to 0.5 mg. per ml. caused variable effects upon the release of calcium-47 from isolated frog sartorius muscle. In some experiments there was an increased release of calcium-47 (Fig. 34 , page 150), whereas in others no distinct release was observed. In some experiments the release of calcium-47 was delayed by from 10 to 20 minutes after exposure of the test muscle to the drug. Table 22 (page 173) shows the results of a group of ten experiments. using 100 μg. per ml. of protoveratrine B.

Potassium-42

Protoveratrine B in doses of from 50 μg. to 0.5 mg. per ml. had a variable effect on the release of potassium-42 from isolated frog sartorius muscle. In some experiments there was a slight increase in the release of potassium-42 (Fig. 35 , page 151), whereas in others no such effect was noted. In a few experiments potassium-42 release was delayed by from 10 to 20 minutes after exposure of the test muscle to the drug. Table 33, p. 184 shows the results of a group of ten experiments. using 100 μg. per ml. of protoveratrine B.

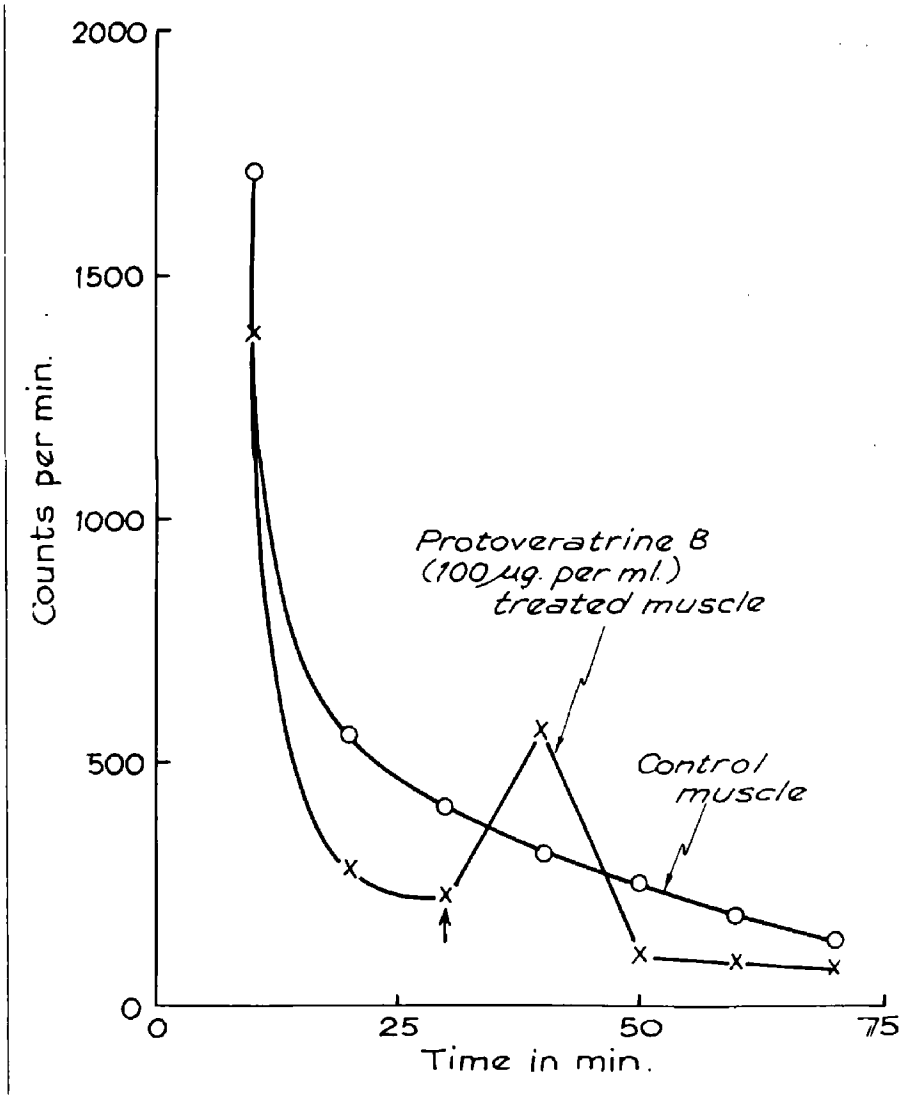


Fig. 34

Effect of protoveratrine B (100 µg. per ml.) on the release of calcium-47 from isolated frog sartorius muscle. The vertical arrow indicates the point of exposure of the test muscle to the drug.

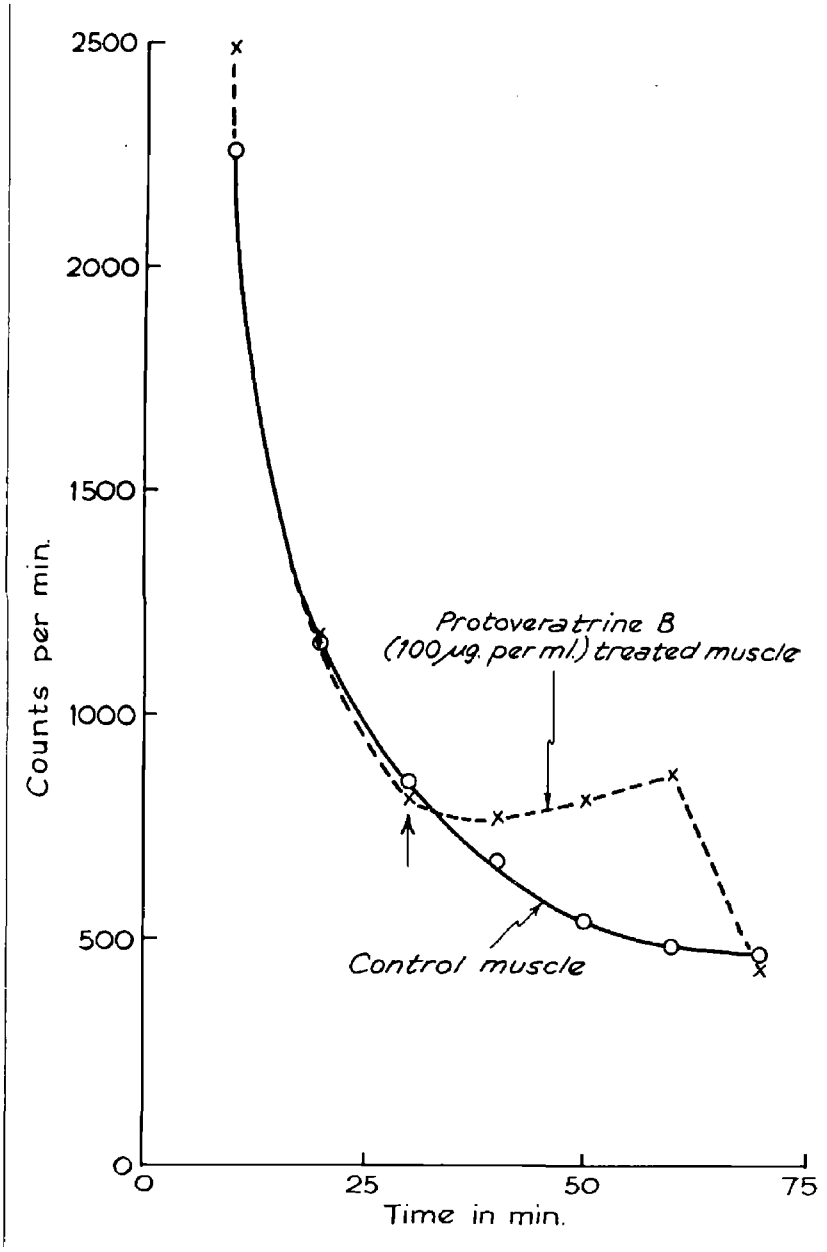


Fig. 35

Effect of protoveratrine B (100 µg. per ml.) on the release of potassium-42 from isolated frog sartorius muscle. The vertical arrow indicates the point of exposure of the test muscle to the drug.

TABLE 1.

Uptake of calcium-47, potassium-42 and sodium-24 by isolated frog sartorius muscle in the presence of suxamethonium (5 mg. per ml.).

Isotope	No. of expts.	Mean percentage uptake of radioactive ions ± S.D.							
		After 30 mins.		After 60 mins.		After 90 mins.		After 120 mins.	
		Test	Control	Test	Control	Test	Control	Test	Control
Calcium-47	10	0.9 ±0.209	0.56 ±0.244	1.37 ±0.151	0.77 ±0.256	1.59 ±0.173	0.94 ±0.249	1.69 ±0.253	1.11 ±0.295
		P, 0.01< 0.001		P, >0.001		P, >0.001		P, >0.001	
Potassium-42	10	0.85 ±0.387	1.68 ±0.707	1.1 ±0.574	2.51 ±0.854	1.24 ±0.781	2.94 ±1.005	1.14 ±0.854	3.02 ±1.148
		P, 0.01< 0.001		P, >0.001		P, >0.001		P,0.01<0.001	
Sodium-24	10	0.19 ±0.033	0.14 ±0.031	0.22 ±0.037	0.16 ±0.043	0.23 ±0.05	0.17 ±0.05		
		P, 0.01< 0.001		P, 0.01<0.001		P,0.02<0.01			

TABLE 2.

Uptake of calcium-47, potassium-42 and sodium-24 by isolated frog sartorius muscle in the presence of decamethonium (200 µg. per ml.)

Isotope	No. of expts.	Mean percentage uptake of radioactive ions ± S.D.							
		After 30 mins.		After 60 mins.		After 90 mins.		After 120 mins.	
		Test	Control	Test	Control	Test	Control	Test	Control
Calcium-47	11	$^{0.82}_{\pm 0.479}$	$^{0.59}_{\pm 0.316}$	$^{1.26}_{\pm 0.624}$	$^{0.78}_{\pm 0.346}$	$^{1.5}_{\pm 0.64}$	$^{0.93}_{\pm 0.4}$	$^{1.65}_{\pm 0.714}$	$^{1.04}_{\pm 0.435}$
		P,0.2<0.1		P,0.05<0.02		P,0.05<0.02		P,0.05<0.02	
Potassium-42	10	$^{0.56}_{\pm 0.346}$	$^{1.04}_{\pm 0.479}$	$^{0.48}_{\pm 0.258}$	$^{1.5}_{\pm 0.556}$	$^{0.48}_{\pm 0.195}$	$^{2.04}_{\pm 0.774}$	$^{0.41}_{\pm 0.141}$	$^{2.21}_{\pm 0.927}$
		P,0.05<0.02		P,>0.001		P,>0.001		P,>0.001	
Sodium-24	10	$^{0.18}_{\pm 0.07}$	$^{0.13}_{\pm 0.03}$	$^{0.29}_{\pm 0.118}$	$^{0.15}_{\pm 0.037}$	$^{0.37}_{\pm 0.123}$	$^{0.16}_{\pm 0.043}$		
		P,0.10<0.05		P,0.01<0.001		P,>0.001			

TABLE 3.

Uptake of calcium-47, potassium-42 and sodium-24 by isolated frog sartorius muscle in the presence of nicotine (1 mg. per ml.).

Isotope	No. of Expts.	Mean percentage uptake of radioactive ions ± S.D.							
		After 30 mins.		After 60 mins.		After 90 mins.		After 120 mins.	
		Test	Control	Test	Control	Test	Control	Test	Control
Calcium-47	10	$^{1.9}_{\pm 0.685}$	$^{0.69}_{\pm 0.23}$ P,>0.001	$^{3.16}_{\pm 1.144}$	$^{0.94}_{\pm 0.289}$ P,>0.001	$^{4.65}_{\pm 1.827}$	$^{1.26}_{\pm 0.298}$ P,>0.001	$^{5.51}_{\pm 2.198}$	$^{1.45}_{\pm 0.316}$ P,>0.001
Potassium-42	10	$^{0.65}_{\pm 0.165}$	$^{1.75}_{\pm 0.458}$ P,>0.001	$^{0.64}_{\pm 0.242}$	$^{2.52}_{\pm 0.6}$ P,>0.001	$^{0.51}_{\pm 0.331}$	$^{3.31}_{\pm 0.884}$ P,>0.001	$^{0.5}_{\pm 0.424}$	$^{3.7}_{\pm 1.216}$ P,>0.001
Sodium-24	10	$^{0.26}_{\pm 0.0916}$	$^{0.17}_{\pm 0.0489}$ P,0.02<0.01	$^{0.31}_{\pm 0.1}$	$^{0.21}_{\pm 0.714}$ P,0.02<0.01	$^{0.3}_{\pm 0.0943}$	$^{0.22}_{\pm 0.0888}$ P,0.1<0.05		

TABLE 4.

Uptake of calcium-47, potassium-42 and sodium-24 by isolated frog sartorius muscle in the presence of ryanodine (50 µg. per ml.).

Isotope	No. of Expts.	Mean percentage uptake of radioactive ions ± S.D.							
		After 30 mins.		After 60 mins.		After 90 mins.		After 120 mins.	
		Test	Control	Test	Control	Test	Control	Test	Control
Calcium-47	9	1.18 ±0.574	0.57 ±0.214	2.12 ±0.768	0.95 ±0.346	2.13 ±0.741	1.24 ±0.412	2.17 ±0.793	1.65 ±0.565
		P,0.01<0.001		P,>0.001		P,0.01<0.001		P,0.2<0.1	
Potassium-42	9	0.81 ±0.242	2.73 ±0.519	0.65 ±0.172	3.46 ±0.714	0.56 ±0.162	3.96 ±1.04	0.52 ±0.273	4.12 ±2.08
		P,>0.001		P,>0.001		P,>0.001		P,>0.001	
Sodium-24	10	0.4 ±0.06	0.17 ±0.037	0.49 ±0.164	0.2 ±0.05	0.44 ±0.16	0.2 ±0.048		
		P,>0.001		P,>0.001		P,>0.001			

TABLE 5.

Uptake of calcium-47, potassium-42 and sodium-24 by isolated frog sartorius muscle in the presence of carbachol (5 mg. per ml.).

Isotope	No. of Expts.	Mean percentage uptake of radioactive ions ± S.D.							
		After 30 mins.		After 60 mins.		After 90 mins.		After 120 mins.	
		Test	Control	Test	Control	Test	Control	Test	Control
Calcium-47	10	0.63 ±0.226	1.23 ±0.547	0.99 ±0.547	1.51 ±0.979	1.29 ±0.624	1.8 ±0.86	1.51 ±0.721	1.99 ±0.734
		P,0.2<0.1		P,0.3<0.2		P,0.3<0.2		P,0.3<0.2	
Potassium-42	10	1.35 ±0.192	1.57 ±0.273	2.17 ±0.287	2.45 ±0.374	2.9 ±0.761	3.37 ±1.014	3.13 ±1.086	4.08 ±1.166
		P,0.1<0.05		P,0.1<0.05		P,0.3<0.2		P,0.2<0.1	
Sodium-24	10	0.16 ±0.052	0.16 ±0.048	0.19 ±0.078	0.2 ±0.088	0.21 ±0.098	0.21 ±0.091		
				P,0.8<0.7					

TABLE 6.

Uptake of calcium-47, potassium-42 and sodium-24 by isolated frog sartorius muscle in the presence of neostigmins (100 µg. per ml.).

Isotope	No. of Expts.	Mean percentage uptake of radioactive ions ± S.D.							
		After 30 mins.		After 60 mins.		After 90 mins.		After 120 mins.	
		Test	Control	Test	Control	Test	Control	Test	Control
Calcium-47	9	$^{0.45}_{\pm 0.19}$	$^{0.48}_{\pm 0.22}$	$^{0.92}_{\pm 0.316}$	$^{0.7}_{\pm 0.269}$	$^{1.43}_{\pm 0.346}$	$^{0.93}_{\pm 0.244}$	$^{1.67}_{\pm 0.435}$	$^{1.01}_{\pm 0.234}$
		P, 0.8<0.7		P,0.2<0.1		P,0.01<0.001		P,0.01<0.001	
Potassium-42	9	$^{0.73}_{\pm 0.412}$	$^{1.47}_{\pm 0.848}$	$^{0.97}_{\pm 0.655}$	$^{2.39}_{\pm 0.748}$	$^{0.98}_{\pm 0.7}$	$^{2.93}_{\pm 0.964}$	$^{0.87}_{\pm 0.774}$	$^{3.1}_{\pm 1.1}$
		P,0.05<0.02		P,>0.001		P,>0.001		P,>0.001	
Sodium-24	10	$^{0.33}_{\pm 0.157}$	$^{0.13}_{\pm 0.05}$	$^{0.42}_{\pm 0.186}$	$^{0.16}_{\pm 0.067}$	$^{0.4}_{\pm 0.151}$	$^{0.17}_{\pm 0.069}$		
		P,0.01<0.001		P,>0.001		P,>0.001			

TABLE 7.

Uptake of calcium-47, potassium-42 and sodium-24 by isolated frog sartorius muscle in the presence of edrophonium (2 mg. per ml.).

Isotope	No. of Expts.	Mean percentage uptake of radioactive ions ± S.D.							
		After 30 mins.		After 60 mins.		After 90 mins.		After 120 mins.	
		Test	Control	Test	Control	Test	Control	Test	Control
Calcium-47	10	0.33 ± 0.141	0.7 ± 0.331	0.42 ± 0.205	0.91 ± 0.36	0.51 ± 0.28	1.12 ± 0.435	0.54 ± 0.304	1.18 ± 0.479
		P,0.01<0.001		P,0.01<0.001		P,0.01<0.001		P,0.01<0.001	
Potassium-42	10	1.0 ± 0.479	1.59 ± 0.953	1.45 ± 0.574	2.26 ± 1.067	1.67 ± 0.685	2.63 ± 1.236	1.66 ± 0.479	2.97 ± 0.509
		P,0.10<0.05		P,0.05<0.02		P,0.05<0.02		P,0.01<0.001	
Sodium-24	10	0.1 ± 0.022	0.13 ± 0.034	0.13 ± 0.031	0.14 ± 0.043	0.14 ± 0.04	0.13 ± 0.038		
		P,0.05<0.02		P,0.4<0.3		P,0.9<0.8			

TABLE 8.

Uptake of calcium-47, potassium-42 and sodium-24 by isolated frog sartorius muscle in the presence of tubocurarine (200 µg. per ml.).

Isotope	No. of Expts.	Mean percentage uptake of radioactive ions ± S.D.							
		After 30 mins.		After 60 mins.		After 90 mins.		After 120 mins.	
		Test	Control	Test	Control	Test	Control	Test	Control
Calcium-47	10	0.56 ±0.195	0.93 ±0.565	0.73 ±0.226	1.08 ±0.489	0.89 ±0.268	1.18 ±0.447	1.11 ±0.346	1.38 ±0.529
		P,0.1<0.05		P,0.1<0.05		P,0.1<0.05		P, 0.2	
Potassium-42	9	1.11 ±0.435	1.22 ±0.509	1.68 ±0.678	1.79 ±0.761	2.08 ±0.894	2.17 ±0.922	2.11 ±1.039	2.17 ±1.1
		P,0.7<0.6		P,0.8<0.7		P,0.9<0.8			
Sodium-24	9	0.17 ±0.056	0.17 ±0.036	0.21 ±0.031	0.2 ±0.031	0.22 ±0.037	0.22 ±0.041		

TABLE 9.

Uptake of calcium-47, potassium-42 and sodium-24 by isolated frog sartorius muscle in the presence of gallamine (4 mg. per ml.).

Isotope	No. of Expts.	Mean percentage uptake of radioactive ions ± S.D.							
		After 30 mins.		After 60 mins.		After 90 mins.		After 120 mins.	
		Test	Control	Test	Control	Test	Control	Test	Control
Calcium-47	9	0.8 ±0.509	0.59 ±0.242	1.1 ±0.556	0.89 ±0.304	1.32 ±0.583	1.12 ±0.316	1.65 ±0.547	1.39 ±0.295
		P, 0.4<0.3		P,0.4<0.3		P,0.5<0.4		P,0.3<0.2	
Potassium-42	10	1.69 ±0.583	2.08 ±0.761	2.48 ±0.655	3.08 ±0.922	3.21 ±0.932	4.05 ±1.264	3.3 ±1.029	4.18 ±1.385
		P, 0.3<0.2		P,0.1		P,0.2<0.1		P,0.2<0.1	
Sodium-24	10	0.1 ±0.0412	0.11 ±0.0529	0.14 ±0.0556	0.13 ±0.07	0.14 ±0.0538	0.13 ±0.0728		
		P,0.7<0.6		P,0.9<0.8		P,0.7<0.6			

TABLE 10.

Uptake of calcium-47, potassium-42 and sodium-24 by isolated frog sartorius musole in the presence of protoveratrine A (100 µg. per ml.).

Isotope	No. of Expts.	Mean percentage uptake of radioactive ions ± S.D.							
		After 30 mins.		After 60 mins.		After 90 mins.		After 120 mins.	
		Test	Control	Test	Control	Test	Control	Test	Control
Calcium-47	9	$^{0.56}_{\pm 0.231}$	$^{1.12}_{\pm 0.685}$ P,0.05<0.02	$^{0.93}_{\pm 0.331}$	$^{1.38}_{\pm 1.06}$ P,0.3<0.2	$^{1.3}_{\pm 0.435}$	$^{1.64}_{\pm 0.984}$ P,0.4<0.3	$^{1.5}_{\pm 0.7}$	$^{2.0}_{\pm 1.18}$ P,0.4<0.3
Potassium-42	10	$^{1.8}_{\pm 0.547}$	$^{1.65}_{\pm 0.387}$ P,0.4<0.3	$^{2.53}_{\pm 0.883}$	$^{2.32}_{\pm 0.624}$ P,0.6<0.5	$^{2.8}_{\pm 1.191}$	$^{2.63}_{\pm 0.8}$ P,0.8<0.7	$^{3.51}_{\pm 1.268}$	$^{2.84}_{\pm 1.04}$ P,0.3<0.2
Sodium-24	10	$^{0.29}_{\pm 0.098}$	$^{0.13}_{\pm 0.094}$ P,>0.001	$^{0.59}_{\pm 0.171}$	$^{0.16}_{\pm 0.026}$ P,>0.001	$^{0.69}_{\pm 0.231}$	$^{0.17}_{\pm 0.034}$ P,>0.001		

TABLE 11.

Uptake of calcium-47, potassium-42 and sodium-24 by isolated frog sartorius muscle in the presence of protoveratrine B (100 µg. per ml.).

Isotope	No. of Expts.	Mean percentage uptake of radioactive ions ± S.D.							
		After 30 mins.		After 60 mins.		After 90 mins.		After 120 mins.	
		Test	Control	Test	Control	Test	Control	Test	Control
Calcium-47	9	$^{0.88}_{\pm 0.435}$	$^{2.75}_{\pm 1.94}$ P, 0.02<0.01	$^{1.21}_{\pm 0.583}$	$^{2.81}_{\pm 1.94}$ P,0.05<0.02	$^{1.52}_{\pm 0.616}$	$^{2.94}_{\pm 1.81}$ P,0.05<0.02	$^{1.88}_{\pm 0.911}$	$^{3.41}_{\pm 1.75}$ P,0.1<0.05
Potassium-42	9	$^{1.95}_{\pm 0.509}$	$^{1.79}_{\pm 0.616}$ P, 0.6<0.5	$^{3.16}_{\pm 0.632}$	$^{2.67}_{\pm 0.824}$ P,0.2<0.1	$^{4.04}_{\pm 0.953}$	$^{3.3}_{\pm 1.245}$ P,0.2<0.1	$^{4.58}_{\pm 0.529}$	$^{4.01}_{\pm 0.922}$ P,0.2<0.1
Sodium-24	10	$^{0.21}_{\pm 0.093}$	$^{0.27}_{\pm 0.104}$ P,0.2<0.1	$^{0.3}_{\pm 0.13}$	$^{0.27}_{\pm 0.095}$ P,0.6<0.5	$^{0.36}_{\pm 0.117}$	$^{0.26}_{\pm 0.105}$ P,0.1<0.05		

TABLE 12.

Effect of suxamethonium (5 mg. per ml.) on the release of calcium-47 from isolated frog sartorius muscle.

No. of Expt.	Muscle	Counts released per min. during a 10 min. immersion period. 10	20	30	40	50	60	70	Total number of counts released by the muscle	Ratio of T/C
1	Test (T)	2117	469	346	4639*	331	296	193	8391	2.14
(266)	Control (C)	2114	856	415	296	117	86	34	3918	
2	Test (T)	2653	1120	426	3597*	255	221	186	8458	1.95
(265)	Control (C)	2110	942	655	204	201	132	92	4336	
3	Test (T)	2513	644	405	4208*	363	296	218	8647	1.84
(264)	Control (C)	2816	771	501	340	108	98	56	4690	
4	Test (T)	7443	2332	1226	3017*	623	293	173	15107	1.78
(154)	Control (C)	3579	1717	1131	1004	427	321	309	8488	
5	Test (T)	2030	830	531	2327*	188	156	132	6194	1.76
(263)	Control (C)	1698	643	380	353	229	129	86	3518	
6	Test (T)	2939	751	532	2036*	300	222	87	6887	1.68
(138)	Control (C)	1871	697	419	331	308	247	221	4094	
7	Test (T)	3272	494	402	1469*	304	189	176	6306	1.57
(137)	Control (C)	1852	749	398	337	289	211	185	4021	
8	Test (T)	2376	582	522	1284*	156	96	60	5076	1.39
(151)	Control (C)	1902	600	378	252	198	162	144	3636	
9	Test (T)	2137	1237	1112	4273*	510	442	363	10074	1.12
(147)	Control (C)	3751	1819	918	762	663	596	439	8948	

*denotes the point of exposure of the test muscle to the drug.

TABLE 13.

Effect of decamethonium (200 µg. per ml.) on the release of calcium-47 from isolated frog sartorius muscle

No. of Expt.	Muscle	Counts released per min. during a 10 min. immersion period. 10	20	30	40	50	60	70	Total number of counts released by the muscle	Ratio of T/C
1 (36)	Test (T)	2354	470	297	432*	239	172	156	4120	1.95
	Control (C)	986	394	279	171	138	78	63	2109	
2 (42)	Test (T)	765	269	228	195*	120	92	59	1728	1.06
	Control (C)	749	250	238	165	88	75	68	1633	
3 (37)	Test (T)	1312	318	230	219*	154	108	94	2435	0.96
	Control (C)	1531	444	180	131	129	68	51	2534	
4 (34)	Test (T)	2305	825	375	369*	237	191	128	4430	0.96
	Control (C)	1841	1420	470	315	310	186	161	4703	
5 (27)	Test (T)	561	133	132	142*	89	77	62	1196	0.91
	Control (C)	826	151	85	82	76	39	23	1302	
6 (36)	Test (T)	2375	578	538	235*	114	106	95	4041	0.78
	Control (C)	2300	1239	526	341	309	225	212	5152	
7 (26)	Test (T)	479	183	76	101*	66	51	46	1002	0.56
	Control (C)	1178	194	119	108	70	66	52	1787	
8 (31)	Test (T)	1497	630	327	216*	184	133	124	3111	0.63
	Control (C)	1928	954	836	526	320	194	114	4872	
9 (43)	Test (T)	383	113	85	78*	75	59	39	823	0.56
	Control (C)	610	207	202	185	151	79	49	1483	
10 (41)	Test (T)	470	147	104	132*	71	67	60	1051	0.54
	Control (C)	628	329	296	201	173	162	156	1945	

*denotes the point of exposure of the test muscle to the drug.

TABLE 14.

Effect of nicotine (1 mg. per ml.) on the release of calcium-47 from isolated frog sartorius muscle

No. of Expt.	Muscle	Counts released per min. during a 10 min. immersion period. 10	20	30	40	50	60	70	Total number of counts released by the muscle	Ratio of T/C
1	Test (T)	2152	667	423	10296*	409	220	176	14343	5.24
(259)	Control (C)	1398	542	297	226	214	37	21	2735	
2	Test (T)	7150	2222	1701	9218*	1069	587	496	22443	3.04
(161)	Control (C)	3949	1143	828	523	466	271	192	7372	
3	Test (T)	1682	622	609	9122*	477	176	112	12800	2.44
(260)	Control (C)	2642	969	912	289	298	90	46	5246	
4	Test (T)	4151	959	793	4687*	477	291	182	11540	2.24
(217)	Control (C)	2555	1295	775	216	107	105	96	5149	
5	Test (T)	2637	717	749	7129*	552	149	78	12011	2.23
(261)	Control (C)	3087	871	530	289	248	191	163	5379	
6	Test (T)	2329	581	349	5097*	341	71	22	8790	1.57
(262)	Control (C)	3019	1192	459	450	178	155	123	5576	
7	Test (T)	3490	1301	696	1361*	583	577	562	8570	1.52
(170)	Control (C)	2090	1065	821	601	360	354	341	5632	
8	Test (T)	4739	648	453	2848*	261	221	157	9327	1.05
(218)	Control (C)	6091	1240	659	446	238	116	78	8868	

*denotes the point of exposure of the test muscle to the drug.

TABLE 15.

Effect of ryanodine (50 µg. per ml.) on the release of calcium-47 from isolated frog sartorius muscle

No. of Expt.	Muscle	Counts released per min. during a 10 min. immersion period. 10	20	30	40	50	60	70	Total number of counts released by the muscle	Ratio of T/C
1	Test (T)	3371	1276	742	626*	702	279	213	7209	2.0
(282)	Control (C)	1953	700	235	248	168	164	132	3600	
2	Test (T)	2741	603	349	196*	280	306	439	4914	1.77
(272)	Control (C)	1607	408	237	221	115	100	78	2766	
3	Test (T)	3276	948	510	364*	371	283	212	5964	1.6
(274)	Control (C)	2107	550	310	327	199	114	98	3705	
4	Test (T)	2288	631	328	262*	519	282	193	4503	1.46
(275)	Control (C)	1813	605	245	181	142	52	36	3074	
5	Test (T)	3816	1587	923	1219*	736	539	523	9343	1.41
(168)	Control (C)	3390	1177	623	463	370	360	237	6620	
6	Test (T)	4558	967	334	518*	1506	167	129	8179	1.32
(216)	Control (C)	4242	774	502	277	195	97	91	6178	
7	Test (T)	9477	2265	839	948*	481	481	360	14851	1.3
(186)	Control (C)	5661	2666	1070	652	515	479	324	11367	
8	Test (T)	4440	1212	818	572*	1270	359	286	8957	1.13
(167)	Control (C)	4080	1394	755	726	455	318	214	7942	
9	Test (T)	2723	988	712	516*	581	292	168	5980	1.04
(215)	Control (C)	2604	845	838	467	440	362	192	5748	
10	Test (T)	2770	725	485	474*	589	240	221	5504	0.92
(281)	Control (C)	3035	986	435	460	439	314	278	5947	

*denotes the point of exposure of the test muscle to the drug.

TABLE 16.

Effect of carbachol (5 mg. per ml.) on the release of calcium-47 from isolated frog sartorius muscle

No. of Expt.	Muscle	Counts released per min. during a 10 min. immersion period. 10	20	30	40	50	60	70	Total number of counts released by the muscle	Ratio of T/C
1	Test (T)	2726	907	776	565*	769	184	154	6081	1.48
(245)	Control (C)	2344	577	335	306	197	198	132	4089	
2	Test (T)	4519	1477	833	797*	759	682	576	9643	1.38
(246)	Control (C)	3265	1035	947	852	355	286	212	6952	
3	Test (T)	2748	654	451	420*	370	286	253	5182	1.2
(251)	Control (C)	2104	701	499	285	291	221	186	4287	
4	Test (T)	2690	1110	676	279*	328	272	186	5541	1.16
(253)	Control (C)	2588	828	415	320	281	197	136	4765	
5	Test (T)	2370	1162	866	593*	378	232	183	5784	1.10
(254)	Control (C)	2500	1030	699	393	254	209	164	5249	
6	Test (T)	3272	831	430	557*	382	256	182	5910	0.94
(244)	Control (C)	3343	1178	789	332	257	192	156	6247	
7	Test (T)	3158	671	450	420*	391	280	232	5602	0.91
(243)	Control (C)	4065	705	473	309	257	187	132	6128	
8	Test (T)	1892	836	517	287*	364	312	259	4467	0.87
(252)	Control (C)	2577	956	629	359	263	186	116	5086	

*denotes the point of exposure of the test muscle to the drug.

TABLE 17.

Effect of neostigmine (100 µg. per ml.) on the release of calcium-47 from isolated frog sartorius muscle

No. of Expt.	Muscle	Counts released per min. during a 10 min. immersion period. 10	20	30	40	50	60	70	Total number of counts released by the muscle	Ratio of T/C
1 (284)	Test (T)	2507	926	378	579*	342	283	196	5211	1.96
	Control (C)	1506	383	361	120	115	92	75	2652	
2 (89)	Test (T)	1118	553	249	475*	165	115	95	2770	1.78
	Control (C)	607	260	175	144	132	120	115	1553	
3 (92)	Test (T)	1044	718	264	660*	544	322	256	3808	1.36
	Control (C)	1558	470	294	182	110	99	86	2799	
4 (83)	Test (T)	2169	718	283	388*	301	224	196	4279	1.26
	Control (C)	2015	523	307	173	171	122	89	3400	
5 (283)	Test (T)	3966	1374	716	1239*	468	392	248	8403	1.22
	Control (C)	3234	1099	789	786	366	343	274	6891	
6 (117)	Test (T)	1143	355	227	284*	171	134	106	2420	1.15
	Control (C)	1130	400	308	88	79	56	34	2095	
7 (88)	Test (T)	736	267	202	335*	215	152	114	2021	0.99
	Control (C)	991	316	208	206	135	118	74	2048	
8 (75)	Test (T)	1852	563	555	614*	267	255	213	4319	0.82
	Control (C)	2360	1100	571	469	283	240	186	5209	

*denotes the point of exposure of the test muscle to the drug.

TABLE 18.

Effect of edrophonium (2 mg. per ml.) on the release of calcium-47 from isolated frog sartorius muscle

No. of Expt.	Muscle	Counts released per min. during a 10 min. immersion period. 10	20	30	40	50	60	70	Total number of counts released by the muscle	Ratio of T/C
1	Test (T)	1082	290	275	2461*	123	105	82	4418	4.6
(111)	Control (C)	533	162	100	76	42	26	21	960	
2	Test (T)	906	401	308	2286*	209	137	89	4336	2.64
(123)	Control (C)	690	361	206	134	120	92	36	1639	
3	Test (T)	1517	663	273	1604*	217	100	73	4447	2.56
(116)	Control (C)	779	366	212	163	106	69	39	1734	
4	Test (T)	575	174	126	1666*	95	52	36	2724	1.94
(110)	Control (C)	688	278	147	101	81	67	42	1404	
5	Test (T)	1685	462	289	2437*	160	99	76	5208	1.67
(100)	Control (C)	1586	589	271	247	186	137	92	3108	
6	Test (T)	1379	653	316	1447*	153	55	32	4035	1.55
(101)	Control (C)	1367	460	239	213	121	115	86	2601	
7	Test (T)	1612	608	275	2027*	226	139	105	4992	1.33
(97)	Control (C)	1742	678	368	297	272	223	154	3734	
8	Test (T)	705	383	226	695*	117	88	76	2290	1.14
(122)	Control (C)	865	408	256	167	115	102	100	2013	
9	Test (T)	1284	304	127	1367*	164	150	45	3441	1.02
(96)	Control (C)	1882	510	321	222	167	141	138	3381	

*denotes the point of exposure of the test muscle to the drug.

TABLE 19.

Effect of tubocurarine (2 mg. per ml.) on the release of calcium-47 from isolated frog sartorius muscle

No. of Expt.	Muscle	Counts released per min. during a 10 min. immersion period. 10	20	30	40	50	60	70	Total number of counts released by the muscle	Ratio of T/C
1 (25)	Test (T)	2500	1192	347	1367*	258	154	119	5937	1.45
	Control (C)	2199	654	567	217	170	162	112	4081	
2 (22)	Test (T)	3447	1144	736	1318*	402	190	168	7405	2.04
	Control (C)	1622	583	569	286	270	188	107	3625	
3 (14)	Test (T)	2264	682	305	448*	213	152	116	4180	1.08
	Control (C)	1855	820	346	324	273	147	98	3863	
4 (19)	Test (T)	2322	472	245	962*	161	145	140	4447	1.0
	Control (C)	2169	666	468	357	256	253	250	4419	
5 (54)	Test (T)	1184	532	401	495*	262	144	132	3150	0.99
	Control (C)	1224	701	397	246	241	223	152	3184	
6 (11)	Test (T)	2986	685	251	279*	125	111	86	4523	0.95
	Control (C)	2985	542	329	280	231	212	174	4753	
7 (50)	Test (T)	2578	601	584	320*	280	186	132	4681	0.93
	Control (C)	2479	969	454	391	285	215	207	4999	
8 (59)	Test (T)	1257	454	361	399*	216	152	117	2956	0.65
	Control (C)	1960	756	368	293	252	233	196	5478	
9 (35)	Test (T)	1514	644	353	204*	168	127	96	3106	0.71
	Control (C)	2883	592	282	256	211	194	150	4348	

*Showing the point of exposure of the test muscle to the drug.

TABLE 20.

Effect of gallamine (4 mg. per ml.) on the release of calcium-47 from isolated frog sartorius muscle

No. of Expt.	Muscle	Counts released per min. during a 10 min. immersion period.							Total number of counts released by the muscle	Ratio of T/C
		10	20	30	40	50	60	70		
1 (194)	Test (T)	4099	1545	1344	757*	745	632	519	9641	2.02
	Control (C)	2111	1169	475	385	290	196	133	4759	
2 (192)	Test (T)	12163	683	543	523*	289	193	157	14551	1.14
	Control (C)	8416	2305	828	488	310	280	162	12789	
3 (288)	Test (T)	4822	1340	1080	1026*	592	496	388	9744	1.02
	Control (C)	4685	1313	1038	834	649	518	462	9499	
4 (285)	Test (T)	6389	1862	1289	1009*	594	532	476	12151	0.9
	Control (C)	6667	2111	1249	1163	892	756	613	13451	
5 (287)	Test (T)	4029	1006	501	500*	382	346	326	7090	0.87
	Control (C)	4289	1408	967	510	373	311	280	8138	
6 (286)	Test (T)	4414	1419	934	773*	518	436	372	8866	0.83
	Control (C)	4599	1916	1051	947	876	713	502	10604	
7 (191)	Test (T)	6689	2384	2316	1271*	575	436	359	14030	0.71
	Control (C)	11547	3822	1244	1200	787	632	519	19751	
8 (193)	Test (T)	3385	1512	1390	364*	296	172	109	7228	0.71
	Control (C)	4665	1774	1863	734	480	372	196	10084	

*denotes the point of exposure of the test muscle to the drug.

TABLE 21.

Effect of protoveratrine A (100 µg. per ml.) on the release of calcium-47 from isolated frog sartorius muscle.

No. of Expt.	Muscle	Counts released per min. during a 10 min. immersion period. 10	20	30	40	50	60	70	Total number of counts released by the muscle	Ratio of T/C
1 (176)	Test (T)	2658	1576	1253	411*	437	442	310	7087	1.68
	Control (C)	2297	1044	623	295	218	203	173	4853	
2 (175)	Test (T)	4345	2020	1570	515*	2242	1285	355	12332	1.6
	Control (C)	3604	1211	1143	547	451	403	340	7699	
3 (202)	Test (T)	10064	1201	823	981*	499	342	296	14206	1.53
	Control (C)	3949	1698	781	765	745	743	562	9243	
4 (200)	Test (T)	4642	1535	978	1196*	436	385	296	9468	1.3
	Control (C)	3037	957	868	848	552	561	462	7285	
5 (199)	Test (T)	3513	1380	1086	1447*	488	442	374	8730	1.24
	Control (C)	3251	1506	768	593	324	300	276	7018	
6 (201)	Test (T)	6049	2805	1774	1115*	1409	690	543	14385	1.04
	Control (C)	6111	4817	1438	446	367	338	292	13809	
7 (306)	Test (T)	2777	857	528	577*	452	380	223	5794	1.02
	Control (C)	3279	1024	635	261	195	168	132	5694	
8 (185)	Test (T)	5240	1868	1081	638*	195	216	182	9420	0.76
	Control (C)	6748	2307	1327	627	513	511	376	12419	
9 (305)	Test (T)	2192	895	384	834*	343	286	247	5181	0.67
	Control (C)	3670	1222	777	653	562	492	356	7732	

*denotes the point of exposure of the test muscle to the drug.

TABLE 22.

- Effect of protoveratrine B (100 µg. per ml.) on the release of calcium-47 from isolated frog sartorius muscle

No. of Expt.	Muscle	Counts released per min. during a 10 min. immersion period. 10	20	30	40	50	60	70	Total number of counts released by the muscle	Ratio of T/C
1	Test (T)	7439	1563	913	1039*	1355	187	152	12648	1.98
(208)	Control (C)	3173	791	728	713	427	336	213	6381	
2	Test (T)	6440	2823	1531	860*	337	253	196	12440	1.9
(183)	Control (C)	3025	1166	792	524	426	359	214	6516	
3	Test (T)	2913	811	576	298*	399	222	180	5399	1.47
(301)	Control (C)	2359	598	377	162	119	30	26	3671	
4	Test (T)	2394	807	370	471*	207	103	98	4450	1.38
(299)	Control (C)	1539	660	379	295	259	41	56	3229	
5	Test (T)	4196	1298	900	372*	348	88	54	7256	0.88
(210)	Control (C)	4273	1362	842	561	432	420	315	8205	
6	Test (T)	1383	284	218	662*	106	92	78	2823	0.79
(303)	Control (C)	1709	548	410	313	248	182	136	3546	
7	Test (T)	5106	1072	336	515*	415	378	316	8138	0.78
(209)	Control (C)	7121	2027	405	233	226	208	192	10412	
8	Test (T)	2252	544	329	349*	100	113	102	3440	0.75
(302)	Control (C)	2240	664	569	489	216	213	180	4571	
9	Test (T)	1985	495	266	188*	220	179	132	3465	0.67
(304)	Control (C)	2104	1058	590	424	398	321	280	5175	
10	Test (T)	2001	746	476	256*	134	344	296	4253	0.63
(300)	Control (C)	3310	1219	786	667	341	231	146	6700	

*denotes the point of exposure of the test muscle to the drug.

TABLE 23.

Effect of suxamethonium (5mg. per ml.) on the release of potassium-42 from isolated frog sartorius muscle

No. of Expt.	Muscle	Counts released per min. during a 10 min. immersion period. 10	20	30	40	50	60	70	Total number of counts released by the muscle	Ratio of T/C
1 (253)	Test (T)	890	301	223	1519*	1094	306	210	4543	1.99
	Control (C)	1072	347	240	203	172	132	120	2286	
2 (XXXIV)	Test (T)	947	398	235	470*	332	237	194	2813	1.68
	Control (C)	609	283	241	170	127	124	118	1672	
3 (148)	Test (T)	1355	630	375	563*	484	412	386	4205	1.68
	Control (C)	765	515	350	297	242	186	152	2507	
4 (146)	Test (T)	9310	7563	7193	8986*	4624	4129	3612	36417	1.64
	Control (C)	8088	2894	2704	2670	2164	1893	1812	22225	
5 (149)	Test (T)	1253	608	536	706*	669	552	476	4800	1.6
	Control (C)	930	578	424	377	276	214	192	2991	
6 (XXXI)	Test (T)	2020	721	521	678*	387	313	224	4864	1.41
	Control (C)	1360	572	561	512	213	122	109	3449	
7 (XXXV)	Test (T)	3976	661	406	477*	373	211	180	6284	0.85
	Control (C)	4760	892	531	410	334	257	159	7343	
8 (147)	Test (T)	2246	1330	1099	1646*	1129	989	872	9311	0.84
	Control (C)	4112	1886	1516	1227	827	714	689	10971	
9 (254)	Test (T)	3589	1661	1126	2240*	1766	890	736	12008	0.82
	Control (C)	6495	2412	1552	1317	1038	958	812	14584	

*denotes the points of exposure of the test muscle to the drug.

TABLE 24.

Effect of decamethonium (200 µg. per ml.) on the release of potassium-42 from isolated frog sartorius muscle

No. of Expt.	Muscle	Counts released per min. during a 10 min. immersion period. 10	20	30	40	50	60	70	Total number of counts released by the muscle	Ratio of T/C
1 (XXII)	Test (T)	400	121	68	353*	218	207	99	1466	2.03
	Control (C)	277	150	120	67	52	31	24	721	
2 (XXIII)	Test (T)	941	226	188	307*	320	133	97	2212	1.65
	Control (C)	757	186	95	92	85	69	54	1338	
3 (XXIV)	Test (T)	1331	494	393	543*	398	282	261	3702	1.58
	Control (C)	894	432	327	263	205	115	110	2346	
4 (XXI)	Test (T)	3884	763	361	552*	684	292	218	6754	1.5
	Control (C)	2897	604	317	214	197	143	127	4499	
5 (XV)	Test (T)	3833	1927	1050	1817*	545	445	90	9707	1.4
	Control (C)	3840	1071	664	520	350	308	180	6933	
6 (XXVI)	Test (T)	1007	624	506	794*	637	328	274	4170	1.39
	Control (C)	791	508	417	367	343	294	269	2989	
7 (XX)	Test (T)	963	185	156	555*	315	203	79	2456	1.35
	Control (C)	995	273	210	121	127	49	45	1820	
8 (XXVII)	Test (T)	461	200	88	302*	177	96	-	1324	1.16
	Control (C)	482	190	160	149	94	63	-	1138	
9 (XXV)	Test (T)	1208	499	399	572*	526	162	150	3516	0.86
	Control (C)	1395	634	631	493	345	289	270	4057	

*denotes the point of exposure of the test muscle to the drug.

TABLE 25.

Effect of nicotine (1 mg. per ml.) on the release of potassium-42 from isolated frog sartorius muscle

No. of Expt.	Muscle	Counts released per min. during a 10 min. immersion period. 10	20	30	40	50	60	70	Total number of counts released by the muscle	Ratio of T/C
1 (18)	Test (T)	566	224	186	783*	552	221	132	2664	2.55
	Control (C)	366	178	113	128	109	85	65	1044	
2 (132)	Test (T)	1420	806	553	1512*	1115	1013	985	7404	2.35
	Control (C)	1140	490	439	343	287	256	192	3147	
3 (134)	Test (T)	2762	1379	1264	2761*	3464	2692	1652	15974	1.97
	Control (C)	2499	1263	1104	949	844	792	656	8107	
4 (17)	Test (T)	499	248	160	1096*	682	185	116	2986	1.85
	Control (C)	588	238	202	191	141	144	109	1613	
5 (130)	Test (T)	3021	1307	1236	1711*	1831	1028	953	11287	1.63
	Control (C)	2301	1094	936	799	727	560	496	6913	
6 (129)	Test (T)	2029	1299	999	1603*	1466	1352	1296	10044	1.5
	Control (C)	1982	1049	935	734	712	693	562	6667	
7 (133)	Test (T)	1778	1632	1195	1944*	1988	1272	1096	10905	1.47
	Control (C)	1444	1381	1062	930	928	888	796	7429	
8 (135)	Test (T)	3488	1537	1236	1941*	1796	1346	1192	12486	1.44
	Control (C)	3377	1424	1141	853	695	603	572	8665	
9 (131)	Test (T)	2244	1050	847	1704*	1687	924	876	9332	1.34
	Control (C)	2097	1357	818	819	769	598	513	6971	
10 (128)	Test (T)	1823	1142	1029	1310*	1017	917	892	8310	1.11
	Control (C)	2046	1222	1153	857	782	693	586	7339	

* denotes the point of exposure of the test muscle to the drug.

TABLE 26.

Effect of ryanodine (50 µg. per ml.) on the release of potassium-42 from isolated frog sartorius muscle

No. of Expt.	Muscle	Counts released per min. during a 10 min. immersion period. 10	20	30	40	50	60	70	Total number of counts released by the muscle	Ratio of T/C
1	Test (T)	2877	1298	975	1114*	4382	5833	3218	19697	3.91
(174)	Control (C)	1308	831	801	667	534	452	440	5033	
2	Test (T)	2086	948	703	671*	7165	5811	2357	19741	3.64
(60)	Control (C)	1674	912	819	596	555	474	394	5424	
3	Test (T)	1084	400	271	298*	1317	1455	1062	5887	3.34
(176)	Control (C)	531	323	257	230	189	116	114	1760	
4	Test (T)	214	126	41	31*	56	300	297	1065	3.1
(61)	Control (C)	104	75	50	43	32	24	15	343	
5	Test (T)	686	362	200	211*	397	1110	389	3355	2.58
(64)	Control (C)	409	257	176	145	133	120	57	1297	
6	Test (T)	520	197	145	155*	1291	452	150	2910	2.38
(65)	Control (C)	518	220	136	130	103	74	41	1222	
7	Test (T)	556	188	166	165*	318	463	526	2382	2.04
(177)	Control (C)	407	188	177	110	100	91	93	1166	
8	Test (T)	1829	885	877	833*	1128	1432	2934	9918	1.83
(59)	Control (C)	1709	809	792	719	491	452	449	5421	
9	Test (T)	1270	522	380	483*	884	1134	1017	5690	1.67
(175)	Control (C)	1116	586	510	346	329	291	220	3398	

*denotes the point of exposure of the test muscle to the drug.

TABLE 27.

Effect of carbachol (5 mg. per ml.) on the release of potassium-42 from isolated frog sartorius muscle

No. of Expt.	Muscle	Counts released per min. during a 10 min. immersion period. 10	20	30	40	50	60	70	Total number of counts released by the muscle	Ratio of T/C
1 (86)	Test (T)	527	293	189	195*	106	120	98	1528	1.27
	Control (C)	404	203	195	124	113	89	72	1200	
2 (90)	Test (T)	3892	1184	1002	1316*	745	495	498	9132	1.17
	Control (C)	3788	1077	701	679	618	472	445	7780	
3 (151)	Test (T)	1324	638	499	764*	480	392	263	4360	1.11
	Control (C)	1449	617	556	441	352	278	213	3906	
4 (150)	Test (T)	1229	620	372	449*	219	163	141	3193	1.09
	Control (C)	1187	552	354	348	241	192	153	2927	
5 (96)	Test (T)	1047	608	401	569*	297	275	216	3413	1.06
	Control (C)	1189	520	407	307	285	263	232	3203	
6 (152)	Test (T)	5375	2334	1664	1878*	989	842	761	13843	1.04
	Control (C)	6548	2308	1567	1228	656	532	412	13251	
7 (95)	Test (T)	1187	536	376	459*	289	254	216	3317	1.01
	Control (C)	954	569	499	378	336	274	252	3262	
8 (89)	Test (T)	1480	753	535	724*	898	330	284	5004	0.88
	Control (C)	2082	962	710	605	522	389	383	5653	
9 (97)	Test (T)	239	196	116	179*	85	84	72	971	0.75
	Control (C)	509	234	175	142	86	80	58	1284	
10 (87)	Test (T)	2650	1334	725	954*	661	564	505	7393	0.6
	Control (C)	4232	2730	1432	1258	1022	834	702	12210	

*denotes the point of exposure of the test muscle to the drug.

TABLE 28.

Effect of neostigmine (75 µg. per ml.) on the release of potassium-42 from isolated frog sartorius muscle

No. of Expt.	Muscle	Counts released per min. during a 10 min. immersion period.							Total number of counts released by the muscle	Ratio of T/C
		10	20	30	40	50	60	70		
1 (69)	Test (T)	1715	756	537	867*	881	531	523	5810	2.32
	Control (C)	798	487	305	249	225	221	212	2497	
2 (251)	Test (T)	950	335	258	597*	828	368	210	3546	1.7
	Control (C)	798	319	293	199	176	155	138	2078	
3 (71)	Test (T)	745	345	257	316*	189	92	72	2016	1.69
	Control (C)	487	291	123	108	79	63	42	1193	
4 (70)	Test (T)	168	43	24	95*	84	44	8	466	1.6
	Control (C)	90	55	40	39	28	22	16	290	
5 (252)	Test (T)	5338	2219	1721	2739*	4041	2779	1465	20302	1.52
	Control (C)	5389	2248	1606	1324	1116	956	721	13360	
6 (80)	Test (T)	200	88	62	76*	57	27	16	526	1.22
	Control (C)	163	64	61	55	37	30	19	429	
7 (68)	Test (T)	440	175	167	235*	217	112	97	1443	1.22
	Control (C)	416	197	165	123	119	85	73	1178	
8 (72)	Test (T)	616	229	165	197*	202	105	75	1589	0.89
	Control (C)	831	293	210	179	147	81	42	1783	
9 (250)	Test (T)	4611	1950	1382	1965*	1944	1488	1256	14596	0.7
	Control (C)	8414	3580	2568	1889	1498	1402	1319	20670	

*denotes the point of exposure of the test muscle to the drug.

Effect of edrophonium (2 mg. per ml.) on the release of potassium-42 from isolated frog sartorius muscle

No. of Expt.	Muscle	Counts released per min. during a 10 min. immersion period.							Total number of counts released by the muscle	Ratio of T/C
		10	20	30	40	50	60	70		
1 (200)	Test (T)	4774	1326	921	796*	666	581	563	9627	1.29
	Control (C)	3350	1241	893	637	627	399	320	7467	
2 (202)	Test (T)	1869	597	533	473*	413	368	293	4546	1.28
	Control (C)	1326	545	458	412	288	267	252	3548	
3 (98)	Test (T)	7408	2581	1718	1226*	1164	1074	943	16114	1.23
	Control (C)	4434	2106	1683	1498	1286	1053	1023	13083	
4 (257)	Test (T)	763	283	191	161*	155	138	109	1800	1.16
	Control (C)	585	231	196	154	151	134	98	1549	
5 (102)	Test (T)	636	335	242	197*	189	134	129	1862	1.12
	Control (C)	636	300	212	143	138	129	97	1655	
6 (119)	Test (T)	234	103	58	46*	32	26	21	520	0.96
	Control (C)	208	86	59	58	57	43	29	540	
7 (97)	Test (T)	645	281	212	191*	179	143	123	1774	0.96
	Control (C)	737	273	208	169	167	161	132	1847	
8 (201)	Test (T)	261	92	69	58*	49	19	-	548	0.94
	Control (C)	307	101	74	47	30	25	-	584	
9 (99)	Test (T)	670	338	240	222*	163	123	98	1854	0.7
	Control (C)	1342	384	241	207	177	168	109	2628	
10 (117)	Test (T)	459	239	216	175*	160	137	120	1506	0.56
	Control (C)	780	454	349	322	291	273	213	2682	

* signifies the point of exposure of the test muscle to the drug.

TABLE 30.

Effect of tubocurarine (1 mg. per ml.) on the release of potassium-42 from isolated frog sartorius muscle

No. of Expt.	Muscle	Counts released per min. during a 10 min. immersion period.							Total number of counts released by the muscle	Ratio of T/C
		10	20	30	40	50	60	70		
1 (255)	Test (T)	617	219	149	132*	103	69	42	1331	1.47
	Control (C)	426	179	103	65	56	46	31	906	
2 (256)	Test (T)	1236	361	230	223*	146	104	92	2392	1.28
	Control (C)	776	319	248	194	133	108	88	1866	
3 (58)	Test (T)	406	154	112	110*	83	73	-	938	1.08
	Control (C)	385	168	103	87	80	43	-	866	
4 (XXXVI)	Test (T)	1680	678	509	375*	325	281	189	4037	0.99
	Control (C)	1780	706	495	396	274	253	181	4085	
5 (172)	Test (T)	3015	1146	863	780*	619	576	492	7491	0.98
	Control (C)	2816	1108	1040	970	607	553	516	7610	
6 (59)	Test (T)	3740	1682	1303	1242*	1008	771	740	10486	0.92
	Control (C)	3785	1918	1548	1281	1024	863	858	11277	
7 (XXXVII)	Test (T)	824	362	270	175*	154	131	120	2036	0.91
	Control (C)	800	392	325	249	199	144	122	2231	
8 (171)	Test (T)	2386	912	515	360*	355	286	214	5028	0.84
	Control (C)	2447	1251	706	512	436	332	316	6000	
9 (173)	Test (T)	5363	1932	1352	981*	958	888	769	12243	0.79
	Control (C)	5793	2835	2042	1448	1205	1114	934	15371	

*denotes the point of exposure of the test muscle to the drug.

TABLE 31.

Effect of gallamine (4 mg. per ml.) on the release of potassium-42 from isolated frog sartorius muscle

No. of Expt.	Muscle	Counts released per min. during a 10 min. immersion period. 10	20	30	40	50	60	70	Total number of counts released by the muscle	Ratio of T/C
1 (115)	Test (T)	3559	1900	1449	977*	953	846	792	10476	1.46
	Control (C)	2359	1229	1059	802	671	532	476	7128	
2 (110)	Test (T)	1951	734	599	597*	563	493	416	5353	1.31
	Control (C)	1360	644	522	442	421	386	312	4087	
3 (111)	Test (T)	4941	2060	1519	1428*	1409	1207	1192	13756	1.1
	Control (C)	4678	1728	1291	1278	1203	1191	1056	12425	
4 (114)	Test (T)	2941	1523	1224	864*	731	605	529	8417	1.03
	Control (C)	2913	1500	1293	817	667	516	409	8115	
5 (117)	Test (T)	2154	967	757	476*	454	400	343	5551	1.03
	Control (C)	1945	934	777	568	457	380	312	5373	
6 (113)	Test (T)	2473	1285	1233	1086*	652	622	576	7927	1.0
	Control (C)	2379	1441	1378	1122	574	516	489	7899	
7 (112)	Test (T)	514	296	286	270*	203	135	121	1825	0.85
	Control (C)	502	437	400	343	279	99	76	2136	
8 (116)	Test (T)	567	358	252	170*	171	152	116	1786	0.76
	Control (C)	836	419	333	234	199	176	132	2329	

*denotes the point of exposure of the test muscle to the drug.

TABLE 32.

Effect of protoveratrine A (100 µg. per ml.) on the release of potassium-42 from isolated frog sartorius muscle

No. of Expt.	Muscle	Counts released per min. during a 10 min. immersion period. 10	20	30	40	50	60	70	Total number of counts released by the muscle	Ratio of T/C
1	Test (T)	508	274	175	196*	292	173	127	1745	1.66
(72)	Control (C)	283	236	149	113	105	88	75	1049	
2	Test (T)	1486	522	349	273*	425	434	232	3721	1.28
(66)	Control (C)	1183	539	313	235	232	221	179	2902	
3	Test (T)	1339	477	378	605*	590	426	198	4013	1.25
(67)	Control (C)	1319	571	400	294	247	199	165	3195	
4	Test (T)	1676	648	492	592*	662	584	383	5037	1.24
(73)	Control (C)	1603	613	488	428	350	282	277	4041	
5	Test (T)	638	448	215	281*	283	196	138	2199	1.2
(70)	Control (C)	700	360	228	155	135	133	111	1822	
6	Test (T)	785	228	161	184*	160	136	86	1740	1.18
(71)	Control (C)	679	199	174	156	99	89	70	1466	
7	Test (T)	2910	1520	1135	1337*	1156	819	612	9489	1.15
(69)	Control (C)	2668	1406	1060	1048	716	663	660	8221	
8	Test (T)	3243	1391	1044	991*	849	590	542	8650	1.06
(68)	Control (C)	2951	1408	1118	802	759	589	527	8154	
9	Test (T)	294	160	98	146*	160	112	71	1041	0.94
(51)	Control (C)	302	198	150	126	120	111	96	1103	
10	Test (T)	505	219	136	104*	130	95	68	1257	0.7
(52)	Control (C)	594	276	234	180	172	169	164	1789	

TABLE 33.

Effect of protoveratrine B (100 µg. per ml.) on the release of potassium-42 from isolated frog sartorius muscle

No. of Expt.	Muscle	Counts released per min. during a 10 min. immersion period. 10	20	30	40	50	60	70	Total number of counts released by the muscle	Ratio of T/C
1 (163)	Test (T)	7380	2683	1972	1768*	1437	1152	1016	17408	1.26
	Control (C)	5117	2104	1738	1526	1249	1092	986	13812	
2 (164)	Test (T)	5900	3005	2112	1922*	2204	1744	1534	18421	1.17
	Control (C)	5242	2687	1790	1779	1585	1374	1296	15753	
3 (78)	Test (T)	2491	1178	810	764*	803	862	438	7346	1.15
	Control (C)	2252	1157	844	662	533	480	457	6385	
4 (79)	Test (T)	4195	1546	1216	985*	1030	693	579	10244	1.15
	Control (C)	4104	1304	953	809	729	530	482	8911	
5 (166)	Test (T)	5478	1854	1408	1439*	1785	1433	1292	14689	1.12
	Control (C)	4896	2076	1285	1274	1271	1209	1111	13122	
6 (167)	Test (T)	4467	2080	1587	1292*	1423	1190	1056	13095	1.11
	Control (C)	4963	1805	1386	1012	967	861	791	11785	
7 (169)	Test (T)	1542	695	523	564*	468	533	492	4817	1.07
	Control (C)	1425	656	636	487	462	418	396	4480	
8 (170)	Test (T)	1638	1038	734	780*	826	632	576	6224	0.97
	Control (C)	1833	1115	786	759	728	649	512	6382	
9 (165)	Test (T)	5161	1897	1383	1404*	1350	1272	1156	13623	0.87
	Control (C)	5830	2567	1946	1484	1310	1283	1122	15542	
10 (168)	Test (T)	5161	2591	1627	1492*	1076	1087	936	13970	0.68
	Control (C)	8062	3771	2621	1886	1524	1443	1214	20521	

* denotes the point of addition of the test muscle to the drug.

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CHAPTER IV

DISCUSSION

The difference between the composition of the interior of the cell and that of its surrounding medium is maintained by a membrane. The living cell depends upon the ability of this membrane to permit the passage of some substances and prevent that of others. There has been much speculation on the structure of the cell membrane because it is neither possible to demonstrate this by conventional optical methods nor to isolate the membrane unchanged for chemical analysis.

Clark (1) has reviewed the structure of the cell and Davson and Danielli (2) have summarised the theories of the nature of the cell membrane as follows:-

(i) The cell membrane consists of a thin layer of fatty material.

(ii) It is a sieve with pores of molecular dimensions.

(iii) The membrane consists of a mosaic made up of areas of more than molecular dimensions, each of which possesses different chemical properties.

(iv) The cell membrane consists of a sieve structure made up of polypeptide chains or proteins superimposed upon a lipid layer.

(v) The membrane is a bimolecular leaflet of fatty molecules.

(vi) The membrane is a lipid layer a few fatty molecules thick, with an adsorbed protein layer at each fat-water interface./

interface.

Mullins (3) observed that it seems difficult to understand how a lipid film membrane can remain stable between two aqueous phases and can show specificity to the passage of certain substances and prevent that of others.

When attempts are made to explain the permeability to ions of a lipid layer in relation to recent studies on the nature and time course of the action potential, it appears that the fatty layer theory is incompatible with the membrane capacity during activity (4).

More recent studies (5-8) however, favour the presence of a membrane with pores. Mullins (3) has, for example, suggested that if one assumes "a membrane with pores two general types may be considered: (a) the random polymer network, a structure of fibrous molecules that are cross-linked so as to yield pores of widely varying size and shape, and (b) the oriented macromolecular liquid membrane, a structure in which the pores are formed by the inability of the macromolecules, in their most efficient packing, to fill all the space in the liquid. The simplest example of (b) is that of macromolecular cylinders in regular haxagonal packing; the pores exist at the junction between any three cylinders and are characterised by a regularity of size but, ... can show a considerable degree of specificity in their interaction with molecules of various sorts."

Katz (9) suggested that the membrane is a very thin lipid sieve/

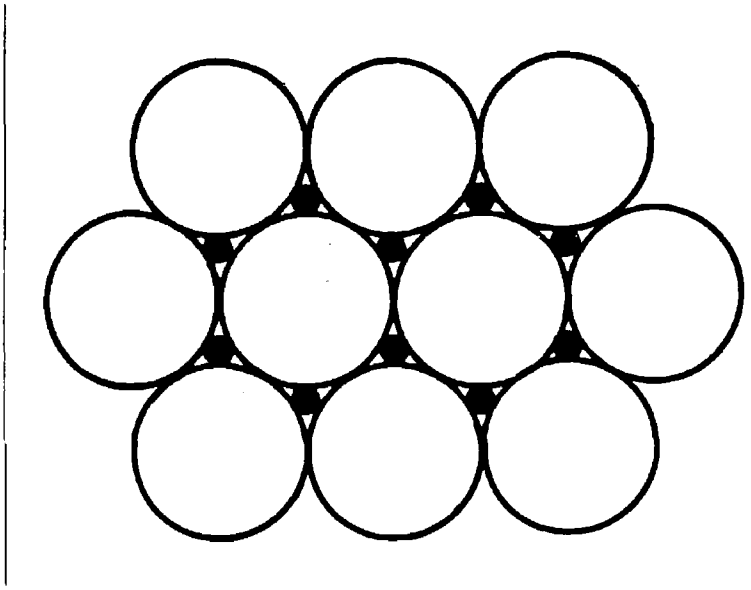

Fig. 36

Macromolecular cylinders, representing structural elements of the cell membrane. The spacing between the macromolecules show the membrane pore formed between any three cylinders being occupied by ions (after Mullins, 3).

sieve which consists almost entirely of insulating dielectric material but contains a few scattered gaps through which water and ions can pass.

Shanes (10) stated that "natural membranes appear to combine the properties of a solvent of low dielectric constant as well as of a porous structure. Available data suggests that the penetration of ions into the untreated membrane is governed by the ease with which water can be removed from them (hydration energies) and their unhydrated diameters; and that permeability changes may be due (a) to changes in the diameters of the channels through which unhydrated, or incompletely or fully hydrated ions move, and possibly (b) to changes in the membrane-ion interaction (solvation)."

Winton and Bayliss (11) stated "the cell membranes of nerves, as of most other living cells as far as is known, consist of radially arranged long-chain fatty acids probably forming parts of lecithin molecules, with superimposed tangential leaflets of protein. This palisade-like structure can probably act as an ionic sieve permitting the passage of some kinds of ion with their attendant water molecules, but not others."

Thus, it appears that we are far from understanding adequately the nature and properties of the cell membrane, yet the existence of such a membrane between the intracellular and interstitial fluid, is however, well established.

It is known that substances in solution tend to move from regions where they are present in high concentration to regions/

regions where they are in lower concentration. They will move in this direction unless they are prevented from doing so by some obstruction, or driven in the opposite direction by some other force. One of the characteristics of most living cells is the fact that the concentration of ions in the interior is quite different from that in the extracellular fluid. Sodium concentration on the outside is usually about ten times as high as that inside the cell and the reverse is true for potassium. The hypotheses advanced to explain this phenomenon has been summarised in Chapter I. Winton and Bayliss (12) stated that owing to the presence within the cell of anions which are unable to diffuse outwards, potassium ions are drawn in and chloride ions are driven out, a steady state being reached when the inflow of potassium ions and the outflow of chloride ions, produced by the electrical potential gradient, is just equal to the outflow of potassium ions and inflow of chloride ions produced by the concentration gradient. The electrical potential difference between the cell interior and the external solution is the resting membrane potential of the cell. Sodium ions are also drawn in by the electrical potential gradient. However, they penetrate the resting membrane far less readily than do potassium ions, so that the rate of inflow is not very large. Those that do enter are actively extruded again by the "sodium pump" and the concentration remaining within the cell is much smaller than that in the external solution.

It is generally assumed (13) that the active state and the associated action potential of muscle and nerve fibres arise/

arise as the consequence of a sudden breakdown of the potassium ion selectivity of the membrane, so that sodium and possibly other ions can move freely through it. There can be no doubt that a sudden increase in membrane permeability takes place during the active state of the membrane. The hypothesis advanced by Hodgkin and Katz (14) to explain this situation assumes that in the resting state the fibre membrane is readily permeable to potassium ions, whereas the permeability to sodium ions is extremely low. The resting potential is determined by the distribution of and permeability to potassium ions. This hypothesis assumes, however, that during activity the membrane acquires a transient high permeability for the sodium ion, many times higher than that for the potassium ion. If the outside medium has a higher sodium ion concentration than the interior of the fibre, as is the case, the preferential sodium ion permeability should clearly lead to a reversal of the sign of the potential. In order for the potential to return to normal it is also necessary to postulate that there exists in the membrane a mechanism which reduces the sodium ion permeability so that it becomes once again potassium ion selective. The hypothesis implies that depolarization of the membrane has two effects:-

(i) it causes a rapid transient increase in sodium ion permeability and

(ii) a delayed but maintained increase in potassium ion permeability.

Hodgkin, Huxley and Katz (15) attempted to devise a theoretical model which would account for the cycle of permeability/

permeability changes during the action potential. They assumed that the sodium ion crosses the membrane in combination with a carrier molecule which bears a negative charge. Each carrier molecule can combine with one sodium ion but not more. This system can transport very little sodium in the resting condition since the sodium carrier compound is attracted to the outside of the membrane by the potential difference across it. The restraining influence of the voltage gradient is removed when the membrane is depolarised and sodium ion transport then occurs at a high rate. External sodium ions combine with the carrier molecules on the outside of the membrane and are then carried across the membrane by thermal agitation. The carrier compound dissociates on the inside, and the uncombined carrier then returns to the outside as a result of thermal agitation. Hodgkin, Huxley and Katz (15) also postulated that external calcium competes with sodium for the carrier and forms a large reservoir of an immobile calcium compound on the outside of the membrane. The calcium compound dissociates as the membrane is depolarised and this process leads to a large increase in the amount of carrier available for the transport of sodium ions in the active state. Frankenhaeuser and Hodgkin (16) suggested similarly that depolarisation acts by removing calcium ions from combination with a sodium ion carrier.

Shanes and his co-workers (17) suggested that the multivalent calcium ion interacts with the cell membrane so as to reduce the number of sites available for the passage of/

of monovalent ions at lower levels of depolarisation, yet more sites apparently become available with higher levels of depolarisation. This can be accounted for if (i) the calcium ion occupies sites that normally become available to the monovalent ions with activity, (ii) in its interaction with the membrane the calcium ion causes more of such sites to appear and occupies them prior to activity and (iii) when depolarisation occurs, particularly above a certain level, the calcium ion is removed from these sites, which then function for the transfer of monovalent ions.

Adelman and Dalton (18) suggested that there are conductance channels for sodium and potassium ions in the membrane. In the resting state these channels are closed by bound divalent calcium ions. The bound calcium ion must be temporarily removed in order for excitation to occur and for any rapid rise of sodium conductance to take place in the channels. Driving current through the membrane from an external source results in a displacement of the calcium ion from its channel-occupying position. The conductance channels are assumed to resemble the macromolecular inter-spaces proposed by Mullins (3). In this case calcium ions and sodium ions are assumed to compete for the same channel as their diameters will be nearly identical at all levels of hydration (18). The larger diameter hydrated potassium ion is assumed to require a larger diameter channel than the sodium or the calcium ion. According to this hypothesis, while the calcium ion and the sodium ion compete for the same channel the mobility of the calcium ion in the channel is much less than/

than that of the sodium ion. This is assumed to be due to the forces established between the divalent calcium ions and the anonic sites on the membrane macromolecules. A channel in which the calcium ion is bound may be considered as maintaining a relatively constant effective diameter. Removal of calcium from the channel momentarily leaves an opening of appropriate size for the entry of sodium ions. The monovalent sodium ion is assumed to be less effective than the divalent calcium ion in maintaining an effective pore diameter which is less than that of the diameter of the hydrated potassium ion. The result of this situation is a rapid entry of sodium ions along its concentration gradient, followed in time by an enlargement of the effective pore diameter, producing a pore no longer suitable for sodium ion entry. Such a situation might arise as a result of competition with the internal potassium ions for these enlarged pores. While the flow of ions in such a channel or pore can be either inward or outward, but not in both directions at the same time, flow reversal must occur so that the potassium ions can move outwards, preventing further sodium ion entry. The entry of sodium ions produces the rising phase of the action potential. Repolarisation is accomplished by the outward movement of potassium ions in these channels. Return of the membrane potential from the peak of the spike towards the resting potential enhances the rebinding of calcium ions to the channel.

An understanding of the action of pharmacological agents on contraction (or contracture) in muscle could not be obtained/

obtained without determining the extent to which alterations in excitability, action potential and contractile mechanism contribute to the effect. It has been recognised that under normal physiological conditions the spike potential triggers off the muscle contraction. Contraction (or contracture) may occur without depolarisation, e.g. in tetraethylammonium treated insect muscle (10). It has been observed (19) that excitation caused by a presynaptic impulse is modified by the local chemical environment of the nerve cells. The higher the calcium ion concentration, the lower is the excitability of the nerve cells. When the calcium ion concentration falls to low values the pre- and post-synaptic cells become more and more excitable. The end result is a repetitive self-excitation in each cell. The shape of the action potential especially in the heart bears no obvious relationship to the strength of the contraction of cardiac muscle, e.g., while the spike is reduced in magnitude and shortened in duration by lowering the sodium ion concentration the contraction of the heart may be greatly improved (10).

Thus, it is clearly possible that there is a chemical link between depolarisation and the shortening of the contractile elements in muscle. Shanes (10) suggested that this link may be the calcium ion. Heilbrunn (20) observed that of all the physiological ions only calcium when injected into the isolated muscle fibre of the frog caused an immediate and pronounced shortening. Frank (21) observed an inward movement of calcium ions when the muscle fibre was depolarised/

depolarised and suggested that the calcium ion acted as a link between the electrical and mechanical events taking place during muscular contraction. Weidmann (22) similarly suggested that calcium ions mediated between electrical and mechanical activity throughout the period of membrane depolarisation.

Many observations (23-30) favour the view that activity brought about in skeletal muscle or cardiac tissue by electrical stimulation, potassium ions or drugs (e.g., ouabain on cardiac tissue) causes an increased movement of calcium ions across the membrane either in the inward or in the outward direction. Bianchi and Shanes (26) pointed out that the movement measured in the outward direction may not actually represent an increase in the transfer across the membrane but is rather an outward component complementary to the inward component of calcium ion movement when calcium ions are released briefly from their binding sites during activity. If an increase in the transfer in the outward direction from intracellular sites does occur it must take place after that of the influx if the calcium ion is to exert its full intracellular effect. Calcium ion entry might be by way of the sodium channel and its exit by way of the potassium channel during the rising and falling phases of the spike. The increased exit of calcium may be a consequence of a substantial increase in the ionised calcium of the myoplasm resulting from the increased entry. Thus, Shanes and Bianchi (31) suggested that the close equality of calcium ion entry and exit during activity in muscle was not/

not inconsistent with a net entry of calcium ion.

Other investigations (32-37) have shown that depolarising agents and contraction (or contracture)-producing substances cause an increase in the release of potassium ions from skeletal muscle. Keynes (38) observed an influx of sodium-24 in nerve during stimulation and Conn and Wood (39) concluded that in cardiac tissue, sodium ion entry into the cell occurs almost entirely during the spike potential and is negligible during the resting potential and the remainder of the action potential.

Analysis of the results presented in Chapter III (pages 96-184) shows that drugs having a depolarising or a contracture-producing effect (suxamethonium, decamethonium, nicotine, ryanodine and neostigmine)caused marked alterations in the movements of calcium-47, potassium-42 and sodium-24 in the isolated frog sartorius muscle at dose levels which also cause a mechanical response (Appendix I, pages 366-392). Carbachol, edrophonium, protoveratrine A and protoveratrine B caused moderate changes in the movement of one or more ions, whereas non-depolarising drugs, for example tubocurarine and gallamine did not induce any significant change in the ion movements.

In the isolated sartorius muscle of the frog calcium-47 and sodium-24 uptake were increased and potassium-42 uptake was decreased by suxamethonium (Fig. 3, page 97, Table 1, page 152), decamethonium (Fig. 4, page 99, Table 2, page 153), nicotine (Fig. 5, page 101, Table 3, page 154), ryanodine (Fig. 6/

(Fig. 6, page 104, Table 4, page 155) and neostigmine (Fig. 8, page 108, Table 6, page 157). Edrophonium depressed the uptake of calcium-47 and potassium-42 (Fig. 9, page 111, Table 7, page 158). Protoveratrine A increased the uptake of sodium-24 and depressed the uptake of calcium-47 (Fig. 12, page 118, Table 10, page 161). Protoveratrine B depressed the uptake of calcium-47 (Fig. 13, page 120, Table 11, page 162). Carbachol (Fig. 7, page 106, Table 5, page 156), tubocurarine (Fig. 10, page 113, Table 8, page 159) and gallamine (Fig. 11, page 115, Table 9, page 160) did not appear to cause any significant alteration in the uptake of calcium-47, potassium-42 or sodium-24 by this tissue.

Suxamethonium (Figs. 14, 15, pages 122, 123, Tables 12, 23, pages 163, 174), nicotine (Figs. 18, 19, pages 128, 129, Tables 14, 25, pages 165, 176), ryanodine (Figs. 20, 21, pages 131, 132, Tables 15, 26, pages 166, 177) and neostigmine (Figs. 24, 25, pages 137, 138, Tables 17, 28, pages 168, 179) caused an increased release of calcium-47 and potassium-42 from the isolated frog sartorius muscle.

Edrophonium (Fig. 26, page 139, Table 18, page 169) increased the release of calcium-47. Decamethonium (Fig. 17, page 126, Table 24, page 175) and carbachol (Fig. 23, page 135, Table 27, page 178) caused an increased release of potassium-42. Protoveratrine A (Figs. 32, 33, pages 147, 148, Tables 21, 32, pages 172, 183) and protoveratrine B (Figs. 34, 35, pages 150, 151, Tables 22, 33, pages 173, 184) had variable effects on the release of calcium-47 and potassium-42./

potassium-42. Tubocurarine and gallamine (Figs. 30, 31, pages 144, 145, Table 20, 31, pages 171, 182) did not cause any significant effect on the release of calcium-47 or potassium-42. Very large doses (1 mg. per ml.) of tubocurarine occasionally showed slight increase in the release of calcium-47, but did not show any change in the release of potassium-42 even in large doses (Figs. 28, 29, pages 142, 143, Tables 19, 30, pages 170, 181). The results are summarised in Table 34.(page 200).

These findings show that compounds capable of producing depolarisation or contracture cause an increased uptake of calcium and sodium ions but depress uptake of the potassium ion in the isolated frog sartorius muscle. At the same time they cause an increase in the release of calcium and potassium ions from this tissue. The lesser effects of edrophonium and carbachol can perhaps be attributed to their low contracture producing potency at the dose levels used, while protoveratrine A and protoveratrine B do not cause the characteristic veratrinic response in non-stimulated muscle.

After King (40) had established the structural formula of tubocurarine, it was thought that the activity of curarising drugs might be due to the presence of two onium groups at a certain optimal distance apart. The important work of Barlow and Ing (41) and of Paton and Zaimis (42) on the polymethylene bis-methonium series which included decamethonium supported this idea and led to the view that there was a cholinergic receptor which had two anionic sites at/

TABLE

Drug	Carbachol		Nicotine		Decamethonium		Suxamethonium		Tubocurarine		Gallamine		Neostigmine		Edrophonium		Ryanodine		Protoveratrine A		Protoveratrine B	
Isotope	Uptake	Release	Uptake	Release	Uptake	Release	Uptake	Release	Uptake	Release	Uptake	Release	Uptake	Release	Uptake	Release	Uptake	Release	Uptake	Release	Uptake	Release
Calcium-47	No change P, 0.3<0.2	No change	Increased P, >0.001	Increased	Increased P, 0.05<0.02	No change	Increased P, >0.001	Increased	No change P, 0.1<0.05	No change	No change P, 0.4<0.3	No change	Increased P, 0.01<0.001	Increased	Decreased P, 0.01<0.001	Increased	Increased P, 0.1<0.001	Increased	Decreased P, 0.05<0.02	Variable	Decreased P, 0.05<0.02	Variable
Potassium-42	No change P, 0.1<0.05	Increased	Decreased P, >0.001	Increased	Decreased P, >0.001	Increased	Decreased P, >0.001	Increased	No change P, 0.8<0.7	No change	No change P, 0.2<0.1	No change	Decreased P, >0.001	Increased	Decreased P, 0.05<0.02	No change	Decreased P, >0.001	Increased	No change P, 0.6<0.5	Variable	No change P, 0.2<0.1	Variable
Sodium-24	No change P, 0.8<0.7	X	Increased P, 0.02<0.01	X	Increased P, 0.01 < 0.001	X	Increased P, 0.01 < 0.001	X	No change	X	No change P, 0.7<0.6	X	Increased P, >0.001	X	No change P, 0.4<0.3	X	Increased P, >0.001	X	Increased P, >0.001	X	No change P, 0.2<0.1	X

at 13 to 15 Angström units apart. It is true that in several series of bis-quaternary muscle relaxants the most potent compounds are those in which the interonium distance is about 13 to 15 Angström units, but there are compounds with shorter interonium distances which are very potent. Thus, it appears that the interonium distance of 13 to 15 Angström units is not a requirement for maximum potency in this group of drugs.

The two major requirements for the interonium distance hypothesis are: (i) all compounds of this group should have a measurable interonium distance and (ii) all should have the same mechanism of action. But the numerous curare-like drugs which are not bis-onium compounds and the qualitative differences in the modes of action of depolarising and non-depolarising drugs may invalidate this hypothesis. Loewe and Harvey (43) suggested the application of an "adumbration" hypothesis to the curarizing activity of the bis-onium polymethylene compounds to help to explain difficulties in the interonium distance hypothesis. This hypothesis applies to compounds which do not possess two onium groups as well as to bis-onium salts. According to it drugs, "the actions of which are often ascribed to spatial competition for a receptor site, the umbrella structure may function by some passive interference due to size and shape of the appendage, for example, an "adumbrating" influence which may hamper the access of substrate or other material to a biologically important site." Both this hypothesis and that postulating spaced receptor sites at an optimal distance apart become more relevant when considered together with Waser's work (44, 45)./

(44, 45).

Waser (44, 45) carried out investigations which were amied at localising the site of action of non-depolarising and depolarising muscle relaxants. Calabash curarine and decamethonium were labelled with carbon-14 and experiments carried out with these compounds. The drugs in aqueous solution were injected into the tail veins of mice and the distribution studied by autoradiographic analysis of the diaphragm. The similarity between the autoradiographs of the two types of drugs demonstrated the direct action of the curarizing (non-depolarising) and depolarising drugs at the end plate region. There were some differences, however, the end plate band of the decamethonium-treated animals showed a blurred appearance whereas clear pictures showing individual end plates were seen with curarine-treated animals. It was suggested by Waser (45) that the diffuse appearance of the end plate band may be explained by fixation of decamethonium not only in the receptor area but farther out on to the muscle membrane around the end plate region. Perhaps the very different chemical structure enables decamethonium, in contrast to curarine, to diffuse from the post-synaptic space into the muscle, which would explain the extended region of depolarisation pointed out by Burns and Paton (46) and Paton and Zaimis (47).

Waser (45) also studied the antagonism of neostigmine to curarine at the level of the end plate region by simultaneous injection of both drugs. He observed that the radioactivity/

radioactivity of the end plate region was not noticeably diminished by low doses of neostigmine. Radiocurarine, however, was lost by the end plates only when a lethal, ten times normal dose of neostigmine was given. This may indicate that the cholinergic receptors blocked by curarine and the cholinesterase blocked by neostigmine are located at different sites. To show the competitive antagonism between acetylcholine and curarine at the same receptor, drugs were given by close intraarterial injection. With different combinations of both drugs the antagonism was apparent by the quenching of the radioactivity in the end plate region.

It was concluded (44, 45) that the receptor sites cannot be in one plane but have a three dimensional structure and cholinergic molecules fit into them. For depolarisation to occur the quaternary nitrogen, carbonyl and ether oxygen will form bonds (polar, covalent, hydrogen and van der Waals bonds) with the corresponding groups of the receptor substance. The receptor site might be a pore in the post-synaptic membrane having an anionic wall and an esteratic site to which the acetylcholine molecule becomes attached. This arrangement possibly causes a change in the macromolecular configuration of the wall resulting in the development of permeability changes in the membrane. On these assumptions the neuromuscular blocking action of curare-like substances could be visualised as due to a covering of the pores of the end plate region by the large molecules of tubocurarine or gallamine, which act by inhibiting the access of acetylcholine to the receptors and thus by preventing/

preventing the flow of ions through the membrane, i.e., a view similar to that of Loewe and Harvey (43). On the other hand the depolarising drugs would attach themselves to the anionic sites and bridge the pore at different chords. The molecules may not cover the whole circular area of the pore but may increase depolarisation by mechanical deformation of the end plate region. Ions can pass on both sides of the molecules, especially when the circular shape of the pore is changed to oval by the strain exerted by depolarising molecules such as suxamethonium or decamethonium attached to opposite parts of the anionic wall.

Chagas (48) has studied the nature of the curare receptor substance in the electric organ of the electric eel (Electrophorus electricus, L.) and the rat diaphragm using carbon-14 labelled gallamine and dimethyl-d-isochondodendrine dimethiodide. The test objects were injected with a mixture of the radioactive and non-radioactive drug. Experiments with rats were performed using groups of 10 animals and pooling their diaphragms after excision. The basis of the experimental approach consisted in analysing the radioactivity of various fractions obtained with different fractionation and separation procedures, thus determining the cell component to which the drug had become attached. A complex was extracted which consisted of the drug and a macromolecular component of the cell. The properties of the complex in relation to the presence of competition with molecules containing quaternary ammonium groups were in close agreement with the phenomenon of curarisation. It behaved like/

like a macromolecular system, being labile in the presence of a saline solution. Extraction with saline solution produced dissociation of the complex, the drug being removed. Chagas (48) suggested that when curare was injected into the blood stream and curarisation occurred a complex was formed between the injected drug and a macromolecular component of the cell. It was shown that the component was not identical with cholinesterase and may apparently be classified as a mucopolysaccharide. The high affinity of all molecules containing the quaternary ammonium group for the purified receptor substance may make it a general component of biochemical cellular structures where cholinergic transmission is involved.

Ehrenpreis (49, 50) pointed out that the complex observed by Chagas (48) dissociated in very dilute salt solution (0.02M) and this raised doubts as to whether the macromolecule responsible for the observed binding was indeed the cholinergic receptor, since curarisation of an electroplax was easily obtained with low concentrations of curare even in 0.18M salt solution (51). Ehrenpreis (49, 50) subjected the electroplax extracts to ammonium sulphate fractionation thereby eliminating non-specific binding. He found such binding to occur with nucleic acid and chondroitin sulphuric acid (a mucopolysaccharide), components present to some extent in extracts of electric tissue but which could be eliminated by ammonium sulphate fractionation.

As a result of his studies Ehrenpreis (49, 50) was able to isolate a protein which was examined for its ability to bind/

bind curare and other related substances. It was observed that (i) there was a qualitative parallelism between the degree of binding of curare and related substances in solution and the affinity of these compounds for the receptor, as measured by their effects on the electrical activity of the intact electroplax (51). The effectiveness of the two types of studies decreased in the following order - curare, dimethylcurare, neostigmine and eserine; (ii) the binding of curare although salt sensitive still occurred at a relatively higher concentration; (iii) decamethonium and neostigmine were not able to displace curare from its complex with the protein in solution.

It has been suggested that the material isolated, which had a protein-like ultra-violet spectrum, may form specific complexes with curare and related compounds and thus has cholinergic receptor-like properties.

In Chapter I the theory advanced by Van Rossum, Ariëns and Linssen (52) on the mode of action of basic types of curariform drugs has been summarised. On the basis of this theory depolarising and non-depolarising (competitive) neuromuscular blocking agents have an affinity towards specific receptors but there is a difference in their intrinsic activity. The depolarising drugs such as suxamethonium have a high intrinsic activity whereas non-depolarising drugs (competitive) such as tubocurarine have a very low intrinsic activity.

Recently/

Recently Paton (53, 54) has presented a rate theory of the basis of drug-receptor interaction. According to this theory "the drug associates with its specific receptors by an ionic exchange with potassium, stimulating in proportion to the rate of association. If it dissociates again rapidly more associations can take place (but not at the initial rate), and a vigorous but fading response can occur. This will ultimately be limited by the supply of intracellular potassium and desensitization develops, extending to other types of receptor also. If the drug dissociates very slowly its occupation of receptors occludes further association and it appears as a specific antagonist whose onset and offset of action and potency depend on its association and dissociation rate constants. Drugs with intermediate dissociation rate constants will exhibit both stimulant and antagonistic actions. The dissociation rate constant determines the character of the drug and the ratio of dissociation to association rate constant its potency." On the basis of this theory the depolarising and non-depolarising drugs may have different dissociation rate constants which will determine the nature of their combination with the specific receptors.

In a recent publication (55) Ariëns and Simonis have maintained that while the intrinsic activity of a drug includes by definition a consideration of the rate of drug receptor association, a measurement involving only this (indicated by Paton (53, 54) as the important determinant in drug activity) would be complicated by the question of drug/

drug transference from the surrounding medium to the biophase.

Suxamethonium and decamethonium are depolarising neuromuscular blocking agents. Their main action on skeletal muscle is to depolarise the motor end plate region. The neuromuscular block produced by these drugs can be explained by a persisting partial depolarisation of the motor end plate region similar to that caused by acetylcholine when it is protected from hydrolysis by an anticholinesterase. Because of the persistent depolarisation which they produce, suxamethonium and decamethonium prevent muscular contraction in response to acetylcholine or stimulation of the motor nerve. With both drugs neuromuscular block is preceded by transient stimulation. It has been suggested (56) that suxamethonium is more selective in its action than decamethonium. Both suxamethonium and decamethonium caused an increased uptake of calcium-47 and sodium-24 but depressed the uptake of potassium-42 in isolated frog sartorius muscle. Both the drugs also caused increased release of potassium-42. Suxamethonium, in addition, increased the release of calcium-47. The results on potassium release confirms the observations of Kraupp and his co-workers (37) and of Klupp and Kraupp (34).

The depolarising nature of suxamethonium and decamethonium would be expected to cause characteristic ionic changes in skeletal muscle as has been postulated by Hodgkin, Huxley and Katz (14, 15). The increased uptake of sodium-24 and the increased release of potassium-42 observed in experiments described/

described on isolated frog sartorius muscle are in conformity with this view.

The major action of nicotine consists of a primary transient stimulation and secondary more persistent depression of all sympathetic and parasympathetic ganglia. Both the initial excitation and the subsequent paralysis are due to a direct action on the ganglion cells. Nicotine does not interfere with the release of acetylcholine in ganglia by cholinergic preganglionic nerve impulses but it renders the ganglion cells temporarily more sensitive and then more resistent to acetylcholine. The finer mechanism of action of nicotine on ganglia has been elucidated by Paton and Perry (57). They have demonstrated that nicotine initially stimulates by depolarising the ganglion cell and subsequently prevents ganglionic transmission by a competitive type of blockade by acetylcholine. The effects of nicotine on skeletal muscle are similar to those on ganglia (58).

Nicotine caused an increased uptake of calcium-47 and sodium-24 but depressed the uptake of potassium-42 in isolated frog sartorius muscle. It also caused increased release of calcium-47 and potassium-42. These ionic changes are similar to that seen with suxamethonium and are probably associated with the depolarising effect of nicotine.

In Chapter I the properties of ryanodine on skeletal and smooth muscle have been summarised. The most remarkable property of ryanodine on mammalian and frog skeletal muscle is to induce an irreversible contracture (59, 60).

Our/

Our present knowledge of the mechanism of muscular contraction suggests that the possible site of action of a drug capable of causing contraction (or contracture) are, (i) at the end plate region, (ii) the muscle cell membrane, (iii) the actual contractile elements or (iv) the metabolic system which supplies energy for muscular contraction and relaxation. It has been observed (59, 60) that tubocurarine and suxamethonium did not affect the contracture produced by ryanodine. Procita (59) obtained neuromuscular paralysis of the tibialis anterior muscle preparation of the cat by tubocurarine administered intravenously. After this ryanodine was given intraarterially and the muscle stimulated directly. It was observed that the muscle progressed into a contracture. Similarly in suxamethonium-treated muscle subsequent administration of ryanodine produced a sustained contracture. Blum and his co-workers (60) induced a contracture of the frog rectus muscle by ryanodine. All attempts to cause recovery of such a muscle by tubocurarine failed. These findings may be regarded as eliminating the end plate region as the site of action of ryanodine. Jenerick and Gerard (61) used ryanodine to alter the membrane potential of single muscle fibres of the frog using concentrations of 0.1 to 1.0 mg. per cent in Ringer's solution. They observed that ryanodine did produce slight (5 mV in one hour at 0.2 mg. per cent; 10 mV at 0.4 mg. per cent; 8 mV after 10 sec. tetanus in 0.4 mg. per cent) depolarisation; but could also produce a contracture even when there was no significant fall in potential (1 to 2 mV). The depolarisation and development/

development of contracture were independent and threshold-potential relations were not measurable because of the contracture. Blum and his co-workers (60) have shown that in frog sartorius muscle the resting membrane potential was unaffected by ryanodine. Procita (59) suggested, however, that the muscle membrane cannot entirely be ruled out as a possible site of action of ryanodine. There are observations (61-63) in the literature which state that the correlation of electrical and mechanical events in frog muscle is not always possible. Liu and his co-workers (63) soaked the sartorius muscle of the toad in iodoacetate solution and then stimulated it. It was observed that the muscle went into a rigor. An indication that the rigor was not accompanied by any considerable depolarisation was obtained by showing that the muscle, soon after the development of rigor, gave a demarcation potential of the usual magnitude on crushing or on application of isotonic potassium chloride. Taylor (62) observed that frog sartorius muscles soaked in caffeine shorten and lose potential, but the two changes were independent. It was found that when the muscle had completed 50 per cent of its shortening the membrane potential was unchanged or lowered by only 5 per cent. Jenerick and Gerard (61) observed that eserine did not alter the membrane potential in frog muscle fibres. The electrical phenomena are but one aspect of the activity of the cell membrane and contractile changes may not necessarily be associated with potential changes. Procita (59) observed that some degree of contractile activity of mammalian/

mammalian muscle in situ was necessary for the production of a sustained contracture with ryanodine. It was found that when ryanodine was injected in the absence of an applied stimulus no sustained contracture was seen. He suggested that the ryanodine molecule was unable to traverse the muscle membrane and gain access to the contractile elements unless the membrane was rendered more permeable by depolarisation. Blum and his co-workers (60) observed that there was tearing of the muscle fibres during ryanodine contracture which could be readily seen by microscopic examination. When a weight was put to the muscle after treatment with ryanodine the muscle was not able to go into contracture and it was seen by a dissecting microscope that many of the muscle fibres were fractured. Membrane potential recorded from such a muscle showed that some of the fibres had normal potentials while many showed low potentials. The low potentials were likely to be produced by the damaged muscle fibres. Blum and his co-workers (60) suggested the existence of a hypothetical substance present in limited quantity in muscle which was necessary for the normal cycle of contraction and relaxation. This was affected by ryanodine in an essentially irreversible manner and at a rate dependent upon the concentration of ryanodine present. When the amount of this hypothetical substance falls below a certain critical level an irreversible contracture ensues, possibly as a result of the extreme instability of the relaxed state in the absence of this substance. This suggestion may explain the phenomenon (59) that in a ryanodine-treated muscle each successive contraction was superimposed/

superimposed upon incompletely relaxed contractile units.

Ryanodine caused an increased uptake of calcium-47 and sodium-24 but depressed the uptake of potassium-42 in isolated frog sartorius muscle. It also caused an increased release of calcium-47 and potassium-42. The ionic changes seen with ryanodine were similar to those caused by depolarising agents such as suxamethonium. This is not compatible with the view (14, 15) that the ionic changes are brought about by depolarisation of the membrane as it has been observed (60) that ryanodine does not apparently cause a significant alteration of the resting membrane potential. It is to be borne in mind, however, that the contracture produced by ryanodine is associated with laceration of muscle fibres (60) and this may well bring about the marked permeability changes seen in the experiments described and which on the surface are similar to those following depolarisation.

Neostigmine and edrophonium are synthetic anticholinesterases. They produce their characteristic effects by enzyme inhibition. They promptly antagonise the neuromuscular block produced by curare and related curarizing alkaloids and non-depolarising drugs. Over and above their action on cholinesterase they have a component of direct action on the end plate region of the muscle fibre (64). On the isolated frog sartorius muscle neostigmine caused contraction (Appendix I, page 369) whereas edrophonium did not show any significant contraction in the dose levels (0.5 to/

to 2.0 mg. per ml.) used. Neostigmine caused an increase in the uptake of calcium-47 and sodium-24 but depressed the uptake of potassium-42 in isolated frog sartorius muscle. It also caused an increased release of calcium-47 and potassium-42. The ionic changes seen with neostigmine were similar to those caused by depolarising agents such as suxamethonium. This may be ascribed to the direct action of neostigmine on the motor end plate region. Edrophonium depressed the uptake of calcium-47 and potassium-42 but did not cause any significant change in the uptake of sodium-24 by isolated frog sartorius muscle. It increased the release of calcium-47 but did not show any alteration in the release of potassium-42. The less marked and indifferent nature of the action of edrophonium on ionic changes may possibly be due to its feeble action on the motor end plate.

Carbachol is a stable ester of choline which is not hydrolysed by cholinesterase. It possesses both the muscarinic and the nicotinic actions of acetylcholine but its muscarinic action is more pronounced. Its effects upon the gastrointestinal and urinary tracts are more marked than those upon the cardiovascular system or respiration. Carbachol did not cause any significant alteration in the uptake of calcium-47, potassium-42 or sodium-24 by isolated frog sartorius muscle. It increased the release of potassium-42 but did not show any change in the release of calcium-47. The lack of change in ionic movement with carbachol may possibly be due to its mainly muscarinic action and the low dose levels used in the experiments described.

Tubocurarine/

Tubocurarine and gallamine are non-depolarising (competitive) neuromuscular blocking agents. On a weight basis gallamine is about one-fifth as potent as tubocurarine. These drugs produce their characteristic action on skeletal muscle by preventing the response to motor nerve impulses. They compete with acetylcholine for the same receptor at the end plate region. Tubocurarine and gallamine did not cause any significant alteration in the uptake of calcium-47, potassium-42 or sodium-24 by isolated frog sartorius muscle and they did not cause a change in the release of calcium-47 or potassium-42. Ionic changes are normally associated with depolarisation of the muscle membrane (14, 15). The non-depolarising character of tubocurarine and gallamine is reflected by the lack of changes in the ionic flux.

It is well known that certain Veratrum alkaloids, notably veratrine, can produce a secondary tetanus following a normal initial twitch in directly or indirectly stimulated skeletal muscle. This secondary phenomenon, the veratrinic response, is associated with a repetitive discharge of impulses in the muscle fibres (65). Kuffler (66) stated that the principal effect of veratrine was to increase the negative after-potential following the conduction of each muscle impulse along the fibre. The negative after-potential was associated with an increase in excitability and when it was sufficiently great additional muscle impulses were initiated. Thus, a single muscle action potential produced a self-sustained train of high frequency after discharges reflected on the myogram as a prolonged tetanus. Veratrine also/

also produced repetitive discharges in peripheral nerve (65).

The accentuation of the negative after-potential may represent a marked slowing of the rate of repolarisation following the passage of an impulse. The theory of the ionic basis of the action potential proposed by Hodgkin, Katz and Huxley (14, 15) suggests that the restoration of the resting potential after the spike is due to an outward shift of potassium ions. The result of a failure of repolarisation could be that the membrane remains in a state of total or partial depolarisation. In the case of muscle this may lead to a state of prolonged contraction and this may account for the characteristic response of the veratrine treated muscle. It could also increase sensitivity to acetylcholine.

Shanes (67) observed that depolarising concentrations of veratrine caused a continuous release of the potassium ion and an uptake of roughly equivalent amounts of sodium ion in frog nerve. Similarly Straub (68) observed that veratridine produced depolarisation in nerve fibres and suggested that its action was due to an increase of sodium and potassium ion permeability. Gordon and Welsh (69) suggested that veratrine gave rise to a repetitive response due to a "delay in the restoration of calcium ions to a surface complex, following the breaking of the chelate linkage of calcium ions to surface polar groups by an initial exciting impulse." Lister and Lewis (70) supporting the view of Gordon and Welsh (69) suggested that protoveratrine and related Veratrum alkaloids interfere with the normal metabolism/

metabolism of calcium on the cell surface, altering membrane permeability and thus ionic balance which is the factor finally responsible for the characteristic actions of the Veratrum alkaloids on muscle and nerve.

Protoveratrine A decreased the uptake of calcium-47 and increased the uptake of sodium-24 but did not cause any significant change in the uptake of potassium-42. Protoveratrine B decreased the uptake of calcium-47 but did not cause any significant change in the uptake of potassium-42 or sodium-24. Both protoveratrine A and protoveratrine B caused variable changes in the release of calcium-47 or potassium-42. The ionic changes obtained with protoveratrine A and protoveratrine B in the experiments described indicate that protoveratrine A and protoveratrine B are capable of causing alterations in ionic movements across the cell membrane. The lesser effects on ionic changes shown by these drugs may possibly be due to the use of non-stimulated frog sartorius muscle.

It has been suggested by Waser (44, 45) that the cholinergic receptor site might be a pore in the post-synaptic membrane. This is an attractive hypothesis and may explain a good deal. There must be some structure on the post-synaptic membrane which permits flux of ions and this might well be a pore with flexible sides or walls which can be blocked by bound calcium as suggested by Adelman and Dalton (18) in the resting state. If it is assumed that the depolarisation produced by depolarising agents such as suxamethonium/

suxamethonium is capable of opening up the pores in the post-synaptic membrane by temporarily removing the bound calcium ions, this may lead to permeability changes with resultant flux of ions inside and outside the membrane. On the other hand the absence of depolarisation in non-depolarising drugs such as tubocurarine cannot open up the pores in the post-synaptic membrane, thus no permeability changes can occur.

It seems likely from the experiments described that when depolarising or contracture producing compounds are applied to frog sartorius muscle calcium ions become more mobile, probably being dissociated from combination with a carrier as suggested by Hodgkin, Huxley and Katz (15) and Frankenhaeuser and Hodgkin (16) or displaced from a site in the membrane as suggested by Shanes and his co-workers (17) and Adelman and Dalton (18). This may result in the break-down of barriers which retain the potassium ion within the cell and sodium ion outside.

Depolarisation of any kind has been suggested (10) to allow calcium ions in the membrane to associate with available anions and to diffuse as ion pairs into the protoplasm where they dissociate. The importance of the calcium content of the membrane lies in providing an adequate source of calcium that will ionize on entering the fibre. The rate of entry or the quantity of ionized calcium entering the myoplasm may be a factor in the rebinding of calcium ions to the membrane.

The/

The results obtained in this study support the view (10, 21, 22) that depolarisation of the membrane or contracture of the muscle is intimately associated with an entry of calcium ion into the muscle fibre and that calcium ions may act as a link between electrical events and contraction (or contracture). They can also be taken as support for the view that the receptor is a pore (45), the physical properties of which are altered by depolarising agents and related drugs and that the calcium ion is an essential link between the mechanical events in contracting muscle and the preceding electrical and humoral changes. The author of this thesis feels that his results not only confirm those of others concerned primarily with the physiology of skeletal muscle but are a logical extension of this aspect to the study of the mode of action of some important drugs.

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CHAPTER V

SUMMARY

In the introductory chapter of this part of the thesis the importance of calcium, potassium and sodium ions on the functioning of excitable tissues, especially in muscle and nerve, has been reviewed. The electrical properties of muscle and nerve and the mechanism of ion exchange across the cell membrane during activity have been summarised. A short account of the normal mechanisms of neuromuscular transmission has been given. The mechanisms of neuromuscular block and the classification of the neuromuscular blocking agents have been reviewed.

In Chapter II of this part the experimental procedures used in the investigation of the uptake of calcium-47, potassium-42 and sodium-24 by isolated frog sartorius muscle and release of calcium-47 and potassium-42 have been described. The method used for analysing the data statistically has also been described in this chapter.

In Chapter III of this part, the changes in the ionic movements produced by suxamethonium, decamethonium, nicotine, ryanodine, neostigmine, edrophonium, carbachol, tubocurarine, gallamine, protoveratrine A and protoveratrine B on isolated frog sartorius muscle have been described. It has been shown that drugs having a depolarising or contracture-producing/

producing action on the frog sartorius muscle caused an increased uptake of calcium-47 and sodium-24 but depressed the uptake of potassium-42. These drugs also caused an increased release of calcium-47 and potassium-42. Suxamethonium, decamethonium, nicotine, ryanodine and neostigmine caused marked changes in the movements of calcium-47, potassium-42 and sodium-24, whereas with other drugs, edrophonium, carbachol, protoveratrine A and protoveratrine B, the changes were not marked. The non-depolarising drugs, tubocurarine and gallamine did not show any significant change in the movement of calcium-47, potassium-42 or sodium-24. The results are summarised in Table 34 (page 200).

In Chapter IV of this section the importance of the cell membrane in relation to the flux of ions has been discussed. The theories of the nature of the receptor site have also been discussed. An attempt has been made to explain the results obtained in Chapter III in the light of the present theories of the action of depolarising and non-depolarising drugs.

P A R T II

STUDIES ON THE PHARMACOLOGY OF PETALINE CHLORIDE, A CONVULSANT ALKALOID FROM *LEONTICE LEONTOPETALUM* LINN.

CHAPTER I

INTRODUCTION

Lovell (1) in his "Compleat Herball" of 1665 mentioned a plant leontopetalon ("lions-leafe") growing "among corne in Italy" and flowering in winter. The root of the plant was "taken in wine" to ease the pain of snake bite and sciatica. It was also used for cleaning and healing "old filthy ulcers." Lovell also stated that "the pouder of the old and greater roots" was used to remove spots from garments. Linnaeus (2) in his "Systema Vegetabilium" gave a short description of the plant genus Leontice. He mentioned five different species of the genus --- (i) Leontice chrysogonum; (ii) Leontice leontopetalum; (iii) Leontice vesicaria Pall; (iv) Leontice altaica Pall and (v) Leontice thalictroides. The genus was also described by other authors. Loudon (3) in his "Encyclopoedia of Plants" mentioned the genus Leontice and observed that it is an abridgment of leontopetalum, its ancient name "because the shape of the leaves was thought to resemble the print of a lion's foot." Lindley (4) in his "Vegetable Kingdom" stated that the roots of the plant Leontice leontopetalum were "used at Aleppo as a substitute for soap" and were "regarded by the Turks as a corrective of overdoses of opium." Post (5) in his "Flora of Syria, Palestine and Sinai" gave a short description of the genus Leontice. He noted that the roots of Leontice leontopetalum were/

were "used as a remedy for epilepsy." Bentham and Hooker (6) in "Genera Plantarum" described the genus Leontice. Willis (7) in his "Flowering Plants and Ferns" included Leontice leontopetalum in the Berberidaceae. Boissier (8) in his "Flora Orientalis" described five different species of the genus Leontice --- (i) Leontice leontopetalum; (ii) Leontice eversmanni; (iii) Leontice minor; (iv) Leontice vesicaria and (v) Leontice altaica. Bailey (9) in his "Standard Cyclopedia of Horticulture" also described five different species of the genus Leontice --- (i) Leontice tuberous, growing from Southern Europe to Central Asia, (ii) Leontice leontopetalum, growing in Italy and the Orient, (iii) Leontice alberti, growing in Turkestan, (iv) Leontice altaica, growing in South Russia, Altai and Siberia and (v) Leontice chrysogonum. He observed that the roots of Leontice leontopetalum were "used ..... against epilepsy." Gunther (10) in his "Greek Herbal of Dioscorides" described two plants --- (i) Leontice chrysogonum and (ii) Leontice leontopetalum, both of which grow in fields amongst wheat.

McShefferty and his co-workers (11) stated that their attention was first drawn to the plant Leontice leontopetalum Linn in 1951 by Dr. W.M. Ford-Robertson and Dr. A.S. Manugian of the Lebanon Hospital for Mental and Nervous Disorders, Asfuriyeh, Beirut. The latter, while making a special study of epilepsy in the Lebanon found that a preparation of the tuberous roots of the plant which grows wild in parts of the Lebanon had a reputation for curing epilepsy. "The 'juice' prepared from the fresh roots, is bitter/

bitter and poisonous. It is this juice which is used in the treatment ..... is regarded as "kill or cure" ..... several cures of confirmed cases of epilepsy have been observed. For treatment, the tuber is dug up after the aerial parts have died down, cut into small pieces and pounded in a mortar. The "juice" is given in a dose of about one teaspoonful three times daily. This dosage is continued for three days and during this time the patient is reported to be more or less in status epilepticus, having convulsions which recur every two or three minutes. The "juice" is prepared from freshly collected roots for each dose. At the end of this initial treatment the patient is given large quantities of an aqueous extract prepared from the marc left after preparation of the "juice." The marc is pounded in water, and glassfuls of the extract thus prepared are given frequently every day, usually for a period of several months. The native prescribers say that this extract stops the convulsive action of the drug, but Dr. Manugian believes that this is not an essential part of the treatment."

Pharmacognosy of Leontice leontopetalum Linn

Nelson and Fish (12) gave a detailed description of the macroscopical appearance of the plant Leontice leontopetalum with brief notes on the history and geographical distribution. They also described the microscopical character of the root tuber (13). "The plant has been reported to inhabit an area including those countries bordering the eastern part of the Mediterranean from Italy, Greece and Turkey through Lebanon,/

Lebanon, Syria, Israel, Jordan and Iraq to north-eastern parts of Egypt. It has also been seen on the island of Cyprus."

"The plant is a perennial herb with large compound leaves, a compound inflorescence of yellow flowers and a large intercalary root tuber. The inflorescence grows to a height of about 29 to 45 cm. while the foliage leaves usually lie close to the surface of the ground both in plants with and without an inflorescence."

"The tuber varies greatly in size and shape but is usually irregularly circular to oval in outline and somewhat flattened dorsiventrally. The maximum size of the tubers of the flowering plants is about 23 cm. in diameter, by about 10 cm. in thickness while the smallest tubers from such plants are about 7 cm. in diameter by about 5 cm. thick. Externally it is covered with a thick brown cork with small protuberances from which fine white rootlets arise. The main stem is attached more or less centrally to the upper side of the tuber and some of the foliage leaves arise around the stem at this point."

Freshly collected tubers when washed, cut into slices and rapidly dried in the sun "consist of irregular slices about 0.5 cm. thick. Externally there is a thick brown cork and the remainder ..... is light brown in colour and consists of starchy parenchyma with vascular bundles appearing as points or striations and the groups of sclereids as/

as protuberances. Often there are dark brown areas lying internal to the cork and these consist of lignified parenchyma. The texture is hard and brittle."

Microscopically "the thick outer layer consists of 1 to 6 bands of lignified, suberised cork cells alternating with bands of phellogen ..... Next to the innermost layer of phellogen is a phelloderm which consist of up to 10 rows of cellulosic parenchyma, the cells of which are devoid of starch and are 32 - 86 - 158μ long and 12 - 31 - 62μ deep. The remainder of the tuber consists of ground tissue in which there are groups of sclereids and numerous vascular bundles.... The collateral vascular bundles ramify throughout the tuber and in any section it is possible to find bundles cut transversely, longitudinally or obliquely." .....

"The yellowish-brown powder has a pleasant odour but has a sternutatory effect and a bitter taste."

Chemical Investigation of the genus Leontice

Plants of the genus Leontice which have been chemically investigated include --- (i) Leontice eversmanni; (ii) Leontice alberti and (iii) Leontice leontopetalum. Orekhov (14) reported that a mixture of crystalline alkaloids was obtained from the roots of Leontice eversmanni, but these have not been completely separated. Orekhov and Konovalova (15) reported that the dried bulbs of Leontice eversmanni contained about 0.4 per cent of alkaloids, from which leontamine/

leontamine was separated. Its hydrochloride, diiodomethylate chloroplatinate and picrate were crystallised and studied. Another alkaloid leontidine was obtained in colourless rhombic plates. Orekhov and Konovalova (16) also reported that the tubers of Leontice eversmanni were extracted first with alcohol and this extract later exhausted first with diethyl ether and subsequently with chloroform. These extracts finally yielded two well characterised alkaloids --- leontamine and leontidine. In addition they obtained a third alkaloid in small amounts in the form of a crystalline picrate. Yunusov and Sorokina (17) reported that extraction of the upper parts of Leontice eversmanni with dichloroethanol and 5 per cent ammonium hydroxide gave 0.87 per cent total alkaloids, from which leontidine was obtained as the free base. The mother liquor yielded pachycarpine, lupanine and leontine. Similar extraction of the tubers of the plant yielded 0.17 per cent total alkaloids from which leontidine hydrochloride, leontamine and leontine were obtained. Extraction of the seeds of the plant in a similar way yielded 1.1 per cent total alkaloids, which in turn gave leontidine hydrochloride and leontine. Platonova, Kuzovkov and Massagetov (18) reported that extraction of the upper parts of Leontice eversmanni with dichloroethanol in the presence of 10 per cent ammonium hydroxide and treatment of the extract with 15 per cent sulphuric acid gave a precipitate of taspine sulphate, which was purified by crystallisation from 10 per cent acetic acid, the yield was 0.18 per cent of the plant weight. The acidic filtrate made/

made alkaline with ammonium hydroxide with cooling and extracted with chloroform yielded 0.56 per cent mixed bases as a dark oil. This in acetone, acidified to congo red with alcoholic hydrochloric acid gave leontidine hydrochloride. The mother liquor after evaporation was distilled in vacuo, yielding pachycarpine isolated as the hydrogen iodide salt, l-lupanine hydrogen iodide, isoleontine isolated as the picrate and leontidine hydrochloride. Leontidine hydrochloride with sodium hydroxide gave free leontidine. On hydrogenation of leontidine over platinum oxide a new base leontidane was obtained. Isoleontine picrate treated with 18 per cent hydrochloric acid, washed with dilute hydrochloric acid and the acid solution treated with sodium hydroxide after removal of picric acid gave free isoleontine. Platonova and Kuzovkov (19) studied taspine. They also reported that reduction of leontine with lithium aluminium hydride gave isoleontane (20).

Yunusov and Sorokina (17) reported that extraction of the tubers of Leontice alberti gave 0.75 per cent alkaloids and extraction of the upper parts of the plant gave 1 per cent alkaloids which on further extraction gave methyl cystine, leontine and leontidine.

McShefferty and his co-workers (11) studied the tubers of Leontice leontopetalum chemically. They obtained a pure quaternary alkaloid "after extraction of the chloroform-soluble alkaloids by precipitating as reineckate. The crude reineckate so obtained was readily purified by chromatography from/

from acetone on alumina and obtained as a pale pink amorphous solid ..... Decomposition of the pure reineckate gave the pure quaternary alkaloid chloride, designated petaline chloride as bright greenish-yellow scales ..... Petaline chloride so obtained, has been formulated $C_{20}H_{22}O_3NCl,H_2O$, is extremely hygroscopic, readily soluble in water, methanol and ethanol, but insoluble in ether and chloroform. All attempts to obtain the chloride in a crystalline state failed, largely due to the facility with which it absorbs moisture ..... A crystalline fraction was finally isolated from the chloroform-soluble alkaloids by dissolving in acid, extracting from acid solution with ether and chloroform to remove impurities, basifying with ammonia and then extracting with ether. A small proportion only of the total alkaloids could be extracted in this way, yielding a clear, pale brown viscous oil, which partly crystallised from ethanol, to give a colourless, crystalline, optically inactive alkaloid, leonticine ..... The oily residue from the ether extraction was distilled under reduced pressure, giving a colourless, oily, strongly basic alkaloid, the properties of which resembled those reported for leontamine."

Pharmacology of alkaloids obtained from plants of the Berberidaceae and related families.

There has been no detailed report on the pharmacological activity of Leontice leontopetalum, its extracts or constituent alkaloids. Certain alkaloids from plants of the/

the Berberidaceae and related families have been reported to exhibit convulsant activity and other pharmacological properties in experimental animals.

According to Malcolm (21) pukateine, an alkaloid obtained from the plant Pukatea belonging to the Monimiaceae family, one of the most characteristic plants of the northern part of New Zealand, has convulsant properties. Pukateine hydrochloride in doses of 0.25 g. per kg. is a spinal convulsant. In the rabbit the convulsions resemble those induced by strychnine. On intravenous injection the blood pressure falls slightly, the heart beats slowly and death results from respiratory failure. Applied to the tongue pukateine causes numbness. The alkaloid itself is inactive owing to its insolubility.

Reynolds and Waud (22) reported the pharmacological actions of capaurine and its O-methyl ether. Capaurine was obtained from the plant Corydalis aurea, a member of the Fumariaceae family which is indigenous to Canada. The base produces paralysis in frogs and convulsions in mice and rabbits, both effects being of a central origin. The heart is depressed as is the smooth muscle of the intestine and the uterus. Capaurine-O-methyl ether is a convulsant substance exhibiting actions on the heart and smooth muscle very similar to those of the alkaloid itself.

Iwakawa (23) reported the pharmacological actions of dicentrine, an alkaloid obtained from the plant Dicentra pusilla. He observed that in frogs as well as in warm blooded animals/

animals dicentrine causes in small doses light narcosis, in moderate doses tonic-clonic convulsions and in large doses paralysis of the respiratory centre preceded in warm blooded animals by a transient stimulation.

Raymond-Hamet (24) reported the pharmacological actions of boldine, an alkaloid obtained from the plant Boldea fragans, a member of the Monimiaceae family. Boldine lowers blood pressure. Large doses provoke violent convulsions. It antagonises but does not reverse the action of adrenaline on the blood pressure.

Schroeder (25) reported the pharmacological actions of cryptopine, an isoquinoline alkaloid of opium, also obtained from the plants Corydalis nobilis and Corydalis ochrolenca. He observed that cryptopine stimulated the central nervous system before paralysing it. He considered that the convulsions, and with them the dilatation of the pupils, were not due to respiratory failure. Heathcote (26) reported that in experiments with isolated organs, cryptopine has a depressant action very similar to that of papaverine but was only one-half to one-third as potent. He found that in the toad cryptopine showed little evidence of stimulation but rather caused paralysis of the central nervous system. Delphant and Blache (27) reported the actions of cryptopine on intact animals. For the dog the lethal dose of the hydrochloride was 80 mg. per kg. which when injected intravenously caused respiratory arrest. Smaller doses produced hypotension, depression of the heart, stimulation of/

of respiration and inhibition of the response of the vagus nerve to faradic stimulation. When injected into the spinal canal it inhibited the reflex response to faradic excitation of the sciatic nerve. For the rabbit the lethal dose was 40 to 80 mg. of the hydrochloride when injected intravenously and for the guinea pig it was 190 mg. per kg. when injected subcutaneously. Mercier (28) observed that a dilute solution of cryptopine (1 in 200,000) in Tyrode's solution decreased the tone of isolated intestine, whereas stronger solutions (1 in 20,000) arrested the movements. On the dog intestine the above concentrations exerted a stimulating action while higher concentrations had a depressing action. Mercier, Delphant and Blache (29) compared the pharmacological actions of papaverine, cryptopine and berberine. They observed that the effects of cryptopine on the intestine, the central nervous system, blood pressure, heart action and respiration were intermediate between those of papaverine and berberine but resembled those of berberine more closely. Luduena (30) studied the pharmacological actions of cryptopine on intact animals and isolated organs. The mean lethal dose of cryptopine hydrochloride for guinea pigs injected intravenously was 160 mg. per kg. In the spinal cat doses of more than 5 mg. per kg. produced hypotension and an increase in respiration. The same effects were produced in rabbits anaesthetised with urethane. Cat, dog and rabbit uteri isolated or <u>in situ</u> were stimulated. The tone of contraction of rabbit intestine <u>in situ</u> was increased by cryptopine but the contractions of isolated rabbit/

rabbit intestine were inhibited.

Hesse (31) was the first to isolate the alkaloid protopine from opium. It has since been obtained from plants belonging to many genera, the best source being Dicentra spectabilis. Protopine in small doses has a narcotic action in frogs, while larger doses abolish reflex activity and show a curare-like action. At times there is evidence of medullary stimulation. According to Bolm (32) large doses (18 to 200 mg. per kg.) of protopine given parenterally to experimental animals induce excitement or convulsions. Small doses slow the heart, lower blood pressure and have a quietening effect. It has an inhibiting action on the isolated frog heart, muscle or nerve and a stimulating action on the guinea pig intestine. According to Andersen and Chen (33) the LD50 (mg. per kg.) for protopine hydrochloride by intravenous injection in mice is 35.9 ± 1.90.

Tobitani (34) reported the pharmacological study of the alkaloid domesticine, obtained from Nandina domestica. He compared the actions of domesticine with those of bulbocapine. Both the alkaloids at first inhibit voluntary movement in the rabbit, induce tremors of the whole body and trismus. Convulsions ensue with hyper-secretion of various glands. Large doses cause paralysis from the start. The actions of both alkaloids are closely related. The range of stimulation is narrower for domesticine and its paralysing action stronger. Nantenine, the methyl ether of domesticine acts on the central nervous system producing increased/

increased reflex activity and on cardiac muscle causing bradycardia and weakening of the heart leading to a fall of blood pressure.

Peters (35) studied the pharmacological actions on frogs and mammals of the alkaloids corycavamine, corycavine and corytuberine extracted from Corydalis tuberosa. His results indicated that the first two alkaloids produced narcosis in the frog followed by paralysis of the spinal cord, and in mammals increased secretion of tears and saliva and epileptiform convulsions without increase of reflex irritability. They also adversely affected the heart. Corytuberine did not produce narcosis but induced increased reflex irritability. In warm blooded animals corytuberine gave rise to tonic convulsions and a small increase in reflex irritability. It slowed the pulse by an action on the vagus. The blood pressure rose and the respiratory rate increased during convulsions.

Glaucine (36), an alkaloid obtained from the plant Corydalis ternata nakai caused slight narcosis in animals. This was interrupted by epileptiform convulsions. It was also depressant to the heart and blood vessels and damaged the striated muscles.

Pharmacology of alkaloids obtained from Leontice leontopetalum Linn.

With the isolation of alkaloids from the tubers of Leontice leontopetalum (11) it was reasonable to suppose that the pharmacological activity of the plant lay therein. The/

The pharmacological properties of the alkaloids were thought likely to be similar to those alkaloids obtained from plants of the same or related families. McShefferty and his co-workers (11) made a preliminary pharmacological investigation of the alkaloids petaline chloride and leonticine obtained from the tubers of Leontice leontopetalum. Petaline chloride, 0.05 mg. caused sedation and slowed respiration in 25 g. mice. Larger doses (0.075 mg.) induced respiratory distress and varying degrees of central nervous system stimulation. Doses of 0.08 or 0.1 mg. caused acute death from respiratory failure. It was concluded that the mean lethal dose of petaline chloride in the mouse was approximately 3.1 mg. per kg. In the rabbit a total dose of 30 to 40 mg. of petaline chloride caused respiratory depression, mild clonic convulsions and death. The approximate mean lethal dose was 15.6 mg. per kg. Petaline chloride was also shown to antagonise the stimulant actions of acetylcholine on the isolated frog rectus abdominis muscle. On this preparation it had about 65 per cent of the potency of gallamine triethiodide and its effects were antagonised by eserine. Leonticine, 0.66 mg. caused respiratory and cardiac embarrassment in 25 g. mice. Doses of 0.7 mg. caused respiratory distress, cardiac disturbances and coma, preceded by powerful clonic spasms of the limbs. Doses of 0.8 mg. caused immediate respiratory failure and cardiac arrest. It was concluded that the mean lethal dose in mice was approximately 33 mg. per kg.

Extracts of the tubers of Leontice leontopetalum are undoubtedly/

undoubtedly complex mixtures and probably contain several pharmacologically potent compounds, each of which may possess different types of activity. Results obtained from a study of one compound can naturally not be taken as expressing the whole activity of the plant, but the bizarre manner in which the extracts are made use of by local inhabitants, the reports of confirmed cures of epilepsy (11) and the convulsant activity of the extracts seemed of sufficient interest to warrant a fuller investigation of the properties of petaline chloride.

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C H A P T E R II

A. MATERIALS

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

1. Acetylcholine chloride is described as acetylcholine.
2. Sodium pentobarbitone " " " pentobarbitone.
3. Atropine sulphate " " " atropine.
4. Histamine acid phosphate " " " histamine.
5. 5-Hydroxytryptamine creatinine sulphate " " " 5-hydroxytryptamine.
6. Tubocurarine chloride " " " tubocurarine.
7. Decamethonium iodide " " " decamethonium.
8. Neostigmine methyl sulphate " " " neostigmine.
9. Gallamine triethiodide " " " gallamine.
10. Adrenaline bitartrate " " " adrenaline.
11. Noradrenaline bitartrate" " " noradrenaline.

The compositions and methods of preparation of all physiological saline solutions used in this investigation are to be found in Appendix II, page 393.

Petaline chloride was obtained in aqueous solution.

C H A P T E R II

B. EXPERIMENTAL

1. Convulsant:Anticonvulsant Activity

(A) Comparison of the convulsive reactions and mortality induced by petaline chloride, leptazol and picrotoxin.

METHOD

The method used was based on that of Goodman and his co-workers (1). Male albino mice weighing 20 ± 1 g., or 25 ± 1 g., were used as experimental animals. They were allowed free access to food and water except during the actual experimental period. To restrict the mouse, a small brass cylinder, one end of which was provided with a centrally perforated lid to allow exposure of the tail, was used. The other end of the cylinder was covered with a wire mesh to ensure adequate ventilation. The cylinder was fixed firmly to a stand in the horizontal position. The mouse was put into the cylinder and the tail taken out through the hole in the lid. The dorsal tail vein was made prominent by application of xylene. Drug solutions were injected into the dorsal tail vein by means of a number 22 needle from a graduated 1 ml. tuberculin syringe. The injections were given rapidly. The duration of injection did not exceed two to three seconds. Immediately after injection the animal was taken out of the cylinder and placed upon a big metal tray and/

and the drug effects noted.

The experiment was started by injecting the smallest dose of the drug solution (petaline chloride, leptazol or picrotoxin) that was likely to produce convulsive reaction. The dose of the drug was gradually increased until 100 per cent convulsive seizures and 100 per cent mortality were obtained. Each mouse was given a single injection and no mouse was used for the second time. In order to ensure reactions of uniform character, the volume of drug solution injected into the mice at any particular dose level was always kept constant. A minimum of 10 mice were injected at any dose level. The convulsive dose for 50 per cent of the animals (CD50) and the lethal dose for 50 per cent of the animals (LD50) were worked out by the conventional method for petaline chloride, for leptazol or for picrotoxin by plotting on graph paper the dose of the drug (in mg. per kg. body weight) as the abscissa and the percentage reaction (convulsive seizure or death) as the ordinate.

(B) Effect of pre-treatment with petaline chloride on the response to leptazol

METHOD

Three groups, each containing not less than 50 male albino mice were used. The weights of the individuals in each group were constant at 20 $\pm$ 1 g., or 25 $\pm$ 1 g. The groups were given respectively 2.5, 5.0 or 8.0 mg. per kg. of petaline chloride by intravenous injection. At the first dose/

dose level no animal showed any convulsive reaction. At the second dose level some animals exhibited convulsive seizures. At the third dose level, a number of animals exhibited convulsive seizures and some of them died. After an interval of 45 to 60 minutes the members of each large group were divided into four groups of about ten in each and each group was given leptazol by intravenous injection. The doses of leptazol used were from 20 to 75 mg. per kg. The CD50 and LD50 were calculated for the pre-treated animals.

(C) Comparison of the effects of petaline chloride and phenobarbitone on electro-shock seizures.

METHOD

The method used was based on that of Swinyard (2) but ear clip electrodes (3) were employed instead of corneal electrodes (4). Seven groups of 10 male albino mice were used, the weights of the members of each group were constant at 10 ± 1 g., 15 ± 1 g., or 20 ± 1 g. The animals were allowed free access to food and water except during the actual experimental period. The mouse was held on its back, the electrodes clipped on to the ears and was then put into a perspex box of dimensions14" x 6" x 6" and the lid closed. The box was provided with holes on all of its sides to ensure adequate ventilation. The current was applied by pressing a switch. A supra-threshold current intensity of 20 mA. applied for five seconds was used to cause tonic extension of the hind limbs, the appearance of which was taken as the end-point. All animals to be used for the tests were treated/

treated with 20 mA. for five seconds, twenty-four hours before the experiment, and those which did not give the end-point were discarded. Four groups of 10 mice were pre-treated with 1.66, 2.5, 3.3. or 5.0 mg. per kg. of petaline chloride by intraperitoneal injection. The remaining three groups were given 4.0, 5.0 or 6.6 mg. per kg. of phenobarbitone by the same route. After an interval of one hour, electro-shock treatment was repeated. The number of mice in each group failing to show the end-point was counted and the percentage protection calculated.

2. Muscle Relaxant Activity

(A) Cat gastrocnemius muscle-sciatic nerve preparation.

METHOD

The method adopted was that described by Lewis and Muir (5). Cats of either sex weighing from 2.0 to 3.0 kg. were used as experimental animals. They were anaesthetised by an intraperitoneal injection of pentobarbitone. The commercial solution containing 60 mg. per ml. pentobarbitone (Nembutal-Abbott) was employed. A dose of 60 mg. per kg. of cat was usually adequate for the production of surgical anaesthesia within ten to fifteen minutes.

The anaesthetised cat was laid upon a warmed operating table. The legs were secured by means of string ties, and the head was extended by passing a 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/

The fascia covering the trachea was divided in the mid-line and the muscles pushed aside. A pair of blunt, curved forceps was passed under the trachea. A strong linen thread was passed round the trachea and a transverse cut made in the latter by means of a scalpel. The cut edge of the partly severed trachea was held by means of a pair of forceps and a tracheal cannula inserted. The cannula was tied securely in place. Cannulation of the trachea was a precautionary measure in case it became necessary (due to drug-induced respiratory depression or failure) to keep the animal alive under artificial respiration. The amount of air entering and leaving the cannula could be controlled by means of an adjustable sleeve.

The external jugular vein (usually of the left side) was then cannulated. To do this, the skin of the left anterolateral part of the neck was carefully stretched with a blunt dissector, and the left external jugular vein exposed. The fascia covering the vein was very carefully removed. A thread was tied around the cephalic end and a bulldog clip applied to the cardiac side of the vein. A small transverse cut was made in the dilated vein by means of a pair of sharp pointed iris scissors. A vein cannula filled with heparin was then inserted through the cut with the pointed end towards the heart. The cannula was connected by means of rubber tubing to a 50 ml. burette containing normal saline, all air bubbles being previously removed from the system. The bulldog clip was then removed. When the saline in the burette ran freely into the vein it indicated that/

that the cannula had been correctly inserted.

After completing the cannulation of the trachea and the external jugular vein, one of the legs, usually the left one, was prepared for indirect stimulation of the gastrocnemius muscle via the sciatic nerve. The gastrocnemius muscle was partially dissected free from the surrounding tissues and the Achilles tendon severed at a point near to its insertion into the calcaneus. A strong linen thread was tied round the free end of the tendon. The limb was held with its long axis perpendicular to the operating table and fixed rigidly by means of two clamps, one at the knee joint and the other at the ankle. 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.

The skin on the outer surface of the thigh was removed. The hamstring muscles were separated by a blunt dissector and the sciatic nerve exposed. A pair of shielded platinum electrodes were placed round the nerve and the latter stimulated using single shocks from a Dobbie McInnes square wave generator at a frequency of 8 to 10 per minute, 15 to 20 volts, pulse width 3 to 4 msec. In any given experiment frequency, voltage and pulse width were constant. In some experiments, however, a tetanizing current of 1,500 square impulses per minute was applied, voltage and pulse width remaining constant at the values stated.

Drugs in aqueous solution were injected into the rubber tubing/

tubing between the cannula in the external jugular vein and the burette by means of a graduated 1 ml. tuberculin syringe. The drugs were washed in with 3 ml. or 4 ml. of normal saline from the burette.

(B) Rat phrenic nerve-diaphragm preparation.

(i) Using Bell's electrode.

METHOD

The method adopted was based on that of Bülbring (6). The electrode described by Bell (7) was used so that the muscle could be stimulated both directly and indirectly.

The electrode described by Bell consists of two perspex supports. The diaphragm support consists of an L-shaped piece of perspex to which is fixed at right angles a second, smaller L-shaped perspex rod carrying electrodes for stimulation of the nerve. The nerve stimulating electrode itself consists of two parallel platinum wires which run across the bottom of a groove cut in the short limb of the L, and are connected to two terminals on the upper end of the perspex rod. The whole assembly can be moved up or down by means of the clamp attached to it. The short horizontal limb of the diaphragm support to which the costal margin of the preparation is tied, carries a platinum wire which acts as one of the electrodes for direct stimulation of the muscle. This wire is connected to a terminal near the top of the diaphragm support. The tendon of the preparation is tied to a platinum wire fixed to the arm of a light isotonic heart lever which records/

records the contractions. This wire both transmits the pull of the muscle and acts as the second electrode for direct stimulation of the muscle, the current passing along the diaphragm between the ribs and the tendon.

To make the preparation, adult rats of either sex were killed by a blow on the head. The throat was cut and the blood allowed to drain out. After this the animal was placed on its back and pinned upon a cork covered dissecting board. The skin over the chest wall on both sides was removed. The abdomen was opened, a small incision made through the diaphragm from the abdominal side at the point of its attachment to the ribs and close to one side of the sternum. One blade of a pair of scissors was inserted through the incision and all the ribs were cut through close to the sternum. This procedure was repeated on the other side of the sternum. The sternum was then carefully dissected out from the mediastinal tissue and removed. A straight cut was then made across the ribs on the right side parallel to the diaphragmatic attachment, starting between the fifth and sixth ribs. All the ribs above this cut were removed. A second transverse cut was made along a curved line just below the attachment of the diaphragm to the chest wall. The right phrenic nerve was identified as it entered the diaphragm. The nerve was very gently pulled medially and the lobes of the right lung cut off near the hilum and then 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, beginning close to the diaphragm and working/

working upwards in the neck. When the nerve had been freed as high in the neck as possible a ligature was tied round it. The nerve was cut above the ligature. A length of about 4 to 5 cm. was usually obtained. After this, two ligatures were inserted through the costal segment which was attached to the diaphragm and lengths of threads were left with the ligatures. The ligatures were about 2 cm. to 2.5 cm. apart with the point of entry of the phrenic nerve lying midway between them. The ligatures were pulled gently to stretch the diaphragm and to observe the direction in which the muscle fibres ran. Two cuts were made parallel to the muscle fibres commencing just outside the costal ligatures, through the ribs and diaphragm, 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 dish filled with Tyrode's solution. A similar preparation could also be made from the left side of the animal. The right sided preparation, however, is 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 fitted 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. A platinum wire was 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 surface. The phrenic nerve was passed into the groove described before so as to lie/

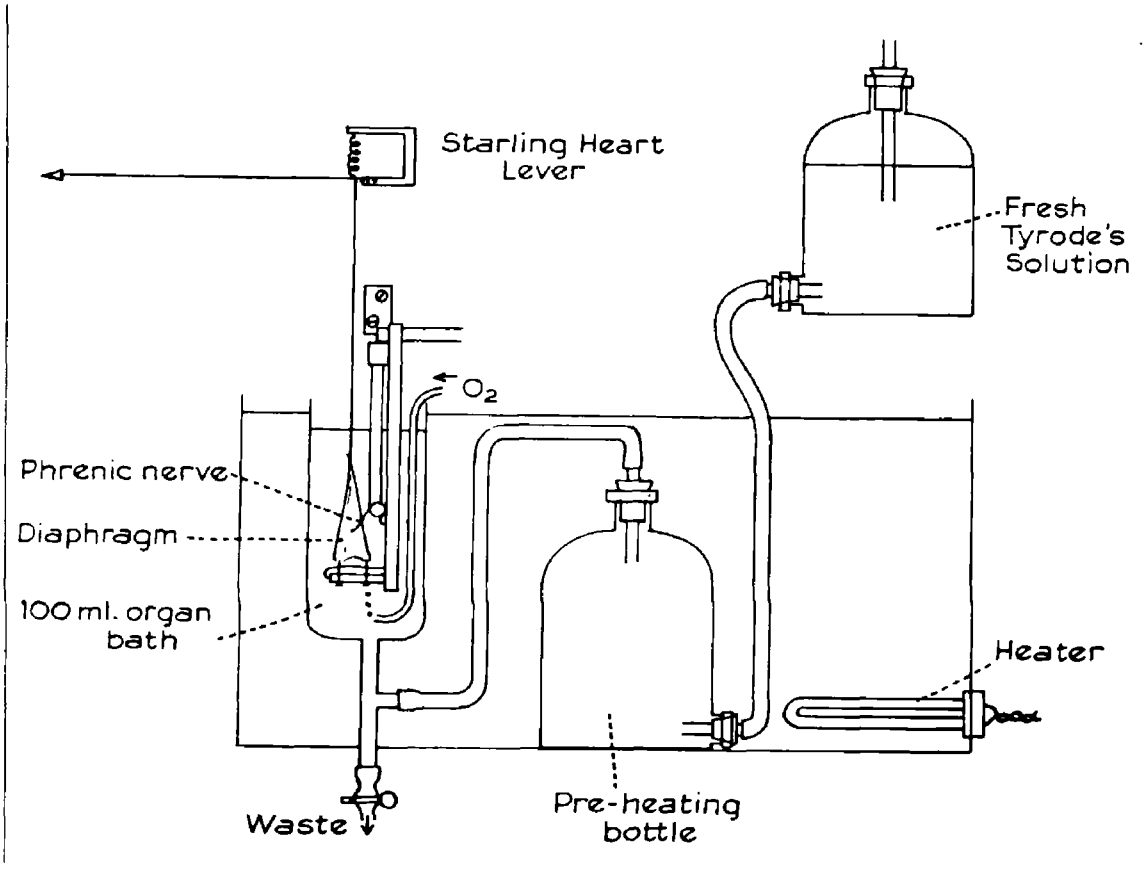

Fig. 37

Diagram of the apparatus used for recording contractions of the rat diaphragm produced by either direct or indirect stimulation of the muscle using Bell's electrode.

lie across the two poles of the electrode. The end of the phrenic nerve was fixed between the electrode holder and the diaphragm support to keep the nerve firmly in place.

The whole assembly was lowered into a 100 ml. organ bath filled with Tyrode's solution containing double the usual amount of glucose. The solution in the organ bath was aerated with pure oxygen or 95 per cent oxygen and 5 per cent carbon dioxide through a gas distribution tube fitted to the bottom of the organ bath. Tyrode's solution was passed from the reservoir to the organ bath through a bottle which was kept in a heated water bath. The temperature of the water bath was maintained thermostatically at 29°C. Fig.37 (page 264) shows the apparatus used in this experiment. Drugs in aqueous solution were added directly to the bath by means of a graduated 1 ml. tuberculin syringe.

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, a frequency of 6 to 8 stimuli per minute, the voltage 8 to 10, pulse width 0.5 to 1 msec., and for direct stimulation the frequency was 6 to 8, voltage 25 to 50 and pulse width 2 msec. In any particular experiment the frequency, voltage and pulse width both for indirect and direct stimulation were kept constant.

At the beginning of the experiment the muscle was stimulated indirectly via the phrenic nerve. After the addition of the drug the contractions of the muscle became gradually/

gradually smaller and smaller and finally stopped. When the response to nerve stimulation failed the muscle was stimulated directly by switching on the stimulator connected to the muscle. After recording a few contractions following direct stimulation of the muscle the stimulator was switched off. The fluid in the organ bath was changed several times and the muscle allowed to recover. The process was repeated again until the response of the muscle became normal.

(ii) Using Collison's fluid electrode.

METHOD

The procedure adopted was essentially that described by Bülbring (6). Adult rats of either sex were killed by a blow on the head. The throats were cut and the blood allowed to drain out. The rat was then laid on its back upon a cork-covered dissecting board to which it was pinned. The skin over the chest was removed and the thorax opened along the right side of the sternum. The frontal part of the right thoracic wall was removed. The mediastinum behind the sternum was severed and a cut was made just above the frontal insertion of the diaphragm. Care was taken not to damage the phrenic nerve which is sometimes attached to the ribs. The frontal part of the left thoracic wall was then removed and the left phrenic nerve exposed. Both lobes of the left lung were removed and the left phrenic nerve carefully freed from fat and other tissues, the utmost care being taken not to injure it. The left abdominal muscles were cut along the costal margin. Holding the last rib by means of a pair of forceps, a segment of the diaphragm was then dissected out. Two converging/

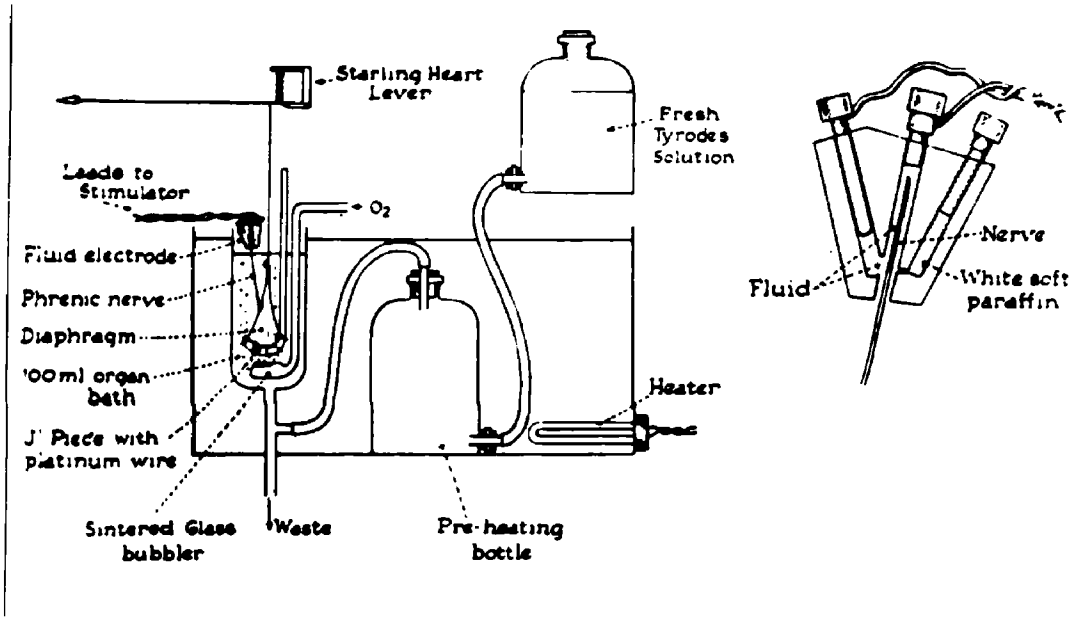

Fig. 38

Diagram of the apparatus used for recording contractions of the rat diaphragm produced by electrical stimulation of the phrenic nerve using Collison's fluid electrode (see inset).

converging cuts were made through the ribs towards the tendinous part of the diaphragm and parallel to the direction of its muscle fibres. The strip of diaphragm was dissected out beyond its tendinous part with about 5 cm. of the phrenic nerve attached to it. The final preparation was fan shaped, about 2 mm. wide at the tendinous end and about 20 mm. wide at the costal margin. The costal margin of the diaphragm segment was fixed to a J-shaped glass rod by means of platinum wires fixed to the concavity of the glass rod. A thread was tied around the tendinous end. The preparation was then set up in 100 ml. organ bath containing double glucose Tyrode's solution. The J-piece held the costal margin of the segment in position at the bottom of the organ bath, while the thread tied to the tendinous end was fixed to a light isotonic heart lever writing upon a moving smoked surface. Tyrode's solution containing double the usual amount of glucose was supplied to the organ bath through a bottle which was placed in a heated water bath while the flow was controlled by means of a spring clip. The temperature of the water bath was maintained at 29°C. A suitable glass gas distribution tube was fixed at the bottom of the bath to provide a vigorous supply of pure oxygen or 95 per cent oxygen and 5 per cent carbon dioxide, with which the bath fluid was aerated in the form of fine bubbles. A thread was tied to the cut end of the phrenic nerve and by means of a fine needle about 1 cm. of the phrenic nerve was drawn into a Collison's fluid electrode (8). The fluid electrode containing the nerve was filled with double glucose Tyrode's solution and the hole at the/

the bottom of the electrode sealed with soft paraffin. By this arrangement the fluid surrounding the nerve at the point of stimulation was not in electrical contact with the fluid surrounding the muscle in the organ bath. The nerve was left sufficiently slack to allow for any movement caused by the contraction of the diaphragm.

Single square wave impulses were applied to the nerve by means of a Dobbie McInnes square wave generator at a frequency of 6 to 10 per minute, voltage 8 to 10 and pulse width 0.5 to 1.0 msec. In any particular experiment the frequency, voltage and pulse width were kept constant. Fig. 38 (page 267) is a diagram of the apparatus used in this experiment.

Drug solutions were added to the organ bath by means of a graduated 1 ml. tuberculin syringe. The drug was allowed to act for three minutes, after which the Tyrode's solution in the organ bath was changed. Between the addition of one dose of the drug and the addition of the next there was an interval of fifteen minutes during which the Tyrode's solution was changed several times.

West (9) observed that the preparation gave a constant response of good magnitude for a long period if the temperature of the bath was lowered from 37°C. to 20°C. He also observed that the rate of recovery of the muscle after the addition of a dose of tubocurarine was greatly accelerated by adding potassium chloride to the organ bath after the tubocurarine had been washed out. In the present series of experiments it was observed that lowering the bath/

bath temperature from 37°C. to 29°C. without the addition of potassium chloride gave a satisfactory recovery and that the magnitude of the muscle contraction was maintained in a satisfactory manner.

Chou (10) using the rat phrenic nerve diaphragm preparation for the assay of curare-like substances, allowed the drug to act upon the muscle for three minutes. In the experiment described here this feature of Chou's technique was adopted because it was thought that if the drug was allowed to act upon the muscle for long enough to produce its maximal effect, then so much time would be needed to wash the drug off the receptors that few comparisons could be made upon one preparation.

(C) Isolated frog rectus abdominis muscle preparation.

METHOD

The method adopted was based on that of de Jalon (11). The muscle was prepared as described by Burn (12). An adult frog was stunned by a blow on the head. It was decapitated and pithed and then laid on its back upon a cork covered dissecting board to which it was pinned. The rectus abdominis muscle was exposed by cutting away the skin of the abdomen. The muscle was then dissected from the point of its insertion into the pelvic girdle to its insertion into the cartilage of the pectoral girdle, freed from the underlying connective tissue and removed from the frog. It was then suspended in a 10 ml. or 20 ml. organ bath by means of two threads/

threads, one 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 platinum wire at the bottom of the oxygen delivery tube. The long thread on the other end was tied to a frontal point writing lever which recorded the contractions of the muscle on a moving smoked surface. The bath contained oxygenated Frog Ringer's solution at room temperature. Fig. 39 (page 272) is a diagram of the apparatus used in this experiment.

Acetylcholine (0.10 to 1.0 μg/ml.) was added directly to the bath by means of a graduated 1 ml. tuberculin syringe and left in contact with the muscle for 90 seconds, the contraction being recorded. Uniform submaximal contractions to the same dose of acetylcholine were obtained before drug effects were studied. Drugs in aqueous solution were added to the bath 30 seconds before a subsequent addition of acetylcholine and the contraction recorded for 90 seconds. In between recording of contractions the muscle was washed four or five times. There was an interval of 7 minutes between successive additions of acetylcholine.

3. Ganglion Blocking Activity

Experiments on the nictitating membrane of the anaesthetised cat.

METHOD

Cats of either sex weighing from 2.0 to 3.0 kg. were anaesthetised with 60 mg. per kg. of body weight of pentobarbitone/

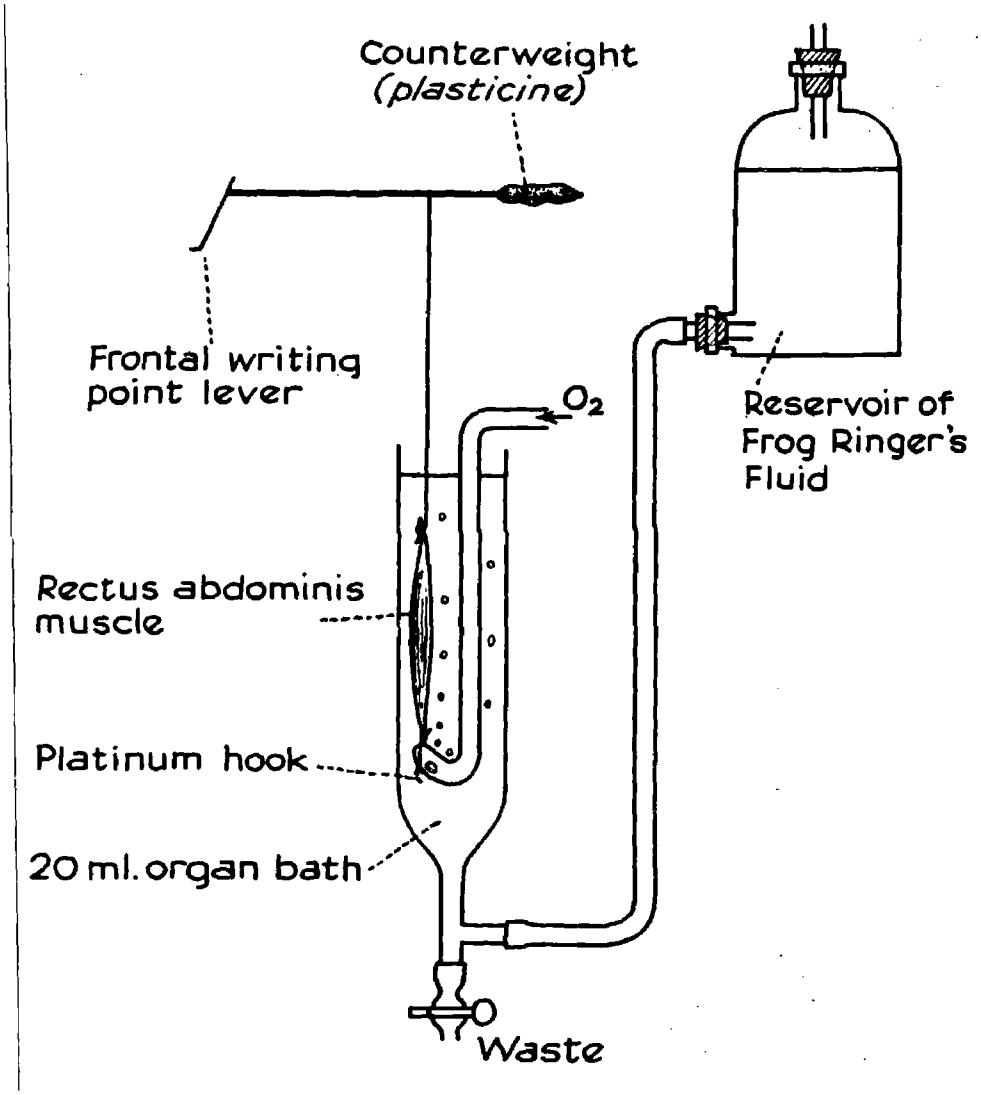

Fig. 39

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

pentobarbitone injected intraperitoneally. The trachea and the left external jugular vein were cannulated in a manner similar to that described before (page 259).

The head of the cat was rigidly fixed by passing a brass rod between the jaws and then tying the jaws firmly together with string. The ends of the rod were gripped firmly in clamps and the latter were supported on uprights fixed to the side of the operating table.

The right cervical sympathetic trunk in the neck was very carefully dissected and separated out from the vagus nerve and the common carotid artery. A fine cotton thread was tied tightly around the cervical sympathetic trunk at as low a point as possible in the neck. The chain was then severed below the ligature and low in the neck. The cut preganglionic cervical sympathetic chain was placed upon a pair of shielded platinum electrodes and kept moist by means of a piece of cotton wool soaked in normal saline.

Using a fine needle, a thin 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 then pulled forward and to one side, making an angle of about 30° to the long axis of the cat. It was then led around pulleys and attached to a frontal point writing lever. The contractions of the nictitating membrane were recorded on a moving smoked surface.

Contractions of the nictitating membrane were elicited by stimulation of the cut end of the preganglionic cervical sympathetic/

sympathetic chain by means of square impulses from a Dobbie McInnes square wave generator at a frequency of 1,000 to 1,200 per minute, 10 to 12 volts, and a pulse width of 0.5 to 1.0 msec. In any particular experiment the frequency, voltage and pulse width were kept constant. Fifteen second bursts were applied at intervals of three minutes.

At the beginning of the experiment a few normal contractions were taken until standard reproducible responses were obtained.

Drug solutions were injected into the left external jugular vein one minute prior to the next period of stimulation.

4. Experiments on Spinal Reflexes

(A) Patellar tendon reflex

METHOD

Cats of either sex weighing from 2.0 to 3.0 kg. were used. Spinalization was carried out under ether anaesthesia as described by Burn (13). Atropine (1.0 mg. per kg.) was administered by intraperitoneal injection and after about 15 minutes the cat was anaesthetised with ether.

The anaesthetised cat was then tied to the warmed operating table and the head extended by passing a thread through the skin at the apex of the lower jaw. The trachea was cannulated in a manner similar to that described before (page 259). The tracheal cannula was connected by means of/

of rubber tubing to a bottle containing ether which could be connected rapidly and easily to an artificial respiration pump. The common carotid arteries on both sides were freed from the accompanying vago-sympathetic trunks and tied as high up in the neck as possible. This was done to reduce the haemorrhage during the operation on the cat. The cat was then turned over on its belly and the legs secured by strings. The head of the cat was, however, kept free. A wooden block was put under the head and the head flexed gently downwards. The fur covering the dorsal neck region was clipped away. The skin was divided in the mid-line by a scalpel. The muscles were rapidly dissected away by a blunt dissector until the bony spine of the second cervical vertebra was exposed. Using a pair of bone forceps the layer of bone covering the spinal cord was nibbled away. Finally, the spinal cord was transected by a sharp scalpel. As soon as the transection was complete artificial respiration was started and ether removed. The brain was destroyed by inserting a blunt probe through the foramen magnum and pushing this up into the brain. Bleeding was arrested by means of cotton wool swabs soaked in hot, normal saline. The cut portion of the spinal canal was plugged with plasticine and the area swabbed clear. The skin at the back of the neck was sewn together with linen thread and the cat turned on its back again. The limbs were secured with strings. One of the external jugular veins was then cannulated in a manner similar to that described before (page 259).

The method adopted for eliciting the patellar tendon reflex/

reflex was based on that described by Schweitzer and Wright (14). One of the hind limbs, usually the left one, was semiflexed at the hip and supported by putting a brass rod under the knee joint. The leg was kept hanging. The fur over the area of the patellar tendon was clipped off. The patellar tendon was tapped mechanically at intervals of 5 seconds or 10 seconds by means of an electrically operated automatic hammer. Alternatively, the patellar tendon was stimulated electrically. To do this, a small incision was made by means of a scalpel to one side of the patellar tendon, and a pair of shielded platinum electrodes were placed under the tendon. Electrical stimulation was applied as single square impulses from a Dobbie McInnes square wave generator at a frequency of 10 or 15 per minute, 25 to 50 volts, pulse width 3 to 4 msec. In any given experiment the frequency, voltage and pulse width were constant.

A thread was tied to the lower end of the tibia and passed over pulleys to a myograph lever, the writing point of which was so adjusted as to record the patellar tendon reflex on a moving smoked surface.

Drugs in aqueous solution were administered by injecting into the rubber tubing between the cannula in the external jugular vein and the burette by means of a graduated 1 ml. tuberculin syringe. These were washed in with 3 ml. or 4 ml. of normal saline.

At least one hour was allowed to elapse before any drug was administered.

(B)/

(B) Crossed extension reflex

METHOD

Cats of either sex weighing between 2.0 and 3.0 kg. were used. Spinalization under ether anaesthesia was carried out as described before (page 275). Cannulation of the trachea and one of the external jugular veins was done in a manner similar to that described before (page 259). The method adopted for eliciting the crossed extension reflex was based on that described by Liddell and Sherrington (15).

The cat was laid flat on its back upon the warmed operating table. The legs were secured by means of strings. One of the hind limbs, usually the left one, was semiflexed at the hip and supported by putting a brass rod under the knee joint. It was fixed in position by putting two screws on the two sides of the knee joint. The skin over the patellar tendon was removed and the tendon detached from the tibial tubercle. The patella was freed from the articular attachments. A strong linen thread was tied to the detached patellar tendon and passed over pulleys to a myograph lever, the writing point of which was adjusted so as to record the crossed extension reflex over a moving smoked surface.

The skin on the outer surface of the opposite thigh was removed. The hamstring muscles were separated by a blunt dissector and the sciatic nerve exposed. The nerve was cut or crushed as low in the thigh as possible. The central end of the cut or crushed nerve was placed over a pair of shielded platinum electrodes and stimulated by square impulses from/

from a Dobbie McInnes square wave generator at a frequency of 10 to 15 per minute, voltage 25 to 50, and pulse width 3.0 to 4.0 msec. In any particular experiment, the frequency, voltage and pulse width were constant. On stimulation of the central end of the cut or crushed sciatic nerve, there was reflex contraction of the extensor muscles of the opposite side.

Drugs in aqueous solution were administered by injection into the rubber tubing between the external jugular vein cannula and the burette. A graduated 1 ml. tuberculin syringe was used. The drug solutions were washed in with 3 ml. or 4 ml. of normal saline from the burette.

At least one hour was allowed to elapse before any drug was administered.

5. Cardiovascular Actions

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

METHOD

Cats of either sex weighing between 2.0 and 3.0 kg. were used. Anaesthesia was induced by intraperitoneal injection of 60 mg. per kg. of pentobarbitone. In some cats chloralose was used as the anaesthetic. A 1 per cent solution of chloralose in distilled water was made. When this solution was used a dose of 8 ml. per kg. (80 mg. per kg.) was usually sufficient to produce surgical anaesthesia in about half an hour.

The/

The anaesthetised cat was laid on its back upon a warmed operating table. The legs were secured by means of strings. The trachea and the left external jugular vein were cannulated in a manner similar to that described before (page 259).

After the cannulation of the trachea and the external jugular vein, an artery cannula was inserted into one of the common carotid arteries, usually the right one. The artery was first separated from the accompanying vago-sympathetic trunk and tied off as high up in the neck as possible. A bulldog clip was then put on to the artery about 3 cm. below the ligature. A thread was placed loosely under the artery and a small transverse cut made in the latter below the ligature by means of a pair of sharp pointed iris scissors. An artery cannula filled with heparin was then inserted through the cut with the pointed end towards the heart. The cannula was connected to a mercury manometer by means of rubber tubing, the space between the mercury and the artery being filled with 25 per cent $^{W}/_{V}$ solution of sodium thiosulphate as an anticoagulant. The air in the system was displaced by pumping the sodium thiosulphate solution through the cannula. After this, the bulldog clip was removed. A writing flag on one arm of the mercury manometer recorded the blood pressure on a moving smoked surface.

Drugs in aqueous solution were injected by means of a graduated 1 ml. tuberculin syringe into the rubber tubing between the cannula in the external jugular vein and the burette/

burette of normal saline. These were washed in with 3 ml. or 4 ml. of normal saline.

B. Experiments on the blood pressure of the spinal cat.

METHOD

Cats of either sex within the weight range of 2.0 to 3.0 kg. were used. Spinalization was carried out as described before (page 275). The left external jugular vein was cannulated and connected by rubber tubing to a burette filled with normal saline. The right common carotid artery was cannulated and connected to a conventional pressure recording system filled with 25 per cent $^{W}/_{V}$ sodium thiosulphate solution as an anticoagulant. A mercury manometer with a writing flag on one arm recorded the blood pressure on a moving smoked surface.

Drugs in aqueous solution were injected by means of a graduated 1 ml. tuberculin syringe into the rubber tubing between the vein cannula and the burette. These were washed in with 3 ml. or 4 ml. of normal saline from the burette.

At least one hour was allowed to elapse before any drug was administered.

C. Experiments on the blood pressure of the anaesthetised rat.

METHOD

The procedure adopted was essentially that described by Dekanski/

Dekanski (16). Albino rats of either sex weighing about 300 g. were used as experimental animals. Anaesthesia was induced by urethane (175 mg. per 100 g. body weight) injected subcutaneously.

After 45 to 60 minutes the rat was laid on its back upon an operating board and the limbs were secured by means of strings. The skin covering the middle of the neck was cut away by a pair of scissors. The skin on the left anterolateral side of the neck was carefully stretched with the help of a blunt dissector and the left external jugular vein was exposed. The fascia covering the vein was carefully removed. A thread was tied to the cephalic end of the vein and a small bulldog clip was put on the cardiac end. A small transverse cut was made in the dilated vein with the help of a pair of sharp pointed iris scissors. A thin polythene cannula filled with heparin and fitted with a small piece of rubber tubing, the open end of which was closed by a bulldog clip, was inserted through the cut with the pointed end towards the heart. The cannula was fixed to the vein by tying a thread around it. The right common carotid artery was then freed from the surrounding structures and tied off as high up in the neck as possible. A small bulldog clip was placed on the artery a little below the ligature and a small transverse cut was made in the artery by means of a pair of sharp pointed iris scissors. A thin polythene cannula filled with heparin was then inserted through the cut with the pointed end towards the heart. The cannula was fixed to the artery by tying a thread around it. The/

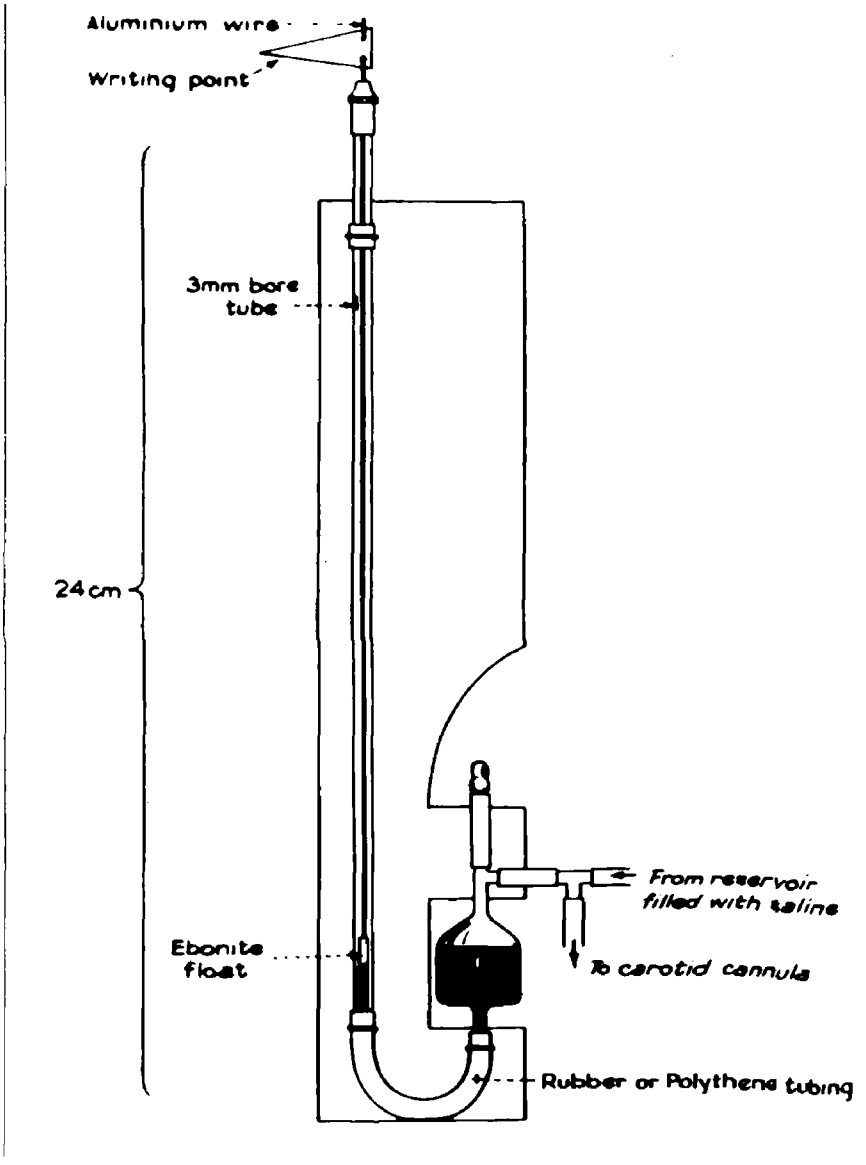

Fig. 40

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

The cannula was then connected by means of rubber tubing to a mercury manometer of the type described by Condon (17), the space between the artery and the mercury being filled with normal saline. Fig. 40 (page 282) shows the diagram of the manometer used in this experiment. Before making the connection extreme care was taken to exclude all air bubbles from the system. The bulldog clip was removed and the blood pressure was recorded upon a smoked surface by a writing flag on one arm of the mercury manometer. In some rats the trachea was freed from the surrounding muscles and tissues and a small transverse cut was made in it by means of a pair of sharp scissors. The partly severed trachea was then held by a blunt pair of forceps and a polythene cannula introduced into the trachea and tied to it by a thread. This acted as a tracheal cannula and eased respiration. Artificial respiration was not used.

Drugs in aqueous solution were administered by injecting by means of a 1 ml. graduated tuberculin syringe into the rubber tubing attached to the cannula in the left external jugular vein and washed in with 0.2 to 0.3 ml. of normal saline from another tuberculin syringe. Extreme care was taken to exclude the air from being injected. The volume of the drug solution injected was kept constant at 0.1 ml.

D. Experiments on the isolated perfused rat hindquarters.

METHOD

In these experiments the pressure at which the physiological fluid passed through the blood vessels was kept/

kept constant and alterations in the rate of outflow of the perfusion fluid which were produced by drugs, were recorded by Stephenson's outflow recorder (18). The vessels were perfused with oxygenated Locke's solution at room temperature.

The method adopted was that described by Burn (19). Rats of either sex were killed by a blow on the head. The throats were cut and the blood allowed to drain out. The rat was then placed on its back upon a cork covered dissecting board. The abdominal cavity was opened by an incision from the sternum to the anus. The rectum, the oesophagus, the superior mesenteric artery and the inferior mesenteric artery were divided between ligatures. The abdominal viscera were then removed. This brought into view the abdominal aorta which was separated from the surrounding structures and tissues and cannulated using a polythene cannula. The body wall and the vertebral column of the rat were then transected above the point of cannulation. The cannula was attached to the perfusion system by means of thin rubber tubing and the hindquarters preparation placed upon a gauze rest within a filter funnel. The outflow from the preparation was led via the funnel to the Stephenson's recorder, which in turn was connected with a float recorder. Changes in the outflow were recorded on a moving smoked surface. Fig. 41 (page 285) shows a diagram of the apparatus used in this experiment.

After setting up the preparation, a uniform outflow record was obtained for at least 15 minutes before drugs were administered. Drugs in aqueous solution were administered/

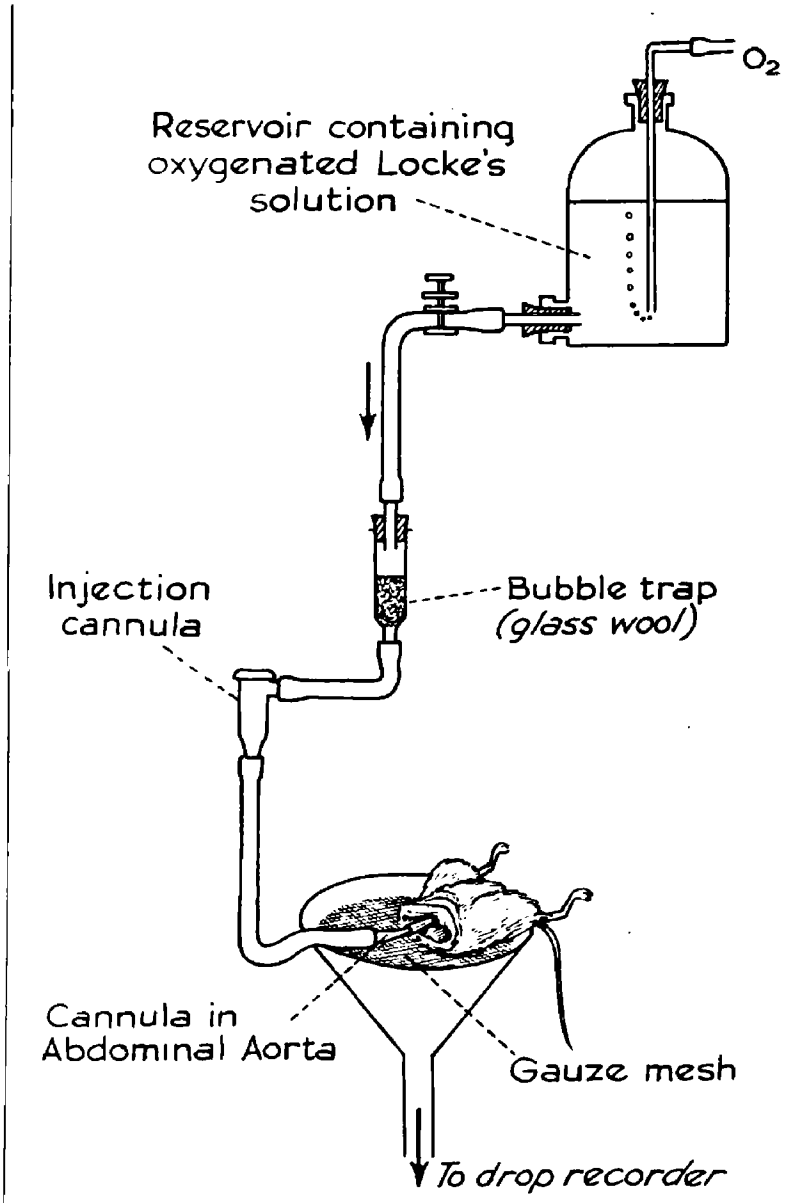

Fig. 41

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

administered by injecting by means of a graduated 1 ml. tuberculin syringe very slowly into the rubber tubing nearest to the cannula.

E. Experiments on the isolated perfused rabbit and kitten hearts.

METHOD

The isolated hearts of both rabbits and kittens were perfused according to the method of Langendorff (20) as described by Burn (21). Wegria (22) in his review on the pharmacology of the coronary circulation made several criticisms of this method. It was 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, which 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 atrium via the ventricle is not constant and in addition cannot be measured satisfactorily. It has also been pointed out that the volume of coronary perfusate may be increased by a purely mechanical massaging effect which cardiac muscle, stimulated by a cardiotonic drug, has upon the coronary vessels. Under these circumstances an increase in outflow might be taken to indicate a coronary dilatation which in fact was not present. For these reasons it/

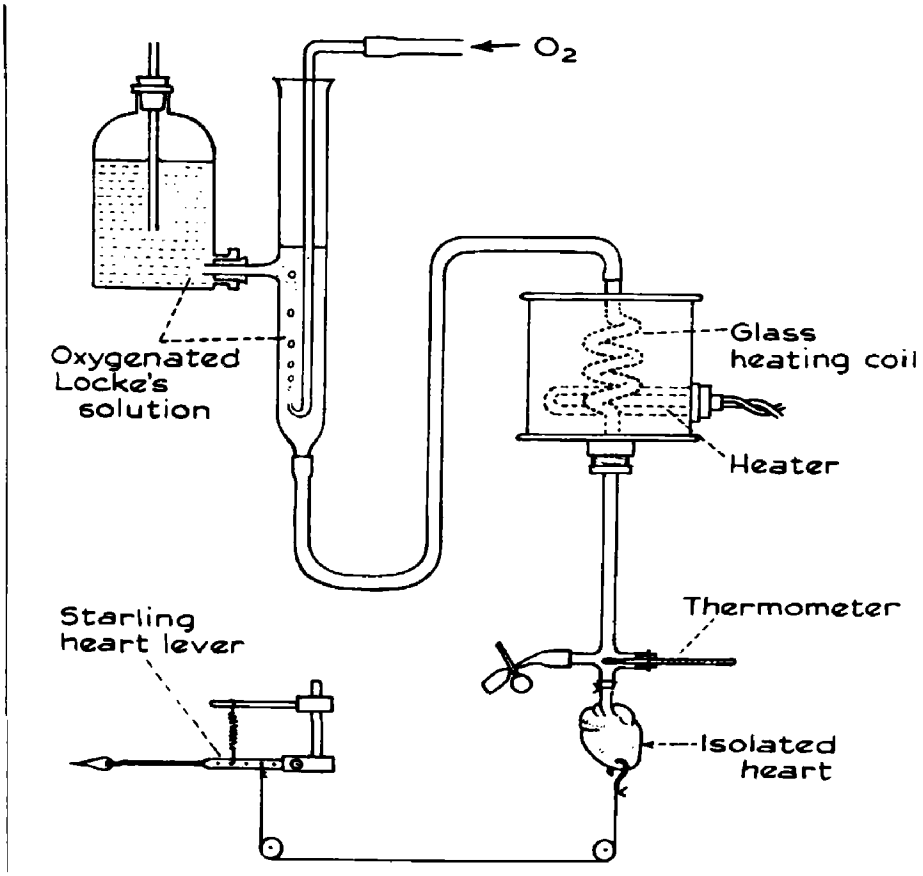

Fig. 42

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

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 still gives useful information about the effects of the drug on cardiac function in vitro. By carefully observing the heart rate, the amplitude of contractions and at the same time measuring the outflow, an estimation of the alterations of cardiac function as well as of the tonus of the coronary vessels can be obtained.

Rabbits and kittens used were within the weight ranges of 1.0 to 2.0 kg. and 0.6 to 1.0 kg. respectively. The animal was killed by a blow on the head. The throat was cut and the blood allowed to drain out. The animal was then placed upon its back on a cork covered dissecting board and the thoracic cavity opened. Great care was taken not to damage the heart with scissors or other instruments. The lungs were separated and the pericardium opened. The heart along with the aorta, was then taken out of the animal and placed in a dish of warmed, oxygenated and heparinised Locke's solution. The temperature of the solution was about 37°C. The heart was squeezed very gently to expel the blood from inside and after this a cannula was tied into the aorta, taking care that its tip was distal to the opening of the coronary artery. The preparation was then set up by connecting the cannula to the perfusion apparatus. Perfusion of Locke's solution containing double the usual amount of glucose, was started at a constant rate of flow, care being taken that no air bubblesentered the aorta. Fig. 42(page 287) shows/

shows a diagram of the apparatus used in this experiment. Any blood remaining in the preparation was rapidly washed away and as a rule the heart started to beat immediately. After about 30 minutes when the contractions became regular a bent entomological pin was inserted into the tip of the ventricle and was connected by a thread over pulleys to a Starling's type heart lever which recorded the contractions of the heart on a moving smoked surface. Doubling the normal amount of glucose in the perfusion fluid gave a more active preparation which did not fatigue so easily. The Locke's solution from the reservoir passed through a spiral glass tubing placed in a heated water bath, the temperature of which was maintained thermostatically at 37°C.

Drugs in aqueous solution were administered by injecting into the perfusion cannula by means of a graduated 1 ml. tuberculin syringe. The heart rate was counted by inspection of the tracing or by direct observation. The cardiac outflow was measured by Stephenson's outflow recorder (18).

In some experiments petaline chloride was dissolved in Locke's solution to give a concentration of 200 μg. per ml., and perfusion of the isolated heart carried on with this solution.

F. Experiments on the isolated auricles of the guinea-pig.

METHOD

These experiments were carried out in order to study the action of drugs on cardiac muscle in vitro. Adult guinea-pigs/

pigs of either sex were killed by a blow on the head. The throats were cut and the blood allowed to drain out. The thoracic cage was opened and the hearts removed as rapidly as possible and immersed in warmed, heparinised and oxygenated Locke's solution. Using a fine pair of scissors the ventricles were carefully removed and the auricles placed in a dish filled with warmed and oxygenated Locke's solution. All extraneous tissues were dissected carefully until only the horse-shoe shaped auricles remained. The auricles were then suspended in a 10 ml. organ bath by means of threads tied to the apices. One thread was connected to the bent hook fixed at the base of the organ bath and the other was attached to a Starling's heart lever set up so as to record the contractions of the auricles upon a moving smoked surface. The bath was well oxygenated through a hypodermic needle fixed to the base of the organ bath. Locke's solution was passed into the organ bath through a spiral glass coil which was placed in a heated water bath, while the flow was controlled by a spring clip. The temperature of the water bath was maintained thermostatically at 29°C. After about 20 to 30 minutes the contractions of the auricles usually became regular and the experiment was started. The rate of the auricles was estimated by inspection of the tracing, or by direct observation. Drugs in aqueous solution were added to the organ bath directly by means of a graduated 1 ml. tuberculin syringe. The drug effects were noted for 1 or 2 minutes, after which the fluid in the bath was changed. Sufficient time was allowed for the auricles to regain their normal regular/

regular rhythm and amplitude before the next addition of the drug.

In those experiments where the Locke's solution was changed to low calcium Locke's solution, two spiral coils were provided. These were joined by means of a glass Y-piece to the inlet tube of the organ bath. Normal Locke's solution or low calcium Locke's solution could be passed to the organ bath from the respective reservoirs by opening up the spring clip which controlled the flow of the particular solution.

6. Effects on Smooth Muscle

A. Experiments upon isolated guinea-pig ileum

The method adopted was a modification of that described by Guggenheim and Loffler (23). Adult guinea-pigs of either sex were fasted overnight. Next morning they were killed by a blow on the head. The throats cut and the blood drained out. The abdominal cavity was opened and the ileocaecal junction found. A piece of ileum about 10 cm. long was removed from a point near to the ileocaecal junction and placed in a dish containing Tyrode's solution. The contents of the ileum were washed out by inserting a pipette into the end away from the ileocaecal junction and perfusing the gut with Tyrode's solution. Great care was taken not to distend the gut. The mesentery was removed from the gut using a fine pair of scissors, care being taken not to damage the ileum itself. The final inch of the ileum was discarded as it contains a large lymph node and any other portion of the/

the ileum containing lymph nodes was also discarded. A piece of ileum about 3 cm. long taken from a point nearest to the ileocaecal junction was used for the experiment. A small loop of cotton thread was sewn to one end of the piece of ileum and a long piece of cotton thread was sewn to the other end. The cotton threads were tied to the wall away from the side to which the mesentery was attached. The gut was then set up in a 5 ml. organ bath containing oxygenated Tyrode's solution. The Tyrode's solution was passed into the organ bath from the reservoir through a spiral glass coil which was placed in a heated water bath. The flow of Tyrode's solution was controlled by a spring clip. The loop of the thread sewn to the gut was fixed to a hook attached to the base of the organ bath while the long thread sewn to the other end was attached to a modified frontal point writing lever, which in turn recorded the contractions of the gut on a moving smoked surface. The fluid in the bath was oxygenated by passing oxygen through a hypodermic needle fixed to the base of the organ bath. The temperature of the water bath was maintained thermostatically at 37°C. Fig. 43 (page 293) is a diagram of the apparatus used in this experiment.

Spasmogens were added to the bath directly by means of a graduated 1 ml. tuberculin syringe every 3 minutes and left in contact with the tissues for 15 or 30 seconds, after which the fluid in the organ bath was changed. Spasmogens used were (i) acetylcholine (1.0 µg. per ml.); (ii) histamine (0.3 µg. per ml.); (iii) 5-hydroxytryptamine (1.0 µg. per ml.); (iv) barium chloride (0.6 mg. per ml.) and (v) potassium chloride/

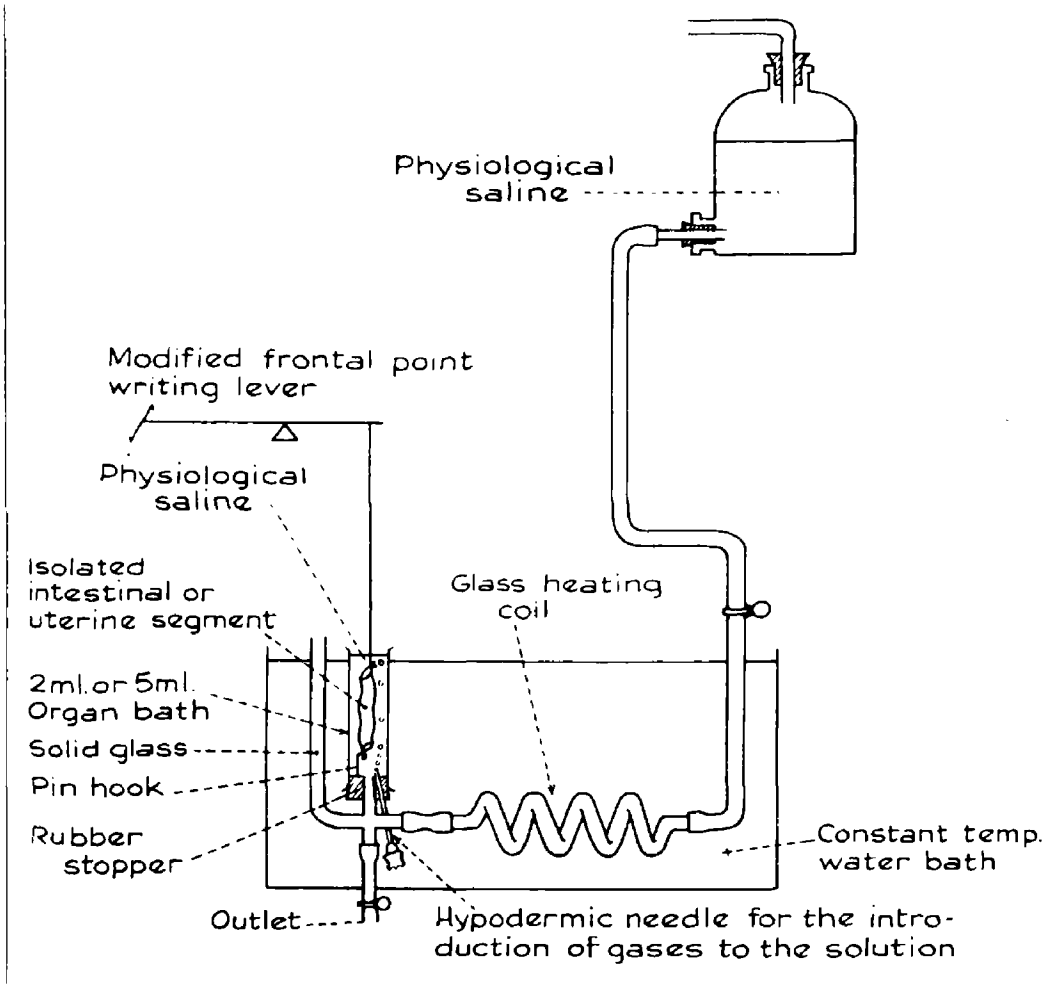

Fig. 43

Diagram of the organ bath used for experiments upon isolated strips of guinea-pig ileum or rat uterus.

chloride (5.0 mg. per ml.). Reproducible submaximal contractions were produced by the spasmogen at the beginning of the experiment. When the response to the spasmogen became constant, drugs in aqueous solution were added directly to the bath by means of a graduated 1 ml. tuberculin syringe, 30 seconds before the next addition of the spasmogen. Any changes in the normal response of the spasmogen were noted. When the drug had a direct effect upon the tissue this was noted. Contractions were allowed to return to their normal level before the next addition of the drug.

B. Experiments upon isolated rat uterus

The method adopted was a modification of that described by Amin, Crawford and Gaddum (24). Virgin female rats weighing 150 to 180 g. were given subcutaneous injections of stilboestrol in arachis oil (0.10 mg. per 100 g.) 24 hours before use. On the following day the animals were killed by a blow on the head. The throats cut and the blood allowed to drain out. The abdominal cavity was opened and both horns and the body of the uterus removed and placed in a dish containing de Jalon's solution. All fat and extraneous tissues were carefully removed. A piece of one horn of the uterus about 2.5 cm. long was taken and used for the experiment. Threads were tied to both ends of the uterus and at one end a loop was made with the thread. It was then suspended in a 5 ml. organ bath containing de Jalon's solution aerated with pure oxygen or a mixture of 95 per cent oxygen and 5 per cent carbon dioxide through a hypodermic needle/

needle fixed to the base of the organ bath. The de Jalon's solution was passed from the reservoir into the organ bath via a spiral glass coil which was immersed in the heated outer water bath. The flow of the de Jalon's solution was controlled by a spring clip. The loop of the thread at one end of the uterus was fixed to the hook at the base of the organ bath, while the thread on the other end of the uterus was attached to a frontal point writing lever which recorded the contractions of the uterus on a moving smoked surface. The temperature of the water bath was maintained thermostatically at 29°C. Some uteri showed much spontaneous activity which could be reduced by lowering the temperature of the water bath or by decreasing the calcium content of the de Jalon's solution. In some tissues these alterations did not reduce the spontaneous activity and these, therefore, had to be rejected.

Spasmogen (acetylcholine 0.1 to 0.5 µg. per ml.) was added directly to the bath by means of a graduated 1 ml. tuberculin syringe every 4 minutes and left in contact with the tissue for 30 seconds, after which the fluid in the organ bath was changed. Reproducible submaximal contractions were produced by the spasmogen at the beginning of the experiment. When the response to the spasmogen became constant, the drug solution under investigation was added directly to the organ bath by means of a graduated 1 ml. tuberculin syringe 30 seconds before the next addition of the spasmogen. Any change in the response of the spasmogen was noted. When the drugs showed a direct effect on the tissue this/

this was also noted. The contractions were allowed to return to normal levels before the next addition of the drug.

7. Toxic Effects on the Rabbit

METHOD

Rabbits of either sex weighing from 2.25 to 2.75 kg. and not having been previously used for experimental purposes were used. They were placed in individual wooden bleeding boxes with the head protruding through the opening at the front. The animals were allowed to settle quietly in the box for some time. The ear vein was made prominent by application of xylene. Petaline chloride in aqeuous solution was injected into the vein from a graduated syringe. Immediately after the injection the rabbit was taken out of the box and the effects of the drug noted.

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C H A P T E R III

RESULTS

Convulsant:Anticonvulsant Activity

(A) Comparison of convulsive reactions and mortality induced by petaline chloride, leptazol and picrotoxin.

Tables 35 and 36 show the range of doses used for petaline chloride, leptazol and picrotoxin. The CD50 were found to be approximately 6.6 mg. per kg., 40 mg. per kg., and 1.8 mg. per kg., respectively for petaline chloride, leptazol and picrotoxin (Fig. 44 , page 303 and Fig. 45 , page 304). The LD50 was found to be approximately 9.2 mg. per kg., 56 mg. per kg., and 3 mg. per kg., respectively for petaline chloride, leptazol and picrotoxin (Fig. 46 , page 305 and Fig. 47 , page 306). Petaline chloride is a more potent convulsant and is more toxic than leptazol, but it has less convulsant potency and it is less toxic than picrotoxin.

(B) Effect of pre-treatment with petaline chloride on the response to leptazol.

Table 37 shows the doses of petaline chloride used for pre-treating the animals and the dose range of leptazol used to investigate the effect of the latter on pre-treatment. The CD50 of leptazol were found to be respectively 42.8 mg. per kg., 43.5 mg. per kg., and 35.0 mg. per kg., for the three groups of pre-treated mice. The LD50 of leptazol was found respectively/

Petaline chloride dose mg./kg.	per cent convulsing	per cent mortality
4	10	-
6	40	-
8	70	20
10	100	70
12	100	100
Leptazol dose mg./kg.	**per cent convulsing**	**per cent mortality**
30	13	-
40	51.4	11.4
50	83.3	22.2
60	92.8	57.1
80	100	100

TABLE 35

Convulsant activity and mortality in mice after petaline chloride or leptazol.

Picrotoxin dose mg./kg.	per cent convulsing	per cent mortality
1	-	-
1.5	30	-
2	60	10
2.5	90	30
3	100	54.5
4	100	90
Petaline chloride dose mg./kg.	**per cent convulsing**	**per cent mortality**
4	10	-
6	40	-
8	70	20
10	100	70
12	100	100

TABLE 36

Convulsant activity and mortality in mice after picrotoxin or petaline chloride.

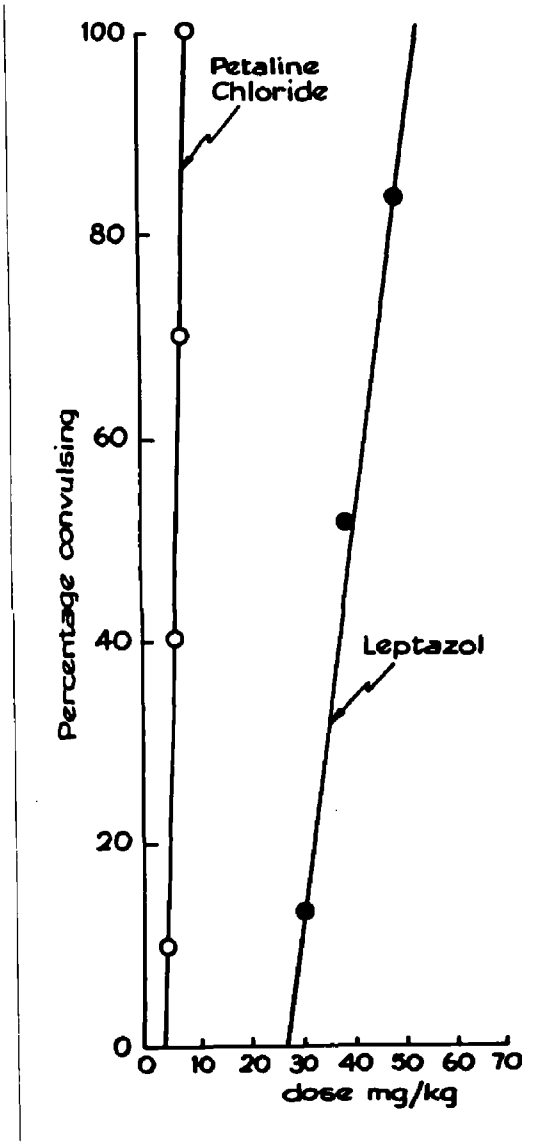

Fig. 44

Convulsant activity of petaline chloride and leptazol in the mouse.

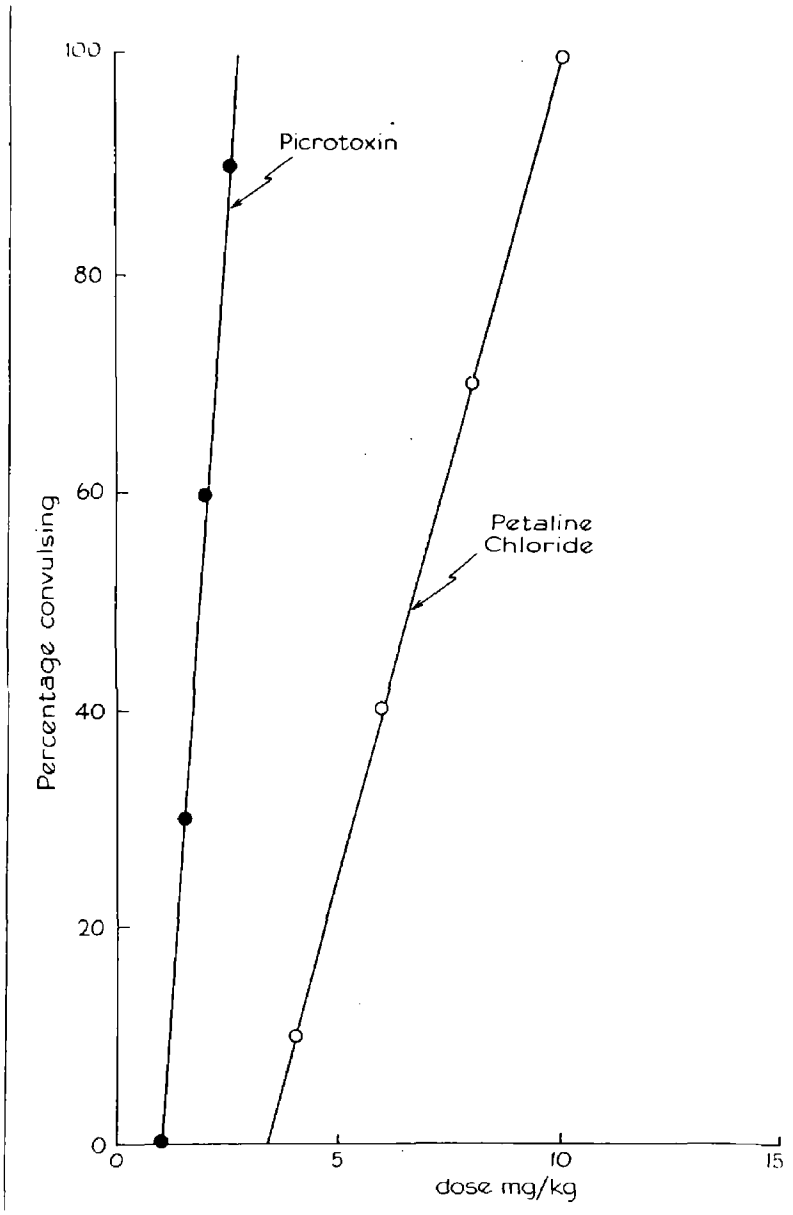

Fig. 45

Convulsant activity of picrotoxin and petaline chloride in the mouse.

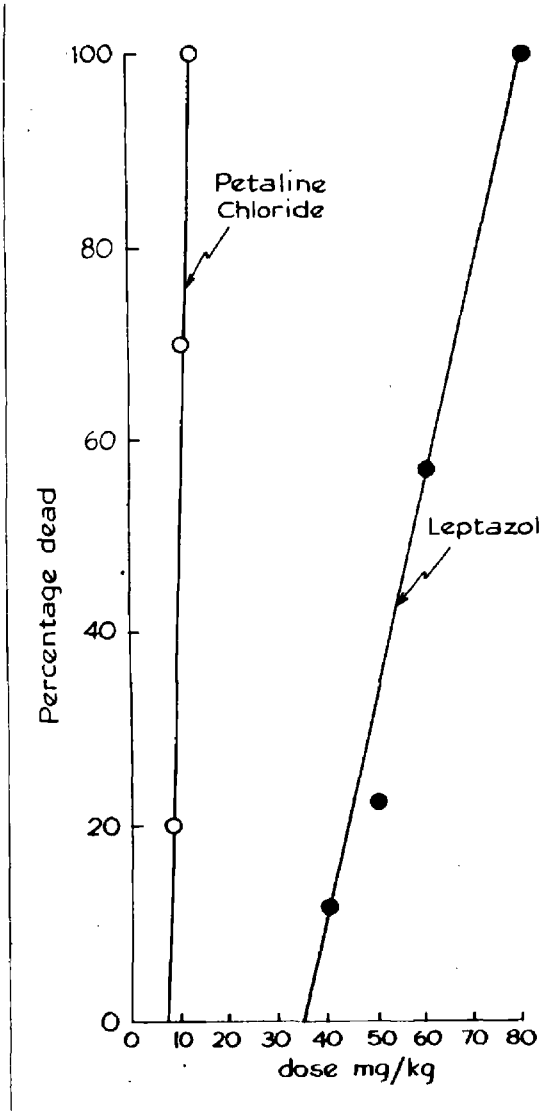

Fig. 46

Mortality in mice from petaline chloride and leptazol.

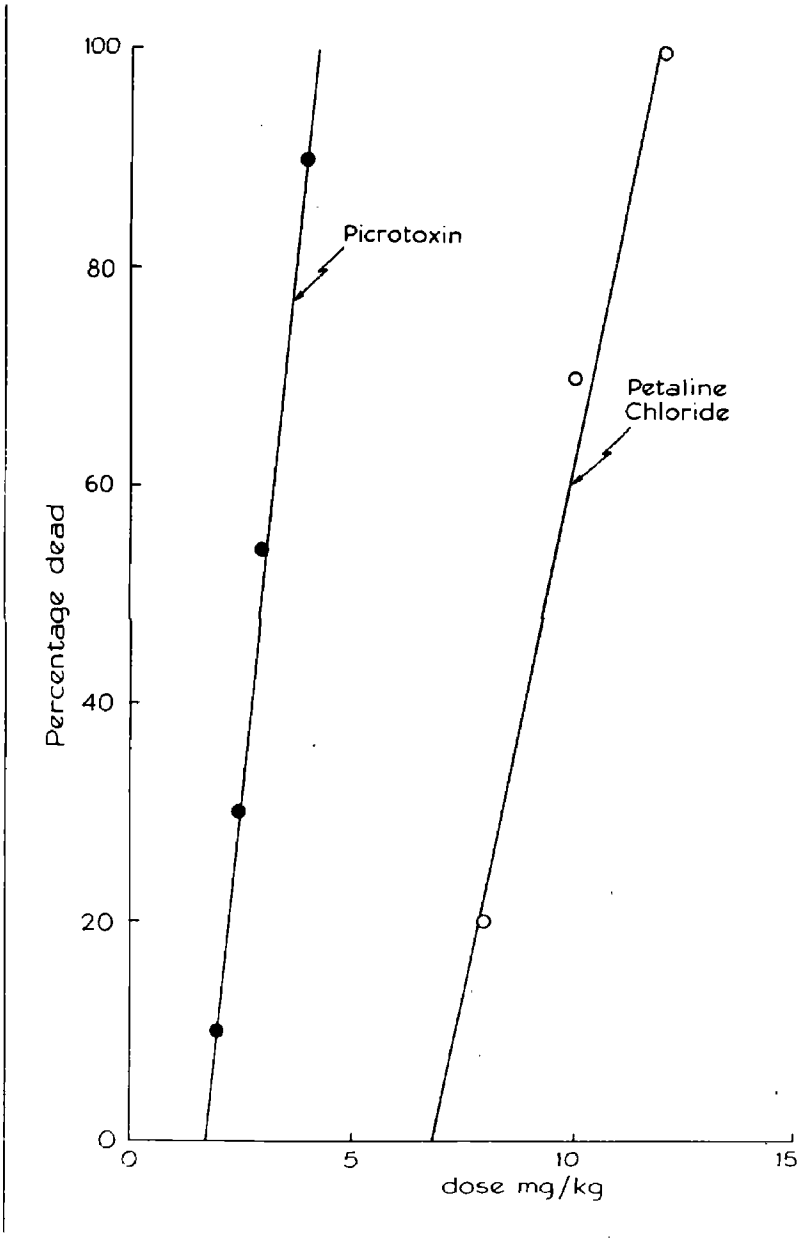

Fig. 47

Mortality in mice from picrotoxin and petaline chloride.

respectively to be 64.0 mg. per kg., 70.7 mg. per kg., and 48.0 mg. per kg., for the three groups of pre-treated animals. Fig. 48 (page 309) and Fig. 49 (page 310) show the effect of pre-treatment with 5 mg. per kg., or 8 mg. per kg., petaline chloride on the convulsant activity and mortality due to leptazol. Lower doses of petaline chloride may give some protection, but higher doses increase both the convulsant activity and toxicity of leptazol.

(C) Comparison of the effects of petaline chloride and phenobarbitone on electroshock seizures.

Table 38 shows the doses of petaline chloride and phenobarbitone administered and the percentage protection conferred by these compounds. Fig. 50 (page 312) shows a comparison of the percentage protection obtained by treatment with petaline chloride or phenobarbitone. Petaline chloride is apparently capable of conferring some protection but it is very much less potent than phenobarbitone. At higher doses petaline chloride protection was reduced.

Muscle Relaxant Activity

(A) Cat gastrocnemius muscle-sciatic nerve preparation.

(i) Neuromuscular blocking activity.

Petaline chloride reduced the twitch height of the gastrocnemius muscle in response to indirect stimulation via the sciatic nerve. There was incomplete or complete neuromuscular block depending upon the dose administered. The block was completely reversible.

Petaline/

Leptazol dose mg./kg.	Petaline chloride dose mg./kg.			Petaline chloride dose mg./kg.		
	2.5	5.0	8.0	2.5	5.0	8.0
	per cent animals convulsing			per cent mortality		
20	-	-	0	-	-	-
25	0	-	10	-	-	0
30	-	0	-	-	-	-
37.5	20	40	-	0	-	-
40	62.5	-	68.75	-	-	12.5
50	90	60	100	10	0	58.3
60	-	-	-	42.8	-	70.5
62.5	-	100	-	71.4	30	-
75	100	-	-	80	60	-

TABLE 37

Convulsant activity and mortality from leptazol given 45 to 60 minutes after petaline chloride.

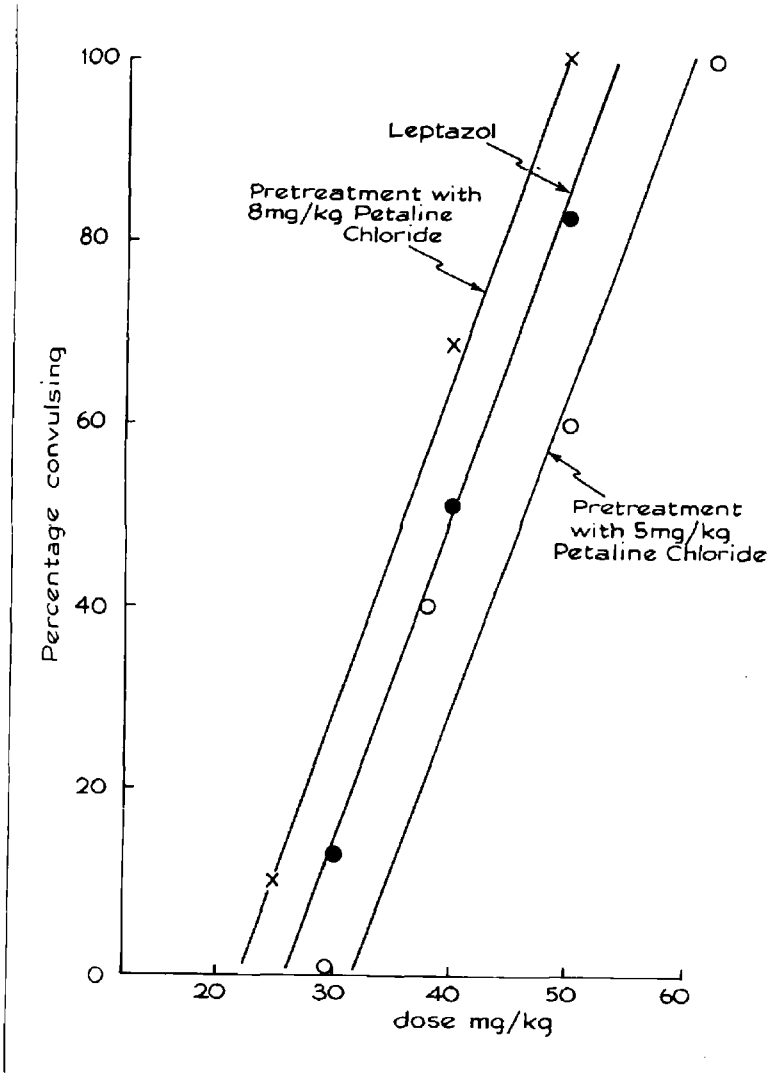

Fig. 48

Effects of pre-treatment with petaline chloride (5 or 8 mg./kg.) on the convulsant activity of leptazol.

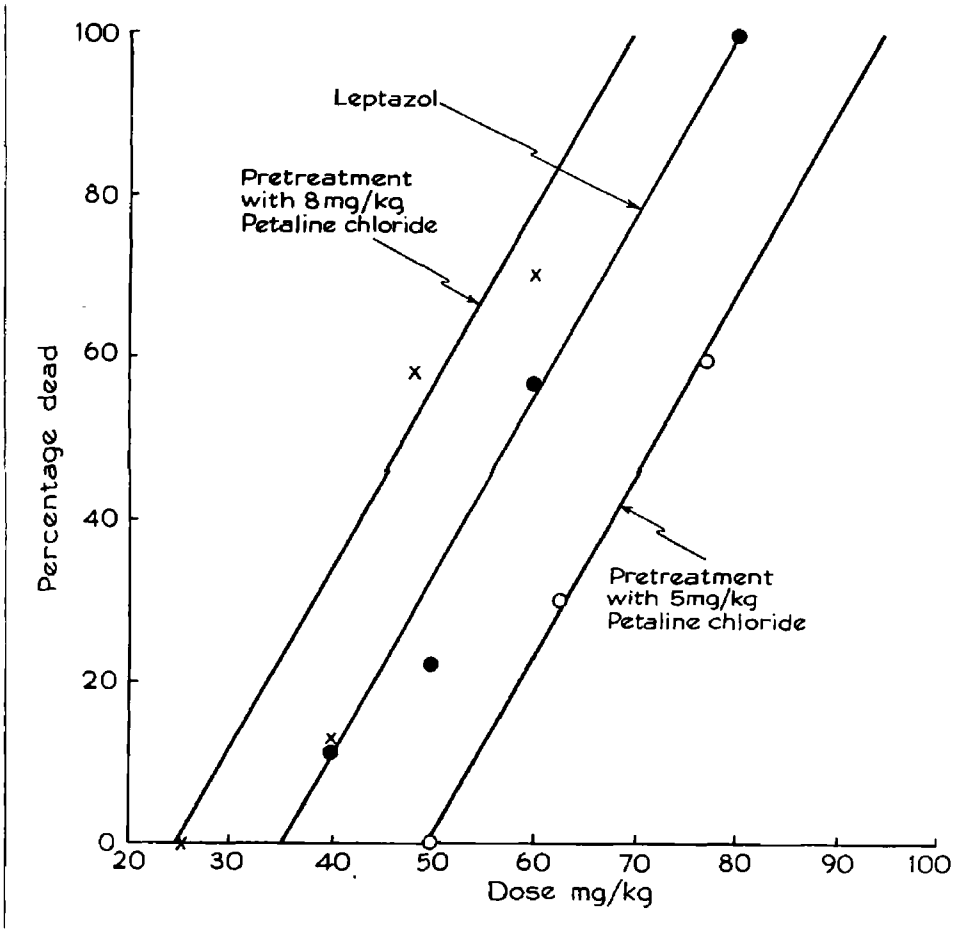

Fig. 49

Effects of pre-treatment with petaline chloride (5 or 8 mg./kg.) on the mortality due to leptazol.

Petaline chloride dosage mg./kg.	per cent protection
1.6	10
2.5	25
3.3	37.5
5.0	20

Phenobarbitone dosage mg./kg.	per cent protection
4	20
5	50
6.6	80

TABLE 38

Protection of mice from electroshock seizure by pre-treatment with petaline chloride or phenobarbitone.

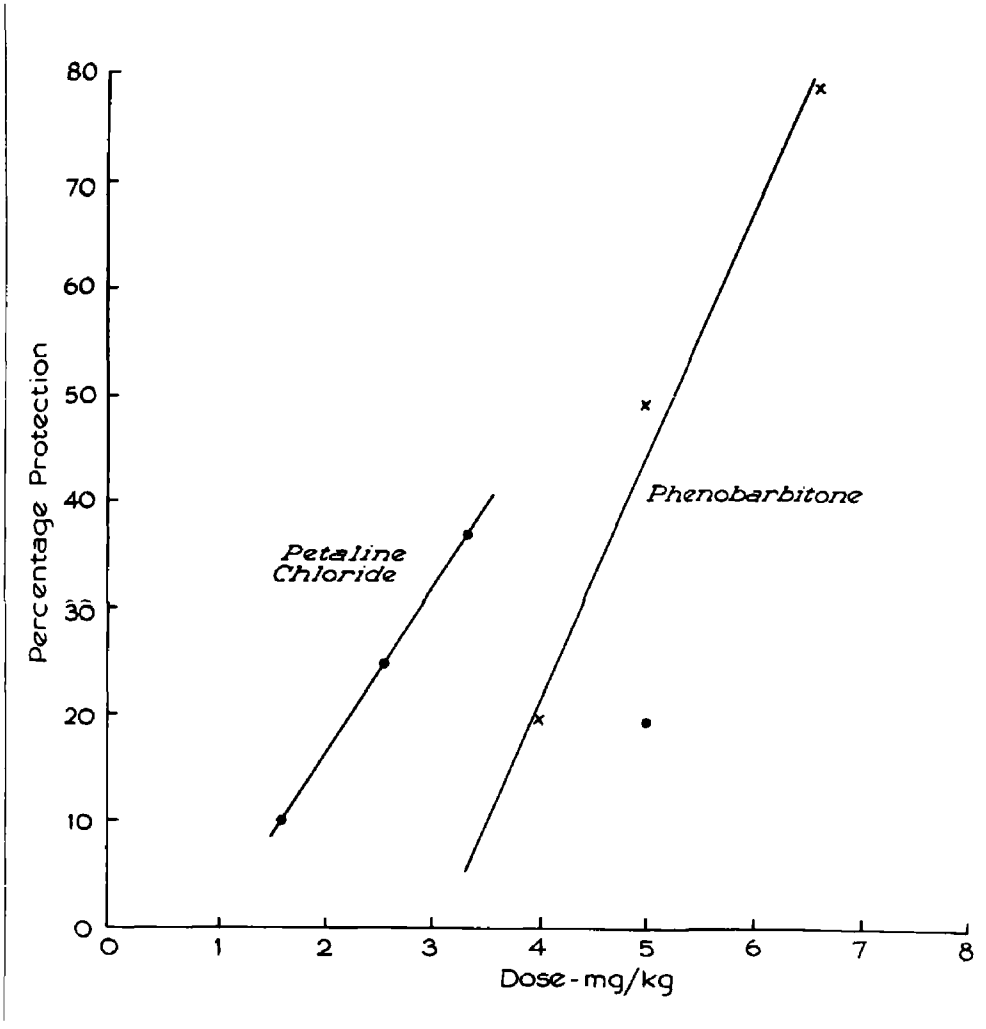

Fig. 50

Protection of mice from electroshock seizures by treatment with petaline chloride or phenobarbitone.

Petaline chloride caused about 50 per cent reduction of the amplitude of the muscle twitch when doses from 3.0 to 5.0 mg. per kg. were injected, whereas 10 to 12 mg. per kg. was needed to cause complete neuromuscular block. The doses needed to produce these effects were comparable in magnitude with the doses of tubocurarine required to produce similar effects on twitch height. Petaline chloride was much less potent than tubocurarine, and in order to produce equivalent effects doses were needed which were from between twenty to twenty-five times greater than those of tubocurarine.

Recovery from the petaline chloride block occurred in about 15 to 20 minutes. The maximum degree of neuromuscular block was usually reached within 2 to 3 minutes. Subsequent doses of petaline chloride did not prolong the neuromuscular block. Fig. 51 (page 314) shows the neuromuscular block caused by petaline chloride and tubocurarine.

(ii) Effect of indirect tetanization of the partially blocked muscle.

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

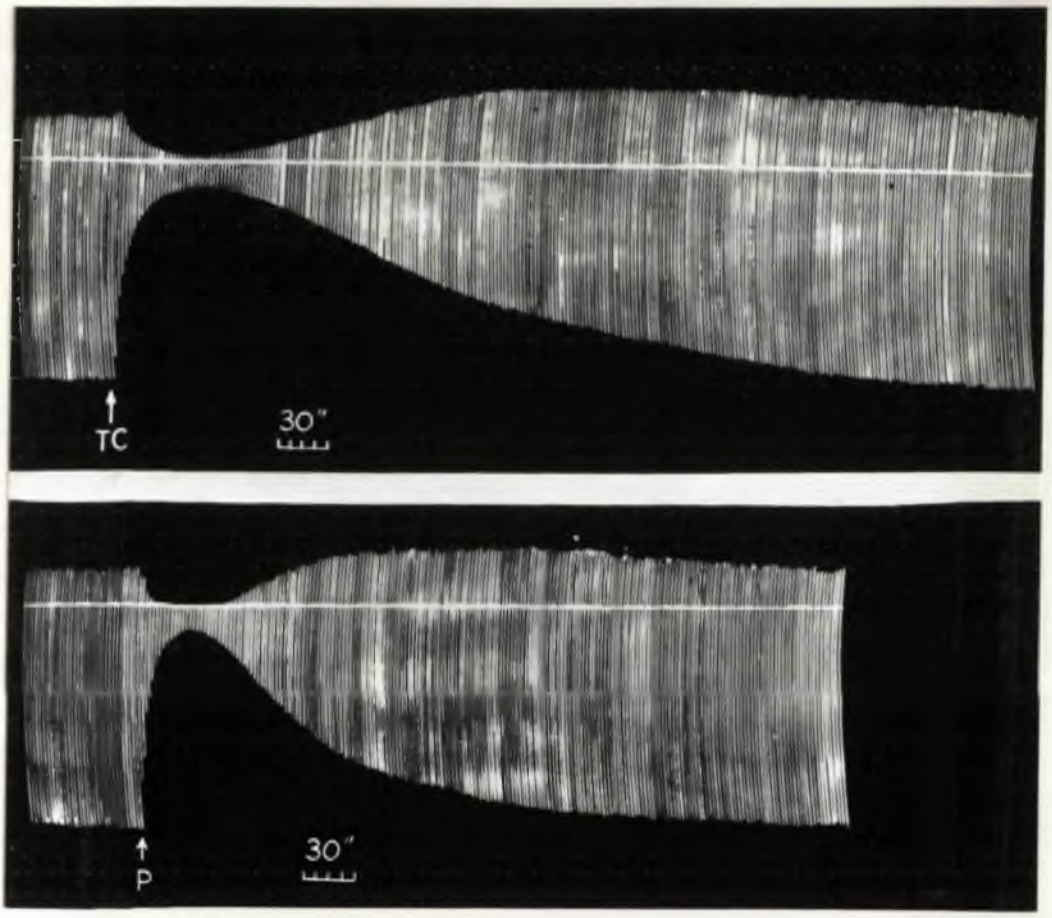

Fig. 51

Cat gastrocnemius muscle-sciatic nerve preparation. Pentobarbitone anaesthesia (60 mg. per kg.). Indirect stimulation via sciatic nerve. Contraction downwards. Drugs in aqueous solution administered intravenously.

At TC, 0.50 mg. per kg. tubocurarine.
At P, 10.0 mg. per kg. petaline chloride.

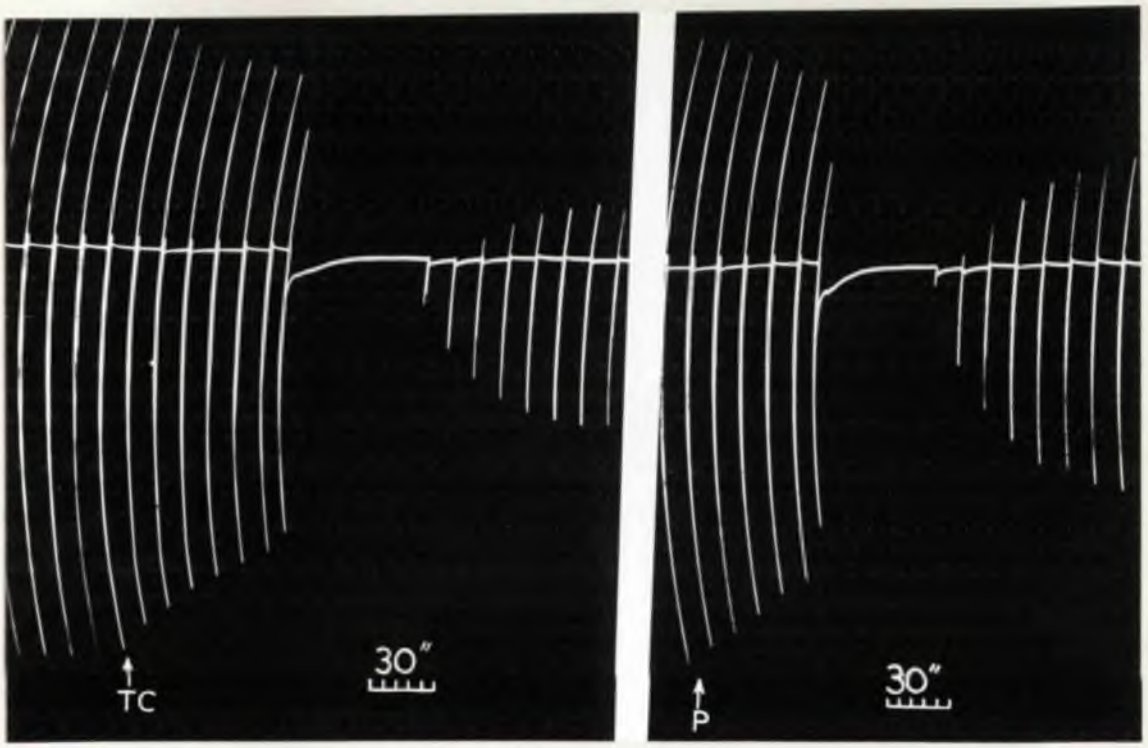

Fig. 52

Cat gastrocnemius muscle-sciatic nerve preparation. Pentobarbitone anaesthesia (60 mg. per kg.). Comparison of the effects of tubocurarine and petaline chloride on the response of muscle to indirect tetanization via the sciatic nerve. Tetanization by square impulses, 15 volts; 3 msec; 1,500 per minute during incomplete neuromuscular block. Drugs in aqueous solution administered intravenously.

At TC, 0.20 mg. per kg. tubocurarine.
At P, 10.0 mg. per kg. petaline chloride.

block has been produced (1, 3, 4).

When a tetanizing current was applied to the nerve of a petaline chloride-treated muscle, the tetanus was not maintained - an effect similar to that seen when tubocurarine is used. Fig. 52 (page 315) shows the typical effects of tetanizing petaline chloride and tubocurarine-treated muscles.

(iii) Drug antagonism

The neuromuscular block produced by tubocurarine or other substances having a similar mechanism of action is antagonised by depolarising drugs (5, 6, 7), anticholinesterases (8), edrophonium (9, 10), adrenaline and potassium ions (11). Anticholinesterases increase the neuromuscular blocking activity of depolarising agents (12, 13). Edrophonium has either no effect on or potentiates a depolarisation block (7, 14). The antagonistic effect shown by neostigmine to the neuromuscular block caused by different types of muscle relaxants is one of the major differentiating factors in the determination of their mechanism of action. Neostigmine antagonises the block caused by the competitive type of neuromuscular blocking agents (8, 15, 16), while it has a potentiating effect upon the effects of the depolarising drugs (17).

Edrophonium and neostigmine were used to compare the type of antagonism to the block produced by petaline chloride and tubocurarine. The antagonists were injected into the cat at the point of maximal neuromuscular block.

Edrophonium (1.0 mg. per kg.) and neostigmine (0.1 mg. per kg.) antagonised the neuromuscular block produced by petaline chloride/

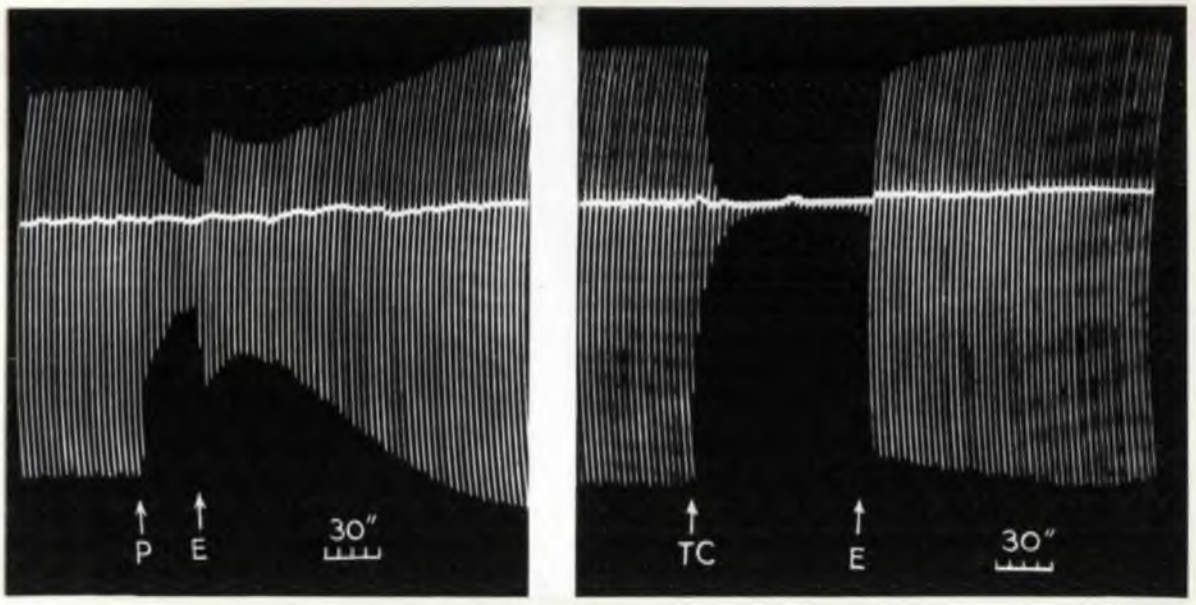

Fig. 53

Cat gastrocnemius muscle-sciatic nerve preparation. Pentobarbitone anaesthesia (60 mg. per kg.). Indirect stimulation via sciatic nerve. Contraction downwards. Drugs in aqueous solution administered intravenously.

At P, 4.4 mg. per kg. petaline chloride.
At E, 1.0 mg. per kg. edrophonium.
At TC, 0.133 mg. per kg. tubocurarine.

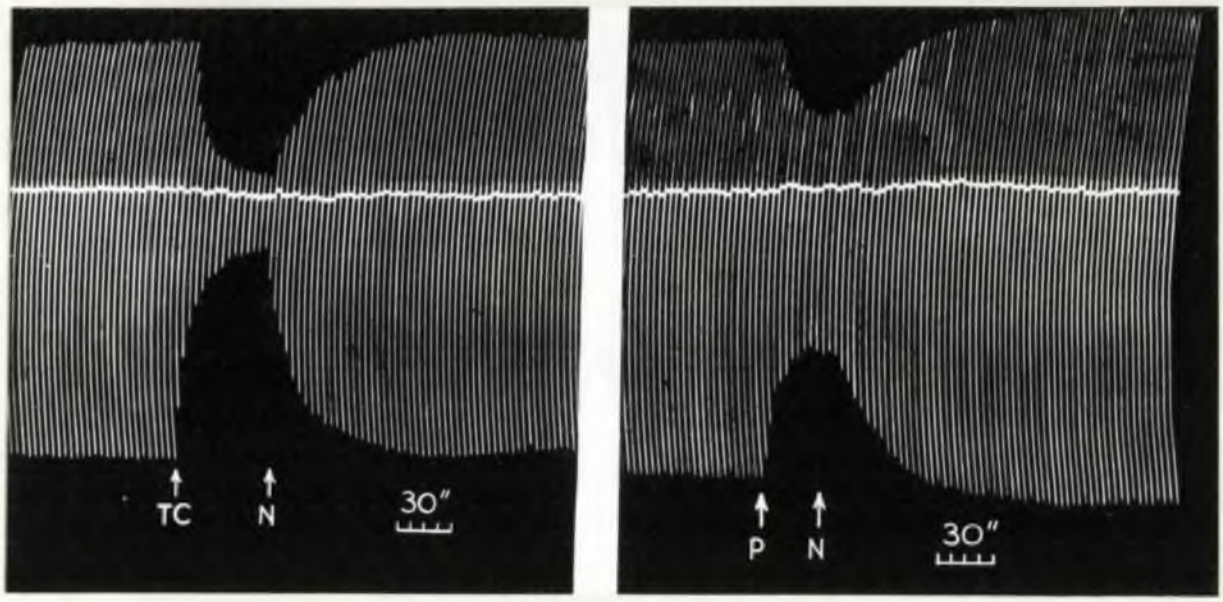

Fig. 54

Cat gastrocnemius muscle-sciatic nerve preparation. Pentobarbitone anaesthesia (60 mg. per kg.). Indirect stimulation via sciatic nerve. Contraction downwards. Drugs in aqueous solution administered intravenously.

At TC, 0.133 mg. per kg. tubocurarine.
At N, 0.10 mg. per kg. neostigmine.
At P, 4.4 mg. per kg. petaline chloride.

chloride and tubocurarine. Fig. 53 (page 317) and Fig. 54 (page 318) show the antagonism caused by edrophonium and neostigmine on petaline chloride and tubocurarine-treated muscles.

(B) (i) Rat phrenic nerve-diaphragm preparation using Bell's electrode.

Petaline chloride in the dose range of 0.20 to 0.30 mg. per ml. gradually reduced and finally abolished the response of the rat diaphragm to indirect stimulation via the phrenic nerve. The time taken was about 5 to 7 minutes. Directly stimulated muscle, using a higher voltage and a wider pulse, produced regular contractions. In this respect the effect was similar to that observed when tubocurarine was used. A typical experiment is shown in Fig. 55 (page 320).

(ii) Rat phrenic nerve-diaphragm using Collison's fluid electrode

Petaline chloride in the dose range of 0.20 to 0.30 mg. per ml. reduced the twitch height of the rat diaphragm in response to indirect stimulation via the phrenic nerve. The effect was always reversible on washing. Tubocurarine was much more potent than petaline chloride. On this preparation petaline chloride was found to be more or less equipotent to gallamine. Fig. 56 (page 321) and Fig. 57 (page 322) show the effects of petaline chloride, tubocurarine and gallamine on the rat phrenic nerve-diaphragm preparation.

(C)/

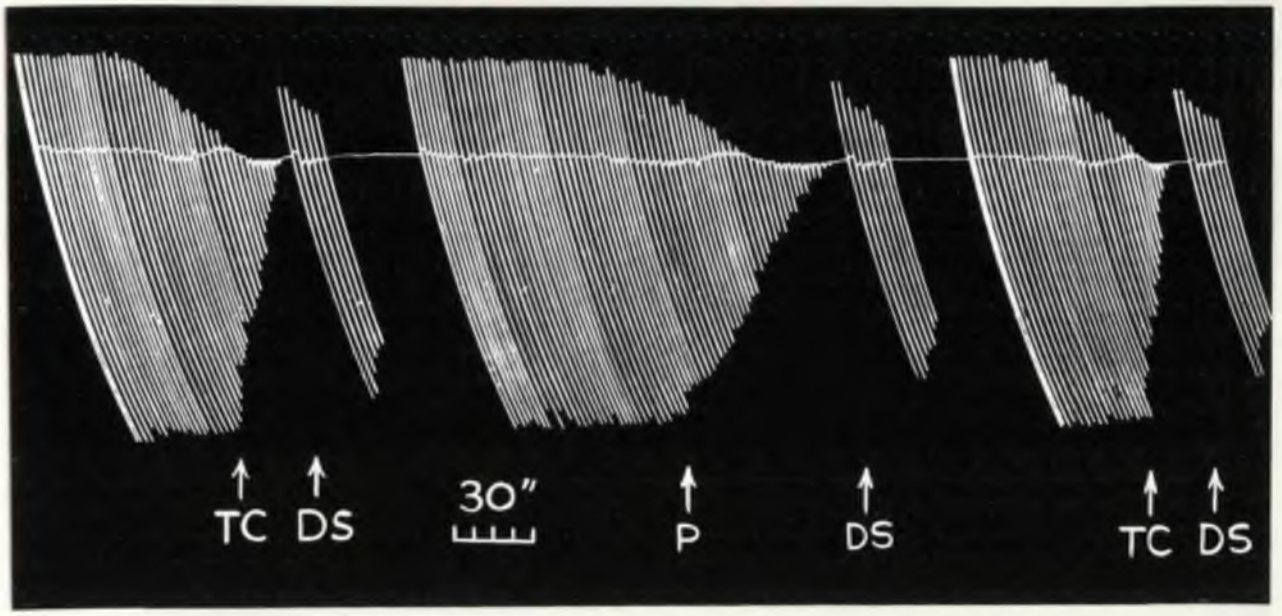

Fig. 55

Rat phrenic nerve-diaphragm preparation. Contractions induced by indirect stimulation of the phrenic nerve or by direct stimulation of the muscle using the electrode described by Bell.
Bath 100 ml., temperature 29°C., bath fluid, oxygenated "double glucose" Tyrode's solution.

At TC, 3.0 μg. per ml. tubocurarine added to bath.
At P, 0.2 mg. per ml. petaline chloride added to bath.
At DS, direct stimulation of the muscle.

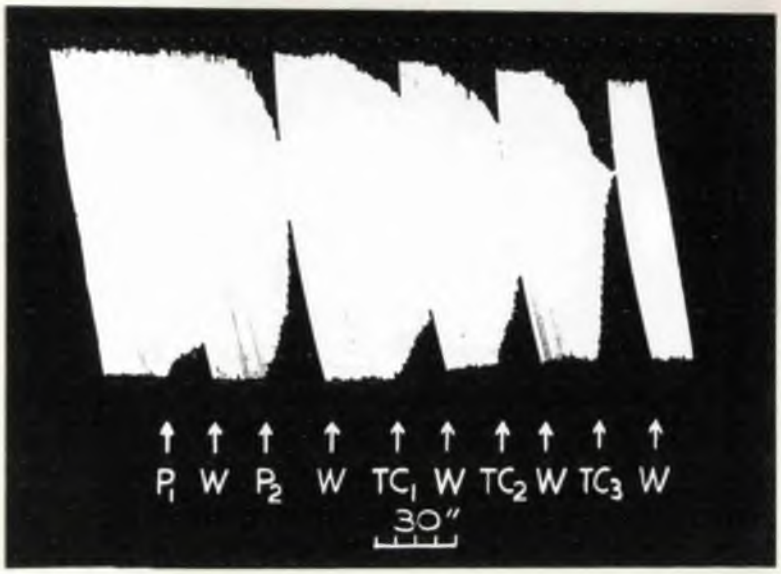

Fig. 56

Rat phrenic nerve-diaphragm preparation using Collison's fluid electrode. Contractions due to indirect stimulation via the phrenic nerve.

Bath 100 ml., temperature 29°C., bath fluid, "double glucose" Tyrode's solution gassed with 95 per cent oxygen and 5 per cent carbon dioxide.

Drugs in aqueous solution left in contact with the muscle for 3 minutes.

At P_1,	0.15 mg. per ml. petaline chloride
At P_2,	0.25 mg. per ml. " "
At TC_1,	1.0 μg. per ml. tubocurarine.
At TC_2,	2.0 μg. per ml. "
At TC_3,	3.0 μg. per ml. "
At W,	Wash out.

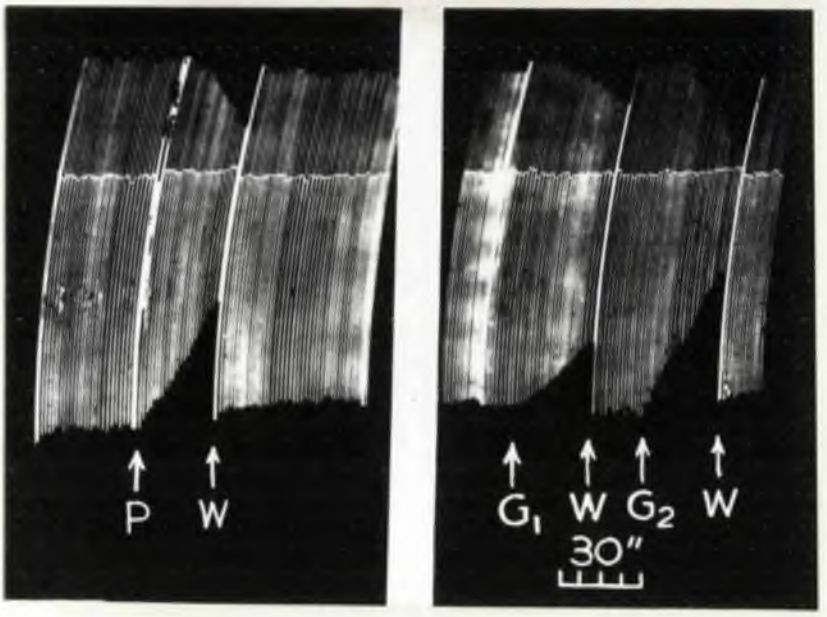

Fig. 57

Rat phrenic nerve-diaphragm preparation using Collison's fluid electrode. Contractions due to indirect stimulation via the phrenic nerve.
Bath 100 ml., temperature 29°C., bath fluid, "double glucose" Tyrode's solution gassed with 95 per cent oxygen and 5 per cent carbon dioxide.
Drugs in aqueous solution in contact with the muscle for 3 minutes.

At P, 0.30 mg. per ml. petaline chloride.
At G_1, 0.20 mg. per ml. gallamine.
At G_2, 0.30 mg. per ml. "
At W, Wash out.

(C) Isolated frog rectus abdominis muscle preparation.

Petaline chloride did not possess any direct stimulant effect on this preparation at doses of up to 20 µg. per ml. In the dose range of 5 to 20 µg. per ml. petaline chloride caused graded inhibition of contractions induced by 0.10 to 1.0 µg. per ml. acetylcholine. Fig. 58 (page 324) shows a typical experiment. Fig. 59 (page 325) shows a comparison between petaline chloride and gallamine on the frog rectus abdominis muscle. On this preparation, petaline chloride was found to have about 50 per cent of the potency of gallamine (Fig. 60 , page 326).

Ganglion Blocking Activity

(A) Nictitating membrane of the anaesthetised cat.

Petaline chloride in doses of from 2.0 to 4.0 mg. per kg. did not produce any direct effect upon the nictitating membrane of the anaesthetised cat. In the dose range of 2.0 to 4.0 mg. per kg. petaline chloride caused some reduction of the height of contractions of the nictitating membrane in response to stimulation of the preganglionic fibres of the cervical sympathetic. The height of contraction returned to normal within the subsequent second or third contraction. Fig. 61 (page 327) shows a typical effect.

Experiments on Spinal Reflexes

(A) Patellar tendon reflex

Petaline chloride in the dose range of from 10 to 15 mg. per/

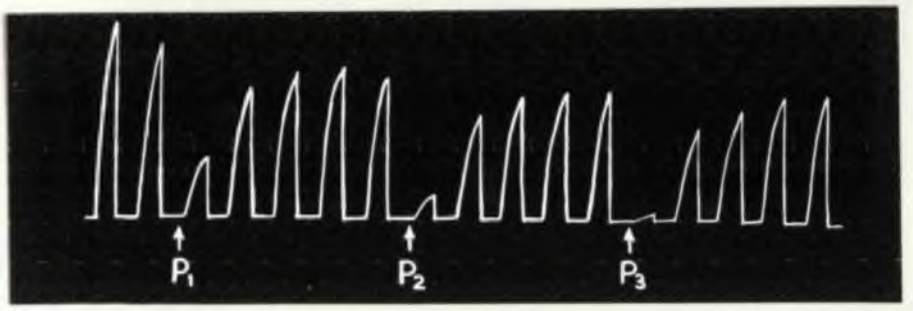

Fig. 58

Isolated frog rectus abdominis muscle preparation.
Bath 10 ml., bath fluid oxygenated Frog Ringer's solution at room temperature.
All contractions due to 0.10 μg. per ml. acetylcholine.
Addition of acetylcholine was preceded 30 seconds earlier by:-

At P_1, 10 μg. per ml. petaline chloride.
At P_2, 15 μg. per ml. " "
At P_3, 20 μg. per ml. " "

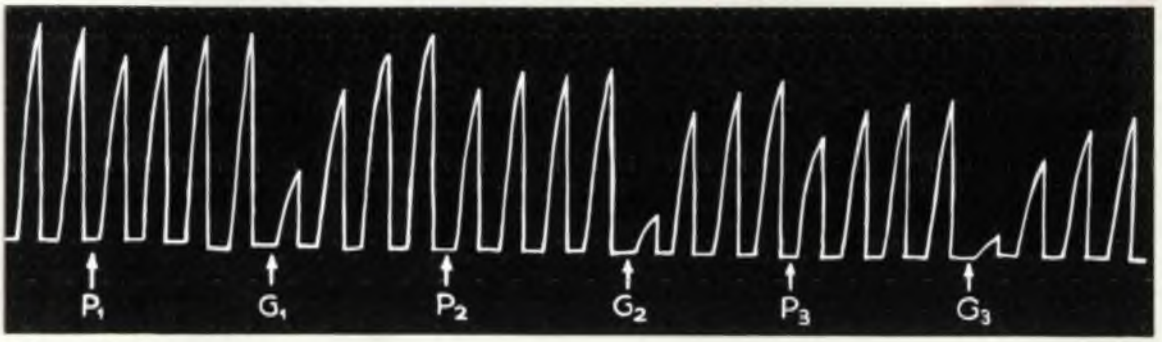

Fig. 59

Isolated frog rectus abdominis muscle preparation.
Bath 10 ml., bath fluid oxygenated Frog Ringer's solution at room temperature.
All contractions due to 1.0 μg. per ml. acetylcholine.
Addition of acetylcholine was preceded 30 seconds earlier by;-

At P_1, 5 μg. per ml. petaline chloride.
At P_2, 7.5 μg. per ml. " "
At P_3, 10 μg. per ml. " "
At G_1, 5 μg. per ml. gallamine
At G_2, 7.5 μg. per ml. "
At G_3, 10 μg. per ml. "

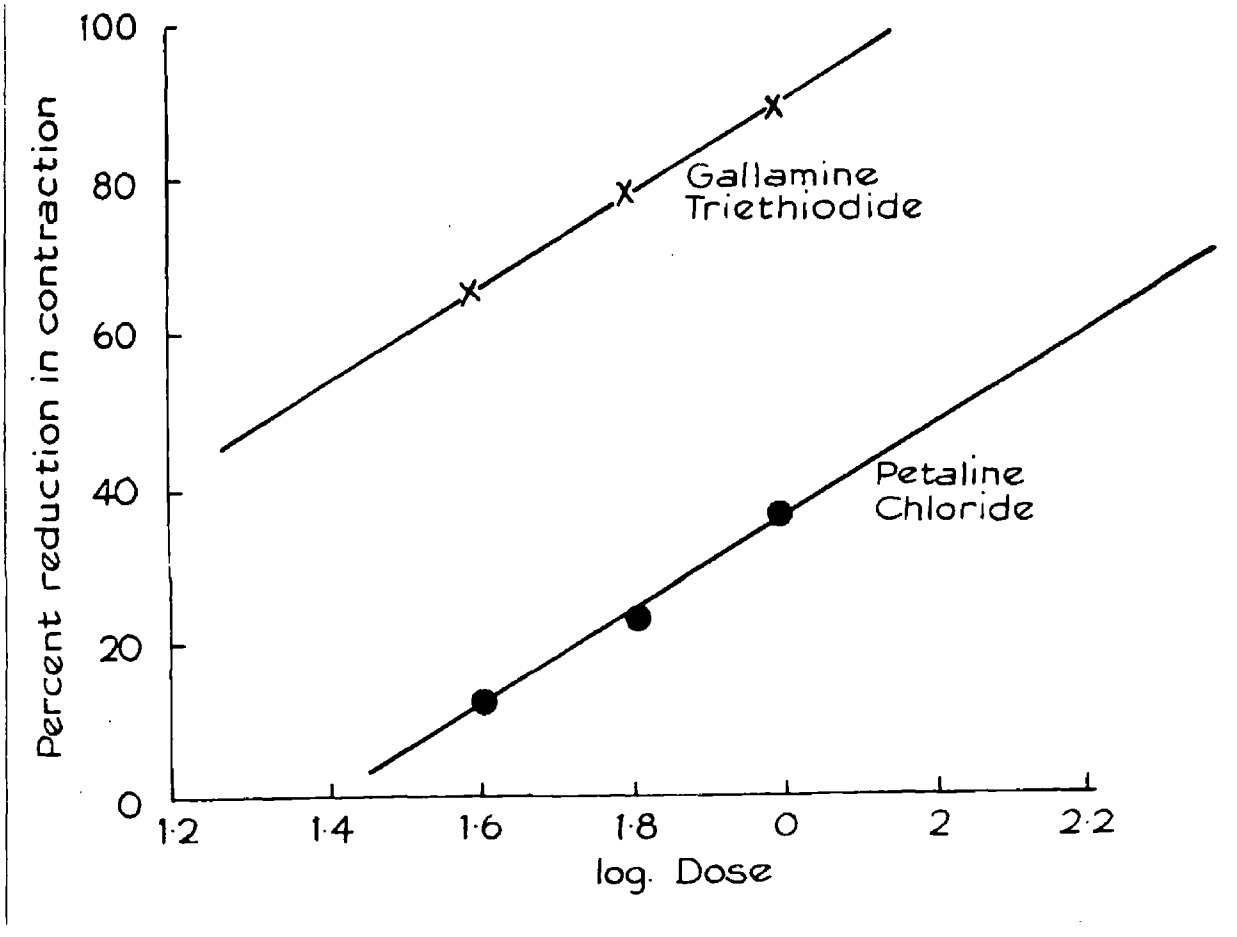

Fig. 60

A comparison of the effects of gallamine and petaline chloride on acetylcholine-induced contractions of the frog rectus abdominis muscle.

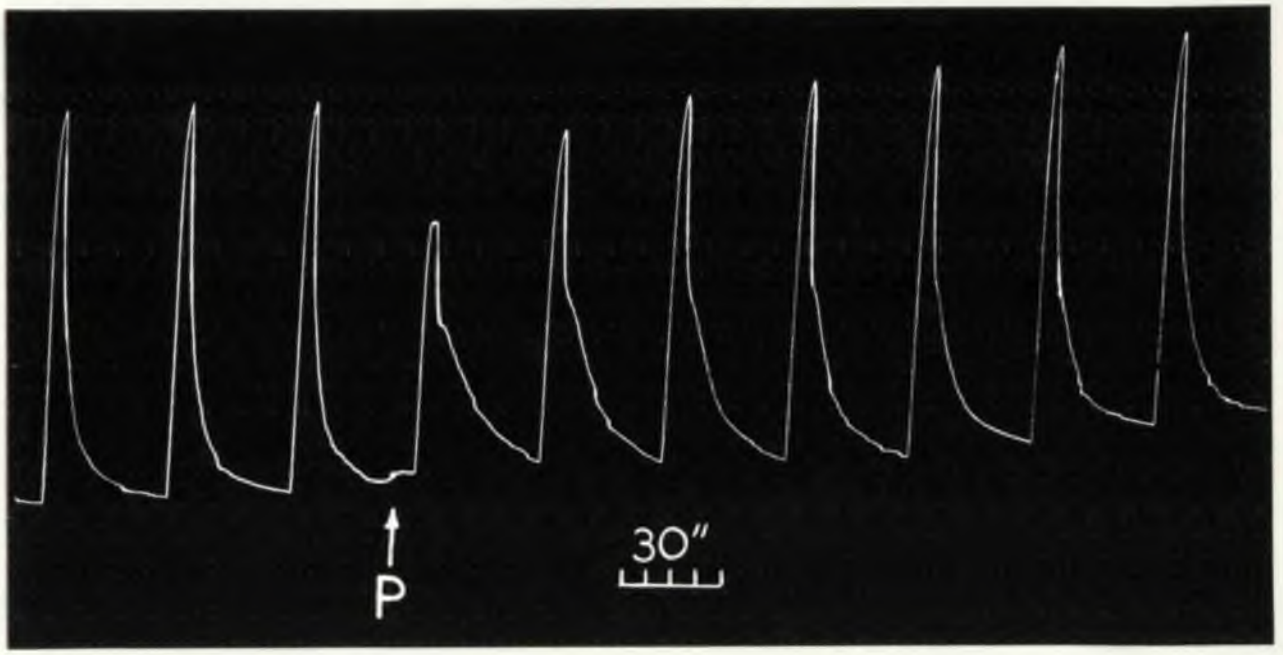

Fig. 61

Contractions of the nictitating membrane of the pentobarbitone anaesthetised (60 mg. per kg.) cat induced by electrical stimulation of the preganglionic fibres of the cervical sympathetic using square impulses at a frequency of 1,000 impulses per minute, 10 volts, pulse width 1 msec, for a period of 15 seconds.

Drugs in aqueous solution administered intravenously.

At P, 2 mg. per kg. petaline chloride.

per kg., depressed the patellar tendon reflex of the spinal cat. The onset of depression was rapid (about 2 minutes) and the recovery was complete in about 15 to 20 minutes. Fig. 62 (page 329) shows the results of a typical experiment. A similar effect was seen with tubocurarine in a dose range which produces neuromuscular block (0.1 to 0.5 mg. per kg.). Fig. 63 (page 330) shows the effect of tubocurarine on the patellar tendon reflex of the spinal cat. The depression produced by tubocurarine was much more prolonged than that produced by petaline chloride.

(B) Crossed extension reflex

Petaline chloride in the dose range of from 10 to 15 mg. per kg., depressed the crossed extension reflex in the spinal cat. The onset of effect was rapid reaching a maximum in about 2 minutes. The recovery was complete in about 15 to 20 minutes. The effect of petaline chloride on this reflex was compared with that of mephenesin (20 to 40 mg. per kg.), which was less effective on this preparation. Fig. 64 (page 331) shows a comparison of petaline chloride and mephenesin on the crossed extension reflex of the spinal cat. Tubocurarine in a dose range (0.1 to 0.5 mg. per kg.) which produces neuromuscular block caused prolonged depression of the crossed extension reflex. Fig. 65 (page 332) shows the effect of tubocurarine on the crossed extension reflex of the spinal cat.

Cardiovascular Actions

(A)/

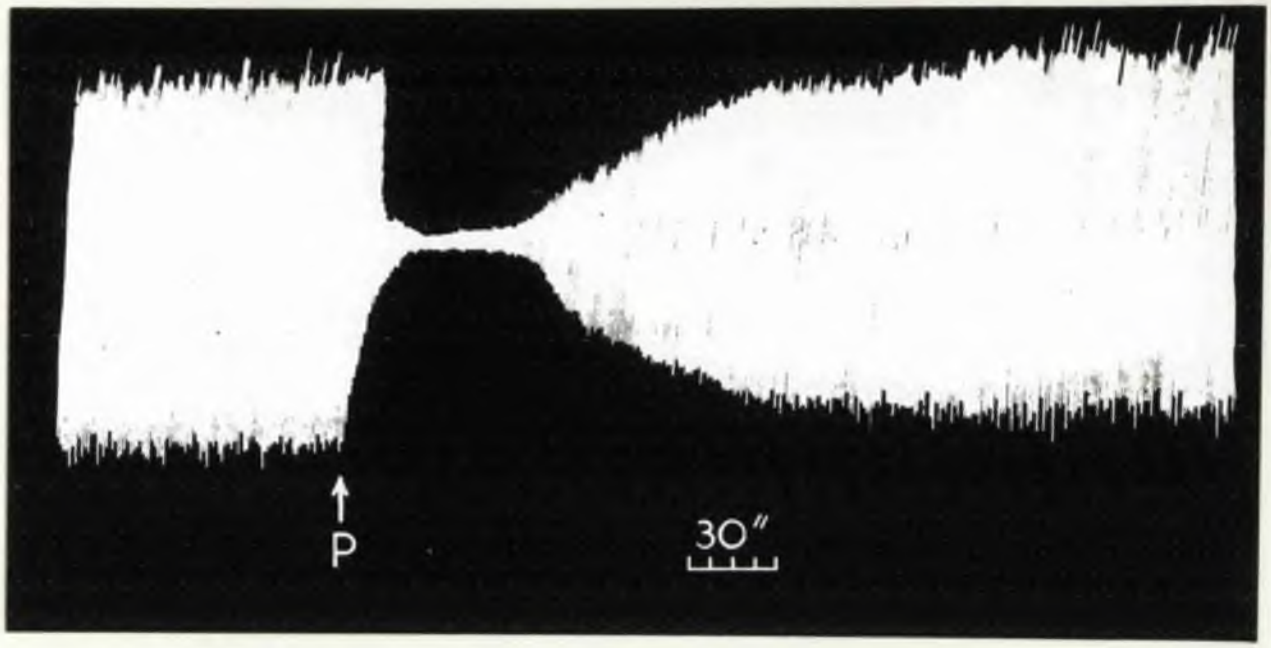

Fig. 62

Reflex response to electrical stimulation of the patellar tendon of the spinal cat.
Frequency 15 per minute, voltage 50, pulse width 3 msec.
Drugs in aqueous solution administered intravenously.
At P, 15 mg. per kg. petaline chloride.

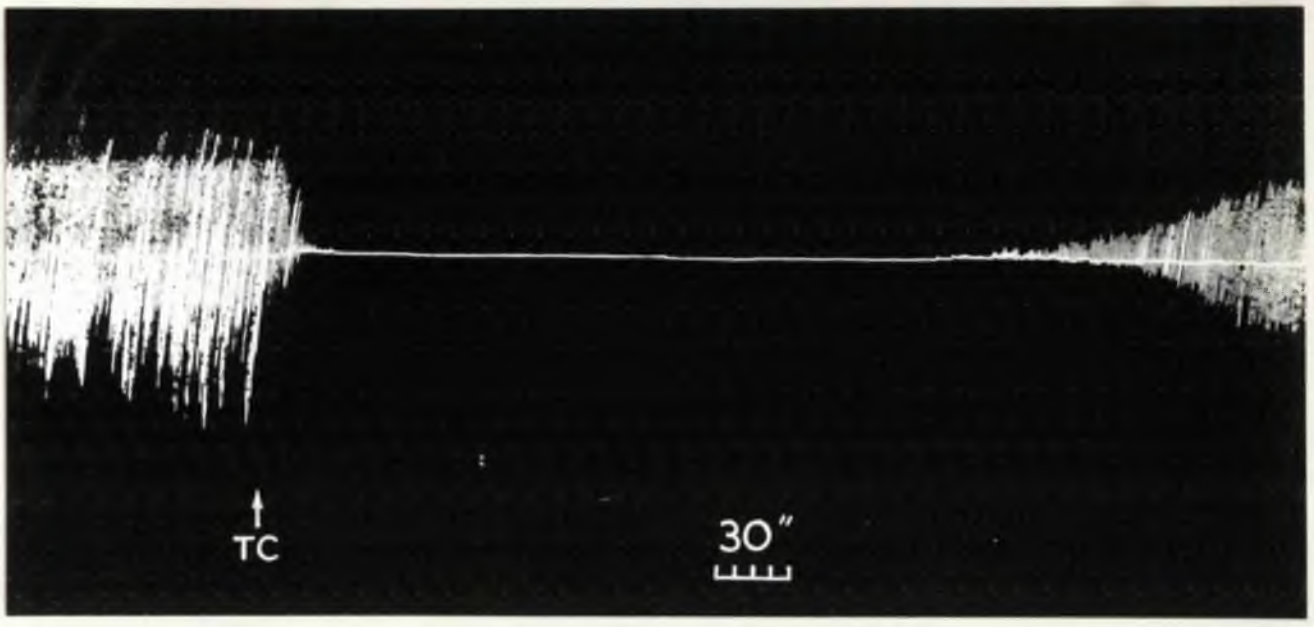

Fig. 63

Reflex response to electrical stimulation of the patellar tendon of the spinal cat.

Frequency 15 per minute, voltage 50, pulse width 3 msec.

Drugs in aqueous solution administered intravenously.

At TC, 0.20 mg. per kg. tubocurarine.

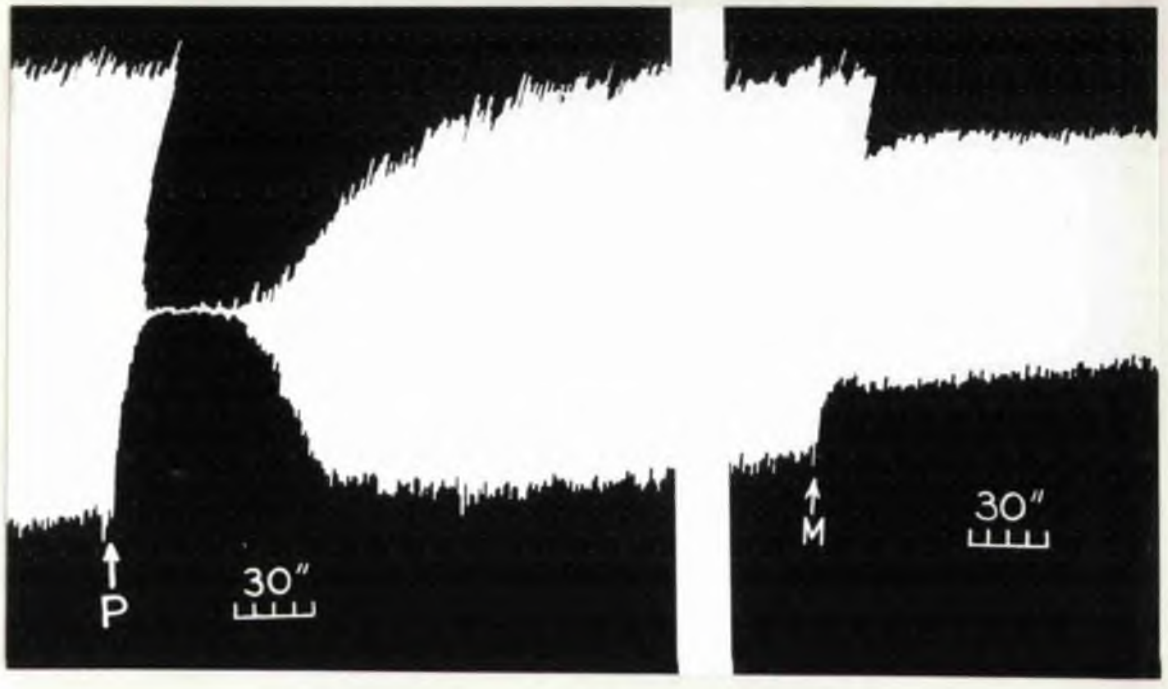

Fig. 64

Reflex response to electrical stimulation of the cut central end of the contralateral sciatic nerve of the spinal cat. Frequency 15 per minute, voltage 50, pulse width 4 msec. Drugs in aqueous solution administered intravenously.

At P, 12 mg. per kg. petaline chloride.

At M, 40 mg. per kg. mephenesin.

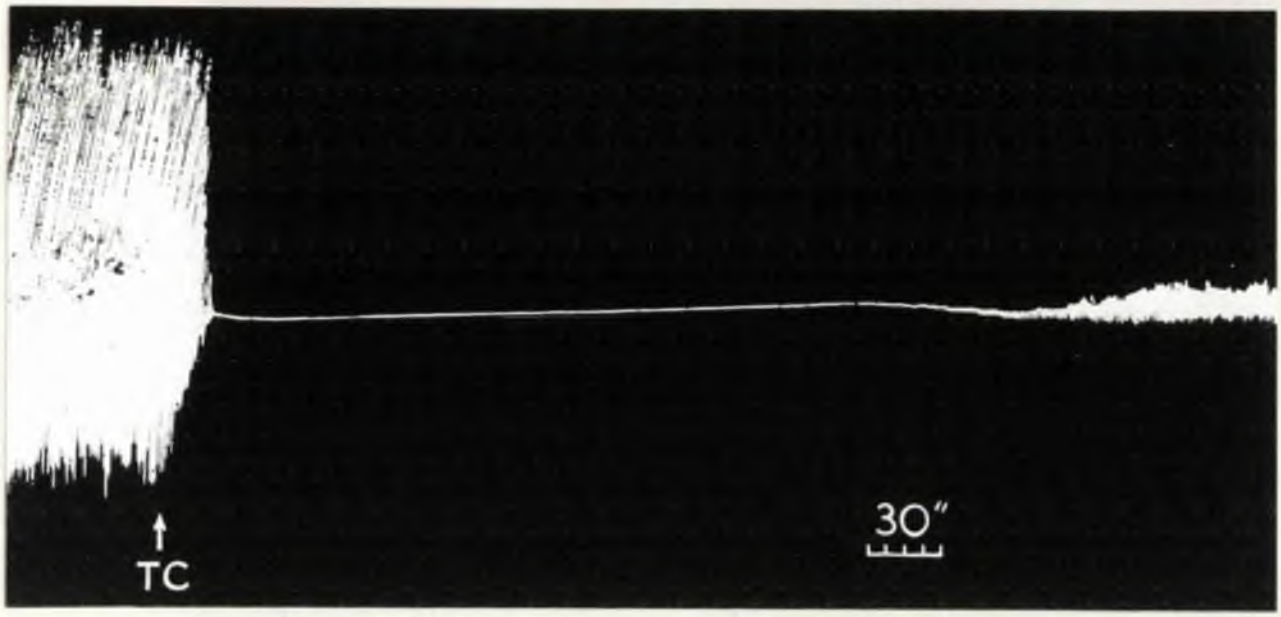

Fig. 65

Reflex response to electrical stimulation of the cut central end of the contralateral sciatic nerve of the spinal cat. Frequency 15 per minute, voltage 50, pulse width 4 msec. Drugs in aqueous solution administered intravenously.

At TC, 0.20 mg. per kg. tubocurarine.

(A) Blood pressure of the anaesthetised cat

Petaline chloride in doses of from 2.0 to 4.0 mg. per kg., produced an immediate fall of blood pressure. The level returned to normal in from 5 to 10 minutes. Fig. 66 (page 334) shows the effect of petaline chloride on the blood pressure of the cat anaesthetised with pentobarbitone. In some experiments there was a slow secondary fall after the blood pressure level had returned to normal. This was only seen with higher doses of petaline chloride.

(B) Blood pressure of the spinal cat

In the spinal cat petaline chloride caused no fall in blood pressure in the dose range (2.0 to 4.0 mg. per kg.) which produced a fall in anaesthetised cats. Even at doses of up to 8.0 mg. per kg. petaline chloride, there was no effect on the blood pressure level of the spinal cats.

(C) Blood pressure of the anaesthetised rat

Petaline chloride in doses of from 20 to 60 μg. per 100 g. caused an immediate but short-lived fall in the blood pressure level of rats anaesthetised with urethane. In some experiments when higher doses of petaline chloride were administered there was a gradual secondary fall. Fig. 67 (page 335) shows the effect of petaline chloride on the blood pressure of an anaesthetised rat.

Petaline chloride in the dose range of from 30 to 40 μg. per 100 g. caused slight antagonism to the pressor effects of adrenaline or noradrenaline (0.05 to 0.10 μg. per 100 g.). Fig./

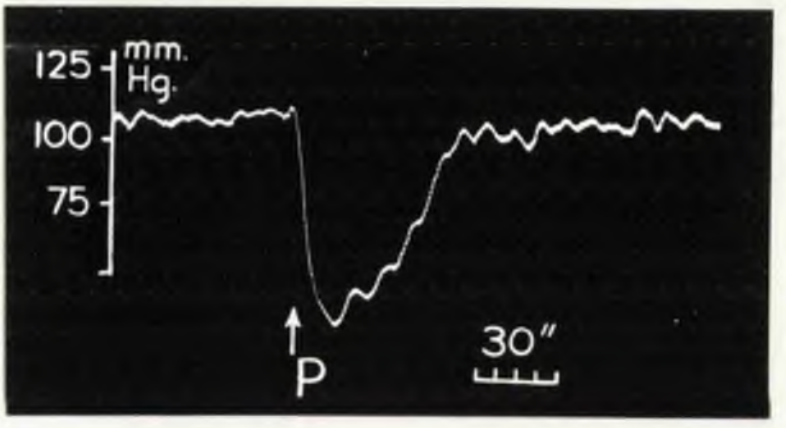

Fig. 66

Record of carotid arterial blood pressure of a pentobarbitone-anaesthetised (60 mg. per kg.) cat.

Drugs in aqueous solution administered intravenously.

At P, 2.0 mg. per kg. petaline chloride.

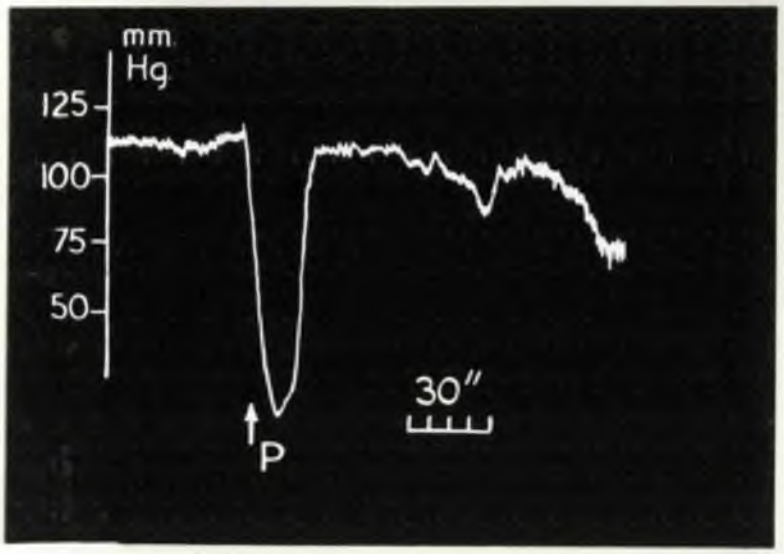

Fig. 67

Record of carotid arterial blood pressure of urethane-anaesthetised (175 mg. per 100 g.) rat.
Drugs in aqueous solution administered intravenously.
At P, 58 µg. per 100 g. petaline chloride.

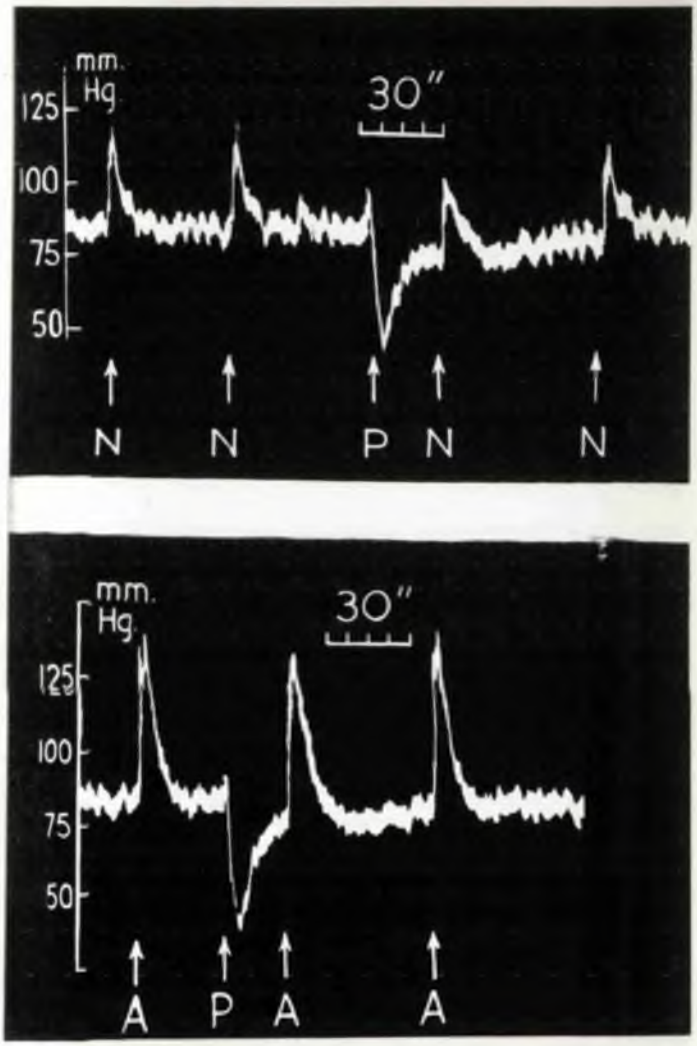

Fig. 68

Record of carotid arterial blood pressure of the methane anaesthetised (175 mg. per 100 g.) rat.

Drugs in aqueous solution administered intravenously.

At N, 0.066 μg. per 100 g. noradrenaline.

At P, 33.3 μg. per 100 g. petaline chloride.

At A, 0.066 μg. per 100 g. adrenaline.

Fig. 68 (page 336) shows the antagonism between petaline chloride and noradrenaline or adrenaline.

(D) Isolated perfused rat hindquarters

Petaline chloride in doses of 2.0 to 3.0 mg. produced slight vasodilatation in the isolated perfused rat hindquarters preparation. In the same dose range it showed slight antagonism to the vasoconstrictor effects of 1.0 μg. noradrenaline or adrenaline (Fig. 69 , page 338). A dose of 5.0 mg. petaline chloride antagonised the vasoconstrictor action of 3.0 mg. barium chloride. Fig. 70 (page 339) shows the antagonism of petaline chloride and barium chloride.

(E) Isolated perfused rabbit and kitten hearts

No differences were noted in the results obtained when the experiments were carried out with either rabbit or kitten hearts. Petaline chloride in doses of from 2.0 to 4.0 mg. caused a slight increase in the rate and amplitude of the ventricular contractions. The increased rate and amplitude, however, returned to normal in a few minutes (Fig.71 , page340). A similar effect was seen when the heart was perfused with 200 μg. per ml. petaline chloride in "double glucose" Locke's solution. Fig. 72 (page 341) shows the effect of perfusion with petaline chloride on the isolated kitten heart.

(F) Isolated guinea-pig auricles

Petaline chloride in the dose range of from 200 to 400 μg. per ml. increased the rate and amplitude of the auricular contractions./

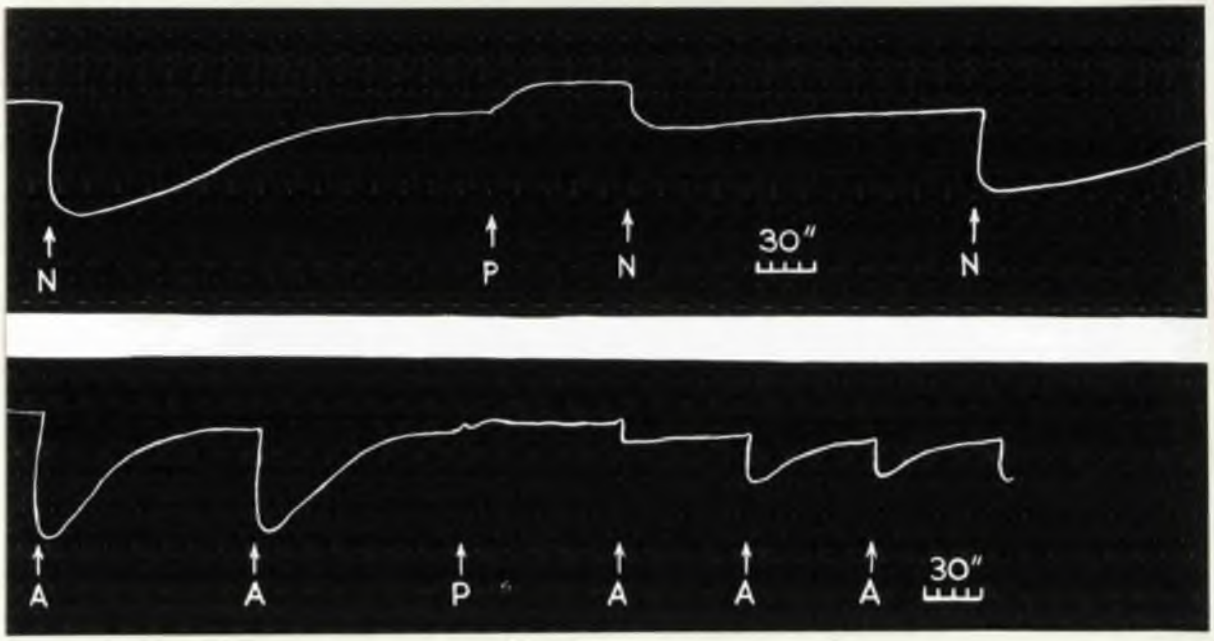

Fig. 69

Perfused rat hindquarters. Perfusion with oxygenated Locke's solution at room temperature. Outflow recorded by Stephenson's outflow recorder.

Drugs in aqueous solution injected into the perfusion cannula.

At N, 1.0 μg. noradrenaline

At A, 1.0 μg. adrenaline

At P, 2.0 mg. petaline chloride.

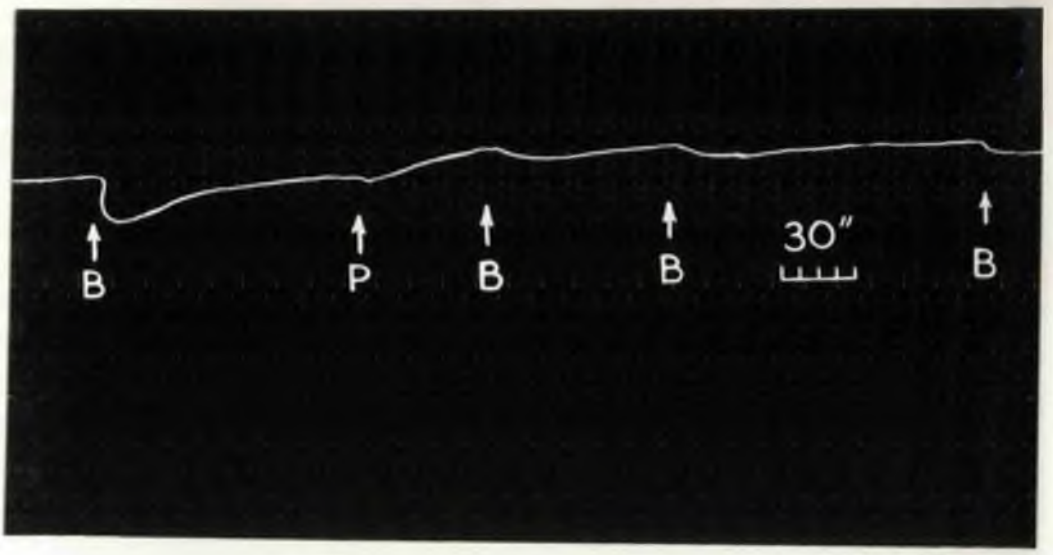

Fig. 70

Perfused rat hindquarters. Perfusion with oxygenated Locke's solution at room temperature. Outflow recorded by Stephenson's outflow recorder.

Drugs in aqueous solution injected into the perfusion cannula.

At B, 3 mg. barium chloride

At P, 5 mg. petaline chloride.

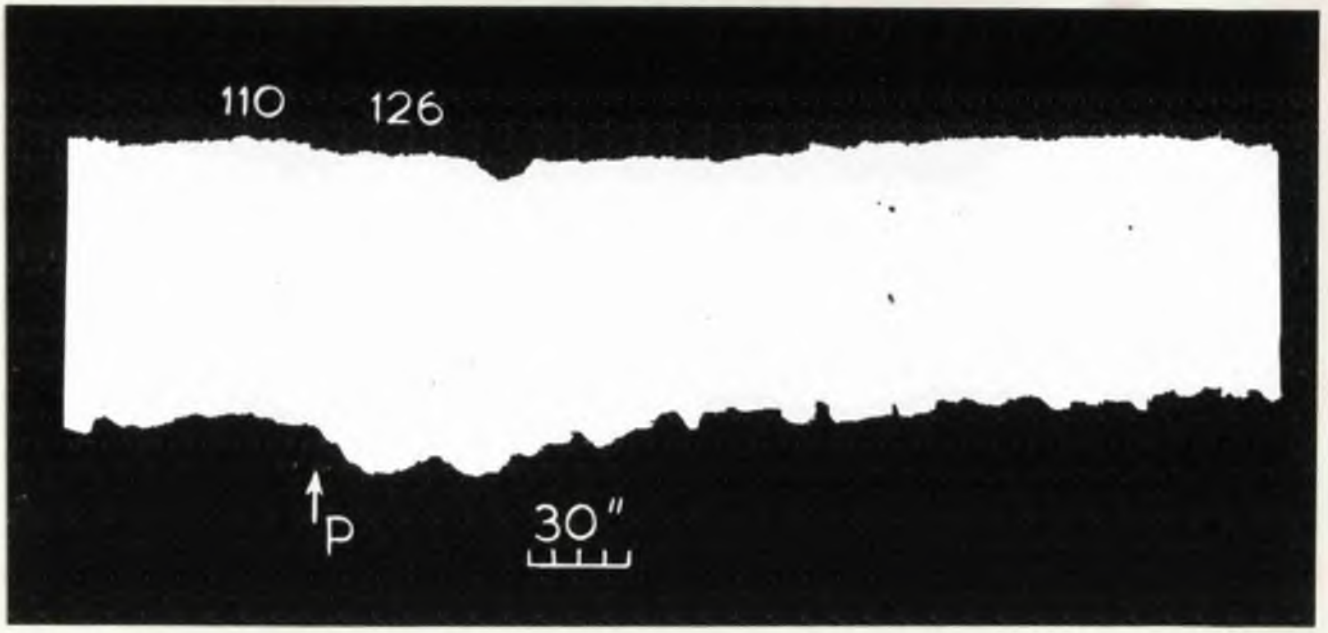

Fig. 71

Contractions of an isolated kitten heart perfused by Langendorff's method with oxygenated "double glucose" Locke's solution at 37°C.

Numbers above the recording indicate rate in beats per minute before and after administration of the drug.

Drug in aqueous solution injected into the perfusion cannula.

At P, 2.0 mg. petaline chloride.

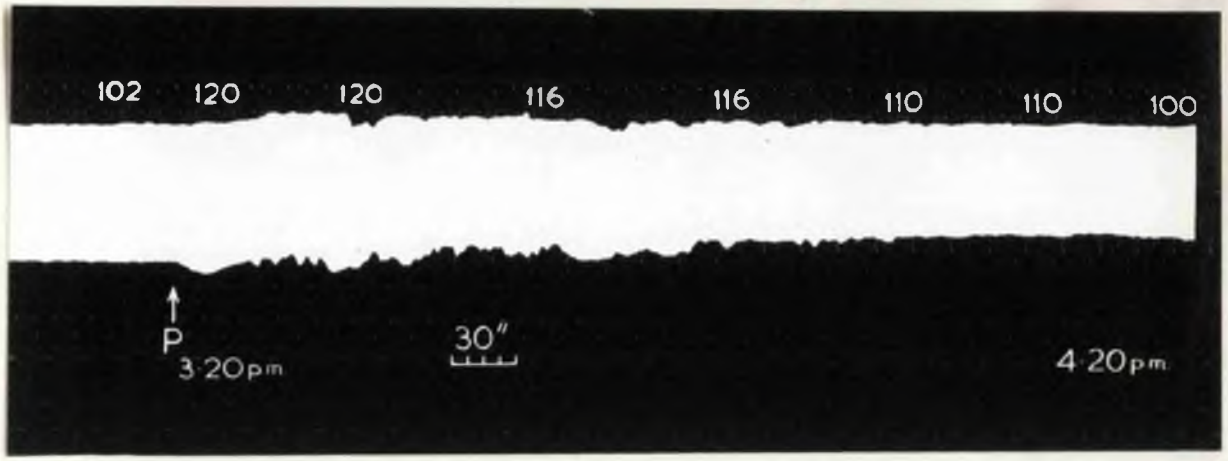

Fig. 72

Contractions of an isolated kitten heart perfused by Langendorff's method with oxygenated "double glucose" Locke's solution at 37°C.

Numbers above the recording indicate the rate in beats per minute before and after administration of the drug.

At P, perfusion started with Locke's solution containing 200 μg. per ml. petaline chloride, and continued for 60 minutes.

contractions. After washing, the rate and amplitude returned to their original levels. Fig. 73 (page 343) shows the results of a typical experiment. Petaline chloride (200 to 400 μg. per ml.) had no antagonism or very slight antagonism, to the effects of 0.10 μg. per ml. of adrenaline or of noradrenaline (Fig. 74 , page 344). Petaline chloride did not show any antagonism or potentiation to the effect of 0.10 μg. per ml. histamine (Fig. 75 , page345). It did, however, antagonise the effect of 0.02 μg. per ml. acetylcholine (Fig. 76 , page 346).

When the auricles were perfused with Locke's solution containing half the usual amount of calcium chloride, the amplitude and rate were reduced and the auricles gradually came to rest. Petaline chloride (200 μg. per ml.) added to the bath restored the rate to normal and the amplitude to supranormal levels. Fig. 77 (page 347) shows the results of a typical experiment. Similar results were obtained with 10 μg. per ml. digoxin (Fig. 77 , page 347).

Effects on Smooth Muscle

(A) Isolated guinea-pig ileum

Petaline chloride when added in small doses to the bath, did not produce any direct effect on the isolated guinea-pig ileum. However, large doses (1.0 to 2.0 mg. per ml.) of petaline chloride caused a slight increase in the spontaneous activity. In doses of 100 μg. to 1.0 mg. per ml. petaline chloride/

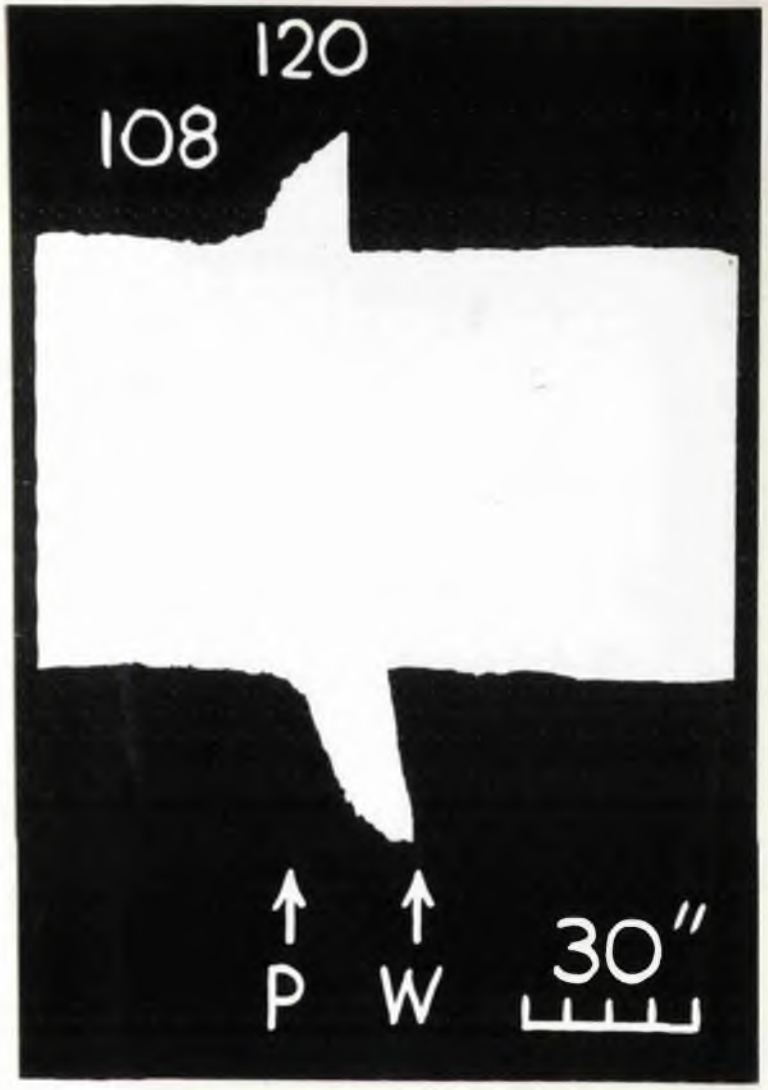

Fig. 73

Isolated guinea-pig auricles. Record of spontaneous contractions.

Bath 10 ml., temperature 29°C, bath fluid, oxygenated Locke's solution.

Numbers above the recording indicate rate in beats per minute before and after administration of the drug.

Drug in aqueous solution in contact with the auricles for 60 seconds.

At P, 200 μg. per ml. petaline chloride.

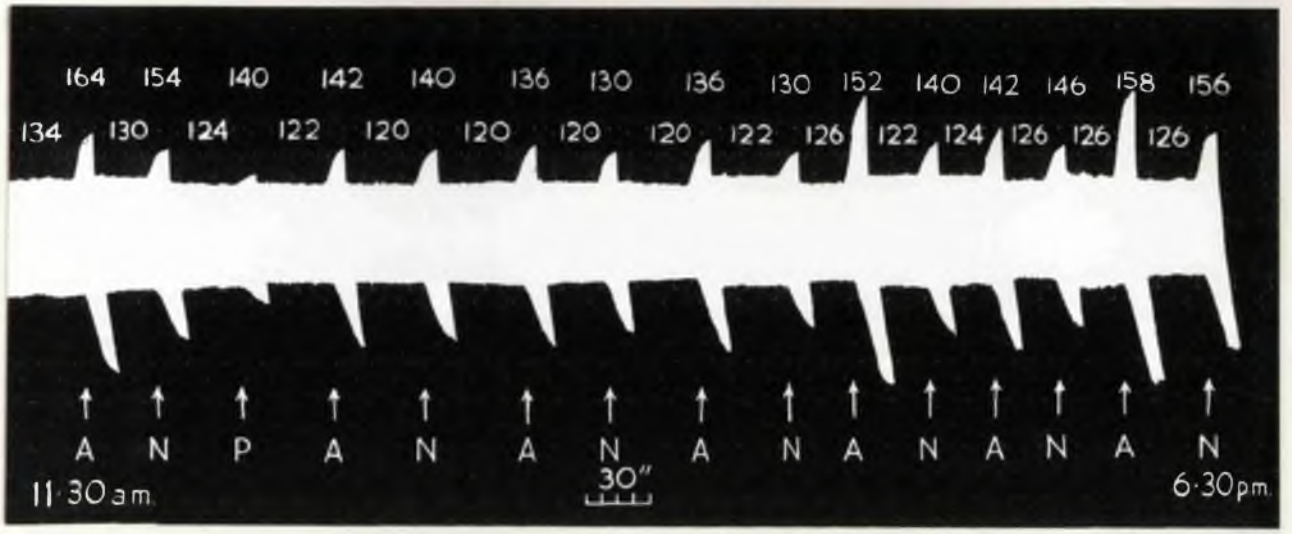

Fig. 74

Isolated guinea-pig auricles. Record of spontaneous contractions.

Bath 10 ml., temperature 29°C., bath fluid oxygenated Locke's solution.

The lower row of figures above the recording indicates the rate in beats per minute before and in between addition of drugs.

The upper row of figures above the recording indicates the rate in beats per minute after addition of drugs.

Drugs in aqueous solution in contact with the auricles for 60 seconds.

At A, 0.10 μg. per ml. adrenaline.

At N, 0.10 μg. per ml. noradrenaline.

At P, 200 μg. per ml. petaline chloride.

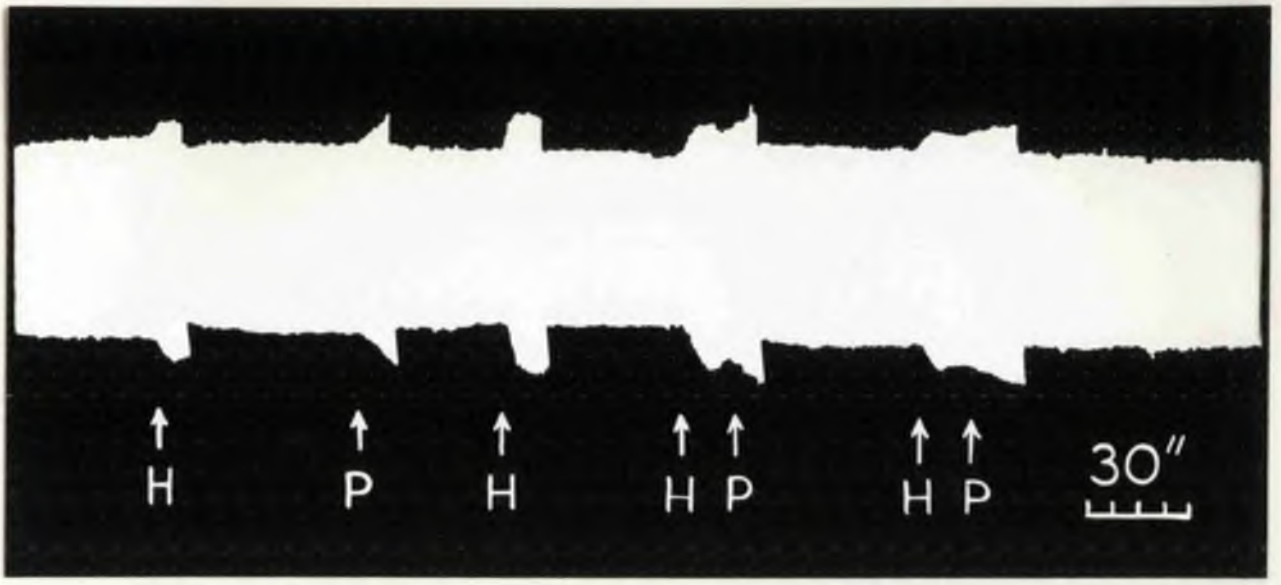

Fig. 75

Isolated guinea-pig auricles. Record of spontaneous contractions.

Bath 10 ml., temperature 29°C, bath fluid oxygenated Locke's solution.

Drugs in aqueous solution in contact with the auricles for 60 seconds.

At H, 0.10 μg. per ml. histamine.

At P, 200 μg. per ml. petaline chloride.

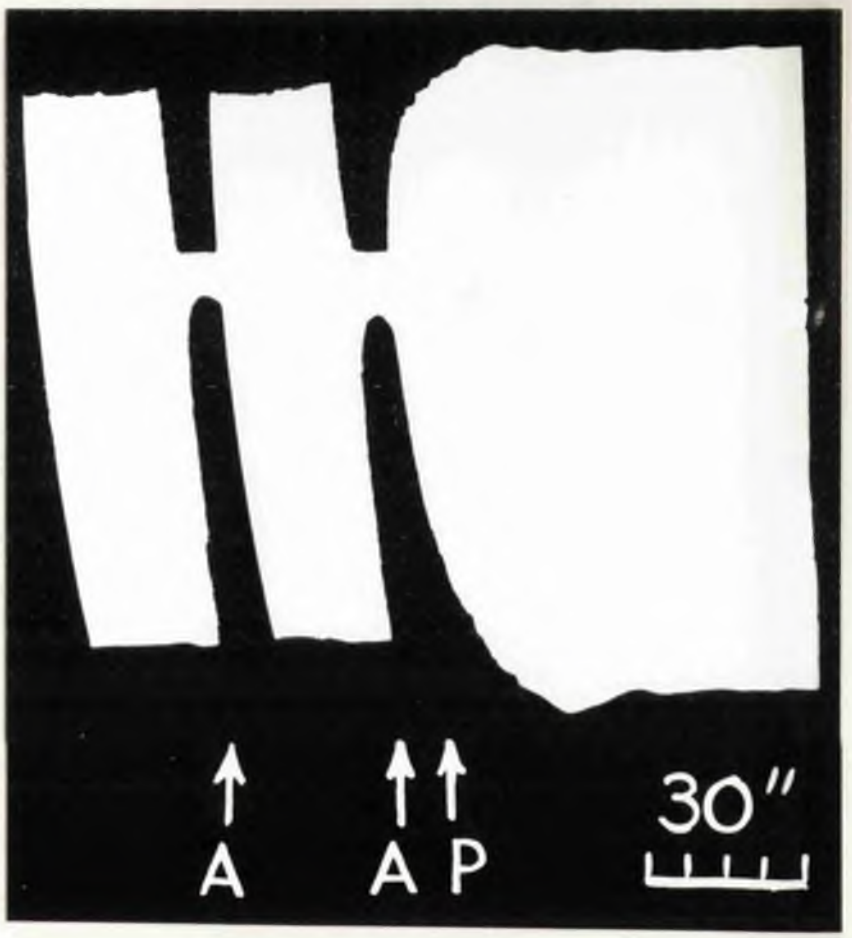

Fig. 76

Isolated guinea-pig auricles. Record of spontaneous contractions.

Bath 10 ml., temperature 29°C, bath fluid, oxygenated Locke's solution.

Drugs in aqueous solution in contact with the auricles for 60 seconds.

At A, 0.02 μg. per ml. acetylcholine.

At P, 200 μg. per ml. petaline chloride.

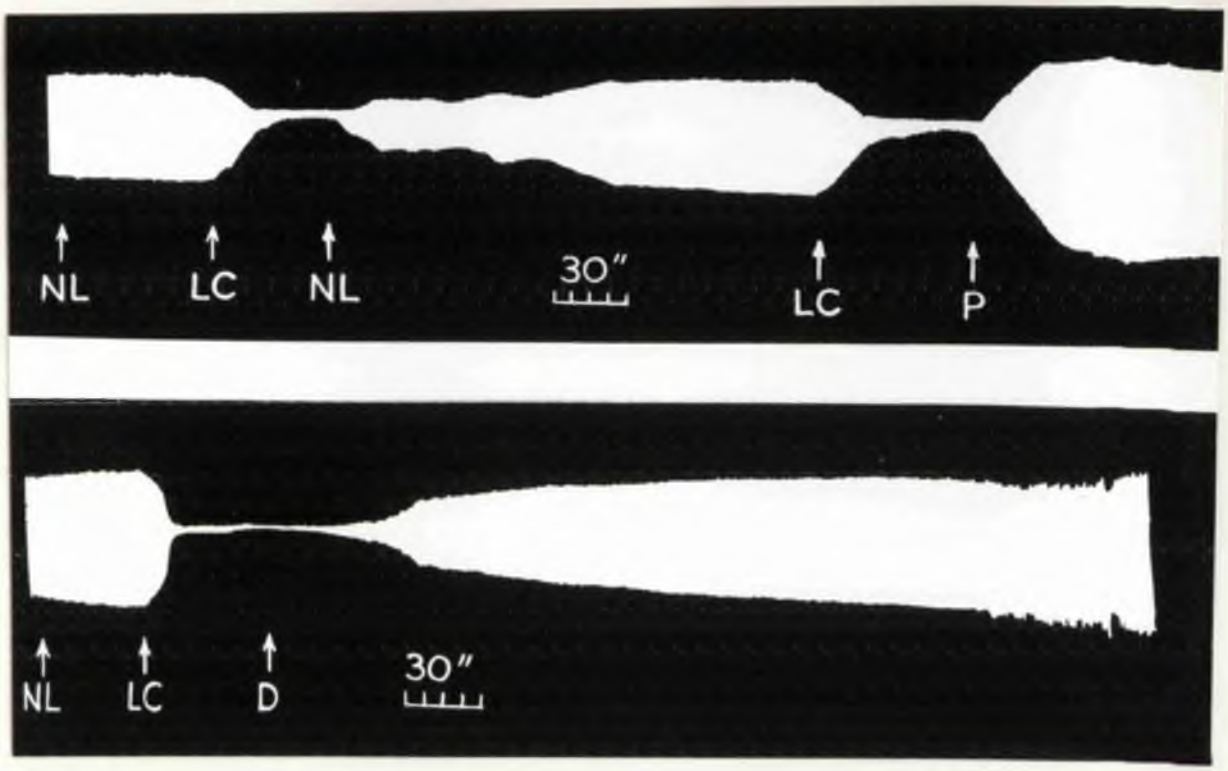

Fig. 77

Isolated guinea-pig auricles. Record of spontaneous contractions.

Bath 10 ml., temperature 29°C, bath fluid, oxygenated Locke's solution.

At NL, Locke's solution.

At LC, the bath fluid changed to "half calcium" Locke's solution.

At P, 200 μg. per ml. petaline chloride.

At D, 10 μg. per ml. digoxin.

chloride antagonised the responses of 1.0 μg. per ml. acetylcholine (Fig. 78 , page 349). Similar doses of petaline chloride antagonised the responses of 0.3 μg. per ml. histamine (Fig. 79 , page 350), 1.0 μg. per ml. 5-hydroxy-tryptamine (Fig. 80 , page 351), 0.6 mg. per ml. barium chloride (Fig. 81 , page 352) and 5.0 mg. per ml. potassium chloride (Fig. 82 , page 353). These effects were reversible on washing and there was a graded inhibition according to the dose.

(B) Isolated rat uterus

Petaline chloride in doses of from 20 to 60 μg. per ml. reduced the height of contractions produced by 0.10 to 0.50 μg. per ml. acetylcholine. There was a graded inhibition according to the dose and recovery to control levels was usually complete. No direct effect was seen. Fig. 83 (page 354) shows the results of a typical experiment.

(C) Toxic effects on the rabbit

Only two rabbits were used. One weighing 2.75 kg. was given 30 mg. petaline chloride by intravenous injection. It immediately developed convulsive jerky movements, respiration stopped and about 3 minutes later the heart ceased to beat. After death the animal was flaccid. Similar effects were seen in a second rabbit weighing 2.25 kg. which was given 35 mg. of petaline chloride. These results are similar to those of McShefferty and his colleagues (18).

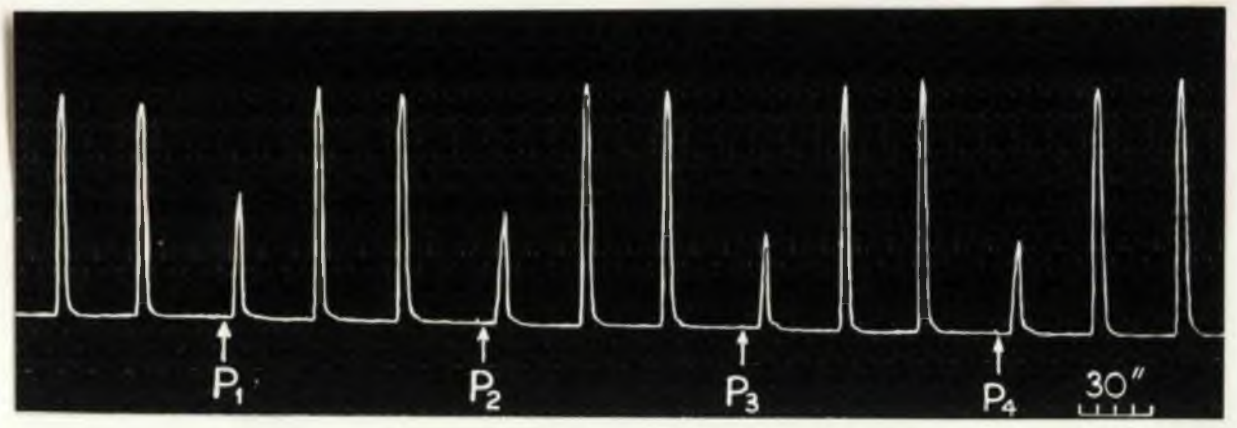

Fig. 78

Contractions of an isolated strip of guinea-pig ileum suspended in 5 ml. of oxygenated Tyrode's solution at 37°C. All contractions due to 1.0 μg. per ml. acetylcholine. Addition of acetylcholine was preceded 30 seconds earlier by:-

At P_1, 400 μg. per ml. petaline chloride.
At P_2, 600 μg. per ml. " "
At P_3, 800 μg. per ml. " "
At P_4, 1 mg. per ml. " "

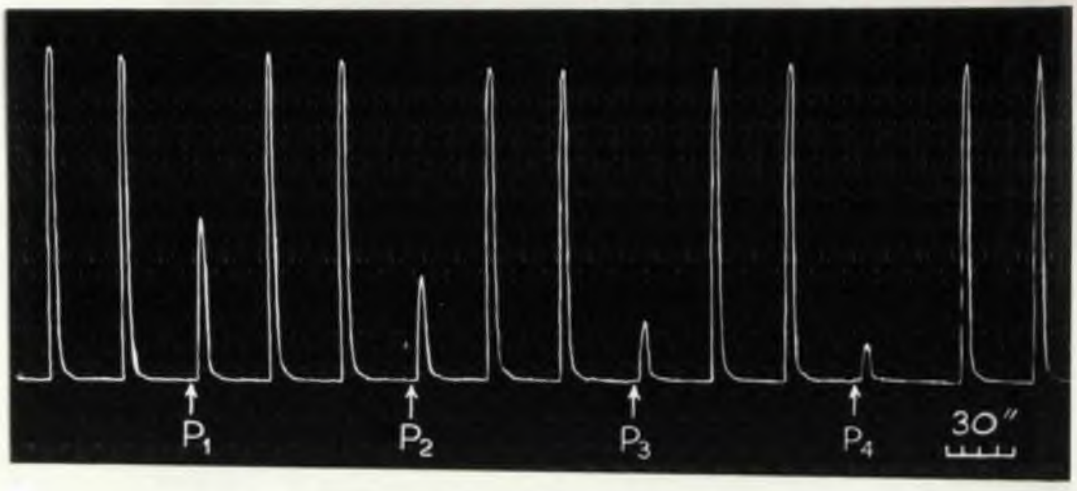

Fig. 79

Contractions of an isolated strip of guinea-pig ileum suspended in 5 ml. of oxygenated Tyrode's solution at 37°C. All contractions due to 0.30 μg. per ml. histamine. Addition of histamine was preceded 30 seconds earlier by:-

At P_1, 200 μg. per ml. petaline chloride.
At P_2, 300 μg. per ml. " "
At P_3, 400 μg. per ml. " "
At P_4, 500 μg. per ml. " "

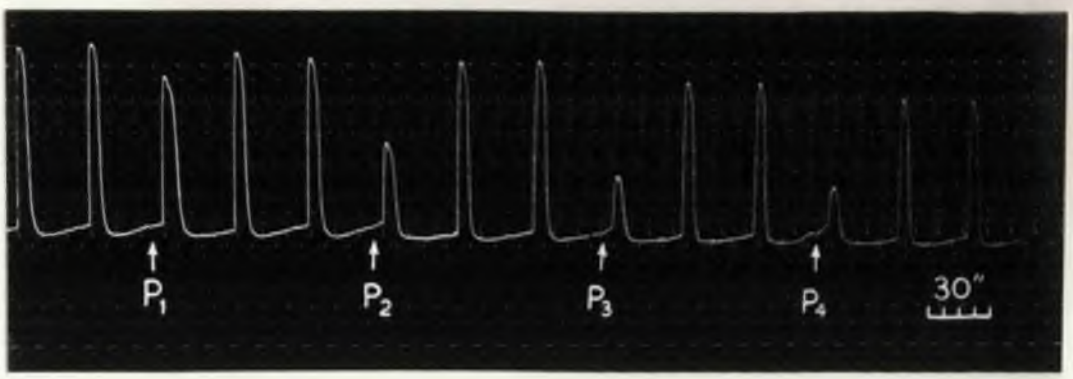

Fig. 80

Contractions of an isolated strip of guinea-pig ileum suspended in 5 ml. of oxygenated Tyrode's solution at 37°C. All contractions due to 1.0 μg. per ml. 5-hydroxytrypamine. Addition of 5-hydroxytryptamine was preceded 30 seconds earlier by:-

At P_1, 100 μg. per ml. petaline chloride.
At P_2, 200 μg. per ml. " "
At P_3, 300 μg. per ml. " "
At P_4, 400 μg. per ml. " "

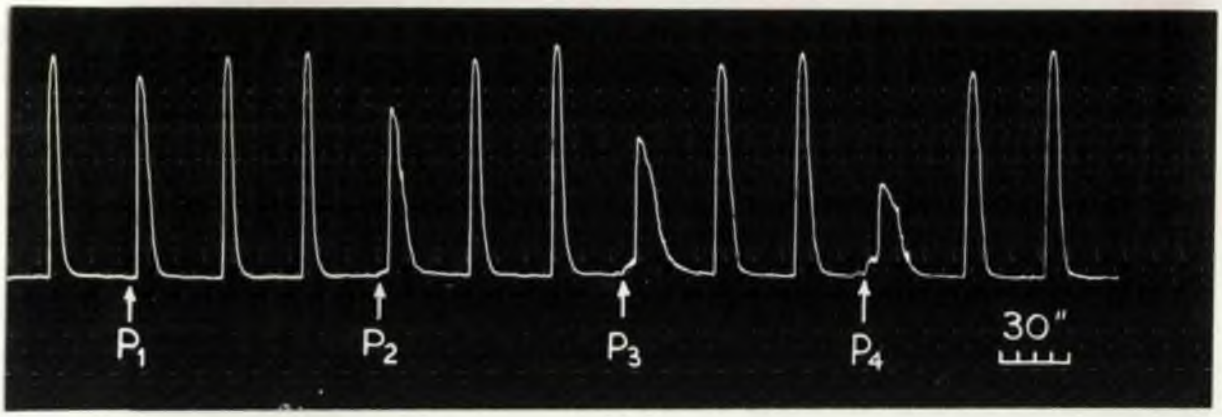

Fig. 81

Contractions of an isolated strip of guinea-pig ileum suspended in 5 ml. of oxygenated Tyrode's solution at 37°C. All contractions due to 0.6 mg. per ml. barium chloride. Addition of barium chloride was preceded 30 seconds earlier by:-

At P_1, 400 µg. per ml. petaline chloride.
At P_2, 600 µg. per ml. " "
At P_3, 800 µg. per ml. " "
At P_4, 1.0 mg. per ml. " "

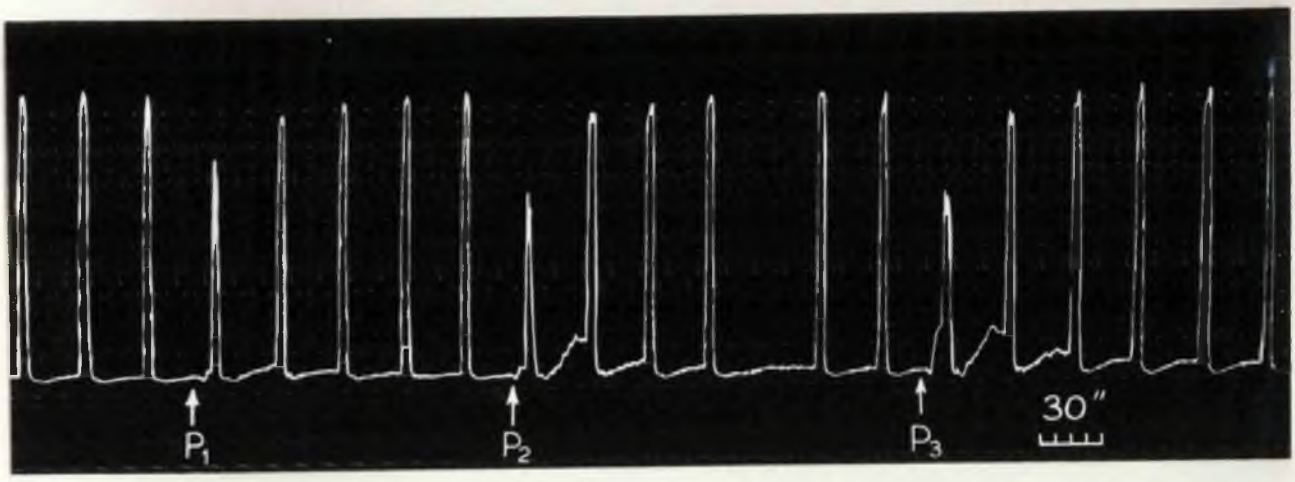

Fig. 82

Contractions of an isolated strip of guinea-pig ileum suspended in 5 ml. of oxygenated Tyrode's solution at 37°C. All contractions due to 5.0 mg. per ml. potassium chloride. Addition of potassium chloride was preceded 30 seconds earlier by:-

At P_1, 600 μg. per ml. petaline chloride.
At P_2, 800 μg. per ml. " "
At P_3, 1.0 mg. per ml. " "

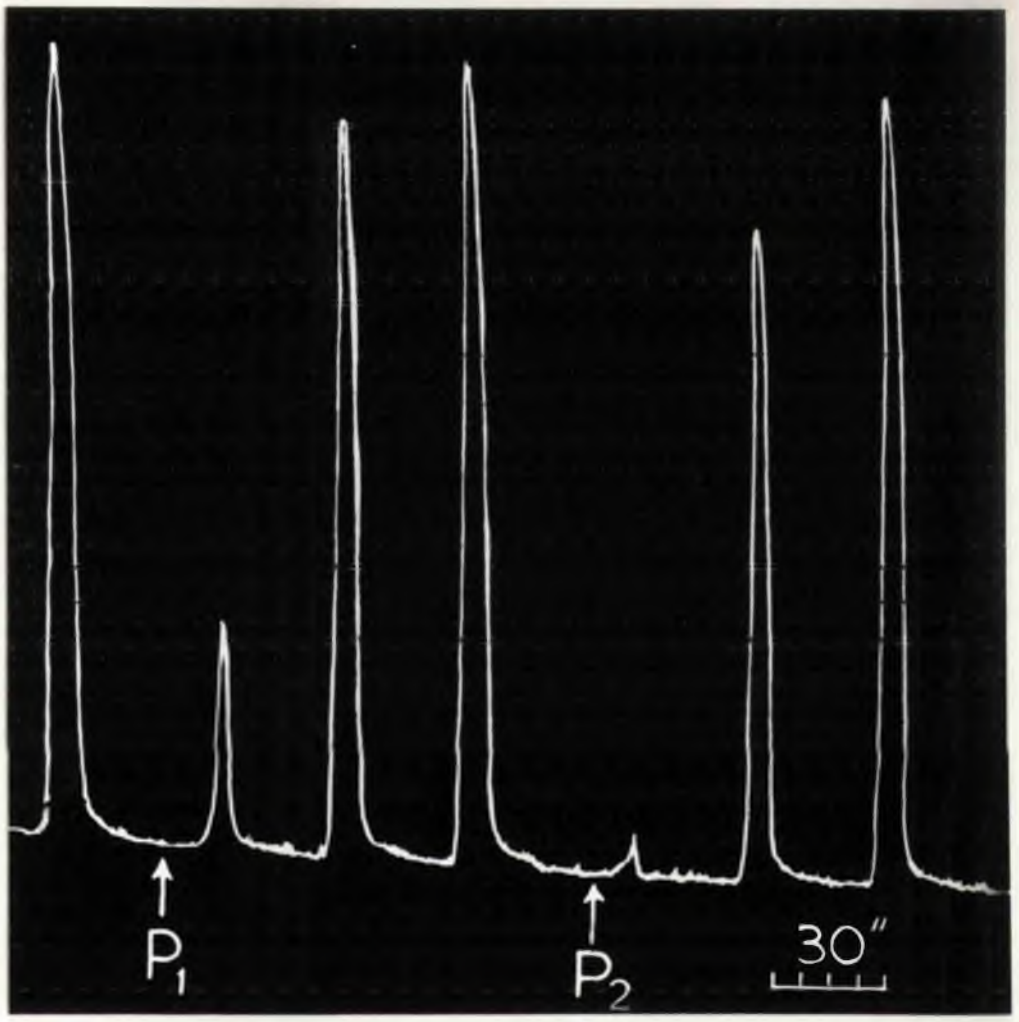

Fig. 83

Contractions of isolated rat uterus suspended in 5 ml. of de Jalon's solution gassed with 95 per cent oxygen and 5 per cent carbon dioxide at a temperature of 29°C.
All contractions due to 0.50 μg. per ml. acetylcholine.
Addition of acetylcholine was preceded 30 seconds earlier by:-

At P_1, 40 μg. per ml. petaline chloride.
At P_2, 60 μg. per ml. " "

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CHAPTER IV

DISCUSSION

Petaline chloride, an alkaloidal salt obtained from Leontice leontopetalum Linn, is a potent convulsant which in mice is apparently 5 to 7 times as powerful as leptazol but is only one-third as active as picrotoxin. The CD50 of petaline chloride in the mouse was 6.6 mg. per kg. (Fig. 44, page 303), whereas those of leptazol and picrotoxin were 40 mg. per kg. (Fig. 44, page 303) and 1.8 mg. per kg. (Fig. 45, page 304) respectively. The convulsant effects of petaline chloride in mice were similar to those observed by McShefferty and his co-workers (1) who in mice obtained varying degrees of stimulation of the central nervous system with a dose of 3 mg. per kg. of petaline chloride. These workers (1) using mice also observed respiratory distress, cardiac disturbances and coma preceded by powerful spasms with 28 mg. per kg. leonticine, another alkaloid obtained from Leontice leontopetalum Linn. In the rabbit petaline chloride produced immediate convulsive movements followed by death, the speed of death depending upon the dose. This confirms the findings of McShefferty and his co-workers (1). Thus, it appears that petaline chloride has distinct convulsant properties. Certain alkaloids from plants of the Berberidaceae and related families have also been reported (2-7) to exhibit convulsant activity in frogs, mice and rabbits.

Lower/

Lower doses of petaline chloride were found to reduce the convulsant activity of a subsequent dose of leptazol. It was observed that by pre-treating mice with 2.5 or 5 mg. per kg. of petaline chloride the CD50 of leptazol was 42.8 or 43.5 mg. per kg. respectively (Fig. 48, page 309), but when the mice were pre-treated with 8 mg. per kg. of petaline chloride the CD50 of leptazol was found to be 35 mg. per kg. (Fig. 48, page 309).

The LD50 of petaline chloride and leptazol were 9.2 and 56 mg. per kg. respectively in mice (Fig. 46, page 305). On pre-treatment of the mice with 2.5 or 5 mg. per kg. of petaline chloride the LD50 of leptazol was now found to be 64 or 70.7 mg. per kg. respectively (Fig. 49, page 310), but on pre-treatment with 8 mg. per kg. petaline chloride the LD50 of leptazol was altered to 48 mg. per kg. (Fig. 49, page 310). Thus it appears that lower doses of petaline chloride may give some protection but higher doses increase both the convulsant activity and toxicity of leptazol.

Similarly, smaller doses of petaline chloride were apparently capable of conferring some protection in mice against electro-shock seizures. It was found that petaline chloride in doses of 1.6, 2.5 or 3.3 mg. per kg. protected mice from subsequent electro-shock by 10, 25 or 37.5 per cent (Fig. 50, page 312), but a dose of 5 mg. per kg. petaline chloride gave only 20 per cent protection (Fig. 50, page 312). It seems that lower doses of petaline chloride may give some protection against electro-shock seizures but larger doses reduced/

reduced the protection conferred. When compared with phenobarbitone, petaline chloride was found to be much less potent in protecting mice from electro-shock seizures.

Petaline chloride has muscle relaxant properties similar to those of tubocurarine, but it is much less potent than tubocurarine. On the cat gastrocnemius muscle-sciatic nerve preparation, petaline chloride was from twenty to twenty-five times weaker than tubocurarine (Fig. 51, page 314). On the isolated rat phrenic nerve-diaphragm preparation petaline chloride was found to be more or less equipotent with gallamine (Fig. 57, page 322). On the isolated frog rectus abdominis muscle preparation petaline chloride had about 50 per cent of the potency of gallamine (Figs. 59, 60, pages 325, 326). The latter result is similar to that obtained by McShefferty and his co-workers (1).

Petaline chloride depressed the patellar tendon reflex (Fig. 62, page 329) and the crossed extension reflex (Fig. 64, page 331) and was apparently more effective than mephenesin with respect to the latter (Fig. 64, page 331). Similar effects on the patellar tendon reflex (Fig. 63, page 330) and crossed extension reflex (Fig. 65, page 332) were seen with tubocurarine in doses which produced neuromuscular block. The depressant effect on the patellar tendon reflex and the crossed extension reflex produced by petaline chloride may be due to its muscle relaxant properties.

Petaline chloride caused an immediate but short lived fall in the blood pressure levels of the anaesthetised cat (Fig. 66, page 334)/

(Fig. 66, page 334) or rat (Fig. 67, page 335) and caused slight antagonism to the pressor effects of adrenaline and noradrenaline (Fig. 68, page 336). It caused some reduction in the height of the contraction of the nicitating membrane in response to electrical stimulation of the preganglionic fibres of the cervical sympathetic in the anaesthetised cat (Fig. 61, page 327). On the isolated perfused rat hind-quarters, petaline chloride produced slight vasodilatation and antagonism to the vasoconstrictor effects of adrenaline, noradrenaline (Fig. 69, page 338) or barium chloride (Fig. 70, page 339). The rate and amplitude of the contractions of the isolated kitten or rabbit heart were increased by petaline chloride (Figs. 71, 72, pages 340, 341). Similar results were obtained in isolated guinea-pig auricles (Fig. 73, page 343) where it did not alter the characteristic actions of adrenaline, noradrenaline (Fig. 74, pages 344) or histamine (Fig. 75, page 345) but antagonised the effect of acetylcholine (Fig. 76, page 346). When the auricles were perfused with Locke's solution containing half the usual amount of calcium chloride, the amplitude and the rate were reduced and the auricles gradually came to rest. Petaline chloride added to the bath restored the rate to normal and the amplitude to supranormal levels (Fig. 77, page 347). Similar effects were seen with digoxin (Fig. 77, page 347).

In the isolated guinea-pig ileum petaline chloride depressed the stimulant actions of acetylcholine (Fig. 78, page 349), histamine (Fig. 79, page 350), 5-hydroxytrymptamine (Fig. 80, page 351), barium chloride (Fig. 81, page 352) and/

and potassium chloride (Fig. 82, page 353). Similarly petaline chloride depressed the stimulant action of acetylcholine on the rat uterus (Fig. 83, page 354).

It is reported (1) that the "juice" prepared by pounding the fresh tubers of Leontice leontopetalum Linn has been used as a native remedy for curing epilepsy. In Chapter I of this part of the thesis the procedure of the treatment has been described. During the period of administration of this "juice" the patient is said to have convulsions which recur every 2 or 3 minutes. The convulsant properties of petaline chloride, an alkaloidal salt obtained from Leontice leontopetalum Linn confirm the convulsant activity of the "juice". Petaline chloride had slight anti-convulsant activity. It produced slight protection to leptazol-induced convulsions and electro-shock seizures in mice. It also had tubocurarine-like muscle relaxant properties. When these actions are taken in conjunction with its cardiovascular actions, in particular the effects on isolated auricles depressed in a low calcium medium, then there may be some basis for its use in folk medicine. It must not, however, be forgotten that the "juice" used undoubtedly contains other substances (1) and the disease reported to have been treated may not have been correctly diagnosed by the native physicians or the sufferer's relatives. Unfortunately other active substances obtained from Leontice leontopetalum Linn (1) were not available for pharmacological investigation at the time of writing this thesis. It is hoped that it will be possible to complete this investigation in the future. The results presented/

presented do not in the writer's view make out any case for using petaline chloride in epilepsy, rather the converse is true.

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CHAPTER V

SUMMARY

In Chapter I of this part of the thesis the earlier literature on the plant genus Leontice has been summarised, and the pharmacognosy of Leontice leontopetalum Linn. described. A description of the method of preparation of the "juice" from the tubers of Leontice leontopetalum Linn. and of its administration for the treatment of epilepsy by native physicians has been given. A short account of the chemical investigation of the plant genus Leontice along with the method used for the isolation of the alkaloidal salt petaline chloride from Leontice leontopetalum Linn. has been described. Pharmacological properties of the alkaloids obtained from the plants of the Berberidaceae and related families and those of Leontice leontopetalum Linn. have been summarised.

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

In Chapter III of this part, the results of the pharmacological investigation of petaline chloride are described. Petaline chloride was found to be a convulsant substance apparently five to seven times as powerful as leptazol but only one-third as potent as picrotoxin. At lower dose levels it reduced the convulsant activity of leptazol and apparently gave/

gave some protection from electrically induced seizures in mice. It had tubocurarine-like muscle relaxant activity and depressed the patellar tendon reflex and the crossed extension reflex. It caused a fall of blood pressure in the anaesthetised cat or rat and increased the rate and amplitude of the isolated intact heart or auricles. On the smooth muscle of the intestine or uterus it antagonised the contractions produced by different spasmogens.

In Chapter IV of this part, the properties of petaline chloride are discussed in relation to the use of the "juice" prepared from tubers of Leontice leontopetalum Linn. in folk medicine.

It is concluded that petaline chloride is a powerful convulsant poison and therefore of very dubious value in epilepsy.

A P P E N D I X I

A P P E N D I X I

Frog sartorius muscle preparation

Experiments were carried out to see whether the drugs used in the experiments described in Part I of this thesis had any direct effect upon the isolated frog sartorius muscle at the dose levels used in the tracer studies. The method adopted was similar to that described in Part II of this thesis for experiments on the isolated frog rectus abdominis muscle (page 270).

Dissection was carried out as described earlier (page 82). Common frogs (Rana temporaria) unselected as to age or sex were stunned by a sharp blow on the base of the head and decapitated. A pithing needle was passed down the spinal canal to destroy the spinal cord and so to prevent reflex movements. The skin covering both thighs and pelvic regions was reflected to expose both ends of the two sartorius muscles. Each muscle was dissected, 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 as close to the pelvis as possible. The muscle was then suspended in a 5 ml. or 10 ml. organ bath by means of two threads, one 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 platinum wire at the bottom of the oxygen delivery/

delivery tube. The long thread on the other end was tied to a frontal point writing lever which recorded the contractions of the muscle on a moving smoked surface. The bath contained oxygenated Frog Ringer's solution at room temperature. Drugs in aqueous solution were added directly to the bath and the contraction recorded for 60 or 90 seconds. In experiments with ryanodine the drug was kept in contact with the muscle for from 3 to 5 minutes.

R E S U L T S

The following drugs caused contraction (or contracture) of the isolated frog sartorius muscle - decamethonium (100 to 300 μg. per ml.), nicotine (0.5 to 1 mg. per ml.), neostigmine (50 to 200 μg. per ml.), suxamethonium (2 to 10 mg. per ml.), carbachol (2 to 10 mg. per ml.) and ryanodine (20 to 100 μg. per ml.), (Figs. 84, 85). The effect of ryanodine was delayed by 2 or 3 minutes.

The following drugs did not show any direct effect on the isolated frog sartorius muscle - edrophonium (0.5 to 2 mg. per ml.), protoveratrine A (200 μg. to 1 mg. per ml.), protoveratrine B (200 μg. to 1 mg. per ml.), tubocurarine and gallamine.

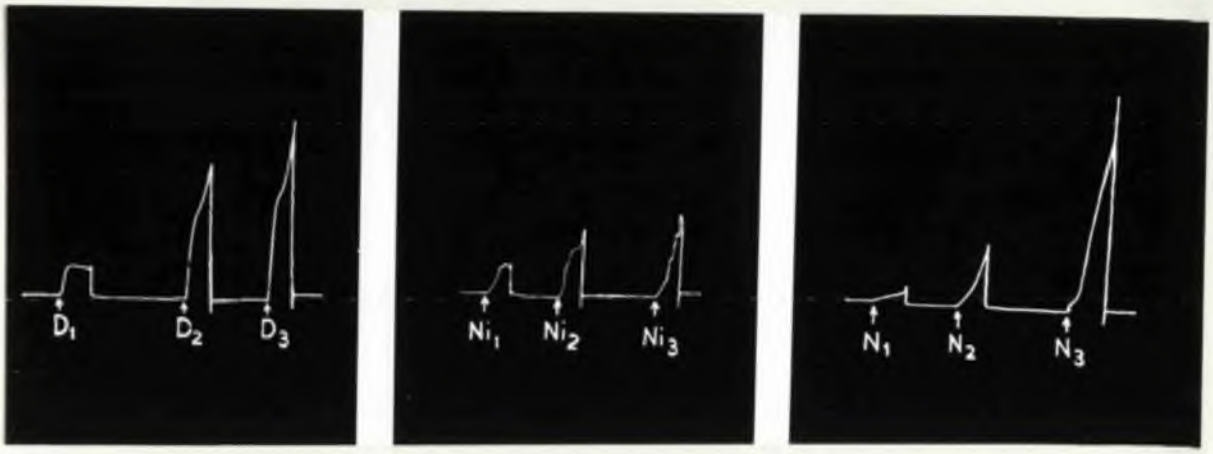


Fig. 84.

Isolated frog sartorius muscle preparations.
Bath 5 ml.; bath fluid, oxygenated Frog Ringer's solution at room temperature.
Contractions due to decamethonium, nicotine and neostigmine in contact with the muscle for 90 seconds.

At D_1, 100 μg. per ml. decamethonium.
At D_2, 200 μg. per ml. "
At D_3, 300 μg. per ml. "
At Ni_1, 0.5 mg. per ml. nicotine
At Ni_2, 0.75 mg. per ml. "
At Ni_3, 1.0 mg. per ml. "
At N_1, 50 μg. per ml. neostigmine
At N_2, 100 μg. per ml. "
At N_3, 200 μg. per ml. "

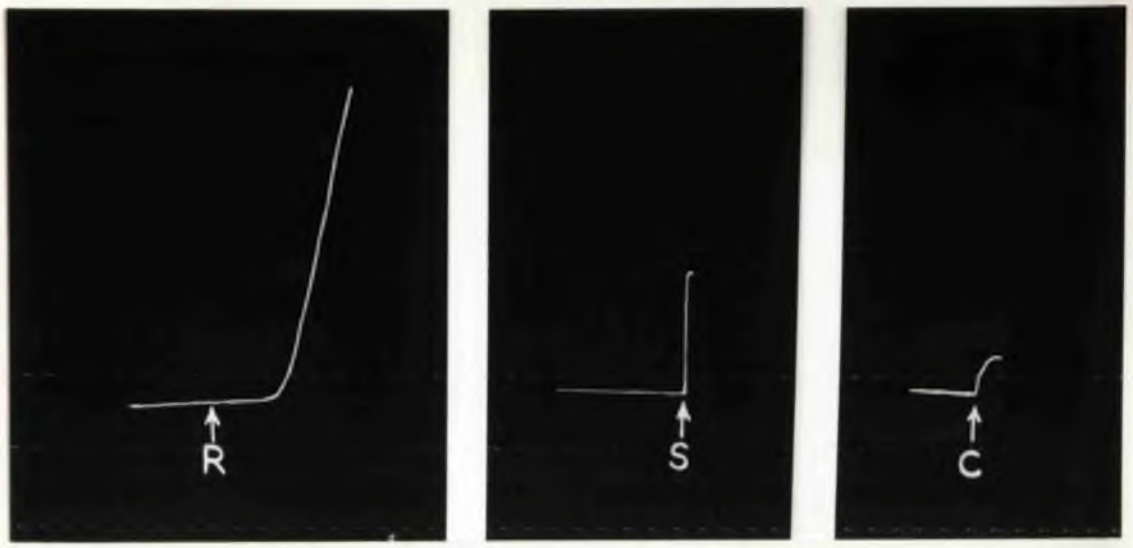


Fig. 85

Isolated frog sartorius muscle preparations.
Bath 5 ml.; bath fluid, oxygenated Frog Ringer's solution at room temperature.
Contractions due to ryanodine for 3 minutes and suxamethonium and carbachol for 60 seconds.

At R, 100 μg. per ml. ryanodine
At S, 2.5 mg. per ml. suxamethonium
At C, 2 mg. per ml. carbachol

TRACER STUDIES

A series of experiments was carried out to see if there was any effect upon the flux of ions in the isolated frog sartorius muscle when the control solutions of the drugs obtained commercially were used. Control solutions of the following commercial drugs were used - suxamethonium, decamethonium, neostigmine, edrophonium and gallamine. Experiments were carried out exactly as has been described earlier (pages 82-93) to study the uptake and release of calcium-47 and potassium-42 and sodium-24 uptake. Along with each drug control solution a control experiment was carried out using Frog Ringer's solution.

R E S U L T S

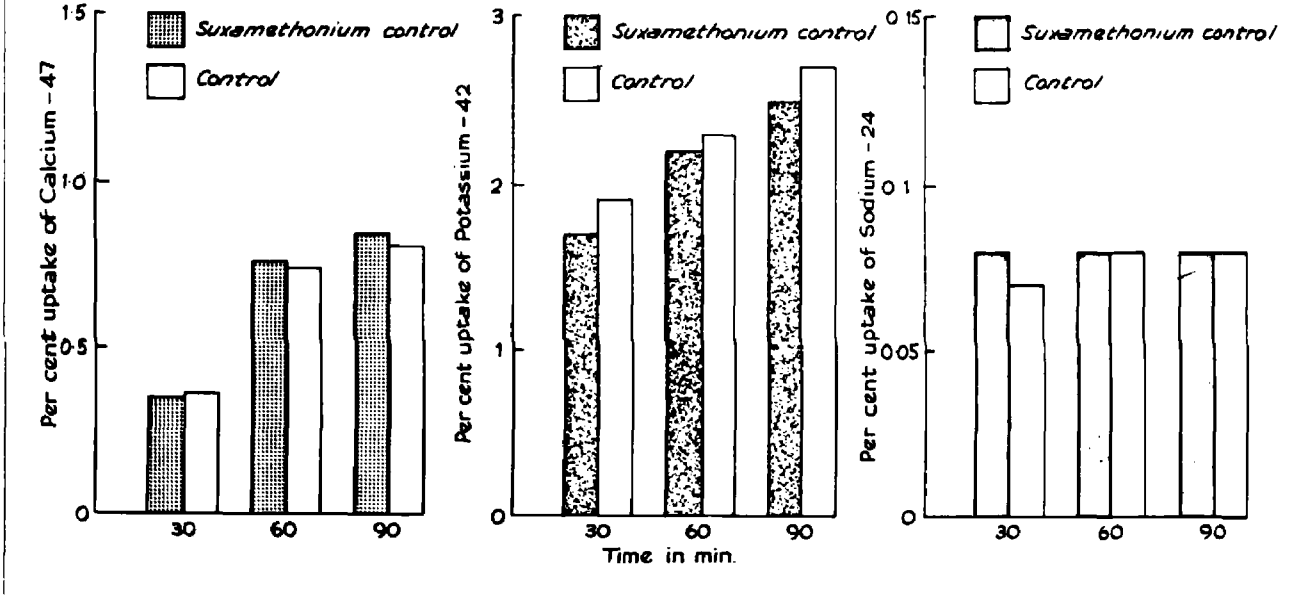
Suxamethonium control solution did not cause any apparent effect on the uptake and release of calcium-47 or potassium-42 or in sodium-24 uptake (Figs. 86, 87, 88).

Decamethonium control solution did not cause any apparent effect on the uptake and release of calcium-47 or potassium-42 or in sodium-24 uptake (Figs. 89, 90, 91).

Neostigmine control solution did not cause any apparent effect on the uptake and release of calcium-47 or potassium-42 or in sodium-24 uptake (Figs. 92, 93, 94).

Edrophonium control solution did not cause any apparent effect on the uptake and release of calcium-47 or potassium-42 or in sodium-24 uptake (Figs. 95, 96, 97).

Gallamine control solution did not cause any apparent effect on the uptake and release of calcium-47 or potassium-42 or in sodium-24 uptake (Figs. 98, 99, 100).



A B C

Fig. 86

Uptake of calcium-47 (A), potassium-42 (B) and sodium-24 (C) by isolated frog sartorius muscle in the presence of suxamethonium control solution.

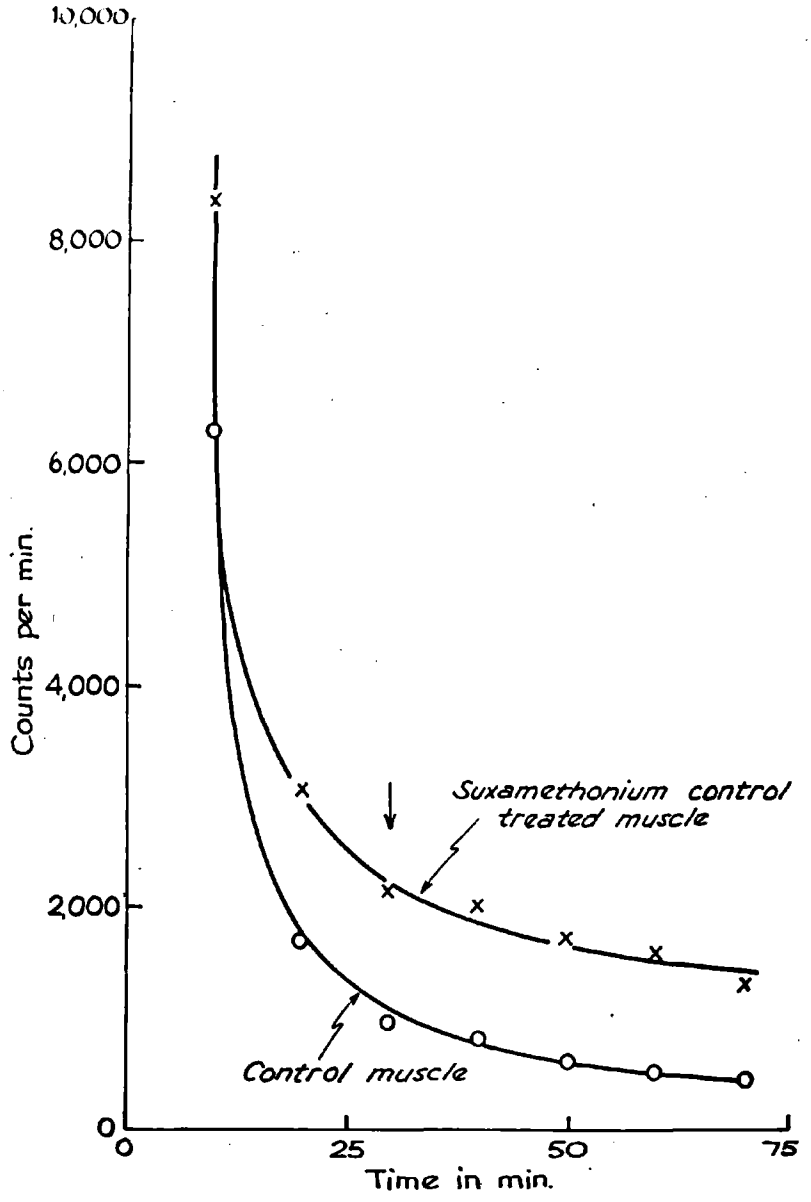


Fig. 87

Effect of suxamethonium control solution on the release of calcium-47 from isolated frog sartorius muscle. The vertical arrow indicates the point of exposure of the test muscle to the suxamethonium control solution.

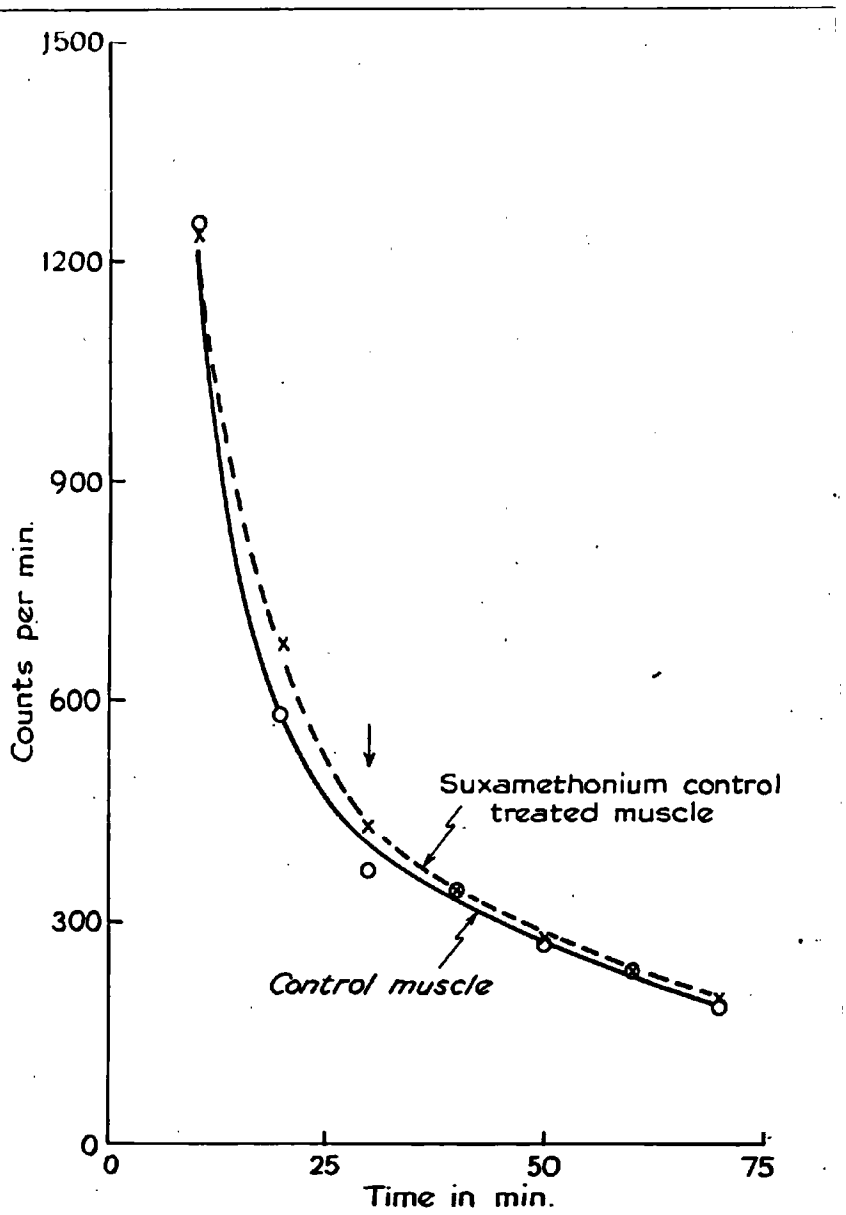
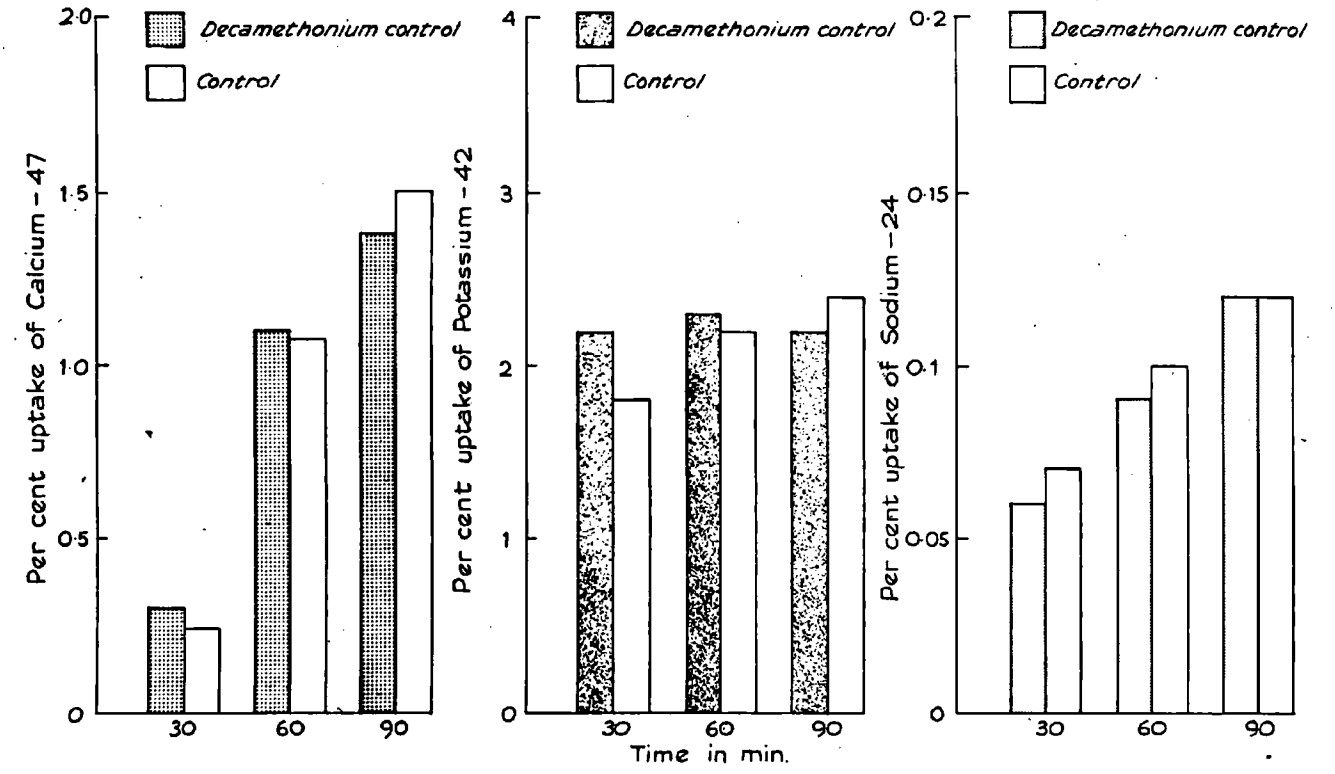


Fig. 88

Effect of suxamethonium control solution on the release of potassium-42 from isolated frog sartorius muscle. The vertical arrow indicates the point of exposure of the test muscle to the suxamethonium control solution.



A B C

Fig. 89

Uptake of calcium-47 (A), potassium-42 (B) and sodium-24 (C) by isolated frog sartorius muscle in the presence of decamethonium control solution.

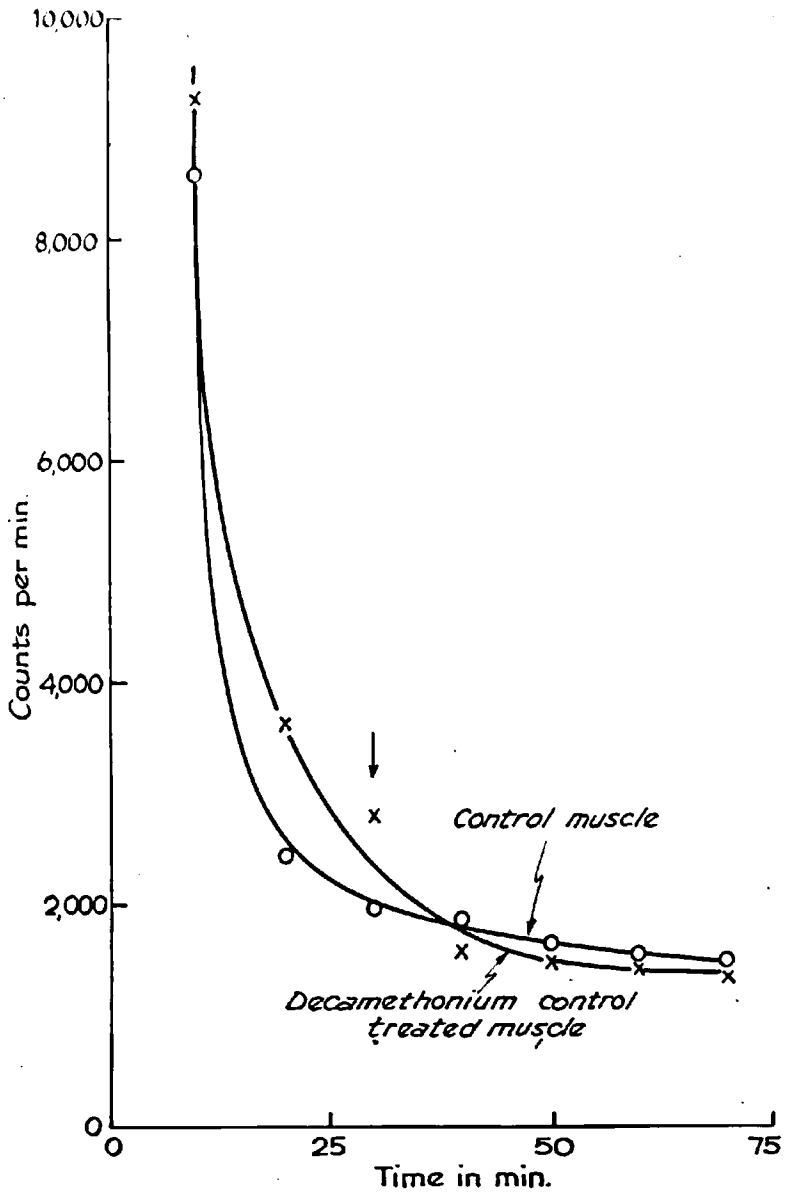


Fig. 90

Effect of decamethonium control solution on the release of calcium-47 from isolated frog sartorius muscle. The vertical arrow indicates the point of exposure of the test muscle to the decamethonium control solution.

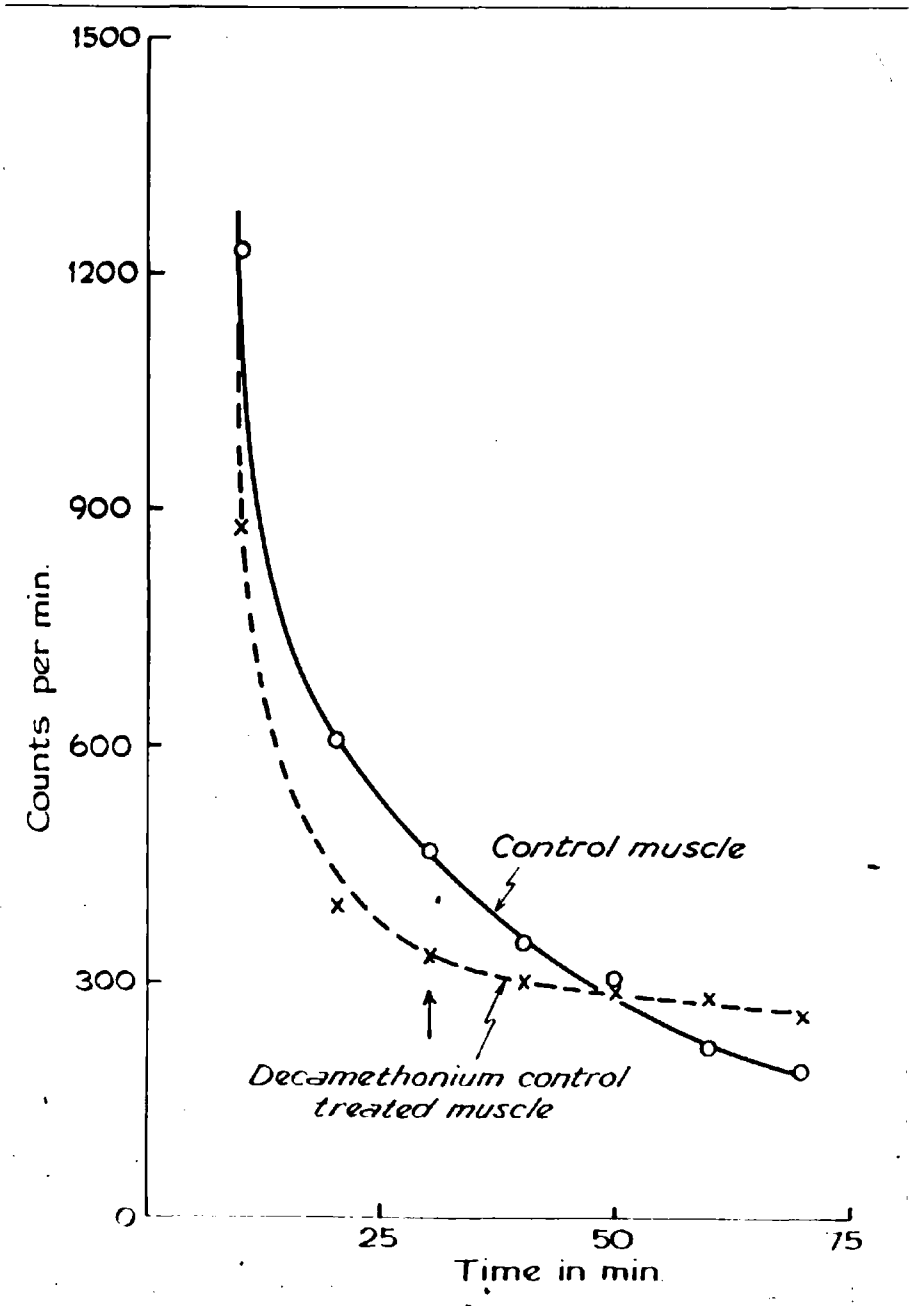
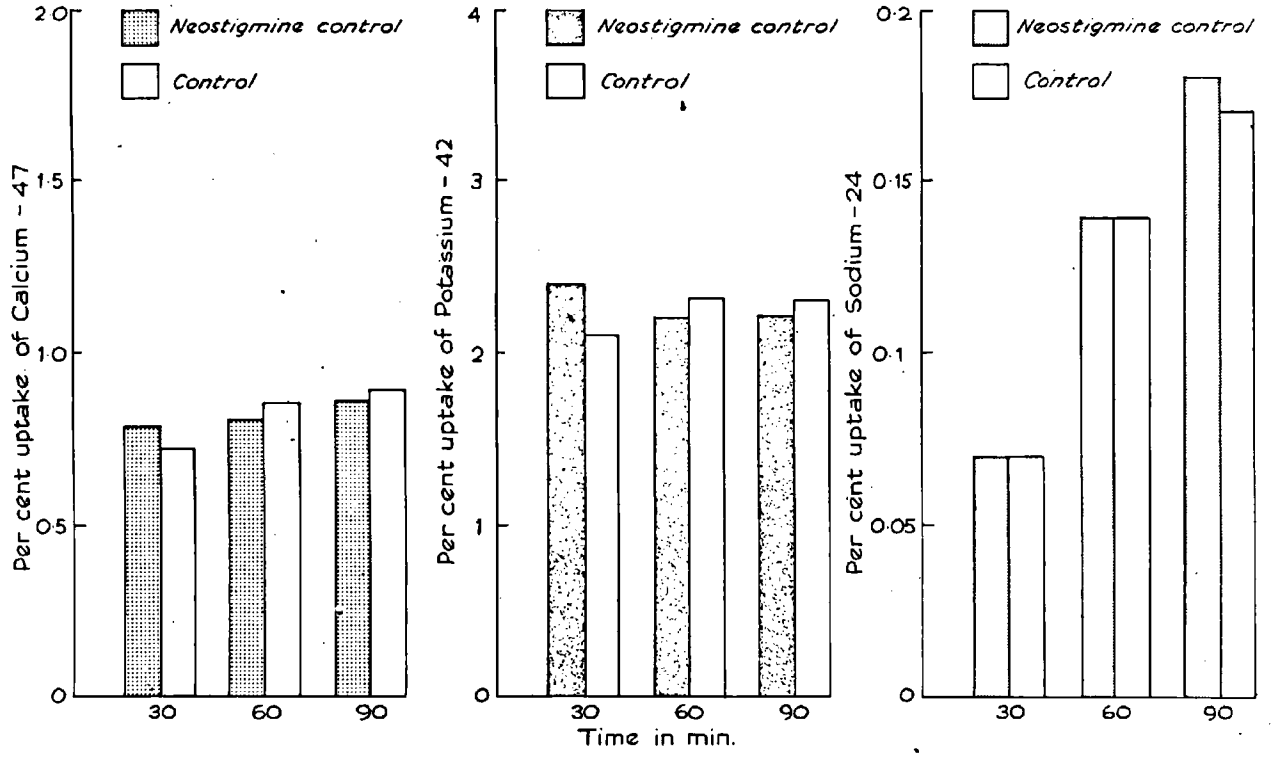


Fig. 91

Effect of decamethonium control solution on the release of potassium-42 from isolated frog sartorius muscle. The vertical arrow indicates the point of exposure of the test muscle to the decamethonium control solution.



A B C

Fig. 92

Uptake of calcium-47 (A), potassium-42 (B) and sodium-24 (C) by isolated frog sartorius muscle in the presence of neostigmine control solution.

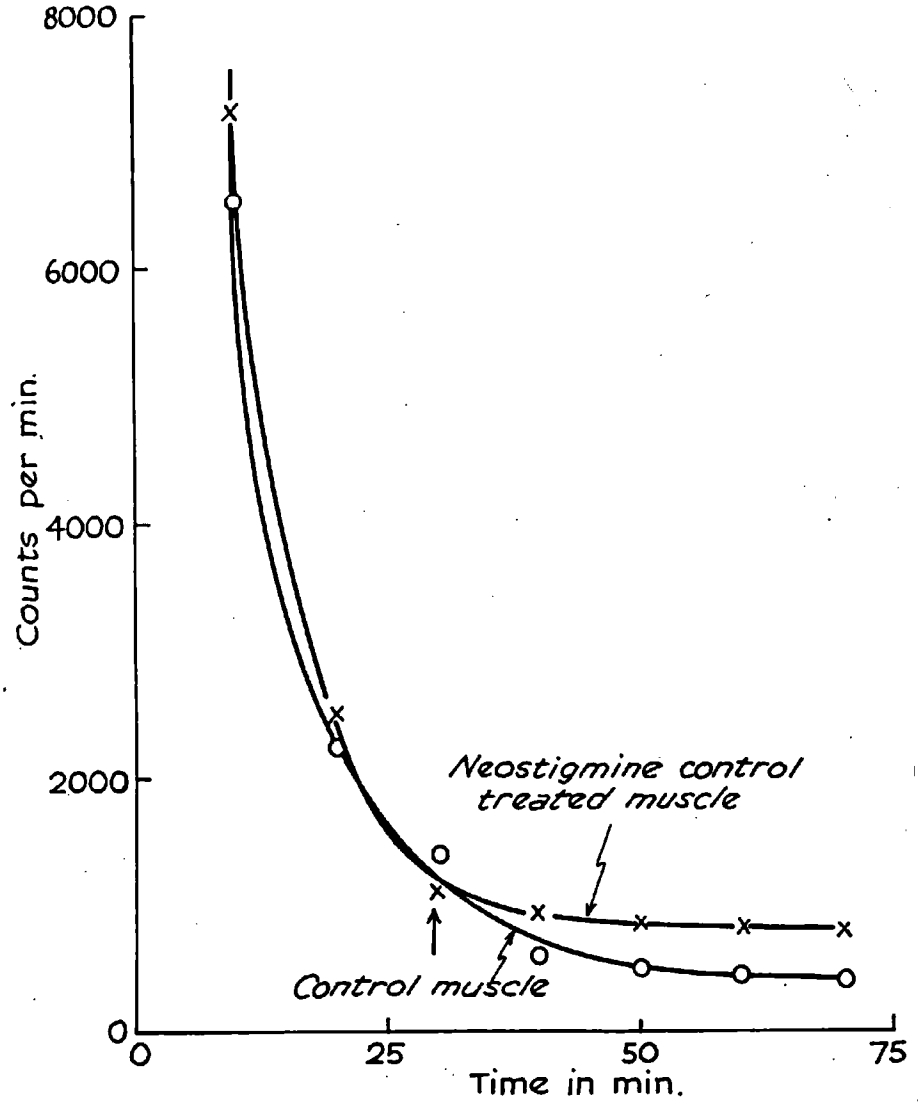


Fig. 93

Effect of neostigmine control solution on the release of calcium-47 from isolated frog sartorius muscle. The vertical arrow indicates the point of exposure of the test muscle to the neostigmine control solution.

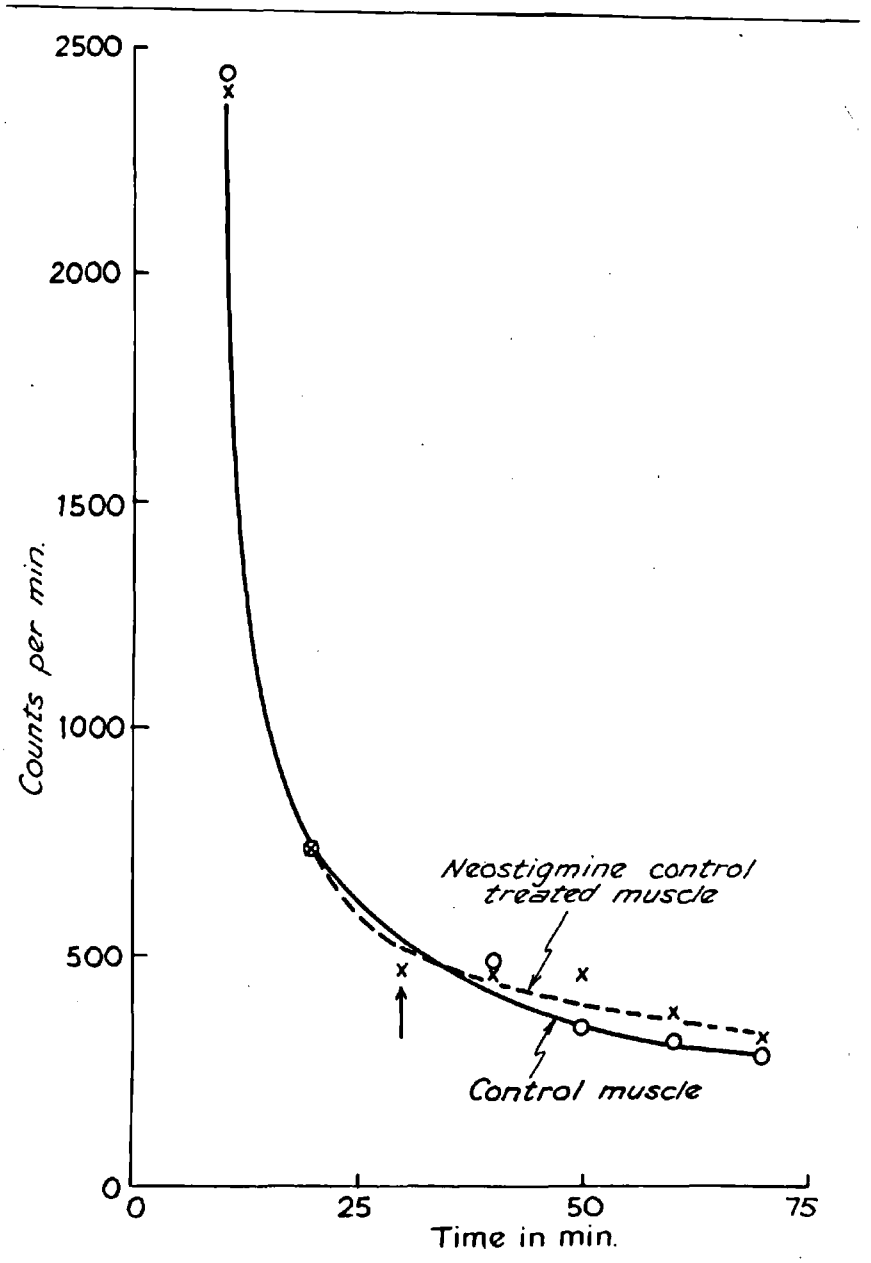
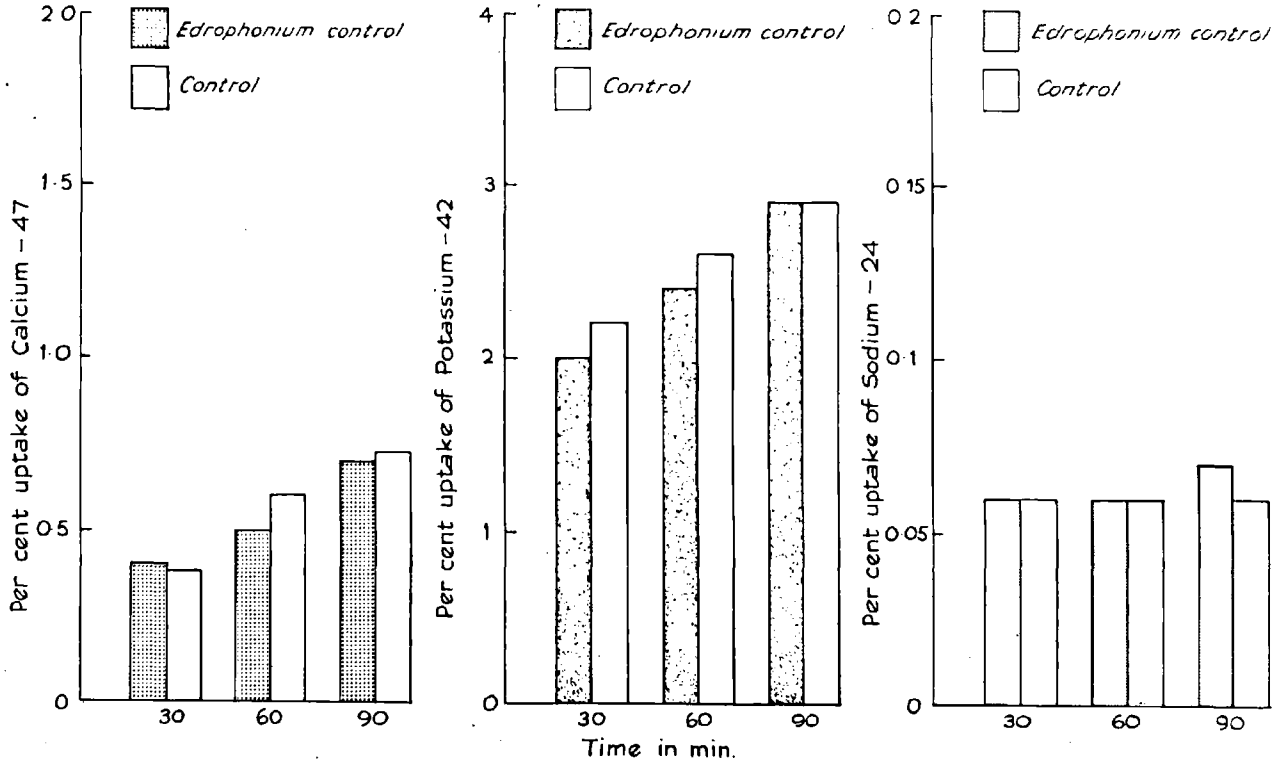


Fig. 94

Effect of neostigmine control solution on the release of potassium-42 from isolated frog sartorius muscle. The vertical arrow indicates the point of exposure of the test muscle to the neostigmine control solution.



A B C

Fig. 95

Uptake of calcium-47 (A), potassium-42 (B) and sodium-24 (C) by isolated frog sartorius muscle in the presence of edrophonium control solution.

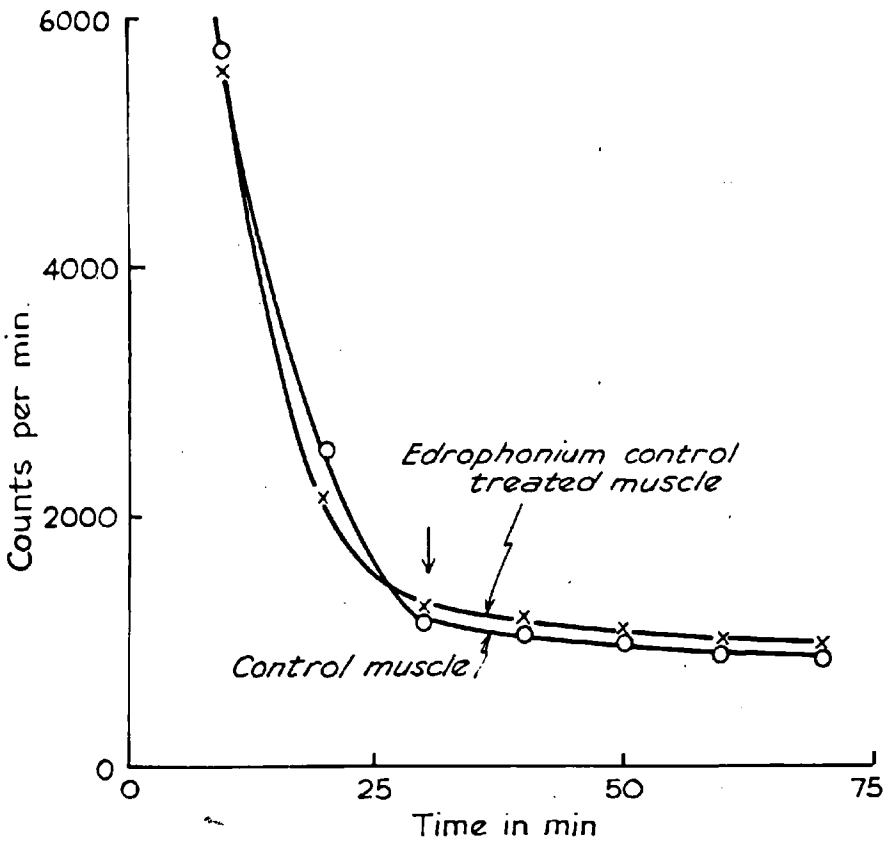


Fig. 96

Effect of edrophonium control solution on the release of calcium-47 from isolated frog sartorius muscle. The vertical arrow indicates the point of exposure of the test muscle to the edrophonium control solution.

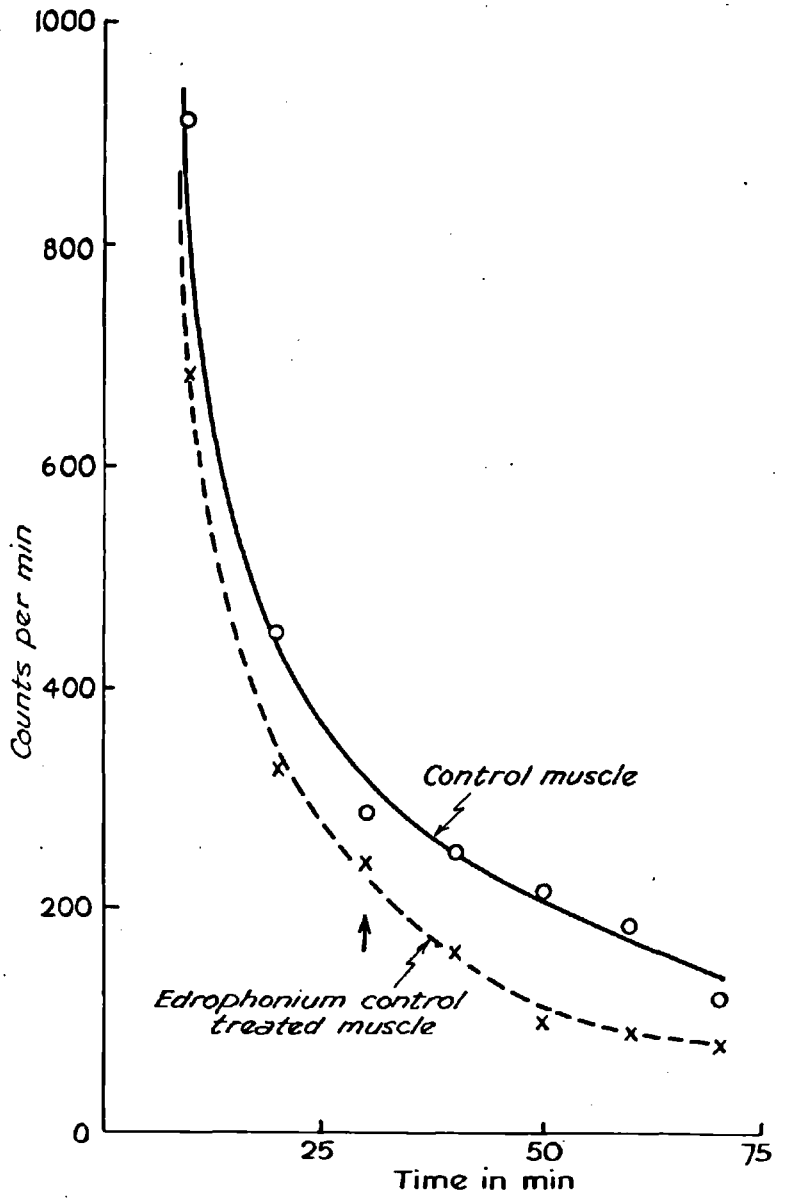
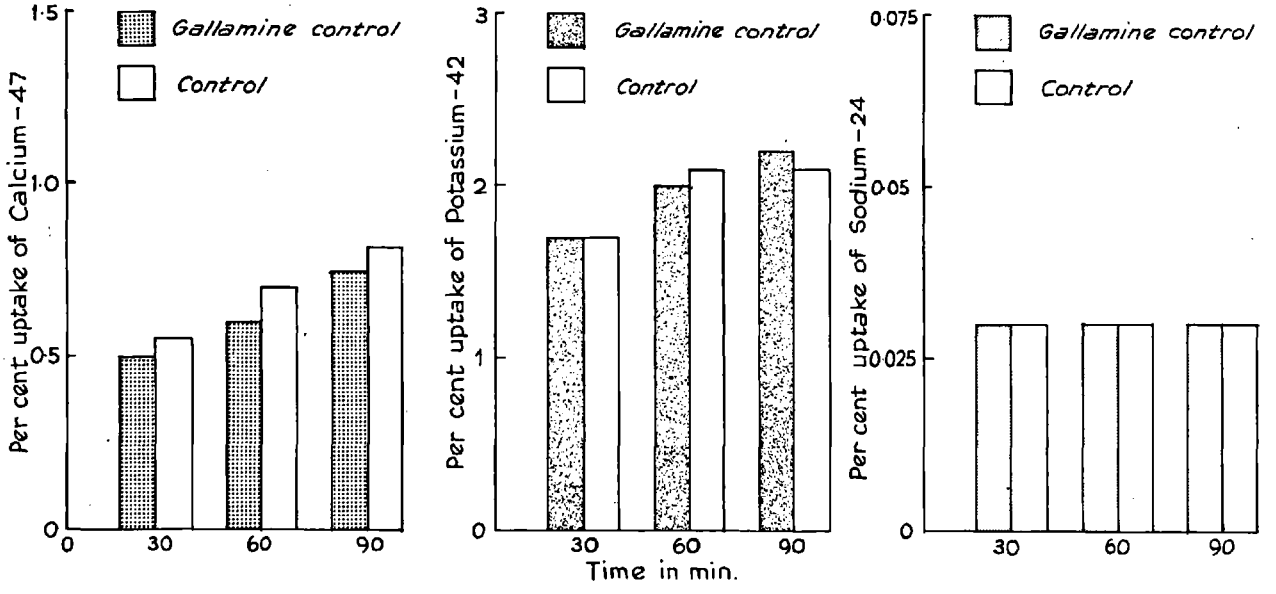


Fig. 97

Effect of edrophonium control solution on the release of potassium-42 from isolated frog sartorius muscle. The vertical arrow indicates the point of exposure of the test muscle to the edrophonium control solution.



A B C

Fig. 98

Uptake of calcium-47 (A), potassium-42 (B) and sodium-24 (C) by isolated frog sartorius muscle in the presence of gallamine control solution.

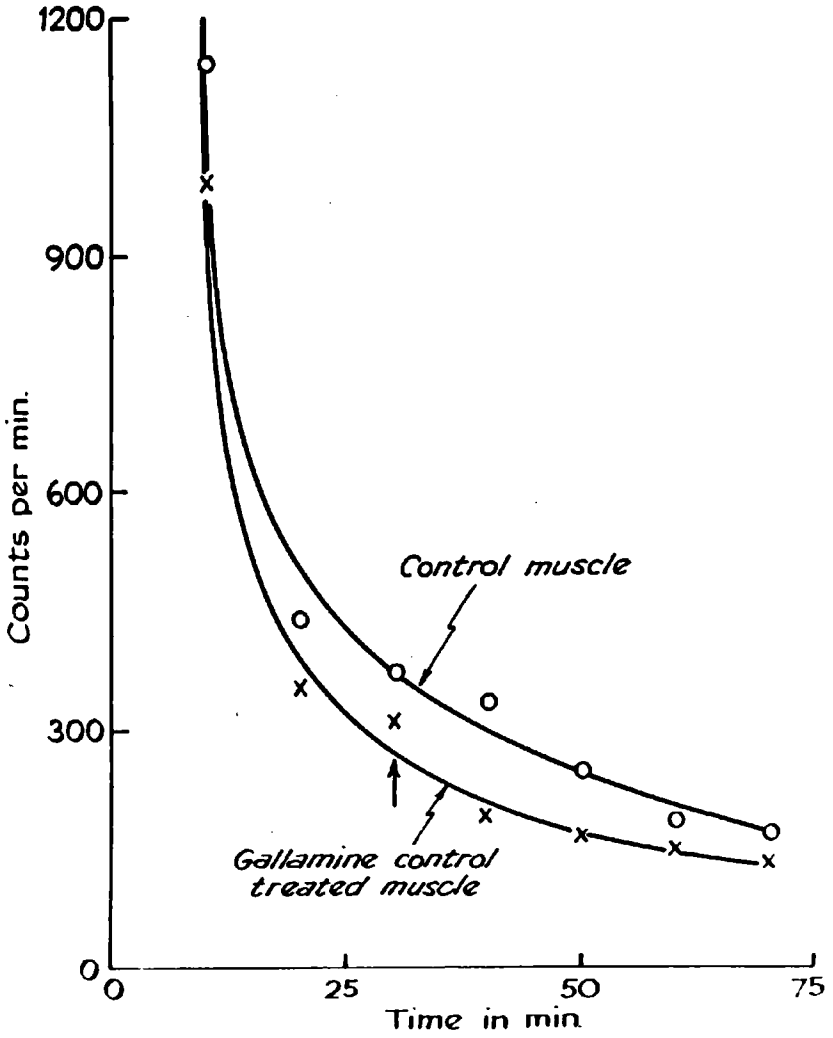


Fig. 99

Effect of gallamine control solution on the release of calcium-47 from isolated frog sartorius muscle. The vertical arrow indicates the point of exposure of the test muscle to the gallamine control solution.

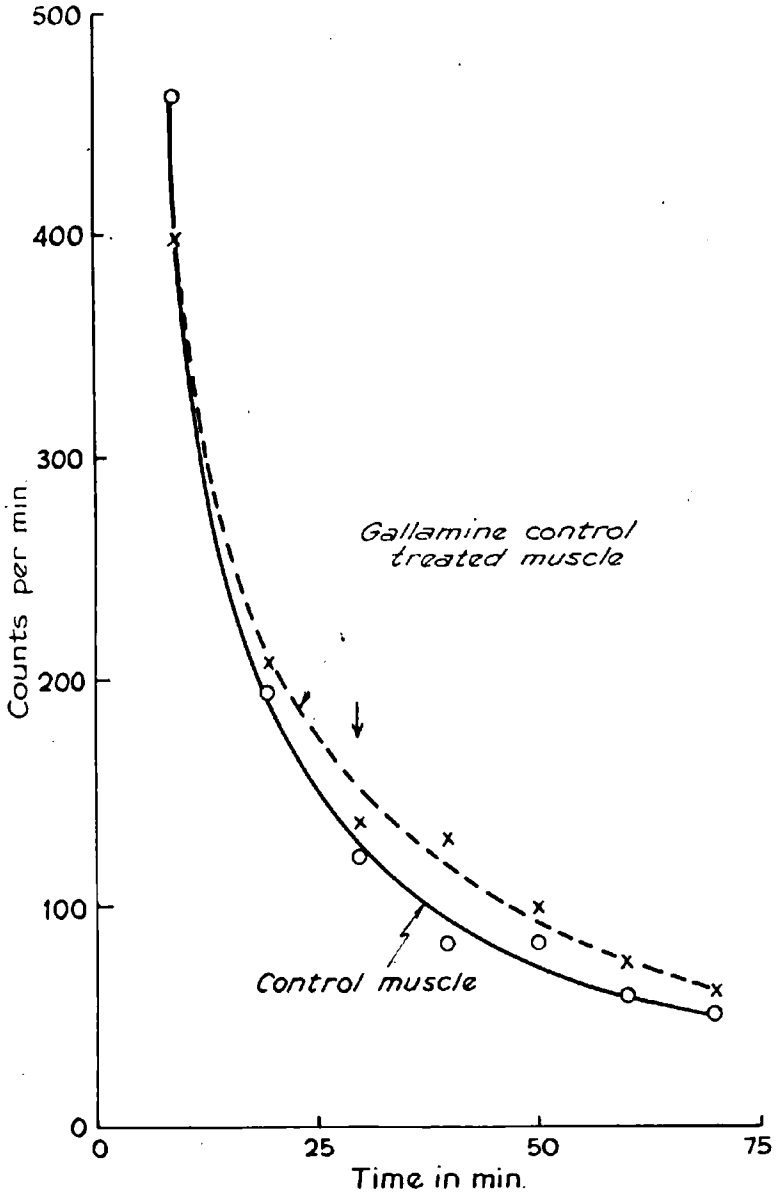


Fig. 100

Effect of gallamine control solution on the release of potassium-42 from isolated frog sartorius muscle. The vertical arrow indicates the point of exposure of the test muscle to the gallamine control solution.

Specimen protocol of experiment to investigate the effect of drugs on Calcium-47 uptake

Muscle weights: Control = 60 mg.
Test = 60 mg.

Radioactivity in bathing solution per ml.

Background counts	Gross counts	Net counts
1398	77017	75619

Muscle soaked in 10 ml. of radioactive solution

Total radioactivity in bathing solution = 756,190 counts

Suxamethonium (5 mg. per ml.) treated muscle

Time (mins.)	Background Counts	Gross Counts	Net Counts	Percentage uptake of radioactivity
30	1475	7263	5788	0.76
60	1442	12220	10778	1.42
90	1507	12398	10891	1.44
120	1556	13429	11873	1.57
150	1544	14353	12809	1.69
Muscles digested in concentrated nitric acid				
	1646	13101	11455	1.51

Control Muscle

Background Counts	Gross Counts	Net Counts	Percentage uptake of radioactivity
1390	4370	2980	0.39
1409	5210	3801	0.5
1556	6441	4885	0.64
1554	7147	5593	0.74
1567	8457	6890	0.91
1609	7765	6156	0.81

Specimen protocol of experiment to investigate the effect of drugs on Potassium-42 uptake

Muscle weights: Control = 68 mg.
Test = 68 mg.

Radioactivity in diluted bathing solution per ml.

Background counts 1103 Gross counts 21825 Net counts 20722

Dilution 10 times

Muscle soaked in 10 ml. of radioactive solution

Total radioactivity in bathing solution = 2,072,200 counts

Decamethonium (200 μg. per ml.) treated muscle					Control Muscle			
Time (mins.)	Background Counts	Gross Counts	Net Counts	Percentage uptake of radioactivity	Background Counts	Gross Counts	Net Counts	Percentage uptake of radioactivity
30	1069	13680	12611	0.6	1045	33844	32799	1.58
60	1351	9964	8613	0.41	1279	48743	47464	2.29
90	1413	10283	8870	0.42	1331	60096	58765	2.83
120	1470	8873	7403	0.35	1385	63532	62147	2.99
			Muscles digested in		concentrated nitric acid			
	1421	5232	3811	0.18	1361	32484	31123	1.5

Specimen protocol of experiment to investigate the effect of drugs on sodium-24 uptake

Muscle weights: Control = 84 mg.
Test = 84 mg.

Radioactivity in diluted bathing solution per ml.

Background counts	Gross counts	Net counts
1205	4244	3039

Dilution 100 times

Muscle soaked in 10 ml. of radioactive solution

Total radioactivity in the bathing solution = 3,039,000 counts

Tubocurarine (200 µg. per ml.) treated muscle					Control Muscle			
Time (mins.)	Background Counts	Gross Counts	Net Counts	Percentage uptake of radioactivity	Background Counts	Gross Counts	Net Counts	Percentage uptake of radioactivity
30	1112	7028	5916	0.19	1056	6013	4957	0.16
60	1448	8043	6595	0.22	1678	7614	5936	0.19
90	1421	8551	7130	0.23	1711	8341	6630	0.22
Muscles digested in concentrated nitric acid								
	1393	7303	5910	0.19	1595	7635	6040	0.2

Specimen protocol of experiment to investigate the effect of drugs on calcium-47 release

Muscle weights: Control = 74 mg.
Test = 74 mg.

Muscle soaked in radioactive Prog Ringer's solution containing calcium-47 for 3 hours

Ryanodine (50 µg. per ml.) treated muscle (Drug in 4th tube)				Control Muscle		
Time (mins.)	Background Counts	Gross Counts	Net Counts	Background Counts	Gross Counts	Net Counts
10	1853	5669	3816	1710	5100	3390
20	1900	3487	1587	1676	2853	1177
30	1920	2843	923	1717	2340	623
40	1987	3206	1219	1782	2245	463
50	1971	2707	736	1745	2115	370
60	1886	2425	539	1818	2178	360
70	1921	2444	523	1755	1992	237
			9343			6620
Ratio of counts released by test Muscles digested in				Muscle/control muscle = 1.41 concentrated nitric acid		
	1943	7086	5143	1767	7636	5869

Specimen protocol of experiment to investigate the effect of drugs on Potassium-42 release

Frog injected with 1 μC. potassium-42

Equilibration period = 2 hours

Muscle weights: Control = 64 mg.

Test = 66 mg.

Neostigmine (75 μg. per ml.) treated muscle (Drug 4th in tube)				Control Muscle		
Time (mins.)	Background Counts	Gross Counts	Net Counts	Background Counts	Gross Counts	Net Counts
10	37	5375	5338	56	5445	5389
20	53	2272	2219	51	2299	2248
30	46	1767	1721	61	1667	1606
40	44	2783	2739	65	1389	1324
50	69	4110	4041	72	1188	1116
60	62	2841	2779	80	1036	956
70	74	1539	1465	83	804	721
			20302			15360

Ratio of counts released by test muscle/control muscle = 1.52

Muscles digested in concentrated nitric acid

	50	23390	23340	36	30261	30225

A P P E N D I X II

A P P E N D I X II

In Table 39 (page 394) are given the formulae of the saline solutions used in the experimental work described in this thesis. All the chemicals used were of "Analar" quality and only glass distilled water was used. In some cases aqueous stock solutions of certain salts were prepared to facilitate the rapid preparation of a saline solution. With the exception of sodium bicarbonate solution these stock solutions could be used for about two weeks after their preparation. Sodium bicarbonate stock solution was freshly prepared every three or four days. Glucose was added in the solid form to each batch of saline.

TABLE 39.

Formulae of Physiological Saline Solutions

Salts (g. per litre)	Frog Ringer's solution	Tyrode's solution	Locke's solution	de Jalon's solution
Sodium chloride	6.5	8.0	9.0	9.0
Potassium chloride	0.14	0.20	0.42	0.42
Calcium chloride anhydrous	0.12	0.20	0.24	0.06
Sodium hydrogen phosphate (dihydrate)	-	0.05	-	-
Sodium bicarbonate	0.20	1.0	0.50	0.5
Magnesium chloride	-	0.10	-	-
Glucose	2.0	2.0	1.0	1.0