STUDIES ON THE

MODE OF ACTION OF RESERPINE.

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

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Studies on the Mode of Action of Reserpine.

PART 2.

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

1). A Note on the Pharmacology of Reserpine, J. Pharm., Lond., (1956), 8, 606.

2). Antagonists of the Action of Reserpine on Smooth Muscle, Nature, Lond., (1956), 178, 859.

3). Action of Reserpine on Isolated Intestinal Muscle, Nature, Lond., (1957), 179, 820.

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

A further publication has been submitted to the Editors of the British Journal of Pharmacology and Chemotherapy.

In addition, part of this work was communicated, jointly with J.J. Lewis, to the Pharmacological Society of Great Britain at London in January, 1957.

CHAPTER 1

CHAPTER 1

INTRODUCTION.

Plants, or plant extracts have been used in the treatment of disease throughout recorded history. The Rigveda of India, believed to have been written six thousand years ago, makes reference to numerous medicinal plants. In spite of the introduction into clinical medicine of many synthetic organic chemicals, the armamentarium of the modern physician still contains many drugs extracted from botanical sources. Among such products, extracts of *Rauwolfia serpentina* Benth. have, in recent years, attracted much interest from pharmacologists, clinicians and chemists alike.

Rauwolfia is a paratropical genus of about 100 species[5,6] consisting of shrubs and small trees. *Rauwolfia serpentina* Benth. (family *Apocynaceae*) is a small erect shrub about three feet high, which is indigenous to India, Burma, Malaya, Siam and Java.

The root of *R. serpentina* has been used for centuries in Indian folk medicine for the treatment of a wide variety of conditions ranging from snake bite and dysentery to various central nervous system derange-

ments, including excitement, maniacal behaviour associated with psychoses, epilepsy and anxiety. Recently, its value in the treatment of psychotic conditions associated with anxiety or hyperactivity has been confirmed[1,2,3].

The use of the drug in human hypertension has been a recent development. Its beneficial effects in this condition were first reported by Chakravarty and his colleagues[4].

In 1952, Müller, Schlittler and Bein[7] reported the isolation from R. serpentina root, of the alkaloid reserpine, which Bein[8] subsequently found to have both the central nervous system depressant and hypotensive actions attributed to extracts of R. serpentina. Of the twenty or more alkaloids which have now been isolated from R. serpentina, reserpine has been the most extensively studied. The literature dealing with its pharmacology and use in clinical medicine is already voluminous. Two recent articles[5,6] review the available literature.

Whilst a detailed account of the pharmacology of reserpine seems unnecessary, a brief outline is clearly indicated.

Even after intravenous administration, the effects of reserpine in man or experimental animals develop slowly. This latency of action can also be seen in certain isolated tissues and organs and is in fact one of the more puzzling features in the actions of the drug.

A sedative and hypnotic action is seen in mice, rats, guinea-pigs, rabbits, dogs and monkeys[8-11]. This is unique in that the subjects can always be easily awakened and, even after large doses of the drug,[8-11] are never anaesthetised. In this respect, its action differs from that of barbiturates or general anaesthetics. It also differs from the latter in producing no distinctive changes in the electroencephalographic pattern either in normal monkeys[11] or human patients with essential hypertension[12].

A subtle manifestation of the action of reserpine is the alteration in the normal behaviour of animals. Thus, the normally aggressive Rhesus monkey may safely be approached and handled after treatment with reserpine[10]. "Sham rage" in cats is also inhibited by reserpine[13].

In rats and mice[14], reserpine antagonises the

central stimulation produced by caffeine, morphine, cocaine and scopolamine. In contrast, the effects of strychnine, nicotine and picrotoxin are unaffected[14], whilst the tonic seizures following camphor and leptazol are potentiated[14,15].

Reserpine causes a slow and relatively prolonged reduction in blood pressure, which is greater in hypertensive than in normotensive human subjects[5]. Reserpine has little effect on the blood pressure of experimental animals if this is already low. Thus, no depressor response could be demonstrated in spinal or decerebrate cats[8]. The reduction of blood pressure is accompanied by a distinct bradycardia, which is unaffected by atropine[7,8,9,10,12]. Since cardiac function in vivo is generally unaffected in man[16] and animals[17], during a simultaneous reduction of peripheral resistance, it has been concluded[6] that the fall in blood pressure is due to peripheral vasodilatation.

Respiration is depressed by reserpine in all animal species studied[9,13]. However, the vagal respiratory reflex and the sensitivity to electrical stimulation of the medullary areas, which are associated with respiratory reflexes, are uninfluenced by reserpine[18].

Reserpine has no ganglion blocking, local anaesthetic or sympatholytic properties either *in vivo* or *in vitro*[8,9,10,16].

Other features, characteristic of the action of reserpine, are prolonged miosis, hypothermia and relaxation of the nictitating membrane[8,9,10,14,17]. There is also stimulation of intestinal activity and gastric secretion, *in vivo*[8-11]: large doses cause diarrhoea.

The pattern of effects seen after reserpine has been compared[14] to a syndrome reported by Hess[19], following electrical stimulation of certain diencephalic structures in the cat. This similarity of the actions of reserpine to those of hypothalamic stimulation, coupled with the absence of a peripheral sympatholytic action, ganglionic blockade or peripheral vagal stimulation with reserpine led Bein and his colleagues[8,9,18] and other workers[10,11,12,13,16,17] to suggest that the action of reserpine was predominantly central. The exact site of this attack has not yet been demonstrated conclusively. It has been pointed out[8,18], however, that many of the effects following the drug can be explained by assuming a partial suppression of sympathetic

predominance. This hypothesis is supported by the finding that relatively small doses of the drug block the reflex pressor response to carotid sinus occlusion in dogs[10,17] and cats[8,10,18]. There is no direct action upon the stretch receptors of the carotid sinus[10,18]. The drug does not affect the pressor response to afferent vagal stimulation in the dog[10] or cat[8,9], or to stimulation of the sciatic or tibial nerve in cats[8,20]. The pressor response following increased intracranial pressure in dogs is not prevented[17]. It has been suggested[13], therefore, that reserpine probably acts at a point higher than the medulla, possibly on the afferent inflow which normally stimulates sympathetic activity in the hypothalamus. Bein suggested[18], following his demonstration of a partial reversal of the carotid sinus pressor reflex block by section of the brain stem, that reserpine stimulated certain normally inhibitory "substrates" in the brain.

That reserpine may not act <u>per se</u> is suggested by the long latency in its action and by the fact that its actions <u>in vivo</u> are seen long after its presence can no longer be detected by physical means[21]. The extreme insolubility of reserpine may be responsible for the latency of action. On the other hand, much attention

has recently been focussed upon certain observations by Brodie and his colleagues[21-25] and others[26-28], who found that reserpine liberated 5-hydroxytryptamine from the brain, intestinal tract, spleen, mast cells and platelets of various animal species. Brodie and his co-workers have postulated, therefore, that reserpine acted by releasing 5-hydroxytryptamine from the brain. Hess, Shore and Brodie pointed out[21] that the typical central effects of reserpine in rabbits appeared to be related, temporally, to the disappearance of 5-hydroxy-tryptamine rather than to the accumulation of reserpine in the brain. The possibility exists that reserpine acts by impairing the capacity of the cells to bind 5-hydroxytryptamine[29].

The suggestion that reserpine acts through the mediation of 5-hydroxytryptamine is not supported by all the evidence available. It is reasonable to expect 5-hydroxytryptamine to possess all, or most of the properties of reserpine if, in fact, it is the actual active substance. This is not so. Thus 5-hydroxy-tryptamine does not facilitate camphor or leptazol convulsions in mice[15]. Parenteral administration of 5-hydroxytryptamine produces effects on respiration,

heart rate, blood pressure and the nictitating membrane which differ from those seen following reserpine[30,31].

5-hydroxytryptamine administered intraventricularly does not cause the miosis, hypotension or relaxation of the nictitating membrane in cats which is characteristic of the action of reserpine[32]. Other anomalies exist. Thus the emesis in pigeons, seen following reserpine[33], is not produced by 5-hydroxytryptamine, despite the fact that the latter is released by reserpine from the brain and other organs of birds[27].

If 5-hydroxytryptamine is a mediator of the action of reserpine, then it follows that all substances releasing this amine should produce the same actions as reserpine. However, amphetamine causes a fall in 5-hydroxytryptamine storage[26] in doses which produce marked central excitation, whilst the histamine liberator, 48/80, which also releases 5-hydroxytryptamine from mast cells, does not evoke the sedation or miosis in rats or guinea-pigs, which is so characteristic of reserpine[28].

In this respect, the symptoms exhibited by patients with carcinoid tumours of the argentaffin cells, resulting in the liberation of 5-hydroxytryptamine from

the tumour cells, are of interest. These patients characteristically show diarrhoea, bronchoconstriction and flushing of the skin, especially of the face. These symptoms have all been observed following reserpine; on the other hand some of the symptoms, for example insomnia and tachycardia, are in direct contrast to the effects of reserpine[34].

Thus it can be seen that whilst certain actions of reserpine may be related to the release of 5-hydroxy-tryptamine, the evidence that release of this amine is responsible for all its effects is inconclusive. It may be, as Bein suggested[35], that the depletion of endogenous 5-hydroxytryptamine produces a suitable "milieu" for reserpine or a metabolite of this compound to exert their characteristic pharmacological actions.

Reserpine decreases the brain concentration of other mono-amines. Holzbauer and Vogt[36] have shown that the nor-adrenaline content of the cat's hypothalamus was reduced by reserpine; this reduction was accompanied by central sympathetic stimulation, since there was a greater fall in medullary amines in the innervated, than in the denervated adrenal gland. Reserpine reduces the nor-adrenaline and adrenaline content of the superior

cervical sympathetic ganglion in rabbits[37]. It was concluded[37] that reserpine incapacitated the sympathetic neurones by causing them to lose their transmitter. The loss of nor-adrenaline was related to the ability of pre- and post-ganglionic stimulation of the cervical sympathetic neurones to retract the eyelid, those with the least loss showing least impairment of function. Recently, Paasonen and Krayer[39] found that reserpine decreased the nor-adrenaline content of the rat heart in situ and suggested that the release of nor-adrenaline may have been a general action of reserpine. The release of nor-adrenaline, however, is not a specific action of reserpine since ether, morphine and insulin also cause a fall in brain sympathin[38].

The intimate mode of action of reserpine has therefore still to be elucidated. There seems little doubt that the alkaloid produces definite effects on the central nervous system. The means by which it does so, however, are not known. Reserpine does release 5-hydroxy-tryptamine and nor-adrenaline from the brain and a number of other tissues, but it remains to be seen whether this is purely coincidental or whether it does, in fact, give some clue to its mode of action within the cell.

The work described in this thesis was undertaken to investigate the actions of reserpine in isolated tissues and organs. Early investigations by others, of the pharmacology of reserpine, included few studies using these preparations. It was felt that the interpretation of data obtained in such experiments would give some information on the action of reserpine at cellular level. It appeared that the fundamental action of reserpine must ultimately involve the biochemistry of the cell, as is probably true of all pharmacologically active materials. With this in mind, the possibility of a biochemical site of action was also investigated.

CHAPTER 1

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EXPERIMENTAL WORK

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Introduction.

Throughout this thesis, the names of certain drugs have been abbreviated. The shortened drug names used are as follows:-

1)	Acetylcholine bromide	is described as		acetylcholine.
2)	Atropine sulphate	"	"	" atropine.
3)	(+)-tubocurarine chloride	"	"	" tubocurarine.
4)	(-)-adrenaline hydrochloride	"	"	" adrenaline.
5)	(-)-*nor*-adrenaline bitartrate	"	"	" *nor*-adrenaline
6)	Histamine acid phosphate	"	"	" histamine.
7)	5-hydroxytryptamine creatinine sulphate	"	"	" 5-hydroxytryptamine.
8)	Hexamethonium bromide	"	"	" hexamethonium.
9)	Decamethonium bromide	"	"	" decamethonium.

The composition and methods of preparation of all physiological saline solutions used in this investigation are to be found in Appendix I (page 181).

In/

In Appendix I are detailed also the methods used in preparing solutions of reserpine.

The common abbreviations for volume and weights of the metric system are used throughout.

EXPERIMENTAL WORK

PART 1.

CHAPTER 2

The concentrations of drugs mentioned in this chapter refer to the weight of the drug per millilitre of physiological saline in the isolated organ bath.

CHAPTER 2

EXPERIMENTS CARRIED OUT USING FROG SKELETAL MUSCLE.

This chapter has been divided into two sections describing experiments with

A), the frog rectus-abdominis muscle preparation.

1) Experiments carried out in early August.

2) Experiments carried out in January and February.

B), the frog sartorius muscle-ischiad nerve preparation.

SECTION A

Experiments on the frog rectus-abdominis muscle preparation.

1) Experiments carried out during the early part of August.

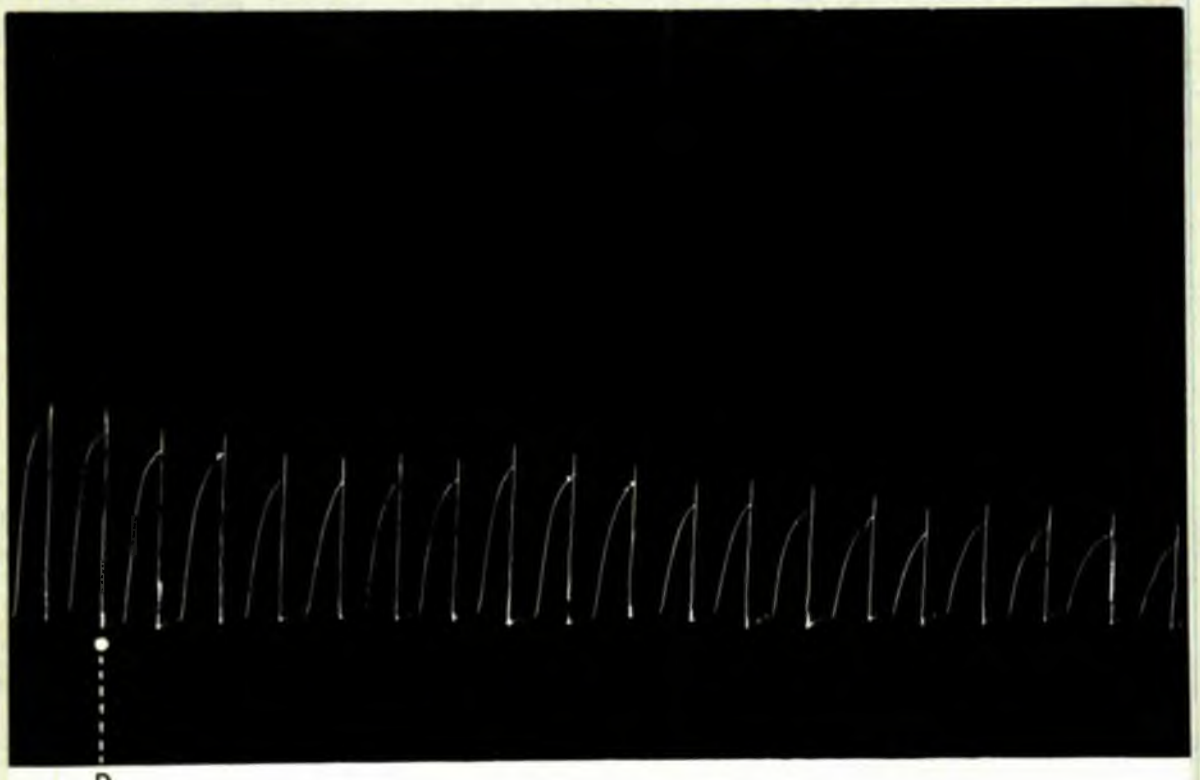
Methods.

The procedure used for preparing the muscle to record drug effects was similar to that described by Burn[1]. Frogs of either sex were decapitated and pithed.

The rectus-abdominis muscle was dissected out and suspended in an organ bath of capacity 12.5 ml. by means of two threads attached to pins which had been hooked through either end of the muscle. One thread was attached to the lower end of a glass tube supplying oxygen to the bath and the other to a modified frontal writing lever which gave a magnification of 1 in 6. The bath contained either 10 ml. of frog Ringer's solution (Appendix I) or 10 ml. of potassium free frog Ringer's solution. Drugs, except reserpine, dissolved in the saline appropriate to each experiment, were added to the bath by means of a 1 ml. graduated tuberculin syringe fitted with a number 12 record fitting hypodermic needle. In all experiments, at least two uniform contractions to the same dose of either acetylcholine or potassium chloride were obtained before the effect of reserpine was studied. The time interval between each dose of either acetylcholine or potassium chloride was six minutes, the resulting contraction being recorded for ninety seconds. The concentration of drugs used to produce contractions of the muscle were 0.1 µg. to 0.3 µg. of acetylcholine and 2 mg. of potassium chloride.

Summary of results. /

Figure 2.1



The inhibition by reserpine, in one experiment, of contractions of the frog rectus-abdominis muscle due to potassium chloride.

All contractions produced by the addition of 2 mg. potassium chloride.

At R, 50 µg. reserpine added.

Summary of results.

Neither reserpine nor the control solution had any direct effect on the muscle and did not modify the responses to either acetylcholine or potassium chloride.

RESULTS.

In this series of experiments, reserpine in concentrations of 10, 50, 100 and 500 µg. had no observable effect on the activity of the muscle. Reserpine neither inhibited nor potentiated acetylcholine-induced contractions of the muscle. A gradual reduction of the response to potassium chloride was seen after 50 µg. of reserpine in one of the nine experiments carried out using this drug (Figure 2,1). Since the contractions did not return to their control level even after two hours and, in addition, the effect was not reproducible in other experiments, it was concluded that the cause in this case was probably not the addition of reserpine.

2) Experiments carried out in January and February./

2). Experiments carried out in January and February.

Methods.

The preparation was set up as described above (page 17). Doses of acetylcholine and potassium chloride were again 0.1 µg. to 0.3 µg. and 2 mg. respectively and the contractions were recorded for ninety seconds. Reserpine was added six minutes after the second of two equal responses to acetylcholine. Due to the persistence of the reserpine effect, the next dose of acetylcholine was added twenty minutes after the addition of reserpine. Atropine and tubocurarine were added thirty seconds before acetylcholine, reserpine or the control solutions.

Summary of results.

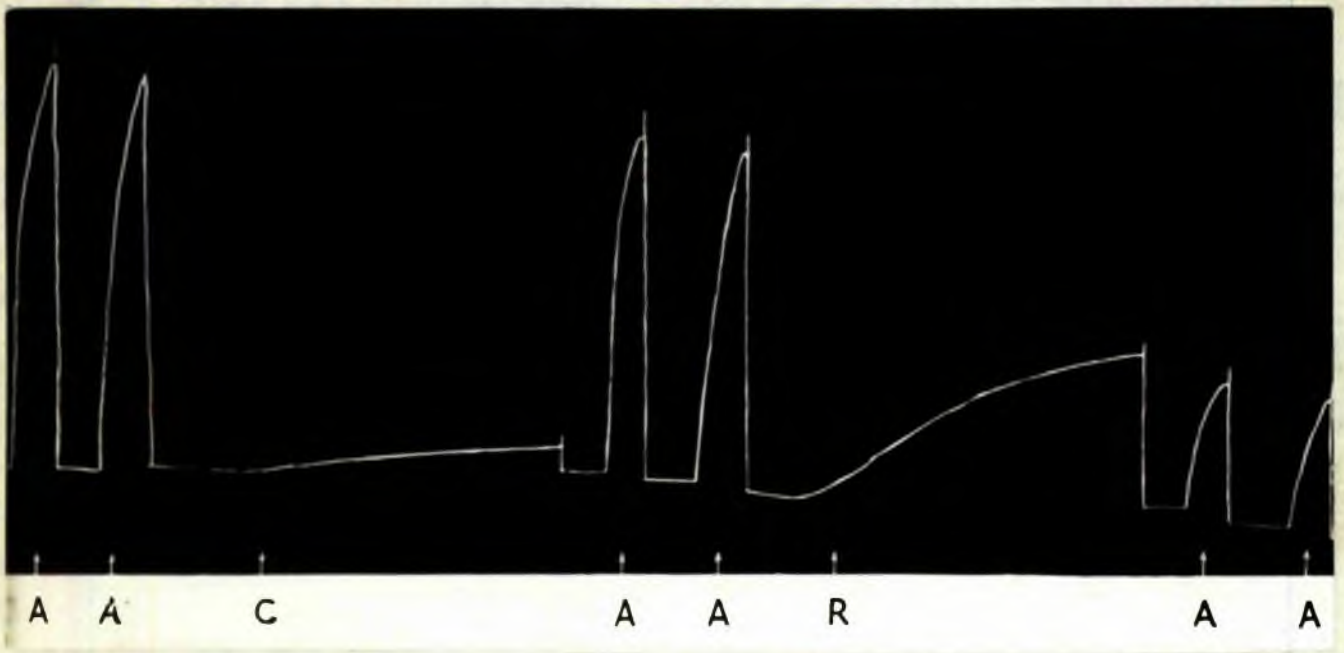
Reserpine produced a slow contraction of the frog rectus-abdominis muscle and reduced the responses to acetylcholine. The control solution occasionally caused a very slight contraction but had no effect on the response to acetylcholine. When citric acid was used instead of ascorbic acid as a solvent for reserpine, contractions were produced by the alkaloid, but not by

the control solution. The magnitude of the stimulant action following the addition of reserpine was unaffected by the presence of tubocurarine or atropine.

RESULTS.

Over the dose range of 10 µg. to 50 µg., reserpine caused the frog rectus-abdominis muscle to contract. Doses below 10 µg. had no stimulant action. 10 µg. did not consistently produce a contraction but doses of 20 µg. and above always caused a contraction of the muscle. There was little variation in the character or magnitude of the responses with varying doses. Figure 2,2 shows the direct effect of reserpine on the muscle. The contractions started two to six minutes after the addition of reserpine and reached their maximum height ten to fifteen minutes later. Relaxation of the preparation after washing was slow. The control solution of ascorbic acid, (Appendix I) in three out of twenty-five experiments, produced a slow contraction of much smaller magnitude than that following reserpine. When reserpine was dissolved in 0.2 per cent citric acid (Appendix I), a contraction of the muscle was produced. The height of the contraction, however, was less than that seen following

Figure 2.2



The direct effect of reserpine on the frog rectus-abdominis muscle and its influence upon acetylcholine-induced contractions.

At A, 0.1 µg. acetylcholine added.

At C, 0.2 ml. ascorbic acid control solution added.

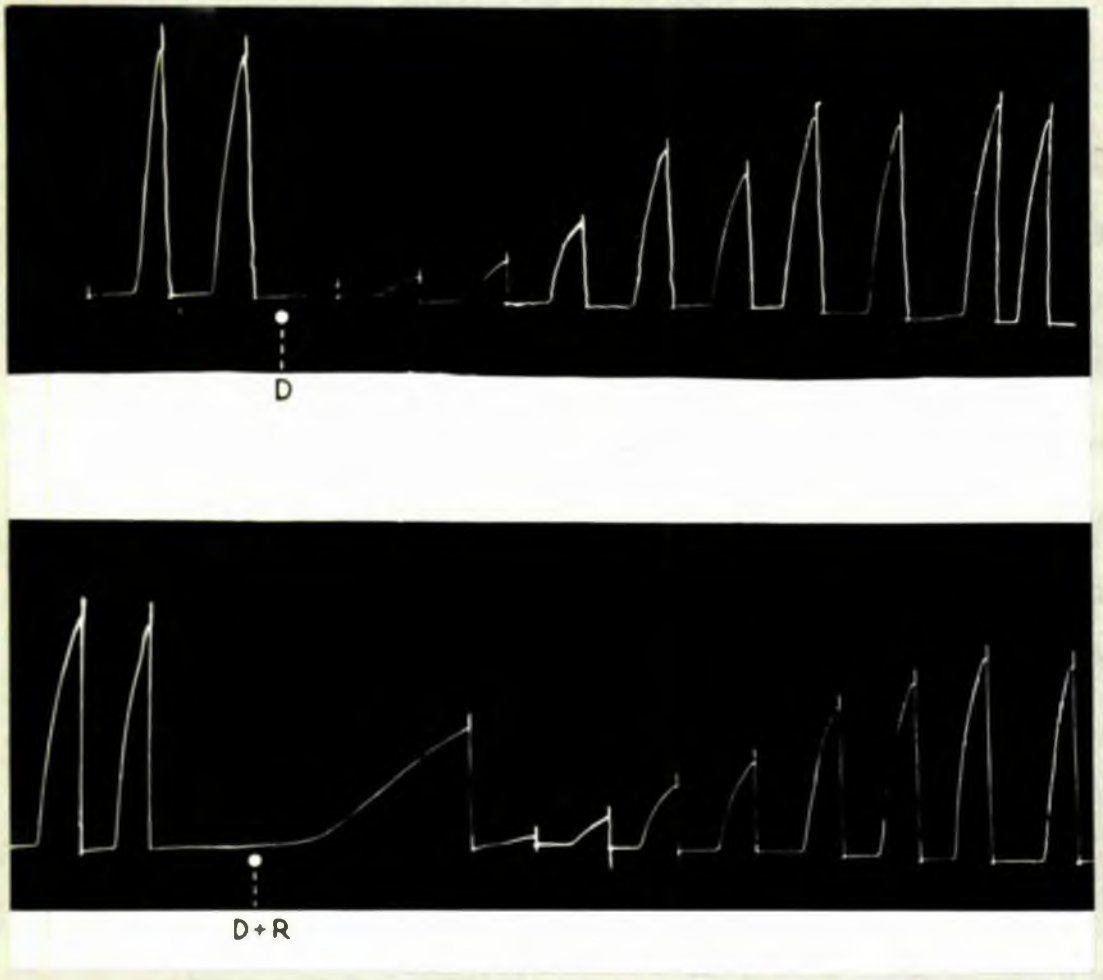
At R, 10 µg. reserpine added.

the same concentration of reserpine dissolved in ascorbic acid solution. The citric acid control solution produced a small contraction in only one experiment out of seven.

The response to a constant dose of acetylcholine was markedly reduced after reserpine and to a lesser degree after the control solution (Figure 2,2). In some cases the control solution did not affect the response to acetylcholine. The response of the muscle to acetylcholine returned to normal after a recovery period of from thirty to ninety minutes. In some cases, however, recovery was incomplete. On no occasion was complete inhibition of the acetylcholine-induced contraction seen.

The stimulant action observed was of interest since, at the time these experiments were carried out, reserpine had been reported to have only depressant actions. It was decided, therefore, to investigate this effect further. Neither tubocurarine nor atropine in concentrations up to 30 µg. had any effect on the magnitude of the contraction due to reserpine. In both cases, the drugs were added to the bath one minute before reserpine. The subsequent inhibition of the responses to acetylcholine, although greater than that normally seen

Figure 2.3



The effect of tubocurarine on the direct action of reserpine on frog rectus-abdominis muscle.

All contractions, unless otherwise indicated, were produced by the addition of 0.2 µg. acetylcholine.

Upper record: At D, 20 µg. of tubocurarine added 1 minute before acetylcholine.

Lower record: At D + R, 20 µg. tubocurarine added 1 minute before 10 µg. reserpine.

after reserpine alone, was no greater than that produced by atropine or tubocurarine alone (Figure 2,3).

In 1956, Barret and his colleagues[2] reported that 50 µg. per ml. reserpine caused a contraction of the frog rectus-abdominis muscle, which was only partially inhibited by 100 µg. per ml. of Intocostrin (a purified extract of Ch. tomentosum) or 50 µg. per ml. of oxyphenonium bromide. Since depolarizing agents, for example acetylcholine, decamethonium bromide and nicotine, had less effect after reserpine, they concluded that reserpine may act as a depolarizing agent with a selective action and that it resembled nicotine in this respect. In further support of their theory, they reported that reserpine, unlike decamethonium, did not cause a contraction of the chick gastrocnemius muscle.

It is well known[3] that the potassium ion is intimately concerned with the metabolic activity of muscle cells. The possibility that reserpine interferes with cellular metabolism, which is developed later in this thesis (discussion of Part 1, page 98), suggested that there might be some interference with intra- and extra-cellular potassium relationships. It was possible, therefore, that the contraction produced by reserpine may have been

associated with a leak of potassium from the cell.

It is appreciated that reserpine was previously found not to affect potassium-induced contractions of the muscle. However, since these experiments were carried out at a time when reserpine had no direct effect on the preparation, the need for an investigation of the possibility of potassium release seemed clear. This relationship is described in Chapter 9, page 131).

SECTION B

Experiments on the frog sartorius muscle-ischiad nerve preparation.

In view of the depression of the effects of acetylcholine on the rectus-abdominis muscle (page 22) it seemed possible that neuromuscular conduction might be affected by reserpine. This possibility was investigated using the isolated frog sartorius muscle stimulated indirectly through its somatic nerve, the ischiad.

Methods./

Methods.

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

Frogs of either sex were decapitated, pithed and pinned in the supine position on a dissecting board. The skin over both thighs was removed and the rectus-abdominis muscles carefully freed at their pelvic insertions. Having thus exposed the upper tendinous attachment of the sartorius muscles, a blunt seeker was used to free both the proximal and distal attachments of the muscle from the underlying tissues. Cotton threads were tied round both ends of the muscle which was then cut free from its attachment to the femur and pelvic girdle. A slight tension was applied to the muscle by means of the threads at each end. At this stage in the dissection, the muscle remained attached to the underlying tissue by connective tissue running along its longitudinal axis. Using the blunt dissection technique the main body of the muscle was freed from the underlying muscles in the leg except for a bridge of connective tissue in which runs the thread-like ischiad nerve.

The ischiad nerve joins the sartorius muscle roughly midway between the two tendinous attachments of

Figure 2.4

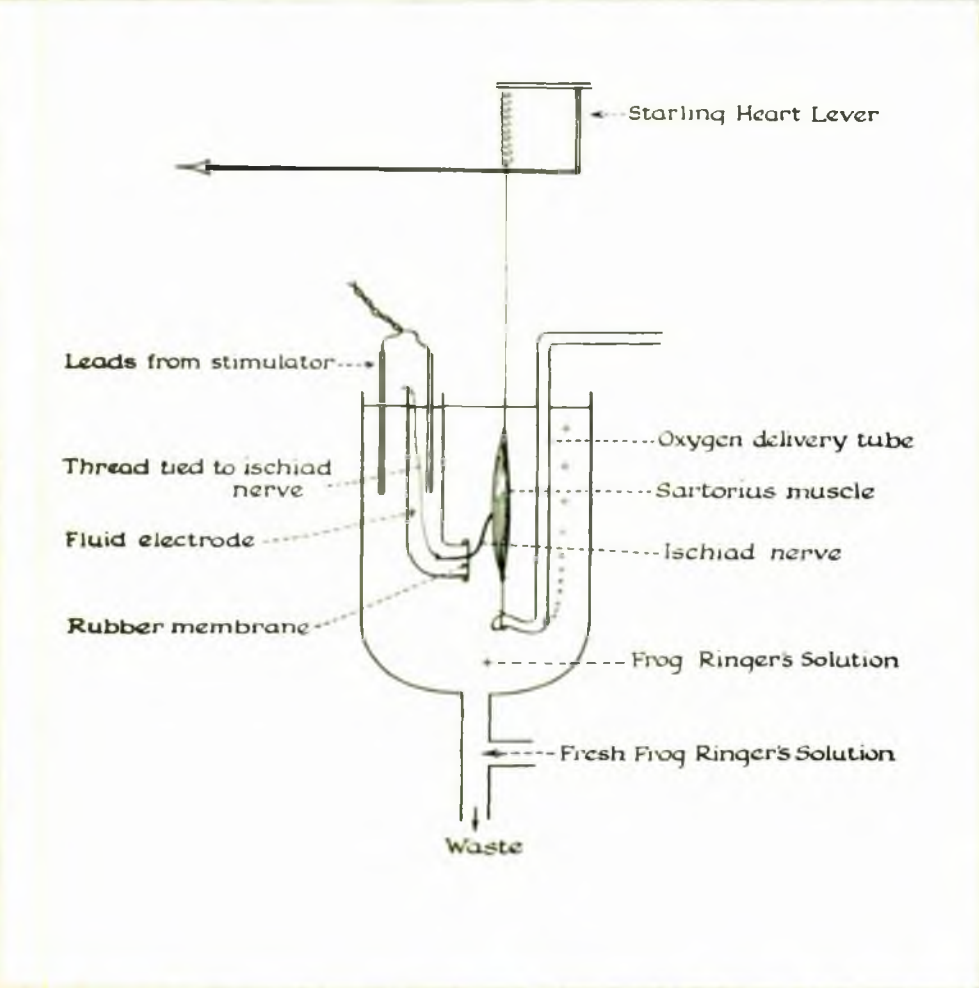
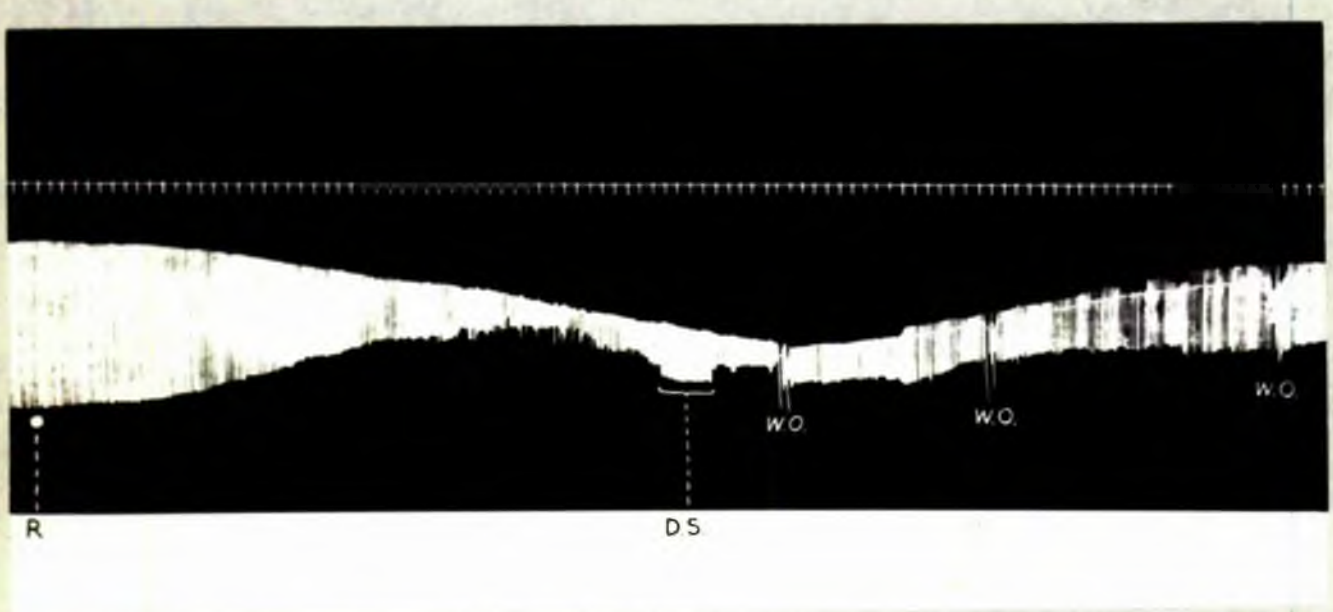


Diagram of the apparatus used for recording contractions of the frog sartorius muscle produced by electrical stimulation of the ischiad nerve.

the latter. Having freed and tied the ends of the muscle as described above, the path of the nerve from the muscle to its junction with the sciatic nerve was traced. At this point, a fine cotton thread was tied round the nerve which was then very gently freed from the connective tissue surrounding it. When the nerve had been freed, the preparation was removed from the leg. By means of a fine needle attached to the thread at its proximal end, about 1 cm. of the nerve was drawn through a rubber membrane into a J shaped fluid electrode filled with frog Ringer's solution. One end of the muscle was attached to an oxygen delivery tube, the other to a Starling heart lever. The complete preparation was then lowered into a bath containing 50 ml. of frog Ringer's solution (Figure 2,4). The muscle was stimulated indirectly through its nerve and also directly by means of a square-wave stimulator delivering 14 impulses per minute. The duration and voltage of the impulses were 1.0 milliseconds and 15 volts respectively. One terminal of the stimulator was connected to the bath fluid, the other to the fluid electrode. It was found that this preparation could be stimulated continuously for up to 5 hours without any signs of fatigue becoming evident.

Figure 2,5



The effect of reserpine upon the indirectly-induced contractions of the frog sartorius muscle.

Upper record, time = 30 seconds, lower record isometric contractions.

At R, 10 µg. reserpine added to the bath.

At D.S., the muscle was stimulated directly.

At W.O., the bath fluid was replaced with fresh frog Ringer's solution.

Contractions of the muscle or increase in tone pulled the lever from top to bottom of the trace.

Summary of results.

10 µg. of reserpine produced a reduction in the amplitude of contractions due both to direct and indirect stimulation of the muscle. Along with this action, the tone of the muscle increased. The reduction in height of the contractions of the muscle, but not the increased tone was also produced by an equivalent volume of the control solution.

RESULTS.

Whilst 2 µg. of reserpine failed to show any effect on the activity of the preparation, 10 µg. produced a gradual reduction of the indirectly-induced contractions. Inhibition was incomplete 20 minutes after the addition of reserpine to the bath (Figure 2,5). At this point direct stimulation was slightly more effective than indirect stimulation of the muscle. Recovery was incomplete two hours after the addition of reserpine. If a second similar dose of reserpine was then given, inhibition of the contractions was complete and direct stimulation had no effect. In three out of five experiments, a gradual increase in tone (indicated by a fall in the base line of the record) was observed

(Figure 2,5). This effect was reversible on washing out the drug. A volume of control solution equivalent to 10 µg. of reserpine had similar effects on the electrically-induced contractions of the muscle, but in no case produced the increase in tone which followed the addition of reserpine.

CHAPTER 2

REFERENCES.

1). J.H. Burn, "Practical Pharmacology", Blackwell Scientific Publications, Oxford, 1952, pg. 2.

2). W.E. Barret, T. Baker and A.J. Plummer, J. Pharmacol., (1956), 116, 5.

3). C.W. Sheppard, Science, (1951), 114, 85.

CHAPTER 3

In section B, the concentrations of drugs refer to the weight of drug per millilitre of physiological saline in the isolated organ bath. In sections A and D, the concentrations of reserpine, posterior pituitary extract and barium chloride refer to the weight of drug per millilitre of the perfusion fluid. In the case of other drugs, the concentrations indicate the weight of the drug injected in 0.1 ml. of physiological saline using a tuberculin syringe.

CHAPTER 3

EXPERIMENTS CARRIED OUT USING PREPARATIONS OF ISOLATED MAMMALIAN VASCULAR AND CARDIAC MUSCLE.

This chapter has been divided into three sections describing experiments with

A), the isolated perfused hearts of the rabbit and the kitten.

B), the isolated auricles of the rabbit and the guinea pig, and

C), the isolated perfused rabbit's ear, and the isolated perfused hind-quarters of the rat.

SECTION A

Experiments with the isolated, perfused hearts of the rabbit and the kitten.

The isolated hearts of both rabbits and kittens were perfused according to the method proposed by

Langendorff[1]. This involves perfusion of the coronary vessels through the aorta. R. Wegria[2], in his review on the pharmacology of the coronary circulation, quotes several published criticisms of the method. It is pointed out that the outflow will give a true picture of the tonus of the coronary vessels only if the aortic valves are competent; this is not always so. In the event of aortic incompetence, some perfusion fluid will leak past the valves into the left ventricle and so to the auricle and the exterior. The increased outflow may therefore exceed the true coronary outflow by the amount of fluid which has passed into the left ventricle. The volume of fluid draining into the right auricle via the ventricle is not constant and, in addition, cannot be measured satisfactorily. It is also pointed out that the volume of coronary perfusate may be increased by a purely mechanical massaging effect which cardiac muscle, stimulated by a cardiotonic drug, has upon the coronary vessels. Under those circumstances, an increase in outflow might be taken to indicate a coronary dilatation which in fact was not present. For these reasons it was decided that in this section the fluid draining from the heart should be described simply as "the cardiac outflow". In spite of the objections raised, it was

felt that the use of the preparation would give some general information on the effect of reserpine on cardiac function *in vitro*. By observing carefully the heart rate, the amplitude of contractions, and at the same time measuring the outflow, an estimate of the state of tone existing in the coronary vessels was obtained.

Methods.

The preparation of the heart for perfusion was the same for both the kitten and the rabbit. Rabbits and kittens used were within the weight ranges of 1 to 2 kg. and 0.6 to 1.0 kg. respectively. The animals were killed by a blow on the back of the neck. The throats were cut and the blood allowed to drain out. They were then placed on a dissecting board and the thoracic cavity exposed, care being taken not to damage the heart with scissors or other instruments. The lungs were removed and a thread was tied loosely round the aortic arch proximal to the source of the innominate artery. The vena cavae and aorta were then cut through and, after removing the pericardium, the heart was placed in a dish of cold Locke's solution (Appendix I) containing a little

heparin. A stream of Locke's solution was washed through the superior vena cava by means of a fine pipette and the heart squeezed gently. After washing, a cannula was tied into the aorta ensuring that its tip was distal to the coronary ostia. The preparation was then set up by connecting the cannula to the perfusion apparatus. Perfusion of oxygenated Locke's solution, containing double the normal concentration of glucose, was started at a pressure of 35 mm. of mercury. Any blood remaining in the preparation was rapidly washed away and the heart started to beat within ten to fifteen minutes. After about thirty minutes, the beat became constant and a supporting thread was tied, by means of a fine needle, through the tip of the left ventricle. A bent entomological pin was inserted into the wall of the right ventricle and connected to a Starling heart lever. It was found that placing the heart directly after dissection in cold rather than warm Locke's solution tended to produce a more strongly beating preparation. The reduction of metabolism so produced may have protected the heart against damage caused during the manipulative procedures prior to cannulation. Doubling the normal glucose concentration of the perfusion fluid also gave a more active prepara-

tion which showed less fatigue. The outflow was measured by means of a Gaddum outflow recorder in series with a three segment rotary key. The timing was so arranged that the maximum flow likely to be achieved did not distend fully the sensitive tambour used with the apparatus.

Locke's solution from the two reservoirs used flowed through heating coils in a water bath maintained thermostatically at 37°C. The two coils were connected by a glass Y piece which was joined to the aortic cannula by a short length of rubber tubing. The temperature drop between the thermostatically controlled water bath and the cannula was never more than 0.2°C.

Reserpine was dissolved in Locke's solution in one of the reservoirs to give the desired concentration. Alternatively a measured volume of the control solution was mixed with Locke's solution in the bottle. The other bottle contained Locke's solution. Unless specifically indicated other drugs were injected by means of a 1 ml. tuberculin syringe (fitted with a number 20 needle) into the rubber tubing attached to the aortic cannula. All drugs were dissolved in Locke's solution so that

0.1 ml. contained the required concentration.

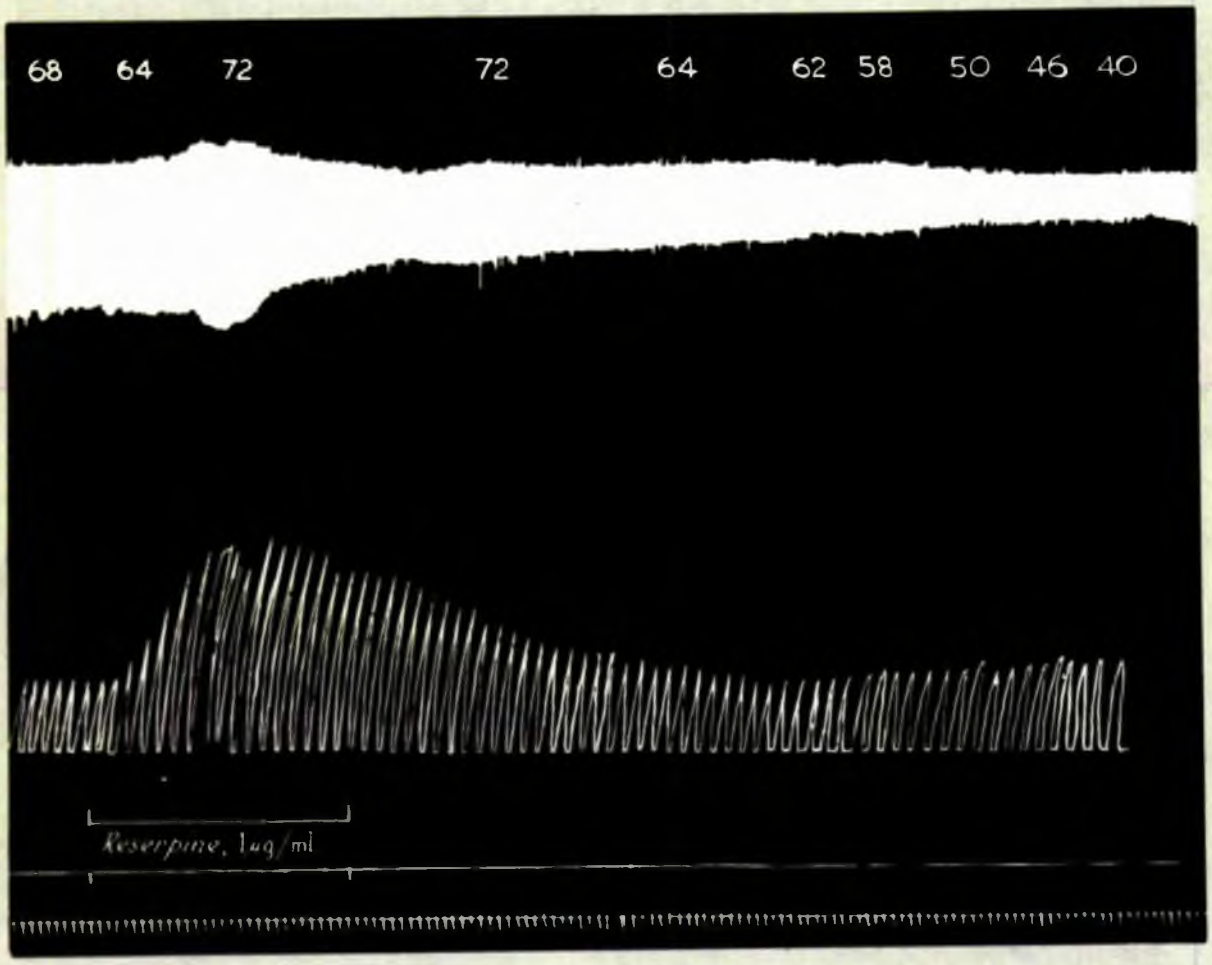
Summary of results.

In concentrations of 0.1 µg. and 1.0 µg. per ml., reserpine caused an increased cardiac outflow which was accompanied by a slight initial increase and followed by a decrease in rate and amplitude. In some cases only the negative inotropic and chronotropic actions were seen. Recovery from these effects was seldom complete, even three hours after the removal of reserpine. The alkaloid generally had little effect on the characteristic actions of adrenaline, nor-adrenaline, histamine or 5-hydroxytryptamine. In some cases, however, the duration of the cardiotonic action of these drugs was reduced by reserpine. The reduction in "cardiac outflow" produced by either posterior pituitary extract or barium chloride was antagonised by reserpine.

RESULTS.

No difference was noticed in the results obtained in experiments with rabbit and kitten hearts. 1 µg. per ml. reserpine produced in most cases a marked increase in

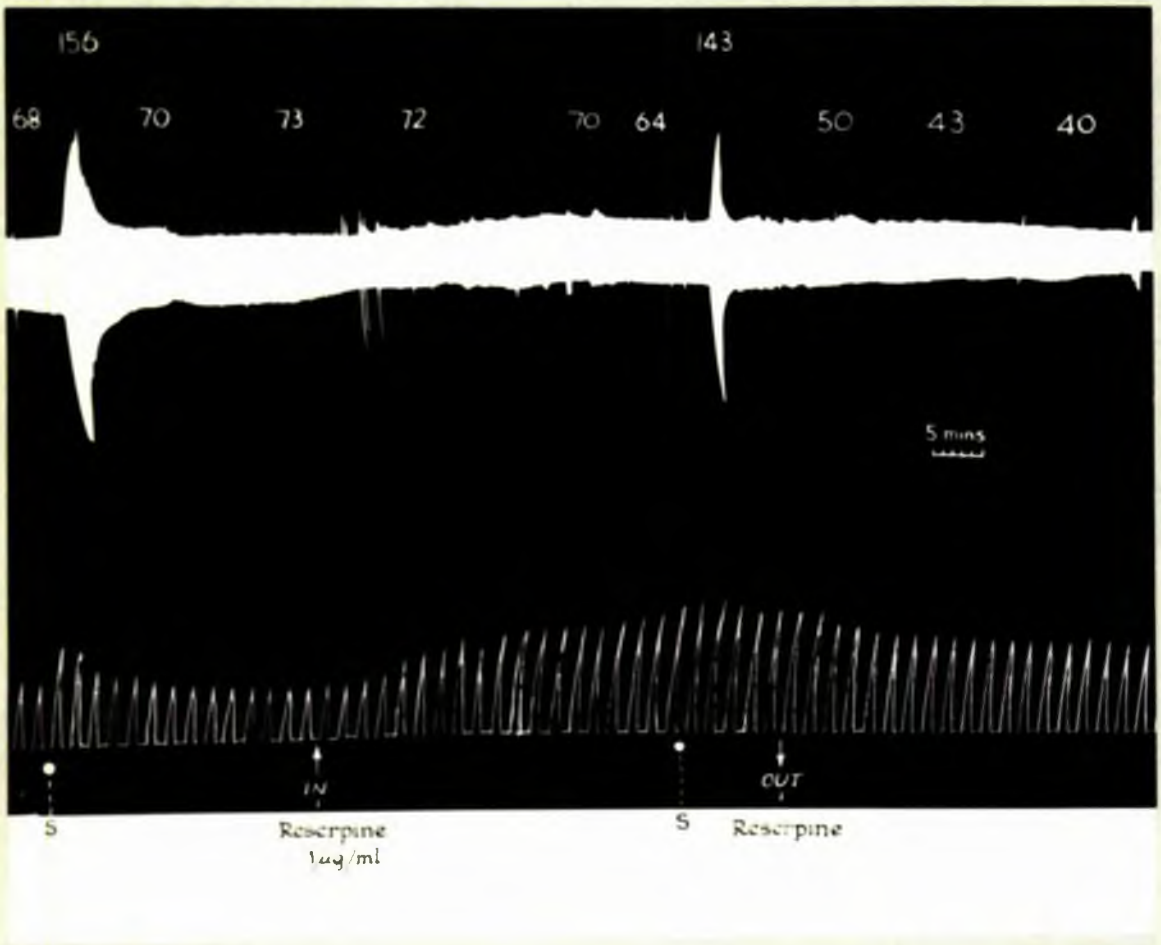
Figure 3.1



The influence of 1 µg. per ml. reserpine upon the rate, amplitude and outflow of the isolated perfused kitten's heart.

Upper record, heart rate in beats per minute;
middle record, amplitude of the beat;
lower record, outflow; lowest record;
time = 60 seconds.

Figure 3.2



The influence of 1 µg. per ml. reserpine upon the duration of effect of 5-hydroxytryptamine on the amplitude of contractions of the isolated, perfused rabbit's heart.

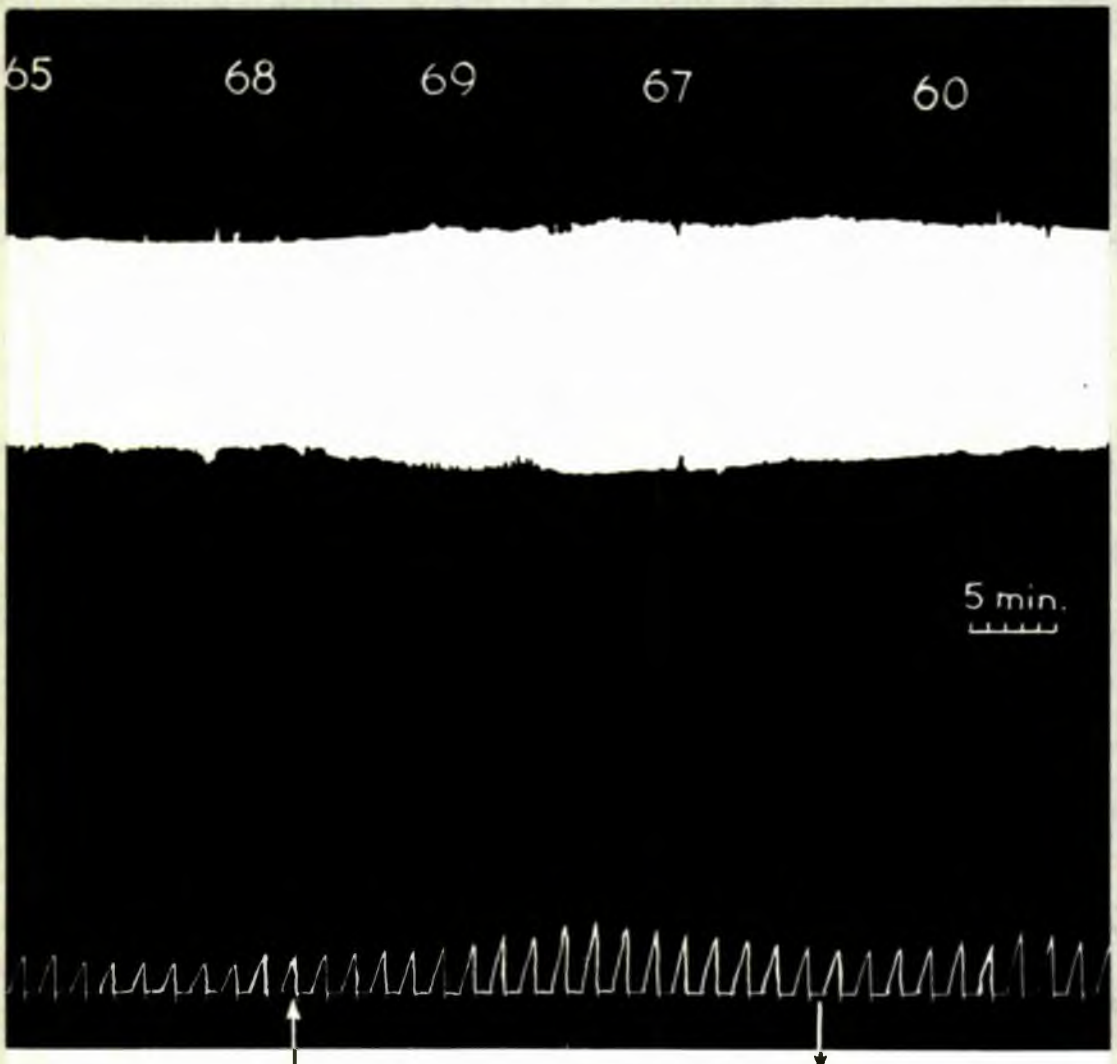
Upper record, heart rate in beats per minute;
middle record, amplitude of the beat;
lower record, outflow.
At S, 10 µg. 5-hydroxytryptamine injected into the perfusion cannula.

outflow. There was an initial increase, followed by a decrease in the rate and amplitude of the contractions, or only a decreased rate and amplitude (Figure 3,1). 0.1 µg. per ml. reserpine produced less effect on the preparation and in some experiments had no observable action on cardiac function. In only one experiment out of twenty was the recovery of the rate and amplitude of the contractions complete after the withdrawal of reserpine. In some cases the outflow did not return to its original level after the withdrawal of reserpine (Figure 3,2). The decrease in rate and amplitude was gradual. Outflow was increased within five minutes of starting the reserpine perfusion. The decrease in rate and amplitude did not become evident until the solution of reserpine had been perfused for at least fifteen minutes.

The control solution of ascorbic acid, equivalent to 1 µg. per ml. of reserpine had qualitatively similar, but quantitatively much weaker effects on the heart (Figure 3,3). A volume equivalent to 0.1 µg. per ml. reserpine had no effect.

The increased rate and amplitude of contraction produced by adrenaline, 5 ng., nor-adrenaline, 1 ng.,

Figure 3.3



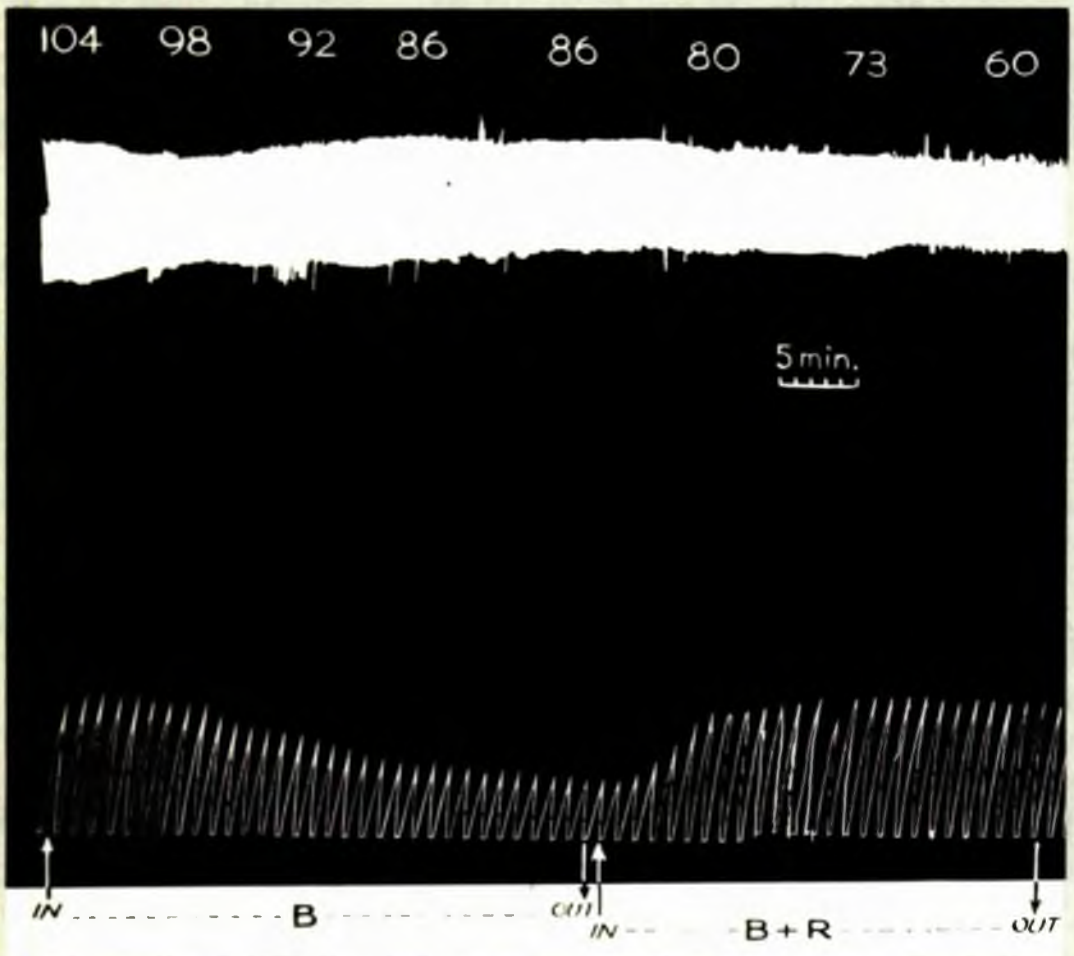
The effect of the ascorbic acid-control solution upon the activity of the isolated, perfused rabbit's heart.

Upper record, heart rate in beats per minute;
middle record, amplitude of the beat;
lower record, outflow.

histamine, 10 µg., or 5-hydroxytryptamine, 10 µg. were not significantly altered by reserpine. In some cases, however, the duration of their effects on the amplitude of the beat seemed to be reduced slightly (Figure 3,2). When considering the possibility of a reduction in the cardiotonic actions of these drugs, the "background" reduction of rate and amplitude had to be considered.

The apparatus used for the work described above was unsuitable for studying the effect of reserpine on the actions of barium chloride and posterior pituitary extract. The injection of either drug into the rubber tubing immediately before the aortic cannula produced only a transient effect on the outflow. A third reservoir was therefore introduced into the perfusion system so that one of four solutions could be perfused through the heart as desired. The solutions used were, 1) Locke's solution, 2) Locke's solution containing either 0.05 i.u. per ml. posterior pituitary extract or 3) 50 µg. per ml. of barium chloride and 4) Locke's solution in which was dissolved the same concentration of posterior pituitary extract or barium chloride together with reserpine, 1.0 µg. per ml. In carrying out each experiment, the normal outflow

Figure 3.4



The antagonism of reserpine to the action of barium chloride on the isolated, perfused kitten's heart.

Upper record, heart rate in beats per minute;
middle record, amplitude of the beat;
lower record, outflow.
At B, preparation perfused with 50 µg. per ml. barium chloride solution.
At B + R, preparation perfused with 50 µg. per ml. barium chloride solution together with 1 µg. per ml. reserpine.

was recorded for about thirty minutes, and then perfusion of either barium chloride or posterior pituitary extract was started. When the resultant decrease in outflow had become constant, perfusion of the same solution containing reserpine was commenced. Using this procedure it was found that the reduced outflow produced by barium chloride and posterior pituitary extract was markedly antagonised by reserpine (Figure 3,4). Reserpine had no influence upon the effects of barium chloride, 50 µg. per ml., or posterior pituitary extract, 0.05 i.u. per ml. upon the rate or amplitude of the contractions.

The results obtained with barium chloride and posterior pituitary extract confirm the observations of Tripod and Meier[3] on the isolated rabbit and cat hearts. These authors obtained results similar to those described above with the cardiotonic drugs mentioned earlier in this section. C. Bianchi[4], using the Langendorff preparation of the rabbit heart found that a concentration of reserpine as low as 0.038 µg. per ml. produced an increased outflow, which he described as vasodilatation. This is at variance with the results described in this section, since the author was unable to demonstrate any observable effect on the preparation with doses lower than 0.1 µg. per ml. Tripod and Meier[3] also report that 0.1 µg. per ml. reserpine,

even when perfused for thirty minutes, failed to produce any effect on the activity of the isolated heart.

SECTION B

Experiments on the isolated auricles of the guinea-pig and the rabbit.

The use of the isolated perfused heart gave an indication of the action of reserpine on cardiac function *in vitro* and on the typical actions of certain cardio-active agents. The impression was gained while carrying out these experiments that reserpine reduced the general vascular support. It seemed important therefore to study the action of reserpine directly on cardiac muscle. Isolated auricular muscle was chosen for this purpose.

Methods.

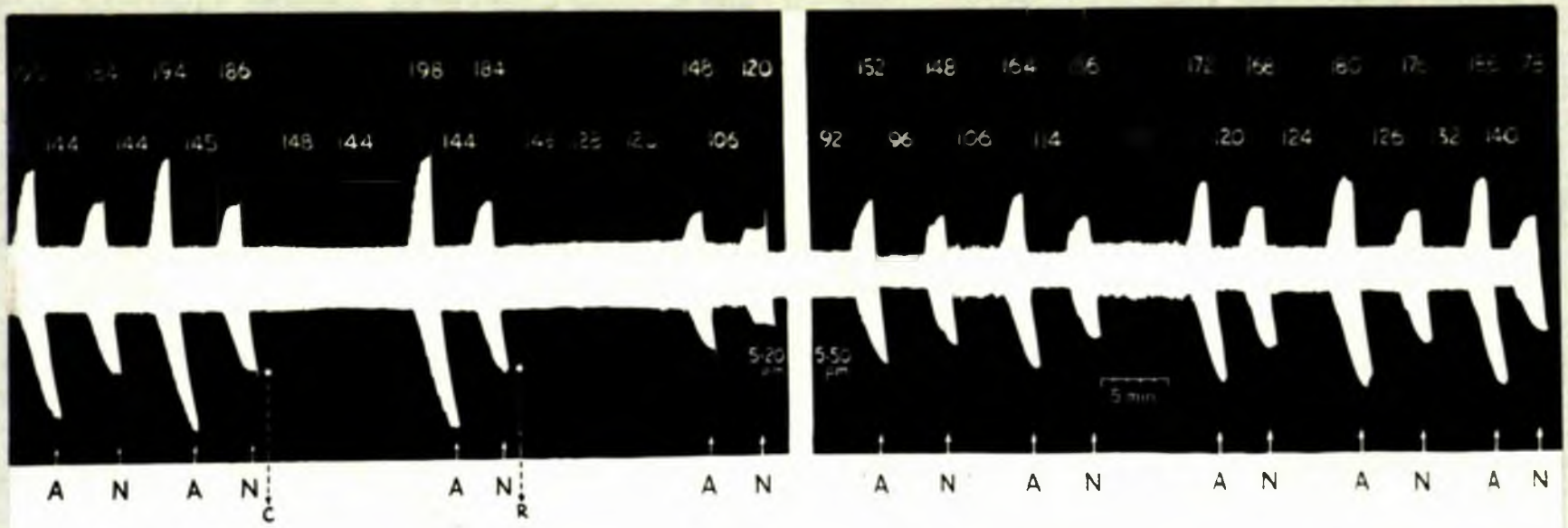
Guinea-pigs and young rabbits were killed by a sharp blow on the back of the head. The throats were cut and the blood allowed to drain out. The hearts were removed as rapidly as possible and immersed in well

oxygenated Locke's solution (Appendix I). Using a pair of fine scissors, the ventricles were removed and the auricles placed upon a cork mat and moistened with Locke's solution. All extraneous tissue was dissected away until the horseshoe-shaped auricles alone remained. They were then suspended in a 25 ml. organ bath by means of two bent pins to which thread was tied. One thread was connected to the oxygen delivery tube at the base of the bath; the other to a Starling heart lever. After about thirty minutes, the beat of the preparation was constant and the experiment begun. Drugs, other than reserpine, were added to the bath in a total volume of 0.1 ml. Locke's solution at ten minute intervals. They were allowed to act for 30 to 60 seconds after which they were washed out. Reserpine was added 5 minutes before each of the stimulant or depressant drugs tested.

Summary of results.

Reserpine reduced the rate and amplitude of the spontaneous contraction of the auricles. The maximum reduction in amplitude usually occurred after the reserpine had been washed out of the bath. The increased rate and amplitude produced by adrenaline, nor-adrenaline

Figure 3.5



The influence of reserpine upon a) the rate and amplitude and b) the response to adrenaline and nor-adrenaline of the isolated guinea-pig's auricles.

Figures above the recording refer to the number of beats per minute; upper row after drug administration, lower row - normal beat..

At A, 0.25 µg. adrenaline added.

At N, 0.1 µg. nor-adrenaline added.

At C, 0.1 ml. ascorbic acid control solution added.

At R, 1 µg. reserpine added.

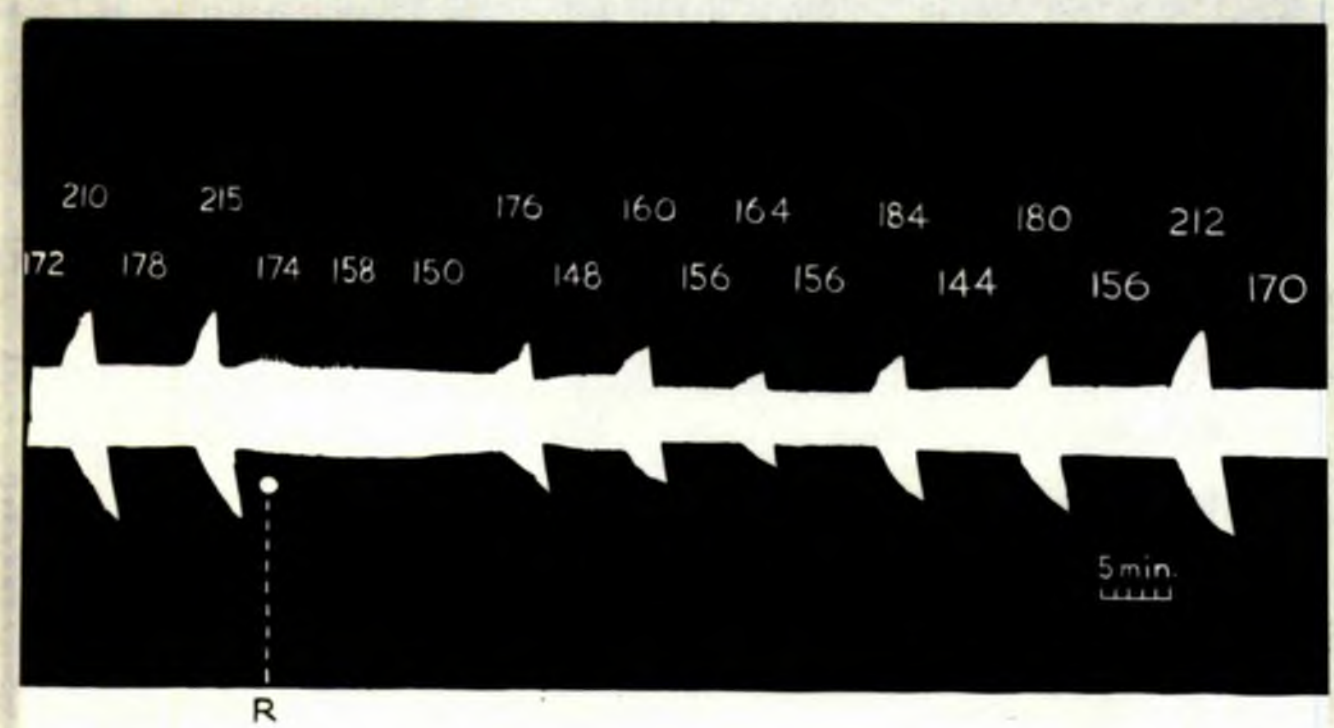
and histamine was reduced by reserpine. This effect was reversible on washing, provided that the time of contact of reserpine with the tissue was not long. In this case the partial recovery was very slow and often took as long as two hours.

RESULTS.

When reserpine in concentrations of 1.0, 2.0 or 10.0 µg. was added to the bath, there was in some cases an almost immediate reduction of the rate and amplitude of the contractions. In most experiments, however, those effects were seen about ten to fifteen minutes after reserpine had been washed out of the bath (Figure 3,5). The alteration in rate and amplitude was usually gradual in onset and tended to be prolonged. There appeared to be little quantitative relationship between the actions of reserpine and the concentrations of the drug which were used.

In order to compare fully the effects of reserpine on the isolated auricles and the isolated perfused heart, the same cardioactive drugs as were used on the heart were also used in this series of experiments.

Figure 3.6



The influence of reserpine upon the response to histamine of the isolated rabbit's auricles.

Figures above the tracing refer to the number of beats per minute; upper row after drug administration, lower row, normal beat.

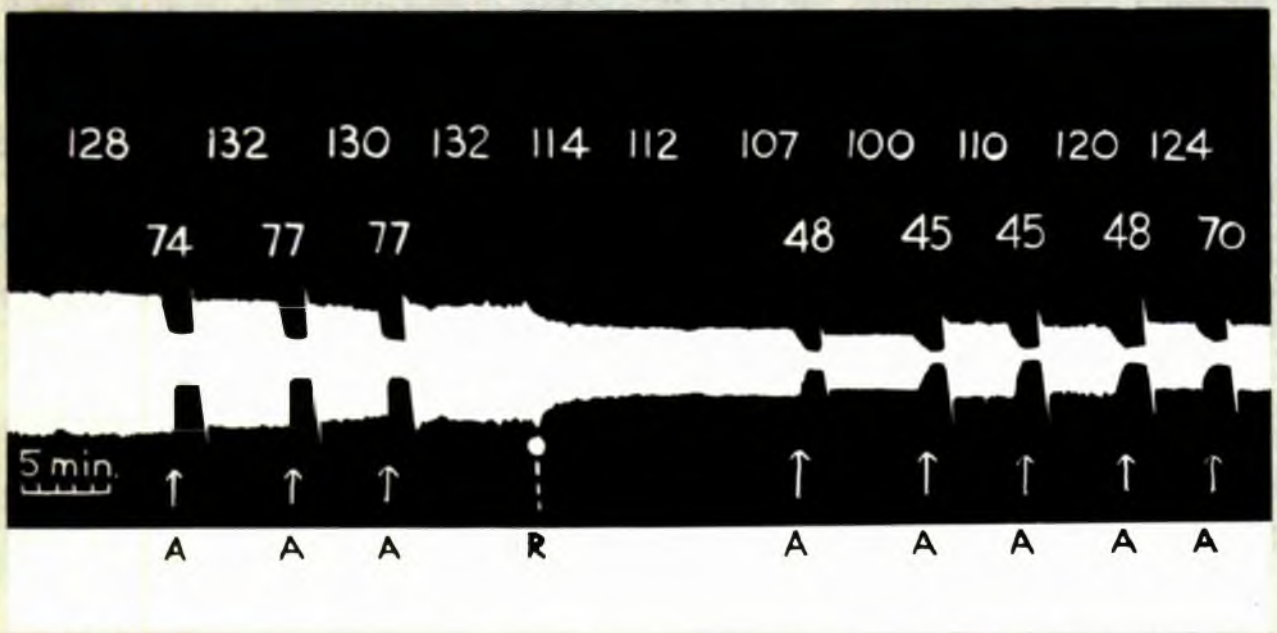
All increases in amplitude were produced by the addition of 0.5 µg. histamine.

At R, 1 µg. reserpine added.

1 µg. of reserpine reduced the increased rate and amplitude produced by adrenaline 0.25 µg., nor-adrenaline 0.1 µg. and histamine 0.5 µg (Figures 3,5 and 3,6). This reduction was seen ten to fifteen minutes after the addition of reserpine. It was completely reversible on washing only if the contact of the alkaloid with the tissue was not longer than ten minutes. If a longer period of contact was allowed, some irreversible change appeared to take place and recovery was incomplete. In some experiments, the responses to nor-adrenaline were more actively antagonised than those to adrenaline (Figure 3,5). It was found impossible to use 5-hydroxytryptamine since the drug appeared to have some toxic action on this preparation and constant responses to the same concentration could not be obtained.

It seemed of importance to test also the effect of reserpine on the response to a drug which reduces the spontaneous activity of the preparation, for example, acetylcholine. It was found that the marked reduction in rate and amplitude produced by 0.01 µg. of this drug was either unaltered by reserpine or apparently, slightly potentiated (Figure 3,7). It must be considered,

Figure 3.7



The influence of reserpine on the response to acetylcholine of the isolated rabbit's auricles.

Figures above the tracing refer to the number of beats per minute; upper row, normal beat, lower row after addition of acetylcholine or reserpine.

At A, 0.01 µg. acetylcholine added.

At R, 10 µg. reserpine added.

however, that the action of reserpine itself was to reduce the activity of the preparation and therefore it is unlikely that any true potentiation of the action of acetylcholine was being recorded. Recovery of the normal response to acetylcholine was usually complete. In some cases, however, the effect of reserpine on the amplitude of the beat was irreversible. This was especially the case after larger doses (Figure 3,7).

SECTION C

The effect of reserpine on the blood vessels of the isolated perfused rabbit's ear and the isolated perfused hind-quarters of the rat.

Having studied the action of reserpine on the isolated heart and auricular muscle preparations, it seemed important to investigate the effect of the alkaloid upon an isolated vascular bed. For this purpose, two preparations of isolated blood vessels were used, namely the perfused vessels of the rabbit's ear and the rat's hind-quarters.

Methods.

In investigations of the effect of drugs on isolated blood vessels, two general types of experiments have been used by various workers. In one, the pressure at which the physiological fluid passes through the vessels is kept constant and alterations in the outflow produced by drugs are recorded. The other method ensures a constant perfusion through the blood vessels by means of a small pump; alterations in the perfusion pressure then give an indication of the changes in the tone of the blood vessels produced by drugs. This method has been used by McQueen, Doyle and Smirk[5] in an investigation of the action of reserpine on the innervated hind limb of the rabbit. In the experiments described in this section, alterations in the outflow of perfusion fluid were recorded on a smoked drum, the perfusion pressure remaining constant.

The perfusion fluid used was that recommended by Page and Green[6] (Appendix I). Gaddum and Hameed[7] observed that the perfusion of this solution rendered the preparation more sensitive to vasoconstrictor drugs than did perfusion with Locke's solution.

Rabbits were killed by a sharp blow on the back

of the neck. It was found that subsequent cannulation was more easily carried out if the fur at the base of the ear was shaved and the path of the dorsal auricular artery traced in ink before killing the animal. Also, if the throat was not cut, the vessels in the ear did not tend to collapse. The animal was placed on a dissecting board and a small strip of skin at the base of the ear removed. The dorsal auricular artery (the central vessel of the ear) was carefully freed from adherent tissue using the blunt dissection technique. A fine polythene cannula, filled with perfusion fluid containing a little heparin, was then tied into the vessel. The ear was severed from the head and connected to the perfusion apparatus (Figure 3,8) by means of fine rubber tubing.

Rats of both sexes, weighing between 200 g. and 300 g. were killed by a blow on the head. The throats were cut and the blood allowed to drain out. The abdominal cavity was opened by means of a longitudinal incision from the sternum to the anus. The rectum, oesophagus and the inferior and superior mesenteric arteries were divided between ligatures. The abdominal viscera were then removed. This brought into view the

Figure 3.8

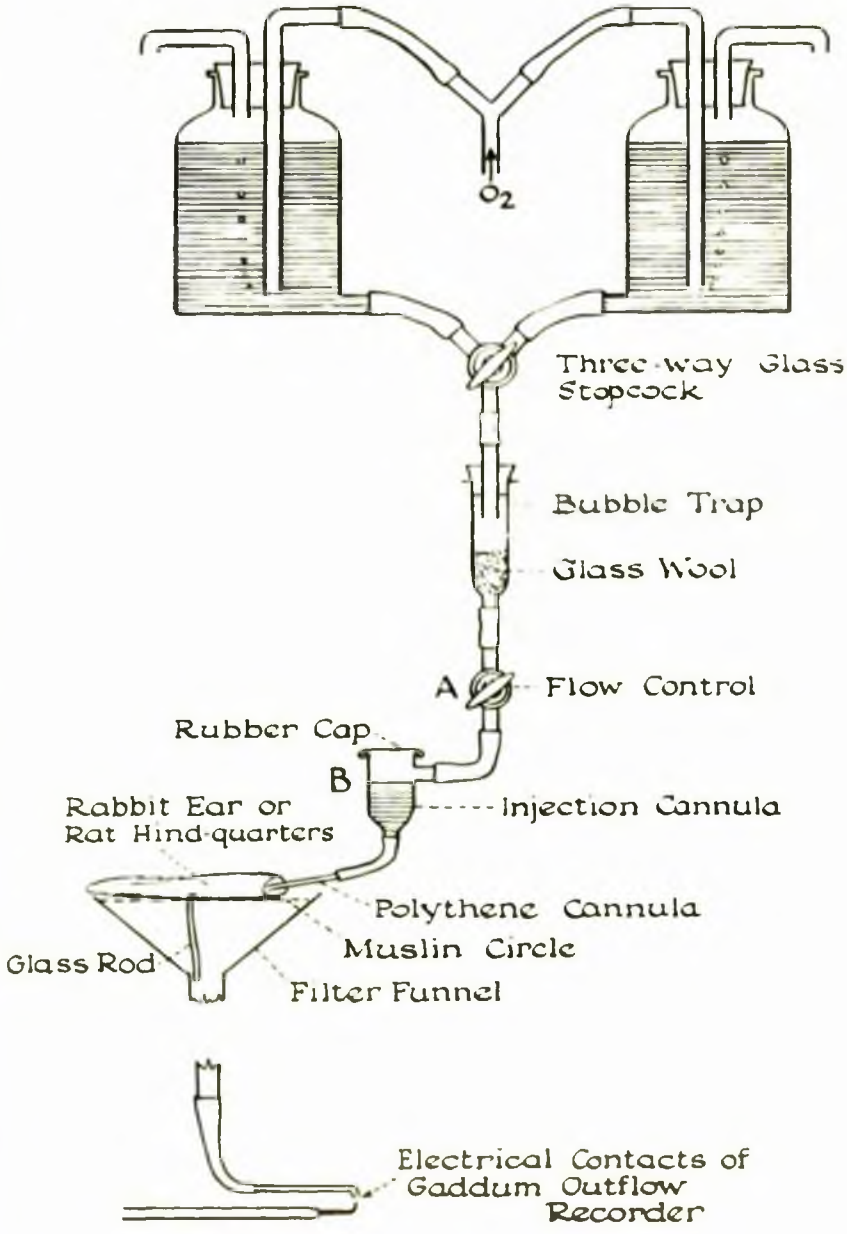


Diagram of the apparatus used for perfusion of the isolated rabbit's ear and rat's hind-quarters.

abdominal aorta, which was cannulated. The body wall and vertebral column were cut through above the point of cannulation and the hind-quarters connected to the same apparatus used to perfuse the rabbit's ear (Figure 3,8).

Two reservoirs were used in the apparatus. These were joined by means of a glass, three-way stop-cock. The rate of flow of fluid from the bubble trap to the injection cannula was controlled by the tap A, and was adjusted to a suitable value at the beginning of each experiment. The injection cannula, the design of which was based upon that suggested by Gaddum and Kwiatkowski[8] (Figure 3,8), allowed the injection of drug solutions at a constant rate. This was achieved by injecting the solution with a tuberculin syringe, fitted with a fine needle, through the rubber cap at a rate such that the level of fluid (B) in the cannula was unaltered during the process. The ear or hind-quarters preparations were placed on a muslin rest lying in a filter funnel. The outflow was led <u>via</u> the filter funnel to the contacts of a Gaddum drop recording assembly[9].

In all experiments reserpine was dissolved

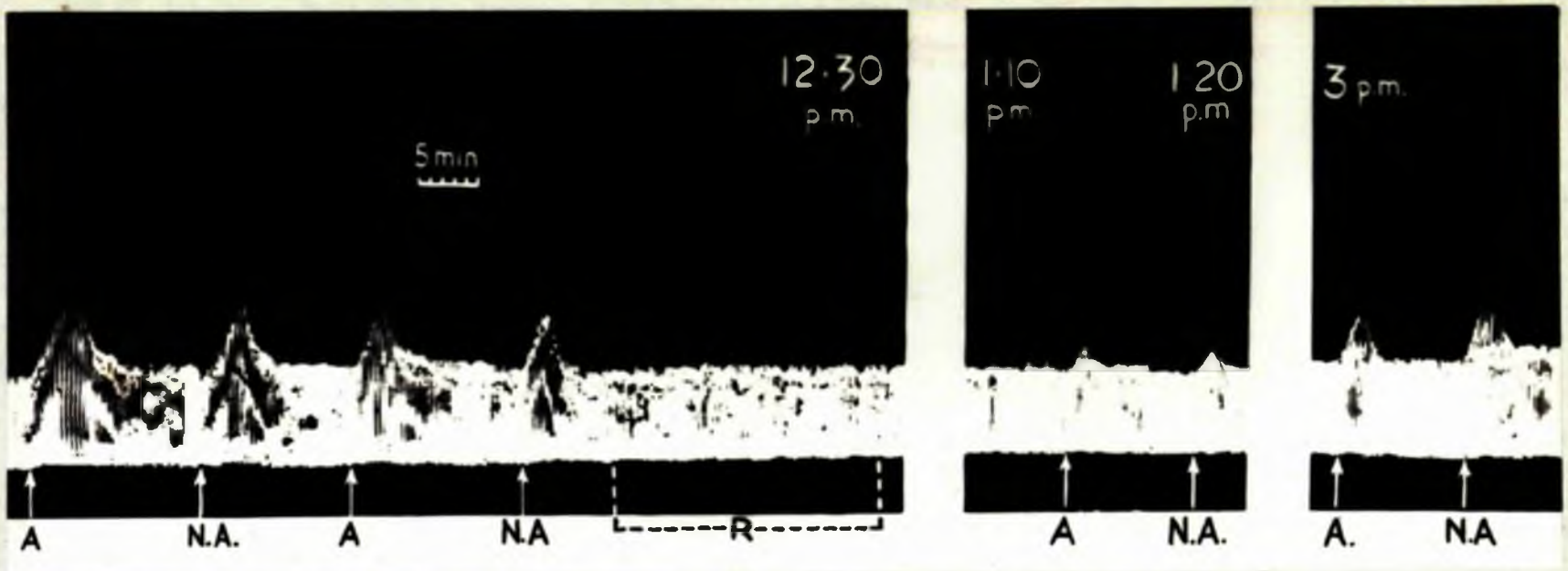
in the perfusion fluid and this solution was placed in one reservoir. The other bottle was filled with the perfusion fluid. Other drugs were dissolved in 0.1 ml. of the perfusion fluid and added at the injection cannula.

After setting up the preparations, a uniform outflow record was obtained for at least fifteen minutes. Drugs were then injected until two similar responses had been obtained to the same concentration of drug. Reserpine solution was allowed to perfuse through the preparation for ten minutes and the same concentration of drug injected again.

Summary of results.

Reserpine at a concentration of 1 µg. per ml. had no observable direct effect on the vessels of either the rabbit ear or the rat hind-quarters even after perfusion for one hour. It did, however, reduce the vasoconstriction produced by the injection of constant doses of nor-adrenaline, adrenaline, 5-hydroxy-tryptamine and histamine. This action was only partially reversed after reserpine perfusion had ceased.

Figure 3.9



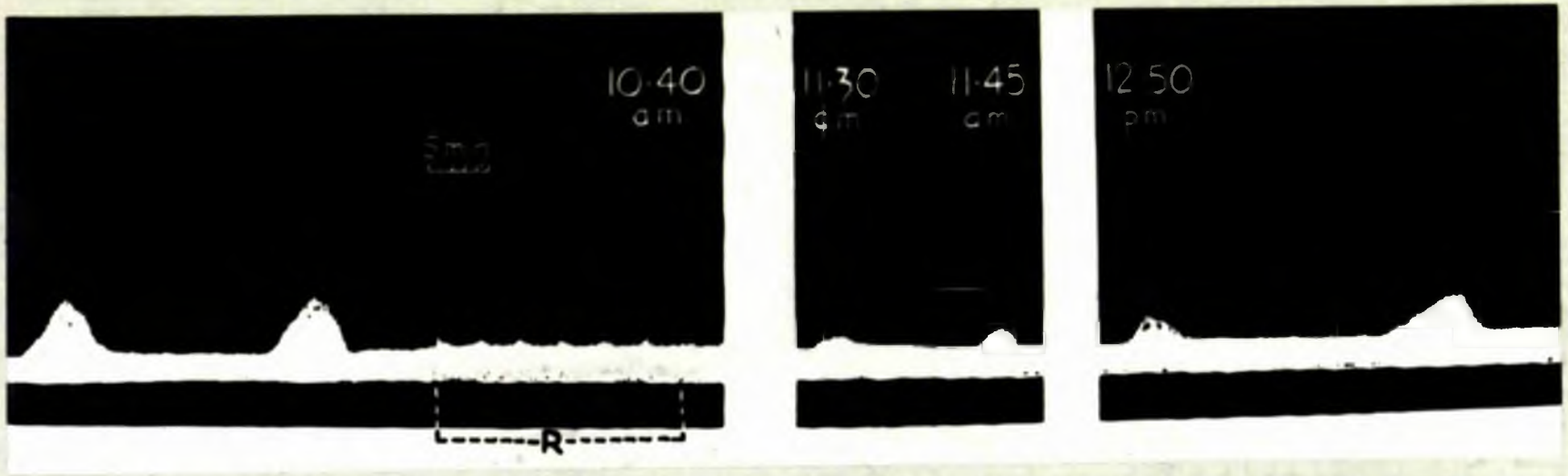
The antagonism shown by reserpine to drug-induced vasoconstriction of the vessels of the perfused rabbit's ear.

At A, 10 ng. adrenaline injected.

At N.A., 2 ng. nor-adrenaline injected.

During period marked R, 1 µg. per ml. reserpine was perfused through the preparation.

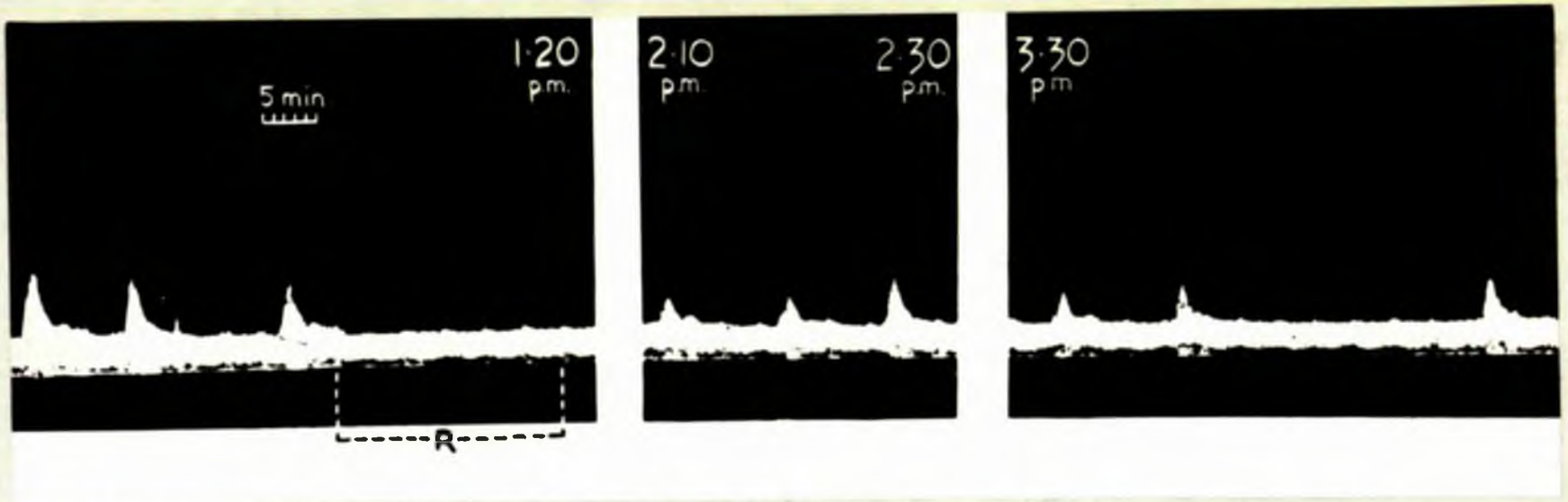
Figure 3.10



The antagonism shown by reserpine to the action of 5-hydroxytryptamine on the vessels of the perfused rat's hind-quarters.

All decreases in outflow produced by the injection of 10 ng. 5-hydroxytryptamine. During period marked R, 10 µg. per ml. reserpine was perfused through the preparation.

Figure 3.11



The antagonism by reserpine to the constrictor action of histamine on the vessels of the isolated perfused rabbit's ear.

All decreases in outflow produced by the injection of 20 ng. histamine.

During the period marked R, 1 µg. per ml. reserpine was perfused through the preparation

RESULTS.

1 µg. per ml. of reserpine did not appear to have any direct effect on the outflow from either preparation. In both the rabbit's ear and rat's hind-quarters, there was a reduction of the vasoconstriction produced by adrenaline 10 ng. to 50 ng. and nor-adrenaline 1 ng. to 10 ng. (Figure 3,9), 5-hydroxytryptamine 5 ng. to 50 ng. (Figure 3,10) and histamine 20 ng. to 100 ng. (Figure 3,11). The constrictor action of histamine was reduced to a lesser degree than that of adrenaline or nor-adrenaline. No difference was observed between the actions of reserpine on the responses to those drugs in the preparations used. In general, the rat hind-quarters preparation was found to be less sensitive to vasoconstrictor agents than was the rabbit's ear. The degree of inhibition was unaltered by increasing the dose of reserpine. Recovery of the response to the vasoconstrictor agents was never complete over the period of observation, which was always for at least three hours after the perfusion of reserpine. The extent and duration of such recovery as did take place was very variable.

The antagonism shown by reserpine to drug-

induced vasoconstriction was unusual, in that it could be demonstrated regardless of the agent used. The pharmacological nature of the vasoconstrictor agents or their supposed sites of action did not appear to alter the effects of reserpine. The apparent non-specificity of this depressant action of the alkaloid was also noticed on the smooth muscle of gut and uterus (Chapter 4, page 52).

Tripod and Meier[3], using the perfused hind-quarters of the rabbit, demonstrated that a sustained vasoconstriction produced by the continuous perfusion of adrenaline and nor-adrenaline was only slightly antagonised by 1 µg. per ml. reserpine. The tone induced by histamine and 5-hydroxytryptamine was somewhat more effectively antagonised. They were unable to report any direct action of reserpine on the isolated blood vessels. McQueen and his colleagues[5,9] however, using the innervated, but otherwise isolated rabbit's hind-quarters, found that the injection of 0.125 mg. reserpine caused an immediate diminution of vasomotor tone. Both groups of workers concluded that reserpine had definite peripheral effects. McQueen and his colleagues added that the hypotensive effect of the

drug could not be ascribed purely to a central action.

In the completely denervated preparation, which was no longer under any neural control, the vessels were maximally dilated. Therefore the observation that reserpine apparently had no direct depressant effect on the vessels of the rabbit ear and rat hind-quarters was not unexpected. The drug-induced tone of the vessels of the rabbit's hind-quarters was only slightly antagonised by reserpine[3]. In all cases, however, some antagonism could be demonstrated. Presumably drugs injected into the cannula were rapidly washed out of the vessels. The period of contact with the receptors ultimately responsible for the vaso-constriction seen would therefore have been short. When these drugs were perfused continuously, it would be expected that the longer period of contact with the receptors would have resulted in a less easily antagonised vasoconstriction. The failure of reserpine to show a very marked antagonism to adrenaline and nor-adrenaline[3] might be explained on this basis. The anatomical differences between the hind-quarters of the rabbit[3] and the rabbit's ear and rat's hind-quarters used by the author may also have played a part in producing the varia-tion in the degree of inhibition of vasoconstriction noticed.

CHAPTER 3

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CHAPTER 4

The concentrations of drugs mentioned in this chapter refer to the weight of the drug per millilitre of physiological saline in the isolated organ bath.

CHAPTER 4

EXPERIMENTS CARRIED OUT IN VITRO USING PREPARATIONS OF ISOLATED INTESTINAL AND UTERINE SMOOTH MUSCLE.

This chapter has been divided into the following sections, describing the actions of reserpine on

A), the isolated ileum of guinea-pig, kitten and monkey,

B), the isolated duodenum of rabbit, kitten and monkey,

C), the isolated colon of rabbit, rat and monkey,

D), the isolated human colon, and

E), the isolated rat's uterus.

Methods.

Excepting when human tissue was used, the experimental procedure was the same for all groups. Virgin female rats, weighing between 120 and 180 g. were brought

into oestrous by subcutaneous injections of 0.1 mg. per 100 g. body weight of stilboestol in arachis oil given 24 hours before use. The animals were killed by a blow on the back of the head, the throats cut and the blood drained out. Monkeys were killed by placing them in an airtight box into which was passed coal gas. The intestines and/or the uteri were removed and placed immediately in the appropriate oxygenated saline. The following physiological saline solutions were used (Appendix I, page 181).

1) Tyrode's solution through which 95 per cent O_2/5 per cent CO_2 was bubbled (for guinea-pig ileum).

2) Locke's solution aerated with oxygen (for rabbit, kitten and monkey intestine). In some experiments, Krebs-Henseleit solution (aerated with 95 per cent O_2/5 per cent CO_2) was used. This was also used for preparations of human gut.

3) De Jalon's solution aerated with O_2 (for rat uterus and intestine).

Figure 4.1

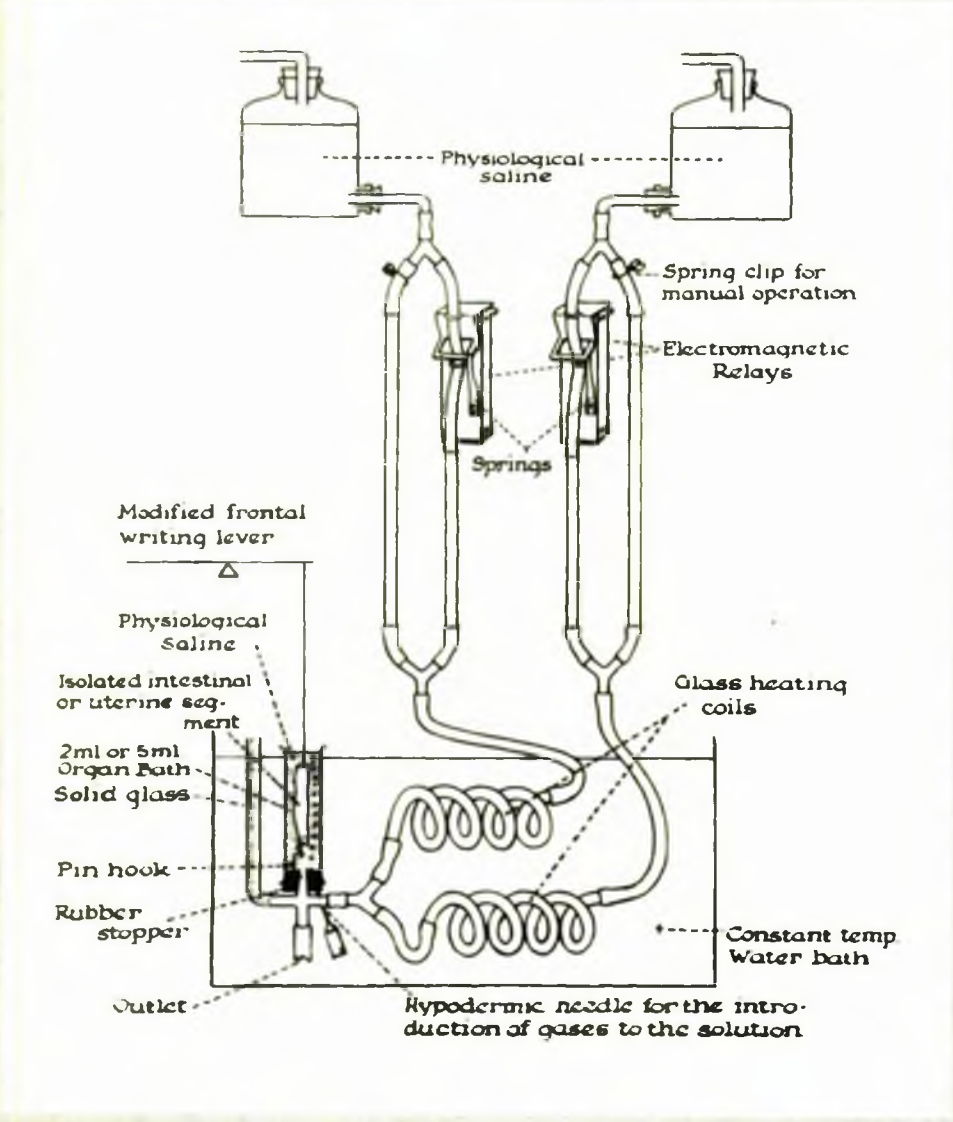


Diagram of the isolated organ bath used for testing the effects of reserpine on isolated gut and uterus.

All the segments used were between 3 cm. and 5 cm. long. They were removed from the following positions in the intestine:

Duodenum: 5 cm. distal from the pyloric sphincter.

Ileum: 2 cm. proximal to the ileo-caecal junction.

Colon: 2 cm. distal from the ileo-caecal junction.

Segments of gut and uterus were freed from fat and other tissue and the contents washed out by means of a stream of physiological saline. Threads were tied to both ends of the segments which were then set up in a 2 ml. or 5 ml. organ bath (Figure 4,1). One thread was attached to a modified frontal-point writing lever, the other to a hook fixed into the base of the bath. Drug solutions were added and the bath washed out automatically using the overflow principle. The inlet tube at the lower end of the bath was connected *via* heating coils to two reservoirs, one of which contained the physiological solution in use, the other a solution of the drug in the same saline. The dose of drug to be used was determined at the start of each experiment, by adding it to the bath by hand. The drug was then dissolved in the physiological

saline to give the dilution required and the automatic apparatus switched on. The electrical controlling equipment (Appendix II, page 184) replaced the saline solution in the bath by the drug solution at three minute intervals. Since the drug solution flowed into the bath for five seconds, there was complete replacement of the solution in the bath. An accessible wander plug bank in the circuit allowed the drug solution to remain in contact with the tissue for 15, 20, 25, 30, 45, 60 or 90 seconds. The contact period chosen was one which allowed the contraction to reach a plateau. At the end of this period, the drug solution was washed out automatically by the inflow of pure saline solution. When the contractions to the drug had become constant reserpine solution was added by hand 60 or 90 seconds before the next addition of drug. This point in the cycle was indicated by a signal light in the circuit which went on 5 seconds before the addition of reserpine solution was due. The contractions were allowed to return to a constant level before the next addition of reserpine.

Segments of human colon, removed during surgery for rectal carcinoma, were placed immediately after

excision in vacuum jars containing chilled Krebs-Henseleit solution. Upon arrival at the laboratory the segment was opened along the line of the mesenteric attachment and the fat and other tissue removed. The mucosa was removed by scraping with a scalpel blade. A 6 cm. length was then suspended in a 100 ml. bath. The bath was aerated with 95 per cent O_2 and 5 per cent CO_2 bubbled through a sintered glass tube, to which the lower end of the tissue was attached. The upper end of the tissue was connected to a modified frontal-point writing lever, recording on the surface of a smoked kymograph paper.

In the case of human gut, drugs were added at 10 or 15 minute intervals by means of a tuberculin syringe. The same dose of drug was added until the response of the tissue was constant. Doses of reserpine were added two minutes before the drug.

Unless otherwise indicated, the bath temperature was maintained thermostatically at 37°C. The pH of all salines used was within the range 7.3 to 7.5 as determined using a glass electrode.

Summary of results.

Reserpine usually had no observable direct effect on the activity of the tissues studied. The dose range used was from 1 μg. to 30 μg. In duodenum suspended in Krebs-Henseleit solution, however, 10 μg. to 30 μg. reserpine had a stimulant action. This effect was not seen when Locke's solution was used. A slight stimulant action was seen on guinea-pig ileum when this tissue showed some spontaneous movements.

In all cases, the contractile response of the tissues to drugs was reduced by reserpine. The degree of inhibition produced varied widely from tissue to tissue and also within each experiment on the same tissue. The inhibition produced at the beginning of an experiment was always smaller than when the same dose of reserpine was given towards the end of the experiment. The contractions due to histamine, acetylcholine, barium chloride and 5-hydroxytryptamine were antagonised to the same extent. On guinea-pig ileum, it was observed that citric acid reduced the degree of inhibition produced by reserpine.

The inhibition of drug-induced contractions following the addition of reserpine usually reached a

maximum after the alkaloid had been washed out of the bath.

Recovery of the responses to drugs was generally complete, but took longer the larger the dose of reserpine employed.

Contractions of the rat uterus, due to acetylcholine, 5-hydroxytryptamine and potassium chloride were reduced by reserpine. In some cases the first addition of reserpine seemed to cause some irreversible change in the tissue since the contractions did not return to the control height.

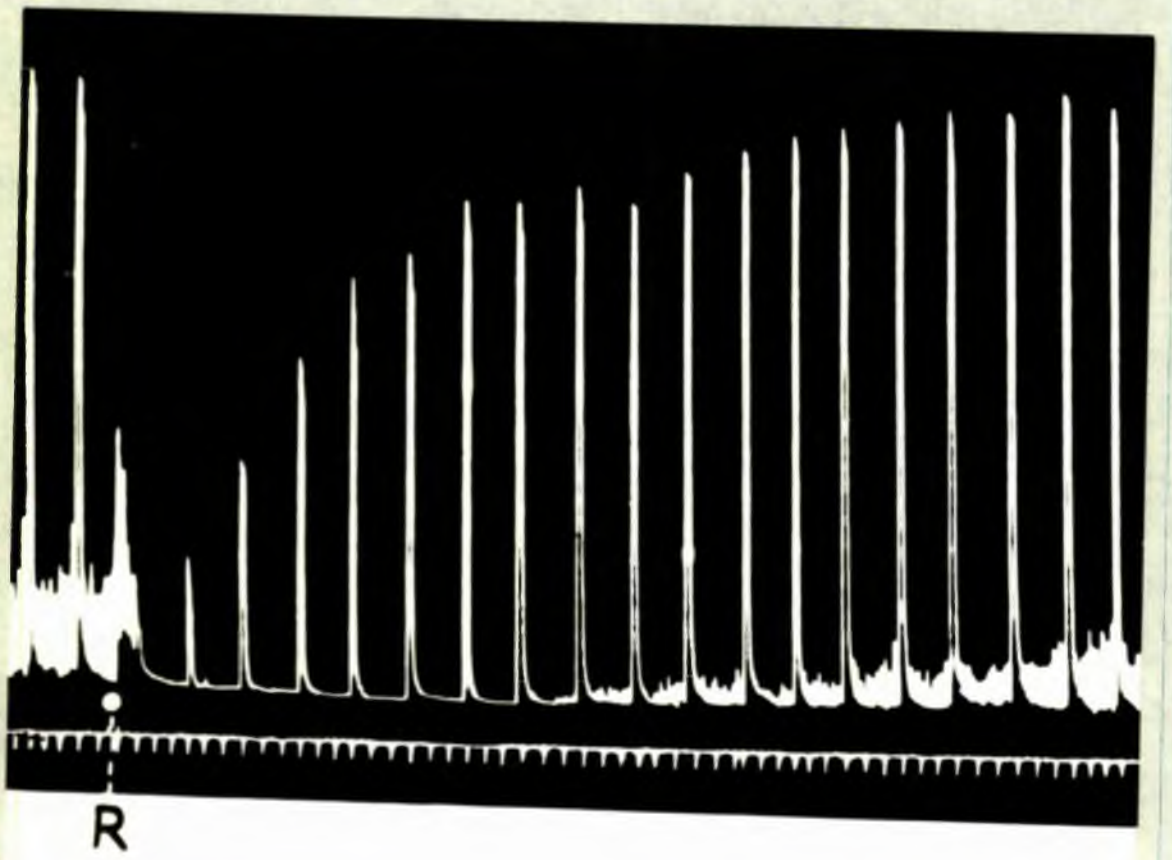
Tone was usually reduced by reserpine. The tone and spontaneous activity of colon and duodenum were reduced and in some cases completely inhibited. Recovery from this effect of reserpine was slow.

RESULTS.

A) The actions of reserpine upon the isolated ileum of guinea-pig, kitten and monkey.

Reserpine in doses of from 1 µg. to 30 µg. normally had no direct action on the quiescent guinea-pig ileum.

Figure 4.2



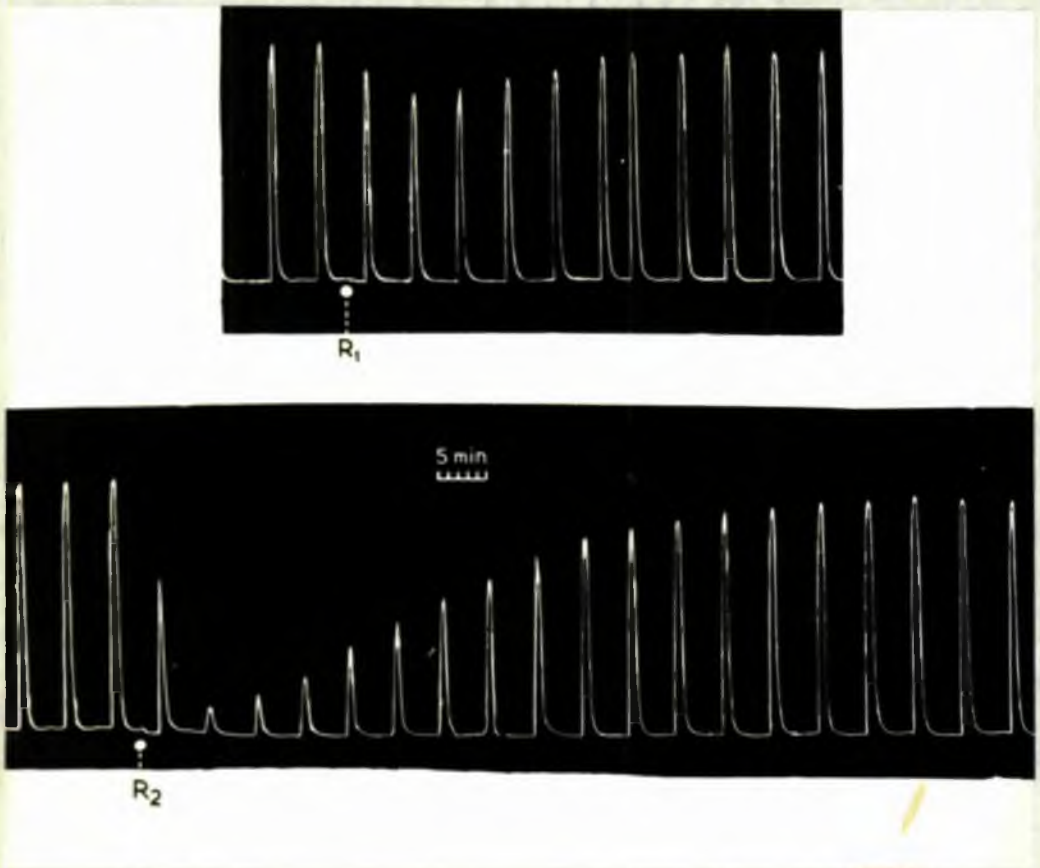
The effect of reserpine on a) the spontaneous activity and b) the response to acetylcholine of isolated guinea-pig ileum.

All contractions (except at R) produced by the addition of 0.5 µg. acetylcholine.

At R, 30 µg. reserpine added.

Time = 60 seconds.

Figure 4.3

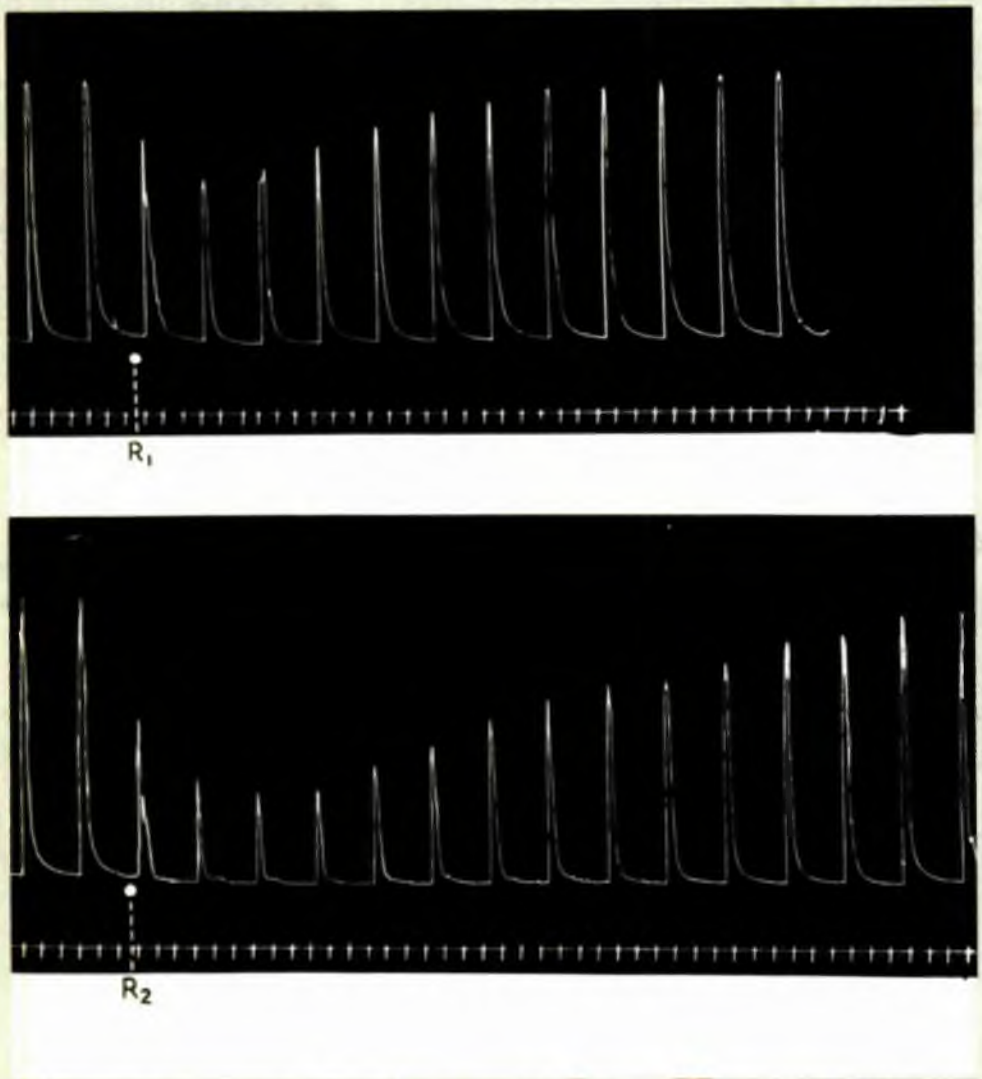


The influence of reserpine upon histamine-induced contractions of the guinea-pig ileum.

All contractions produced by the addition of 0.5 µg. histamine.

At R_1, 4 µg. reserpine added; at R_2, 30 µg. reserpine added.

Figure 4.4



The influence of reserpine upon barium chloride-induced contractions of the guinea-pig ileum.

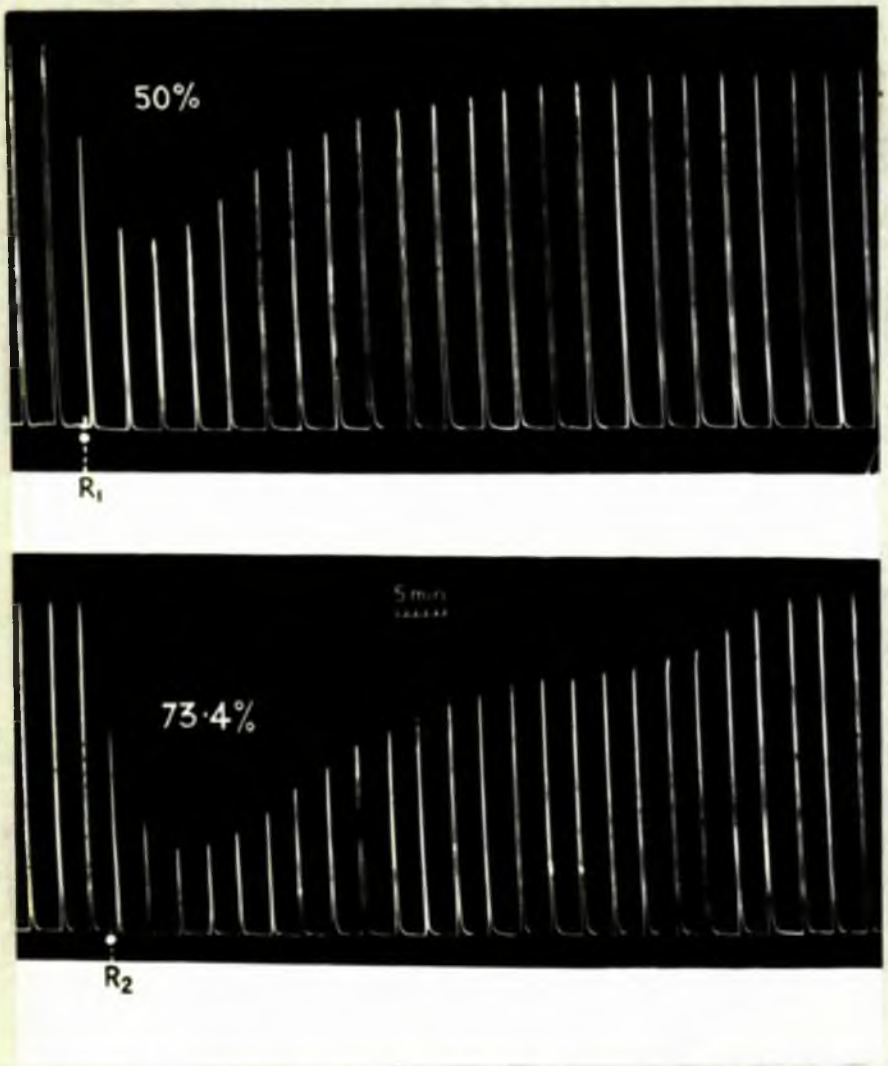
All contractions produced by the addition of 0.5 mg. barium chloride.

At R_1, 10 µg. reserpine added; at R_2, 30 µg. reserpine added.

The slight spontaneous activity sometimes seen with this preparation was, however, completely inhibited. Before this inhibition was seen there was a slight increase in the tone of the preparation (Figure 4,2). The spontaneous activity recovered at the same rate as the response to stimulant drugs. In kitten and monkey ileum, a similar inhibition of spontaneous activity was seen and was accompanied by a reduction of tone (Figure 4,6). Recovery of normal activity and tone in those preparations was generally complete.

The contractions of guinea-pig ileum following the addition of acetylcholine 0.2 µg. to 0.5 µg. (Figure 4,2), histamine 0.2 µg. to 0.5 µg. (Figure 4,3), barium chloride 0.5 mg. (Figure 4,4) and 5-hydroxytryptamine 20 ng. (Figure 4,5), were reduced by 4 µg. to 30 µg. of reserpine. Although the extent of the reduction in amplitude was related to the dose of reserpine used in each experiment, it was found to vary widely from experiment to experiment for the same dose. In addition, the same concentration of reserpine usually produced a more marked inhibition of the contractions following its second and subsequent additions. In many cases, the difference between the percentage inhibition at the

Figure 4.5



Reserpine antagonism of 5-hydroxytryptamine-induced contractions of the guinea-pig ileum.

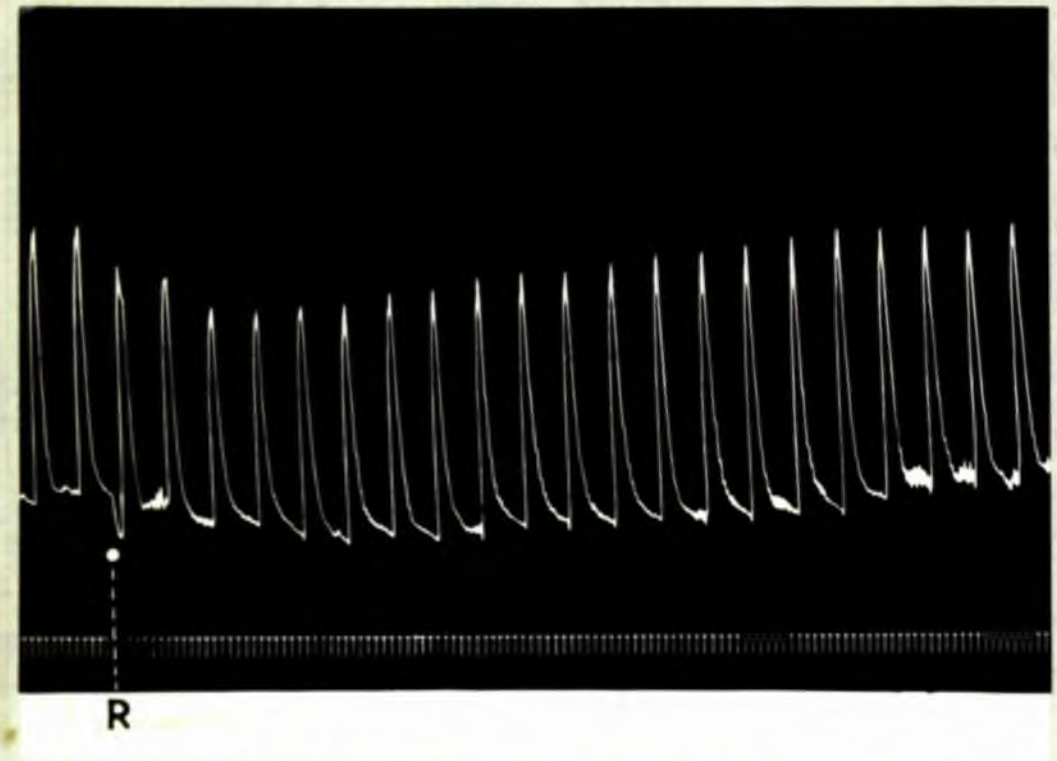
The increased effect of the second addition of the same concentration of reserpine.

All contractions produced by the addition of 20 ng. 5-hydroxytryptamine.

At R_1, 30 µg. reserpine added; at R_2, 30 µg. reserpine added 3 hours after R_1.

Figures refer to maximum percentage inhibition produced by reserpine.

Figure 4.6



The effect of reserpine on acetylcholine-induced contractions of the isolated monkey ileum.

All contractions produced by the addition of 0.2 µg. acetylcholine.

At R, 30 µg. reserpine added.

Time = 60 seconds.

beginning and end of an experiment was marked (Figure 4,5). The average duration of each experiment was seven or eight hours.

The response of monkey and kitten ileum to acetylcholine (0.2 μg.) was reduced by 30 μg. of reserpine (Figure 4,6) but the degree of inhibition was smaller than that seen with guinea-pig ileum. In addition, the reduction of the response to acetylcholine was always accompanied by a fall in tone.

Recovery of the contraction to the control height was generally complete, although the time required for this varied considerably. In general, the larger the dose of reserpine, the longer was the time required for recovery. In those preparations of guinea-pig ileum on which reserpine had least effect, recovery was more rapid than in those in which it had a more marked effect. The maximum inhibition was always produced some time after the addition of reserpine; generally after two to four additions of agonist (Figures 4,2 to 4,6).

Guinea-pig ileum is a readily accessible tissue and a great deal is known about its properties and

reactions to drugs. It was used, therefore, to make a more detailed study of the influence of reserpine upon drug-induced contractions. Log dose-response lines for reserpine-acetylcholine, reserpine-histamine, reserpine, 5-hydroxytryptamine and reserpine-barium chloride were plotted. To obtain a sufficient degree of statistical accuracy, each of the four doses of reserpine used was tested in fifteen individual experiments. Each dose was tested twice in every experiment. Thus thirty values for percentage inhibition were obtained for each dose of reserpine. In calculating the mean percentage inhibition produced by the four doses of reserpine, all values were included. These values, together with the standard deviation of each point, are recorded in Table 4,1.

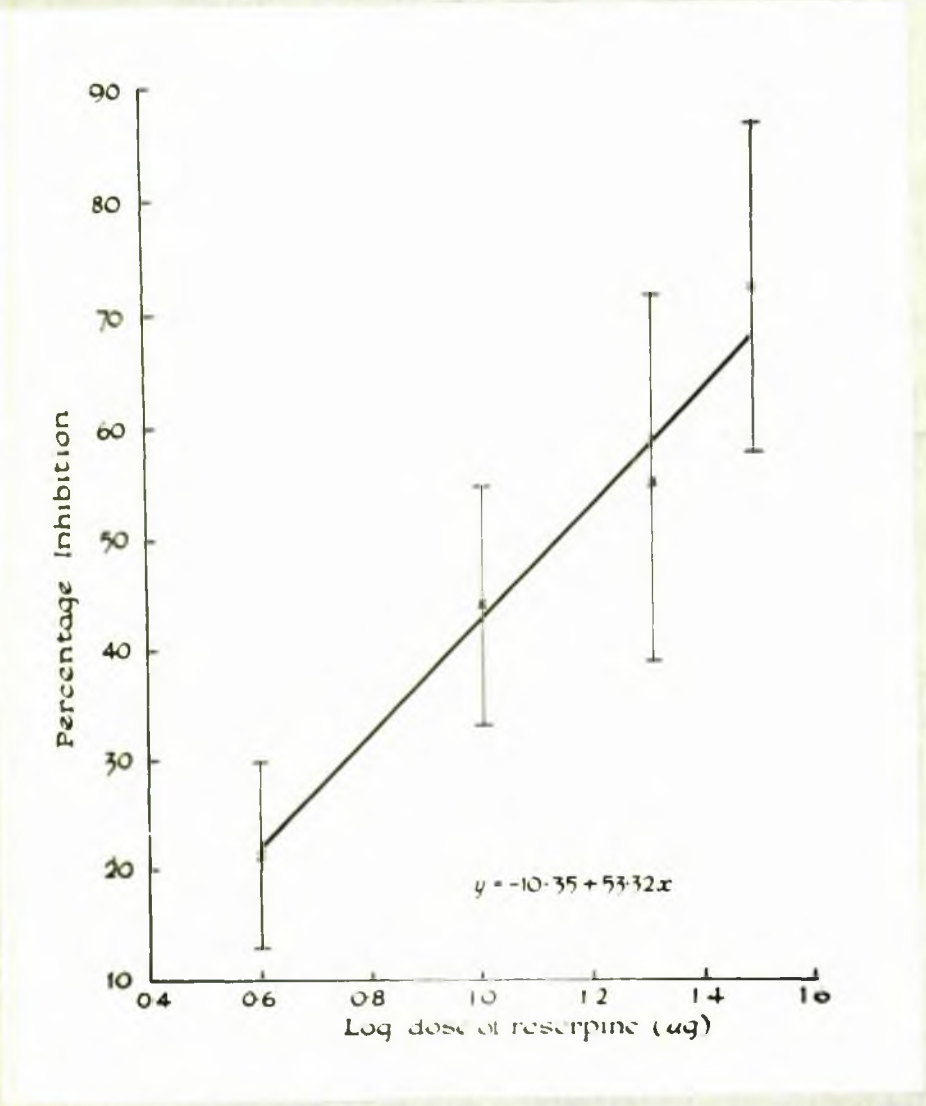
TABLE 4.1

The antagonism of reserpine to acetylcholine, histamine, 5-hydroxytryptamine and barium chloride-induced contractions of guinea-pig ileum.

The values for the percentage inhibitions are the mean of thirty additions of each dose against the four spasmogens used.

Spasmogen.	Dose of Reserpine. Actual (μg.)	Log. dose.	Mean percentage inhibition ± standard deviation.
Acetylcholine, 0.2 μg. to 0.5 μg.	30	1.48	72.4 ± 14.4
	20	1.30	55.3 ± 16.4
	10	1.00	44.1 ± 10.8
	4	0.60	21.4 ± 8.4
Histamine, 0.2 μg. to 0.5 μg.	30	1.48	76.4 ± 15.8
	20	1.30	68.5 ± 14.4
	10	1.00	53.1 ± 12.5
	4	0.60	22.4 ± 10.1
5-hydroxy-tryptamine, 20 ng.	30	1.48	57.1 ± 19.9
	20	1.30	50.0 ± 12.3
	10	1.00	41.0 ± 12.1
	4	0.60	25.5 ± 9.3
Barium chloride, 0.5 mg.	30	1.48	69.5 ± 18.8
	20	1.30	63.8 ± 14.9
	10	1.00	51.0 ± 12.4
	4	0.60	31.3 ± 16.2

Figure 4.7



The antagonism of reserpine to acetylcholine-induced contractions of the guinea-pig ileum.

Relation between log. dose and percentage inhibition.

When plotted on graph paper, the values for the percentage inhibition produced by the same doses of reserpine for the four spasmogens lay close to each other. The slopes of the lines were similar. The log. dose-percentage inhibition line for the reserpine antagonism to acetylcholine is illustrated in Figure 4,7. The standard deviation of each point is indicated on the graph. As can be seen, the scatter about each point was wide. The line is drawn on the regression coefficient derived from the acetylcholine inhibition values given in Table 4,1. This figure was calculated to be 53.32. To compare the four slopes statistically an analysis of covariance was carried out. The results are given in Table 4,2.

TABLE 4.2

Analysis of covariance within the log. dose-percentage inhibition relationship between reserpine and acetylcholine, histamine, 5-hydroxytryptamine and barium chloride.

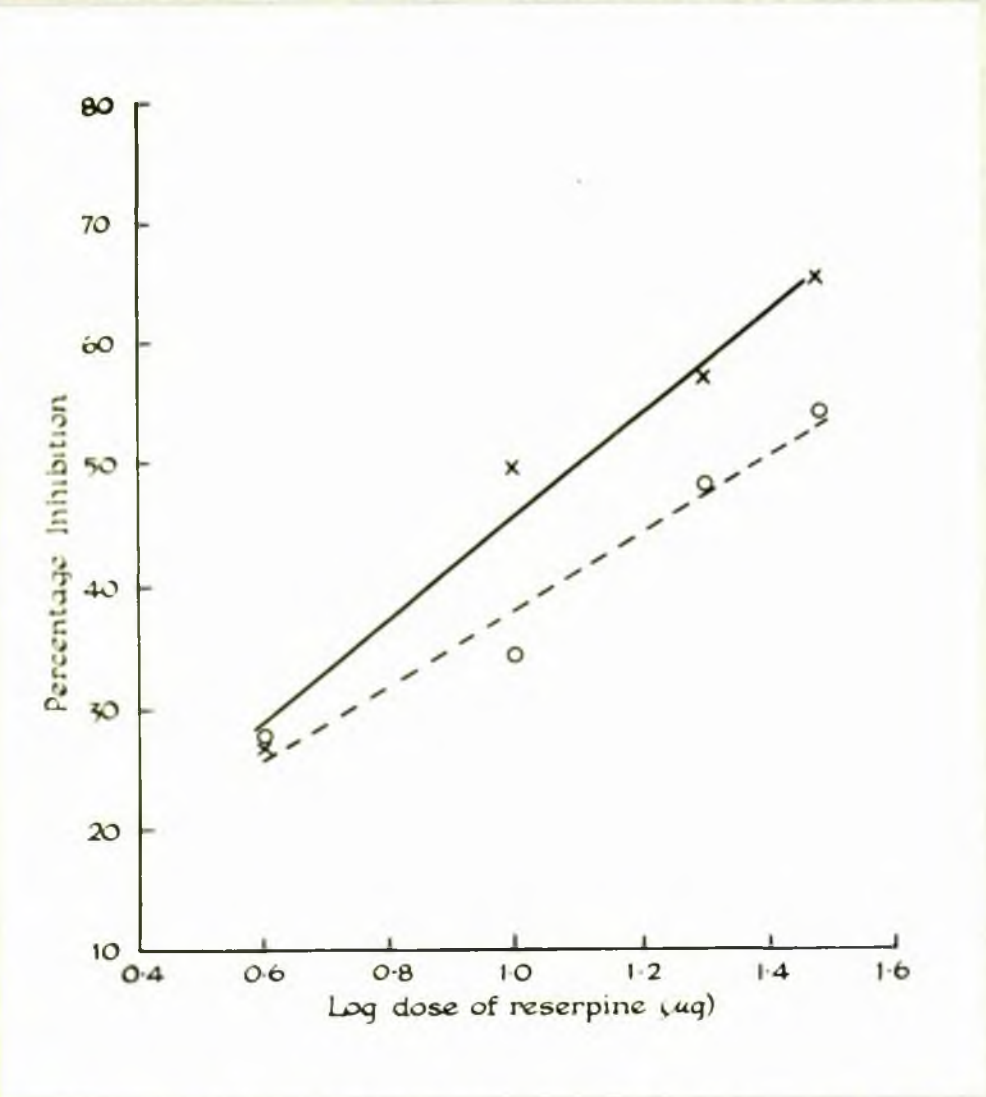
	Sum of squares.	Degrees of freedom.	Mean square.	Ratio (= F)
Differences among individual regression coefficients.	176.00	3	58.67	1.14
Differences among group means.	350.19	3	116.73	2.28
Residual.	410.61	8	51.33	

F (P = 0.05) for 3,8 degrees of freedom[13] = 4.07

It can be seen from the analysis that there is no significant difference (P = 0.05) between either the gradients or the position of the lines.

It appeared that reserpine inhibited drug-induced contractions of gut taken from lighter animals more than those of gut from heavier animals. While the weight of guinea-pigs is an unsatisfactory indication of their age, it seemed possible that gut taken from the lighter and presumably younger animals with a more vigorous metabolism may have been more readily affected by reserpine than that from older ones. It was decided therefore to make an arbitrary division of the weights of the animals used into two ranges, viz. up to and including 300 g. and those above this weight. The weight range of the animals used was 102 g. to 775 g. When this was done it was found that too few animals had been used at certain dose levels to give a sufficient degree of statistical accuracy within the two groups. Further experiments were therefore carried out as described previously (page 52) until each dose of reserpine had been added in at least 14 experiments in each weight range. A log. dose reserpine/percentage inhibition line was constructed for each weight group

Figure 4.7A



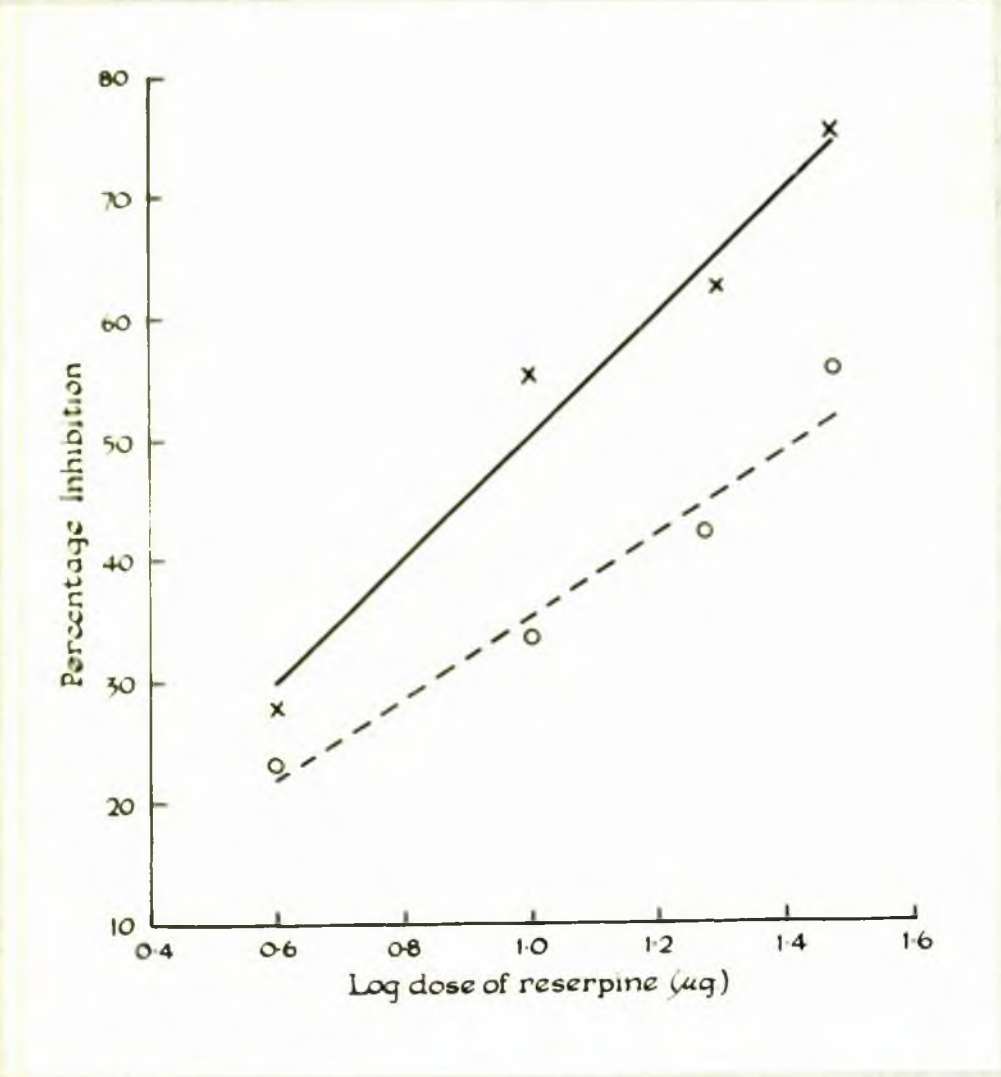
The reserpine antagonism to acetylcholine-induced contractions of the isolated guinea-pig ileum.

Relation between log. dose and percentage inhibition within the weight ranges, 102 g. to 298 g. and 303 g. to 695 g.

- X ——— X -, 102 g. to 298 g.
  Equation is y = 3.42 + 42.30 x

-- 0 ——— 0--, 303 g. to 695 g.
  Equation is y = 6.33 + 31.61 x

Figure 4.7B



The reserpine antagonism to histamine-induced contractions of the isolated guinea-pig ileum.

Relation between log. dose and percentage inhibition within the weight ranges, 110 g. to 300 g. and 312 g. to 775 g.

- X ——— X -, 110 g. to 300 g.
  Equation is y = -0.53 + 50.87 x

- O ——— O --, 312 g. to 775 g.
  Equation is y = 1.12 + 34.32 x

when both acetylcholine and histamine were used to produce the contractions. These are illustrated in Figures 4,7A and 4,7B respectively. For the sake of clarity, the standard deviations of each point have been omitted. They are, however, contained in Table 4,3.

TABLE 4.3

The antagonism between reserpine and the acetylcholine and histamine -induced contractions of ileum from guinea-pigs weighing less than 300 g. and more than 300 g.

Spasmogen	Weight Range	Dose of Reserpine		Mean percentage inhibition ± standard deviation.	Number of additions of each dose of Reserpine.
		Actual (µg.)	Log. dose.		
Acetylcholine, 0.2 µg. to 0.5 µg.	102 g. to 298 g.	30	1.48	65.7 ± 16.4	38
		20	1.30	57.2 ± 13.1	38
		10	1.00	49.5 ± 12.0	38
		4	0.60	26.4 ± 8.2	24
	303 g. to 695 g.	30	1.48	54.1 ± 19.0	34
		20	1.30	48.0 ± 17.2	30
		10	1.00	34.6 ± 10.1	32
		4	0.60	27.4 ± 10.6	26
Histamine, 0.2 µg. to 0.5 µg.	110 g. to 300 g.	30	1.48	75.0 ± 15.2	30
		20	1.30	62.4 ± 12.3	26
		10	1.00	55.2 ± 10.3	26
		4	0.60	28.0 ± 8.4	26
	312 g. to 775 g.	30	1.48	55.3 ± 17.3	32
		20	1.30	42.4 ± 16.4	32
		10	1.00	33.8 ± 14.8	32
		4	0.60	23.4 ± 11.3	30

Figures 4,7A and 4,7B seemed to indicate that reserpine had a more marked effect on the ileum of lighter animals. In addition, the standard deviation of each dose level tended to be greater among the heavier animals. The log. dose-percentage inhibition relationship for the two groups were compared by an analysis of covariance. This was done for both acetylcholine and histamine. The results are given in Tables 4,4 and 4,5. Since unequal numbers of results were obtained, a weighted mean had to be used in the analyses.

TABLE 4.4

Analysis of covariance within the log. dose-percentage inhibition relationship between reserpine and acetylcholine on the isolated ileum of "light" (102 g. to 298 g.) and "heavy" (303 g. to 695 g.) guinea-pigs.

	Sum of squares.	Degrees of freedom.	Mean square.	Ratio (= F)
Differences between individual regression coefficients.	708.14	1	708.14	2.45
Differences between group means.	6828.03	1	6828.03	22.59
Residual.	1209.50	4	302.33	

F (P = 0.05) for 1,4 degrees of freedom[13] = 7.71

TABLE 4.5

Analysis of covariance within the log. dose-percentage inhibition relationship between reserpine and histamine on the isolated ileum of "light" (110 g. to 300 g.) and "heavy" (312 g. to 775 g.) guinea-pigs.

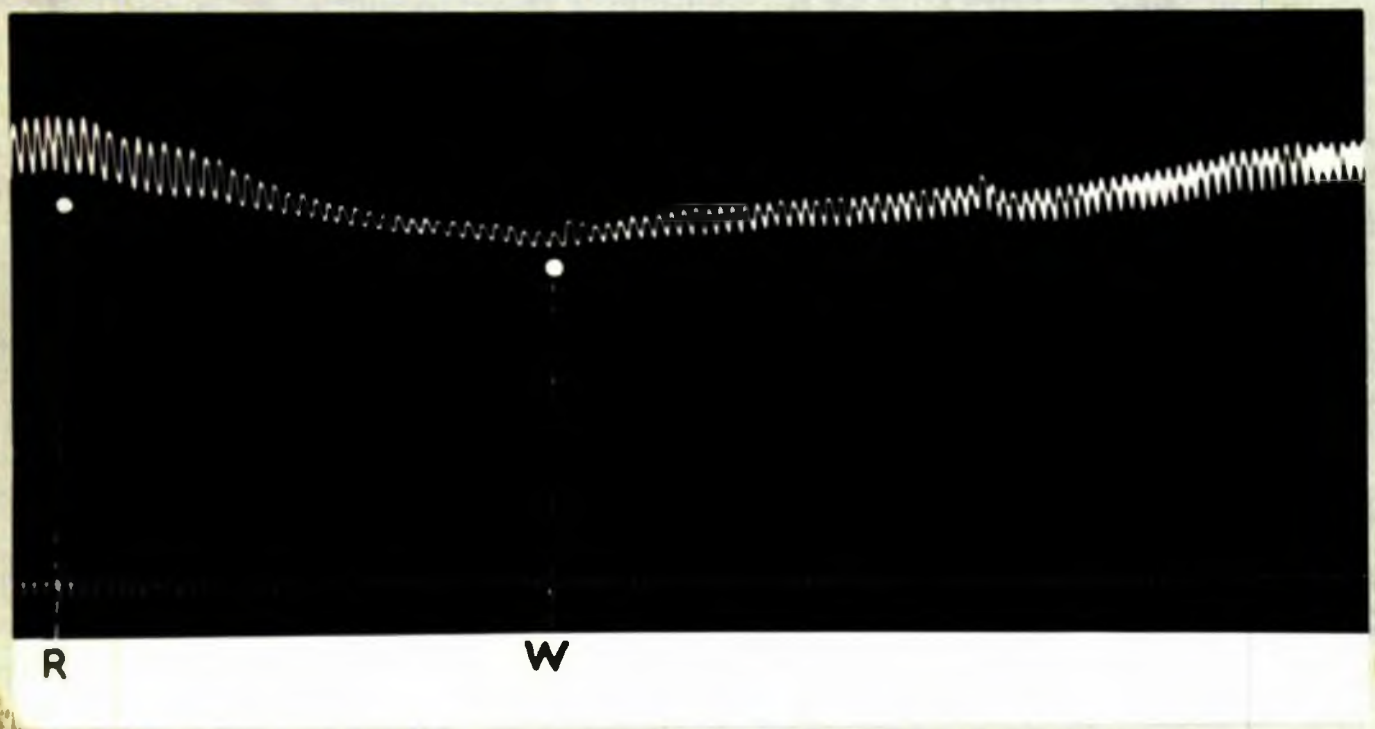
	Sum of squares.	Degrees of freedom.	Mean square.	Ratio (= F)
Differences between regression coefficients.	1759.45	1	1759.45	3.83
Differences between group means.	16642.62	1	16642.62	36.22
Residual.	1838.10	4	459.52	

F (P = 0.05) for 1,4 degrees of freedom[13] = 7.71

From these analyses it can be seen that while no significant differences are to be found between the slopes in each pair, the positions of the lines relative to each other are significantly different. The absence of significant difference between the regression coefficients may be taken to indicate that the mode of action of reserpine is the same in both weight ranges used. Thus it appears that reserpine has a more pronounced effect on the drug-induced contractions of intestinal strips from younger guinea-pigs.

In some of the experiments on guinea-pig ileum, reserpine was dissolved in 0.2 per cent w/v citric acid (Appendix I, page 181). In these cases, the inhibition by reserpine of the responses of the ileum to acetylcholine and histamine was less than when the same concentration of the alkaloid was dissolved in the ascorbic acid solvent. It seemed possible, therefore, that the citric acid was having an antagonistic effect to the action of reserpine. When citric acid was added before reserpine, it was found that the inhibition produced was in fact less than when the same dose of reserpine in the ascorbic acid solvent was used alone. This observation led to the testing of a comprehensive range of intermediates of

Figure 4.8



The influence of reserpine upon the spontaneous activity and tone of isolated kitten duodenum.

At R, 4 µg. reserpine added to the bath.

At W, the bath fluid was changed.

Time = 5 seconds.

carbohydrate metabolism for possible antagonism to the inhibitive effects of reserpine. This is described fully in Chapter 7, page 105).

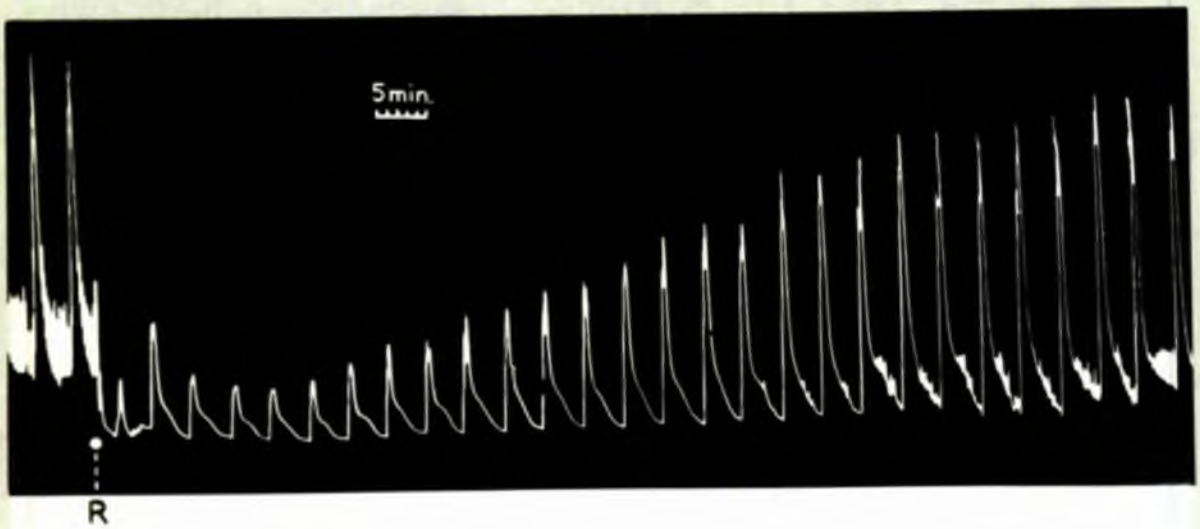
No direct effect on the intestinal segments was seen with the ascorbic acid or citric acid control solutions. Neither control solution had any apparent influence upon the drug-induced contractions of the isolated intestinal strips.

B) <u>The actions of reserpine upon the isolated duodenum of rabbit, kitten and monkey.</u>

4 to 30 µg. reserpine either reduced or inhibited completely the spontaneous activity of rabbit and kitten duodenum, suspended in Locke's solution. At the same time there was a reduction in the normal tone of the intestinal muscle, as recorded by a fall in the level of the lever (Figure 4,8).

The contractions of kitten and rabbit duodenum following the addition of acetylcholine (0.2 µg. and 0.1 µg. respectively) were reduced by 4 µg. to 30 µg. reserpine. A greater inhibition was produced in kitten than in rabbit

Figure 4.9

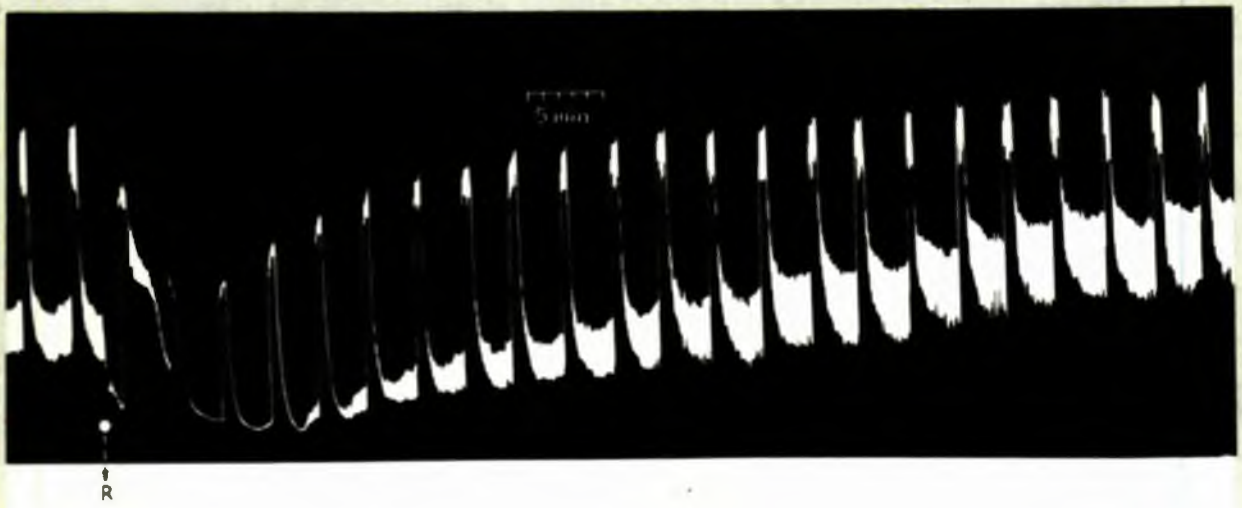


The influence of reserpine upon a) the spontaneous activity and b) acetylcholine-induced contractions of kitten duodenum.

All contractions produced by the addition of 0.2 µg. acetylcholine.

At R, 30 µg. reserpine added.

Figure 4.10

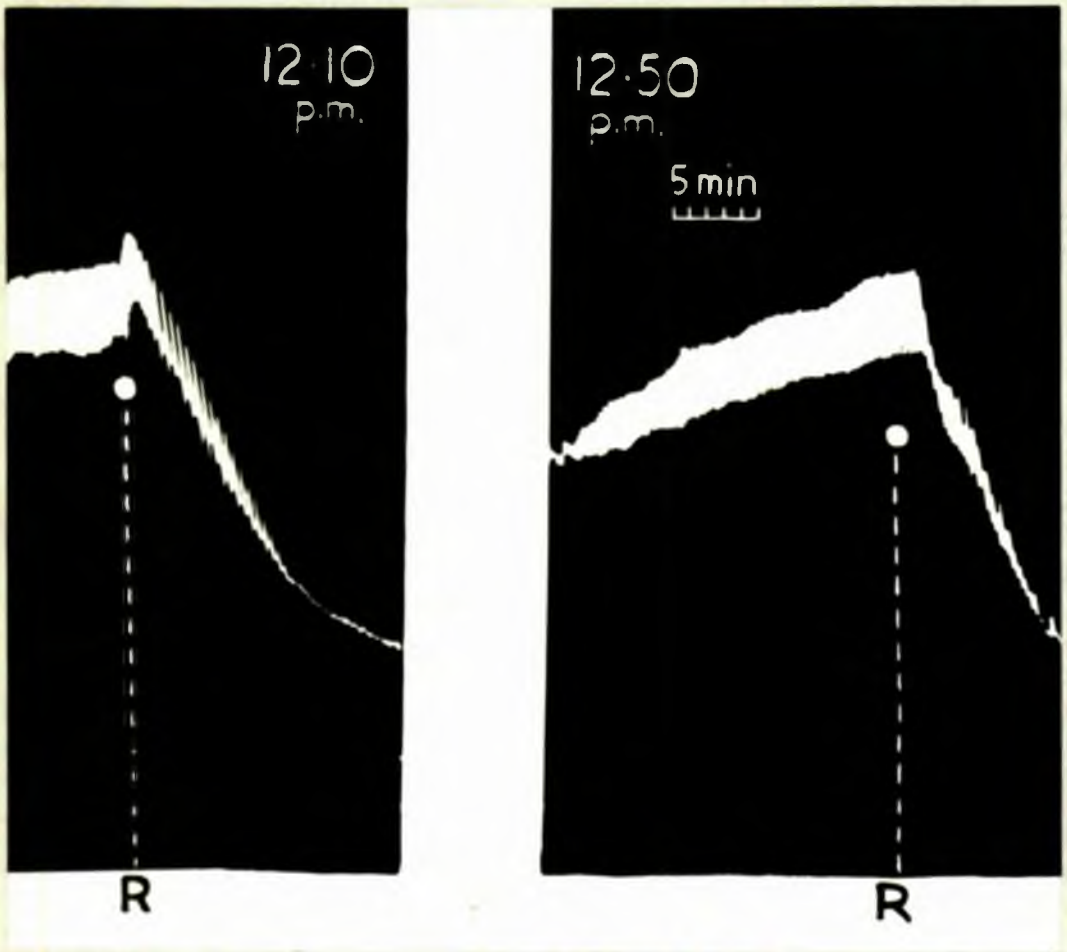


The influence of reserpine upon a) the spontaneous activity and b) acetylcholine-induced contractions of the rabbit duodenum.

All contractions produced by the addition of 0.1 µg. acetylcholine.

At R, 30 µg. reserpine added.

Figure 4.11



The influence of reserpine upon the spontaneous activity and tone of monkey duodenum.

At R, 10 µg. reserpine added.

In the first part of the experiment, the gut was suspended in Krebs-Henseleit solution and in the second it was suspended in Locke's solution.

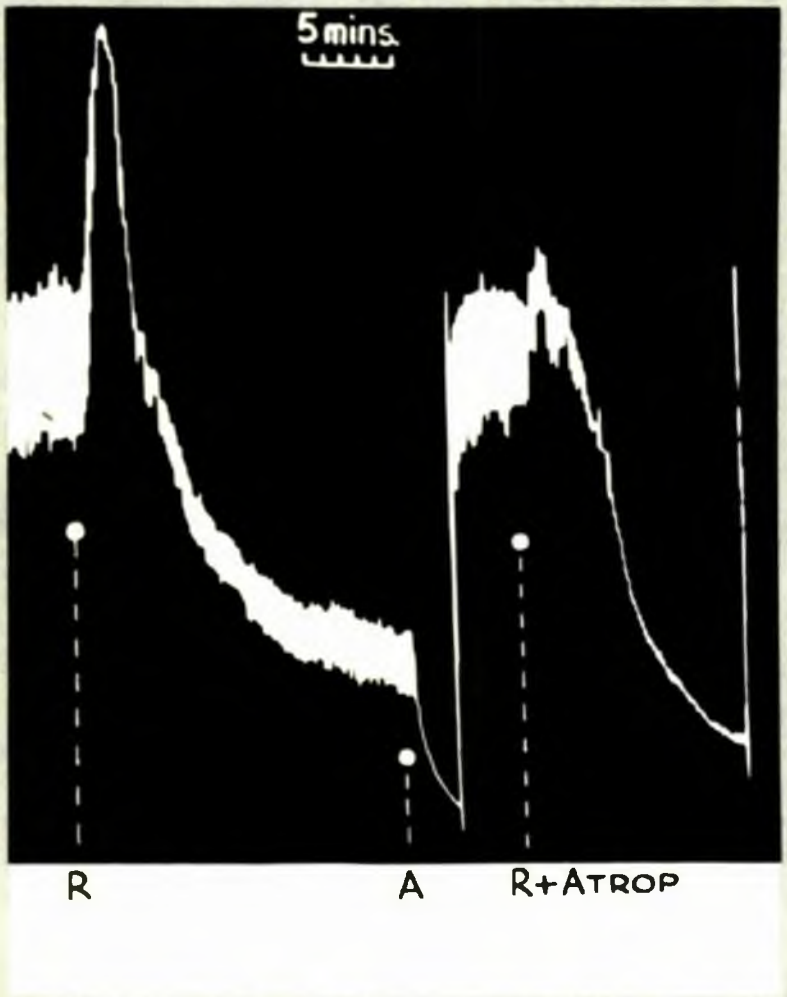
duodenum (Figures 4,9 and 4,10). The spontaneous activity of the former preparation was also more effectively antagonised.

The reduction in tone and spontaneous activity produced by 0.5 µg. adrenaline was unaffected by reserpine at doses of less than 4 µg. which themselves had no direct effect on the gut.

Monkey duodenum was suspended in Krebs-Henseleit solution. 10 µg. to 30 µg. reserpine caused an increase in the tone, accompanied by a slight decrease in the amplitude of the spontaneous movements. About 40 seconds after the addition of reserpine there was a marked fall in tone (Figure 4,11).

The absence of a stimulant action in kitten and rabbit duodenum could have been due to species variation or to the fact that Krebs-Henseleit solution was used with monkey and Locke's solution with rabbit and kitten duodenum. The experiments with rabbit and monkey duodenum were therefore repeated using Krebs-Henseleit solution. It was found that reserpine now caused a stimulant action in intestinal segments from both species. 10 µg. to 30 µg. reserpine caused an increase in the tone of rabbit gut (Figure 4,12)

Figure 4.12



The stimulant action of reserpine on isolated rabbit duodenum and its reduction by atropine sulphate.

At R, 10 µg. reserpine added.

At A, 1 µg. adrenaline added.

At R + ATROP, 10 µg. reserpine added 5 seconds after 1 µg. atropine sulphate.

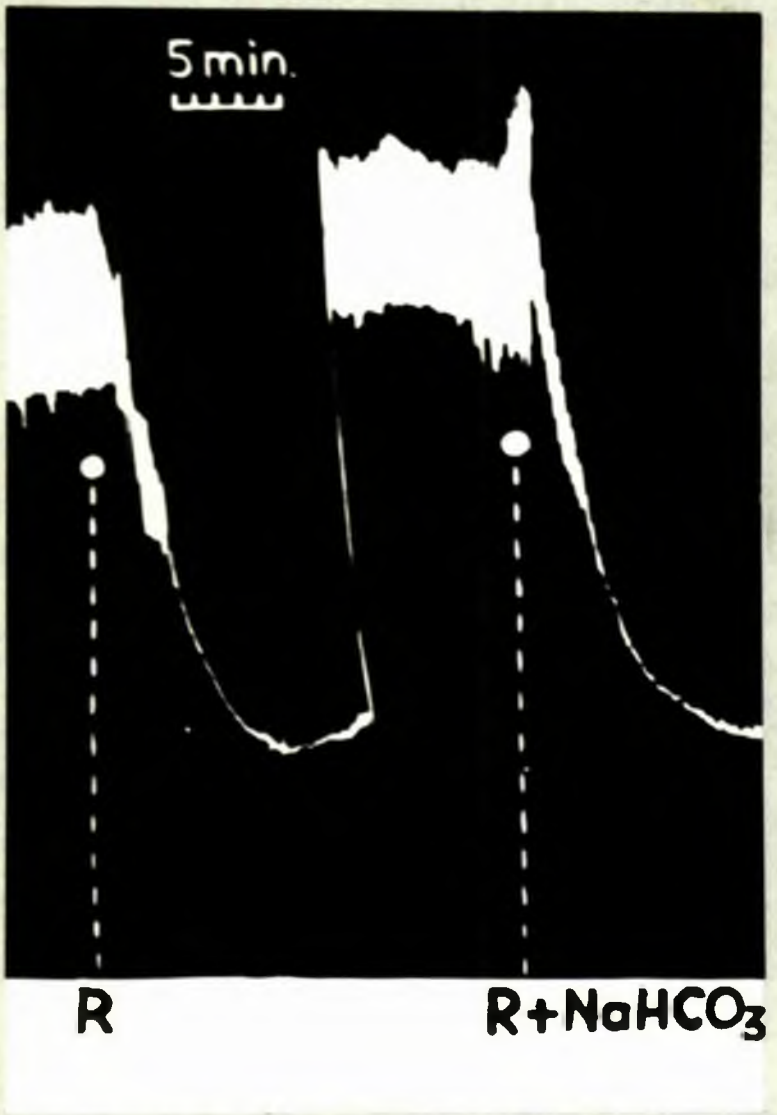
which was more marked than that seen with kitten duodenum. There was normally a delay of about 20 seconds between the addition of reserpine and the appearance of the increased tone. The stimulant action was followed by a reduction in the tone and a reduction in the amplitude of the spontaneous movements. At this point 1 μg. adrenaline produced a further relaxation of the gut and complete inhibition of its spontaneous movements (Figure 4,12). Within the concentration range used, there appeared to be no relationship between dose and stimulant action. Recovery of tone and rhythmic activity was slow, taking normally about one hour. The stimulant action, but not the subsequent reductions in tone and in the amplitude of spontaneous movements, was considerably reduced by 1 μg. atropine (Figure 4,12) or 1 μg. hexamethonium. It was unaffected by 2 μg. of 2-brom-(+)-lysergic acid diethylamide (BOL 148). 1 μg. 5-hydroxytryptamine produced in many cases an increase in tone similar to that following 10 μg. reserpine in the same experiment. Since BOL 148, which is a specific antagonist of the stimulant effects of 5-hydroxytryptamine on rabbit duodenum[1], failed to influence the stimulant action produced by reserpine, but in the same concentration inhibited completely the action of 5-hydroxytryptamine,

it was concluded that reserpine was apparently not causing a release of 5-hydroxytryptamine from the gut. On the other hand, acetylcholine appeared to be involved at some point in the events leading to the contraction, since atropine reduced the effect. It has been reported[2,3] that intestinal preparations kept in the cold lose their power to synthesise acetylcholine. Some segments of gut were therefore kept for 24 hours in glucose free Krebs-Henseleit solution at -1°C. After storage, they were allowed to remain at room temperature for one hour before use. The preparations were then suspended in Krebs-Henseleit solution as described previously (page 52). Reserpine now failed to show a stimulant action. The implication of acetylcholine in the contraction is not ruled out by these experiments, since a number of factors resulting from storage at -1°C, other than decreased acetylcholine synthesis, could be responsible for the absence of contraction. The fact that the ganglion blocking agent, hexamethonium, also prevented the stimulant response to reserpine may point to a site of action at the autonomic ganglia in the intestinal wall.

The production of a stimulant action by reserpine, only when Krebs-Henseleit solution was used, merited further

investigation. Reserpine, dissolved in partially neutralised ten per cent ascorbic acid solution reduced the bath pH (when Locke's solution was used) from 7.4 to 6.9. There was, however, no alteration of pH when Krebs-Henseleit solution was used. The difference in action might be explained in one of three ways. The reduction of bath pH may cause some change in the degree of ionisation either of the reserpine molecule or that of receptors on the cell's surface. It is also possible that qualitative differences in the bath fluids may be responsible. The Krebs-Henseleit solution used has the following qualitative differences from Locke's solution: it contains 0.29 g. per litre hydrated magnesium sulphate and 0.16 g. per litre potassium dihydrogen phosphate. Apart from the presence of these salts in Krebs-Henseleit solution, there are only quantitative differences in salt composition. In three experiments with rabbit duodenum, 0.1 ml. of a solution containing both hydrated magnesium sulphate and potassium dihydrogen sulphate, dissolved in Locke's solution, was added to the bath containing Locke's solution. The concentration of the salts was sufficient to bring their concentration up to that present in Krebs-Henseleit solution. Since they were dissolved in Locke's solution the concentration of the other salts was not

Figure 4.13



The effect of pH upon the stimulant action of reserpine on rabbit duodenum.

Rabbit duodenum suspended in Locke's solution.

At R, 10 µg. reserpine added.

At R + $NaHCO_3$, 0.05 ml. of 5 per cent sodium bicarbonate solution added to the bath, followed 2 minutes later by 10 µg. reserpine.

altered. Ten minutes after the addition of these salts, 10 µg. reserpine was added to the bath. No stimulant action was produced, although the usual inhibition of tone and spontaneous activity was seen. Thus it appeared that the qualitative differences between Locke's and Krebs-Henseleit solutions were not responsible for the variations in action.

The addition of small volumes of 0.2 N hydrochloric acid to Krebs-Henseleit solution in the bath, which reduced the pH to about 6.9, generally had little direct inhibitory effect on the activity of rabbit duodenum provided that it was added slowly. When 10 µg. reserpine was added 2 minutes after this only the reduction in tone and spontaneous activity was produced. However, when sufficient five per cent sodium bicarbonate solution was added to Locke's solution to increase the pH, after the addition of reserpine, to 7.4, a stimulant action was produced (Figure 4,13). It was never as marked as that seen when the entire experiment was carried out in Krebs-Henseleit solution. The acid or alkali was added to the bath slowly to avoid sudden changes in the reaction of the physiological solution, which may themselves have caused alterations in the tone of the intestinal segments[4].

It seemed likely, on the basis of the experiments carried out, that stimulation followed the addition of reserpine only if the bath pH remained unchanged.

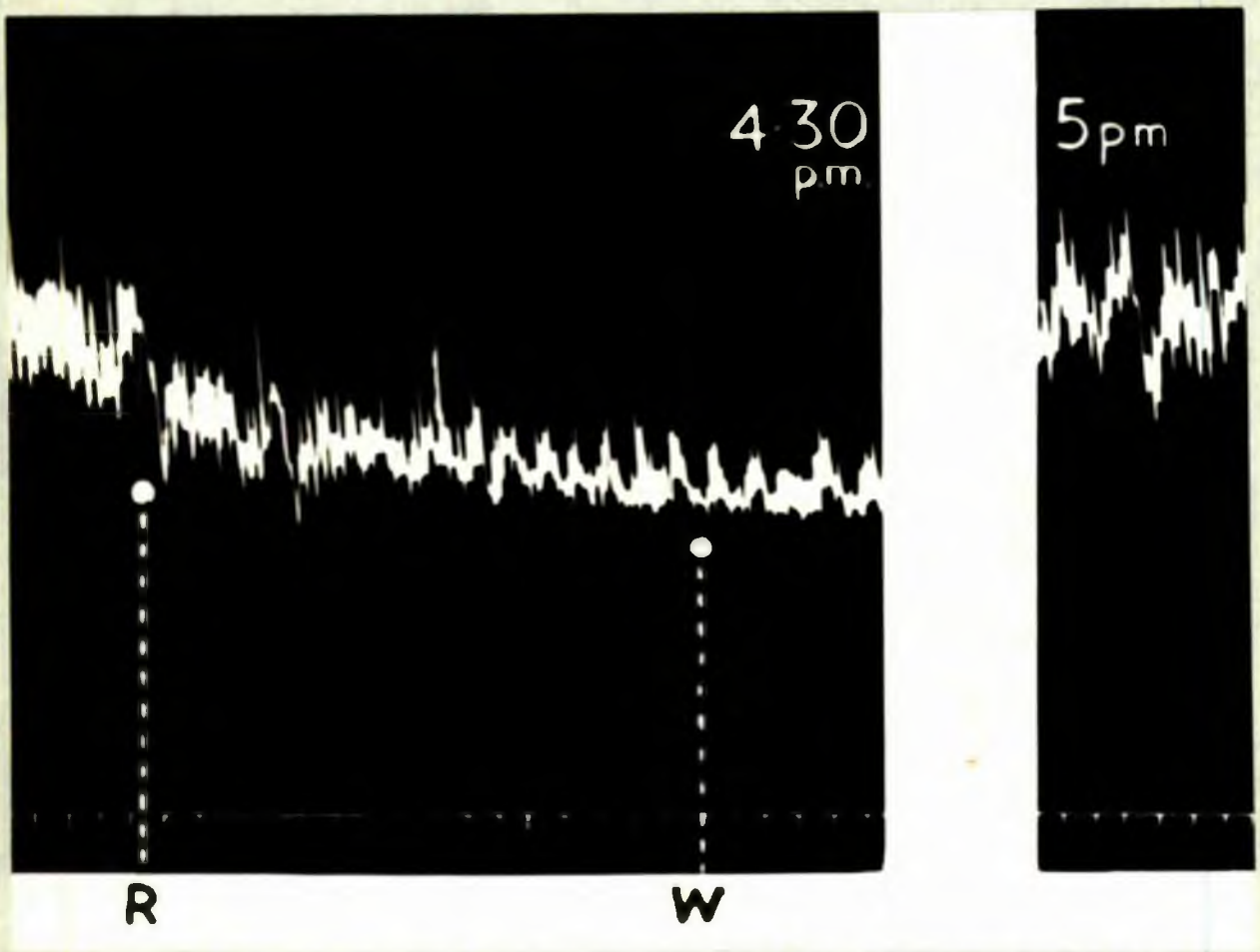
Many reports have appeared in the literature describing the effect of alterations in the pH of the bathing fluid on the activity of isolated rabbit intestinal muscle[4,5,6]. From a survey of the published observations, it was found that slight reduction in pH (from 7.4 to 6.5 or 6.8) caused a decreased tone and reduction of spontaneous activity. The response to acetylcholine was also reduced by a drop in the pH of the bath fluid. Evans and Underhill[4], reported that if the alterations in pH are brought about slowly then the relaxation normally seen is small or completely absent. Slight increases in the reaction of bath fluid (pH 7.4 to pH 8.0) caused an increase in tone. It appears likely therefore that the inhibition by acid of the stimulant actions of reserpine on rabbit intestinal muscle is non-specific.

It has been pointed out that the pH is a most important factor in a consideration of drug action[7]. Many drugs are active in the ionized but not in the unionized form. The percentage of a drug which is ionized depends upon the pKa value of the drug concerned and the pH

of the medium in which it is dissolved. In view of the possibility that alterations in bath pH may have been influencing the ratio of the concentrations of ionized to unionized reserpine, an investigation of this factor was undertaken.

Because of the extremely low aqueous solubility of reserpine, no pKa value in this medium has been reported. A value of 6.06 in 40 per cent methanol has been given[8]. This value was determined by potentiometric titration at 25°C. As a rough estimate of the pKa of reserpine in water, a value of 5 was suggested. Using this value, the percentage of unionized reserpine when using Krebs-Henseleit solution at pH 7.4 was found to be 99.60. (This was calculated according to the formula suggested by Albert[7]). It is reasonable, therefore, to assume that the unionized form of reserpine is the active one (this is true of many alkaloids) or it must be assumed that the ionized moiety is very active and can penetrate cell membranes, which seems unlikely. In Locke's solution at pH 6.9, the percentage of unionized reserpine is 98.96. The reduction in the concentration of the active moiety in Locke's solution (assuming this to be the unionized molecule) is therefore 0.64 per cent. Since all

Figure 4.14



The influence of reserpine upon the spontaneous activity and tone of monkey colon.

At R, 10 µg. reserpine added to the bath.

At W, the bath fluid was replaced with fresh Krebs-Henseleit solution.

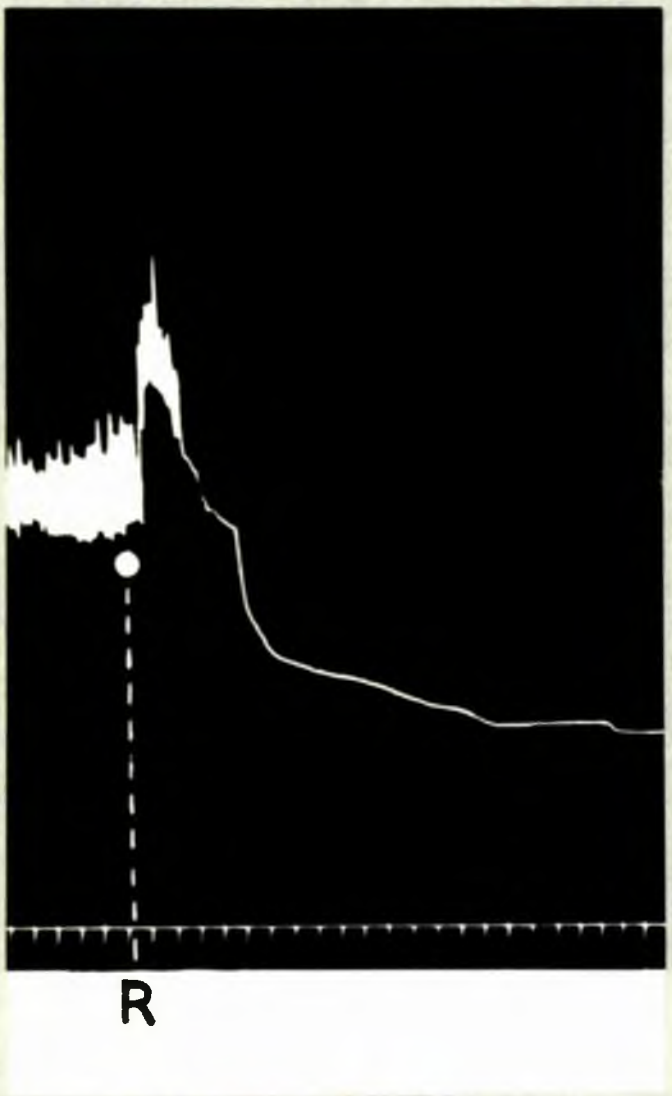
Time = 60 seconds.

experimental evidence obtained appears to point to a non-specific action, it is most unlikely that this small change would account for the lack of stimulant action in Locke's solution.

C) The action of reserpine upon the isolated colon of rabbit, rat and monkey.

Reserpine in doses of from 10 µg. to 50 µg. did not increase the spontaneous activity of rat or monkey colon, suspended in Krebs-Henseleit solution. The reaction of the bath fluid remained at pH 7.4 after the addition of reserpine. The action seen after the addition of, for example, 10 µg. of reserpine was a gradual reduction in the amplitude of the spontaneous movements and in the tone, as indicated by a fall in the recording lever (Figure 4,14). The spontaneous activity was never completely inhibited. In this respect, the colon of the monkey appeared to be more resistant to the action of reserpine than the duodenum (Section B, page 71). The reduction of activity was slow in onset and prolonged. Even after washing out the reserpine from the bath, recovery was slow, but usually complete.

Figure 4.15

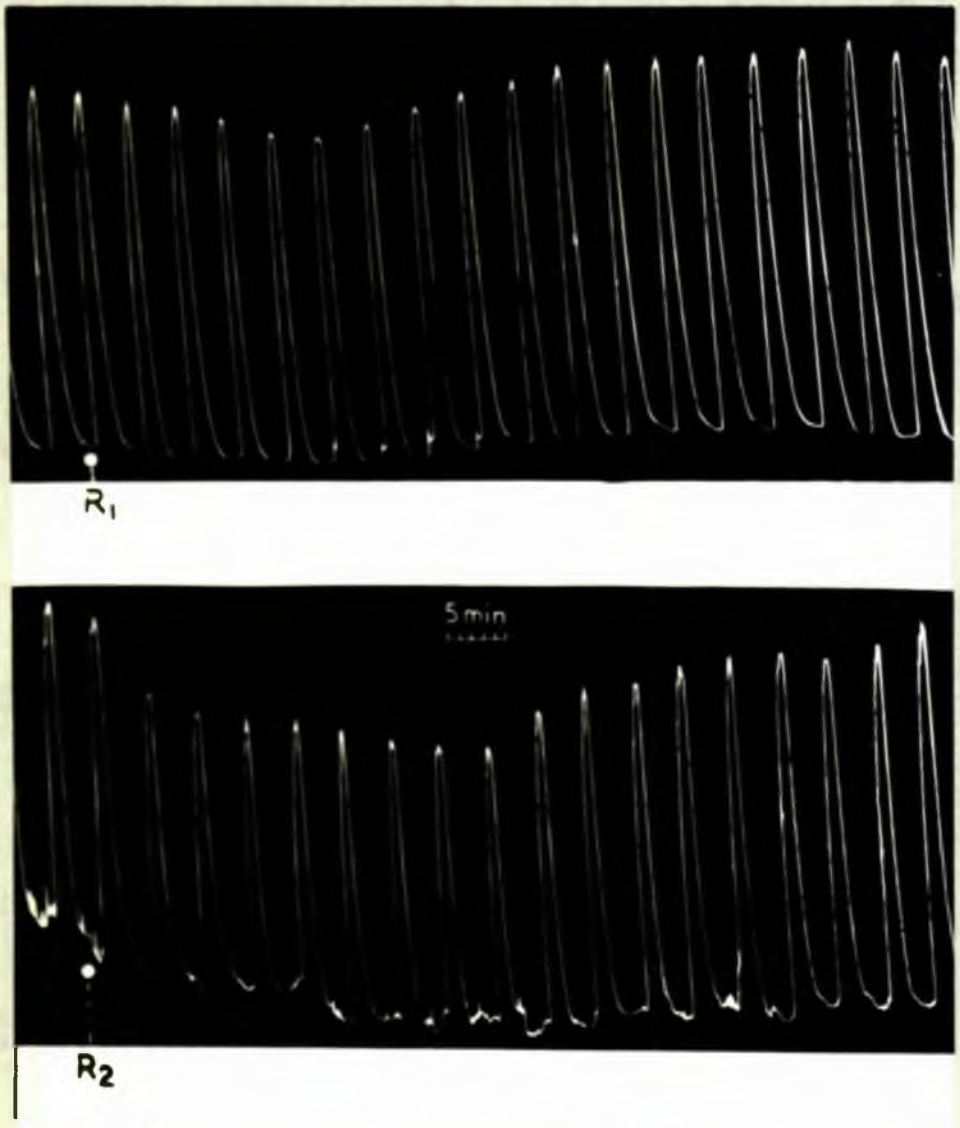


The stimulant action of reserpine in one experiment with isolated rabbit colon.

At R, 10 µg. reserpine added.

Time = 60 seconds.

Figure 4.16



The action of reserpine upon acetylcholine-induced contractions of isolated rat colon.

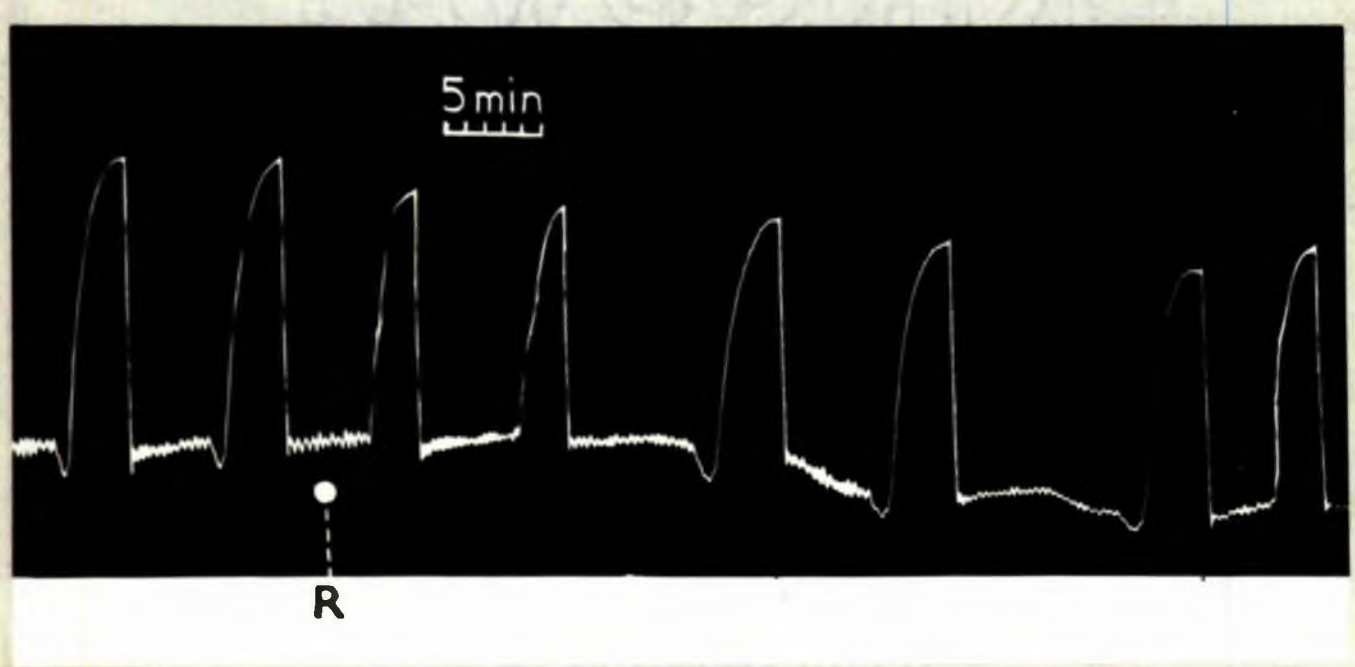
All contractions produced by the addition of 0.05 µg. acetylcholine.

At R_1, 10 µg. reserpine added and at R_2, 30 µg. reserpine added.

On rabbit colon, suspended in Krebs-Henseleit solution 10 µg. to 50 µg. reserpine produced similar effects. In one experiment out of twelve, however, a stimulant action was seen (Figure 4,15). The increase in tone and slight increase in the amplitude of spontaneous activity started about thirty seconds after the addition of reserpine. It was followed by a complete inhibition of activity and a fall in basal tone. Recovery in this case was incomplete.

The contractions to acetylcholine of colon taken from rabbit, rat or monkey were reduced by 4 µg. to 30 µg. reserpine (Figure 4,16). The doses of acetylcholine used were as follows:- monkey colon, 0.1 µg., rat colon, 0.05 µg. and rabbit colon, 0.05 µg. The reserpine inhibition in these cases was not very marked, but the same general characteristics which have been described for guinea-pig ileum (Section A, page 58) could be seen. Thus the maximum inhibition was seen following the third or fourth addition of acetylcholine after reserpine had been washed out of the bath. There was also a reduction of the tone. The maximum inhibition of colon appeared later than with ileum or duodenum. Recovery was complete. There appeared to be some relationship between dose and effect, but the

Figure 4.17



The action of reserpine upon the spontaneous activity, tone and response to acetylcholine of isolated human colon.

All contractions produced by the addition of 1 µg. acetylcholine.

At R, 20 µg. reserpine added.

The drum was stopped for 5 minutes after each "wash out" period.

inhibition produced by reserpine was not very pronounced even with doses up to 50 µg.

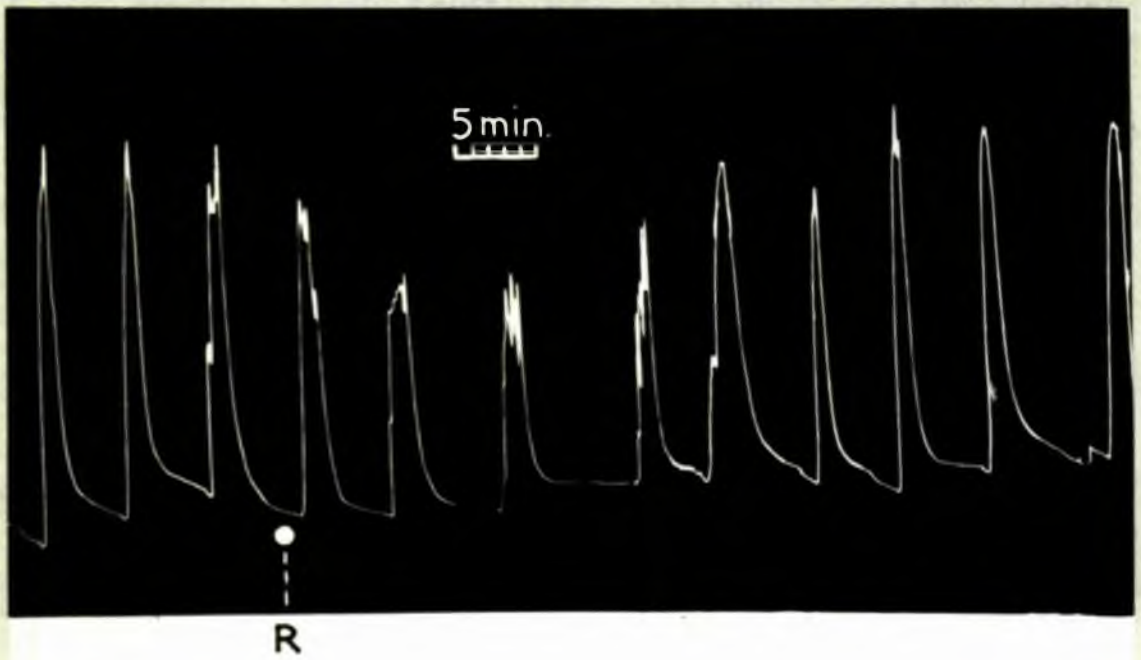
D) <u>The action of reserpine upon isolated human colon</u>.

Only two successful experiments were carried out using human tissue. This was due to the difficulty of obtaining the specimens soon enough after excision. The results must therefore be interpreted with caution. They are included merely for the sake of completeness and because few reports of experiments of this kind are to be found in the literature.

While only healthy tissue (as judged by its external appearance) was used, it is possible that the condition necessitating surgery may have influenced the experimental behaviour of the gut. In both cases the indication for surgery was rectal carcinoma. Both patients were female, aged 75 and 64 years.

In one preparation, 1 µg. acetylcholine was used to produce a contraction of the preparation (Figure 4,17). This dose was added at ten minute intervals. It was

Figure 4.18



The action of reserpine upon histamine-induced contractions of human colon.

All contractions produced by the addition of 10 µg. histamine.

At R, 20 µg. reserpine added.

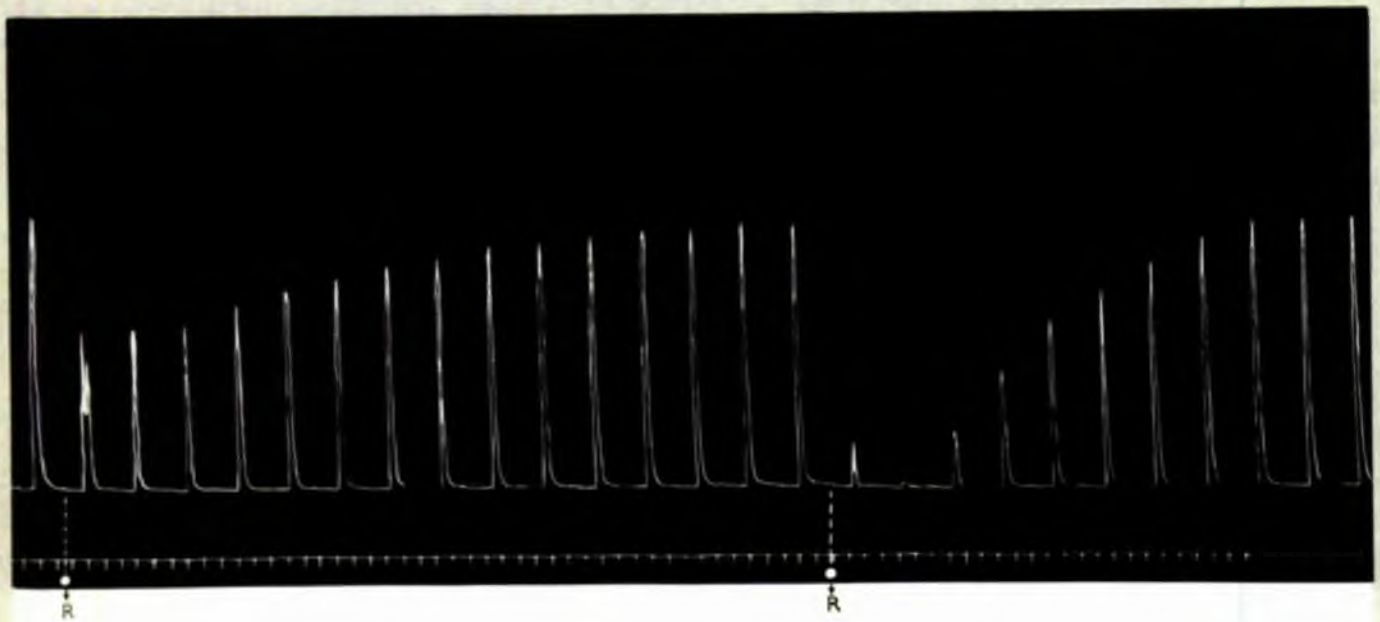
The drum was stopped for 10 minutes after each "wash out" period.

found possible to obtain a reproducible response to the same concentration of acetylcholine. The muscle also showed small, but regular spontaneous contractions. The addition of 20 µg. reserpine reduced very slightly the tone but not the height of the contraction to acetylcholine. The spontaneous activity was also slightly reduced (Figure 4,17).

In the second experiment, the response to 10 µg. histamine, given at 15 minute intervals, was reduced by 20 µg. reserpine (Figure 4,18). Recovery in this case was complete.

Several groups of workers have reported the results of experiments carried out to test the effects of reserpine on isolated intestinal strips[9-12]. These reports indicate that reserpine in concentrations of 1 µg. and 10 µg. had no stimulant effect upon the isolated ileum of guinea-pigs[10,11], rabbits[9,10,11], cats[11], or

Figure 4.19



The influence of reserpine upon acetylcholine-induced contractions of the isolated rat uterus.

All contractions produced by the addition of 0.25 µg. acetylcholine.

At R, 30 µg. reserpine added.

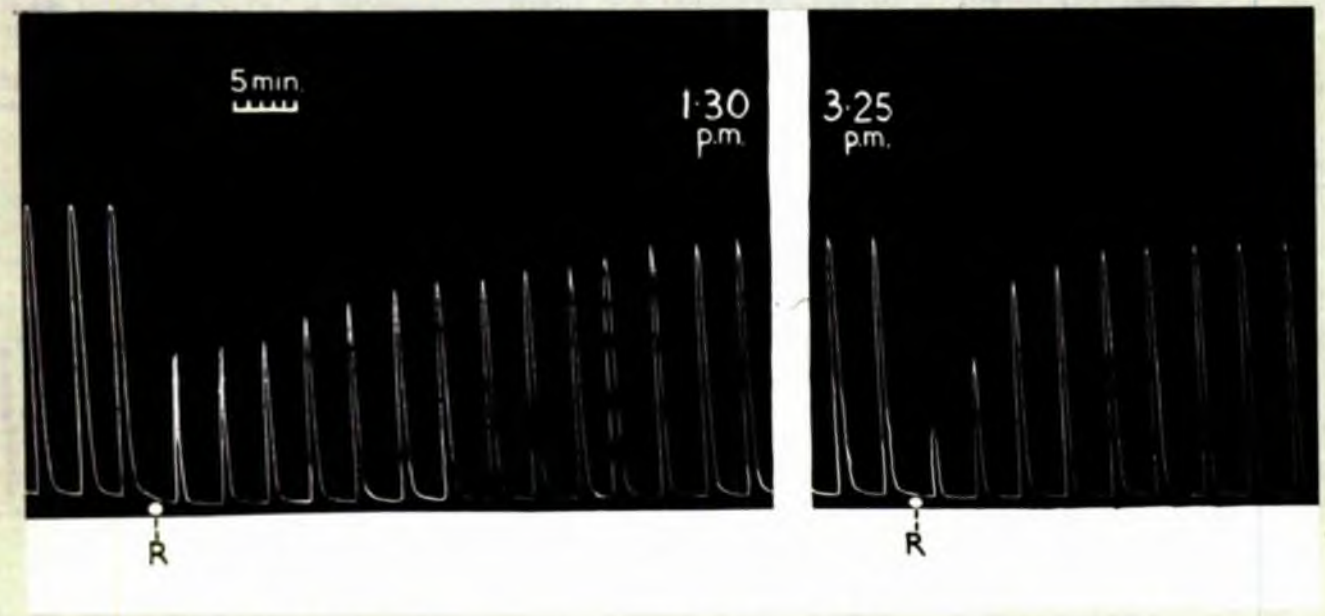
Time = 60 seconds.

dogs[11]. The activity of rabbit, rat, cat and dog colon was also unaltered by reserpine[11,12]. Reserpine, in concentrations above 1 µg. depressed the contractions of both colon and ileum of guinea-pig, rabbit, rat, cat and dog to acetylcholine, barium chloride and histamine. Reserpine showed less antagonism to acetylcholine on the isolated colon than on isolated ileum[11,12].

E) <u>The actions of reserpine upon isolated rat uterus</u>.

No direct action was seen following the addition of reserpine at dose levels from 2 µg. to 100 µg. The contractions induced by acetylcholine, 0.25 µg., 5-hydroxy-tryptamine, 20 ng., or potassium chloride, 2 mg. were reduced by the same concentration of reserpine. The general characteristics of the reserpine-inhibition were the same, regardless of which spasmogen was used. The first addition of reserpine in any experiment did not produce as marked an inhibition of the contractions as did subsequent addition of the same dose (Figure 4,19). It was noticed that the maximum inhibition occurred immediately following the addition of the first dose of reserpine and was not delayed in onset (Figure 4,19).

Figure 4.20



5-hydroxytryptamine-induced contractions of the isolated rat uterus. Incomplete recovery after reserpine.

The effects of subsequent addition of reserpine.

All contractions produced by the addition of 20 ng. 5-hydroxytryptamine.

At R, 30 µg. reserpine added.

The maximum inhibition of contraction following the second and subsequent doses of reserpine was in some cases delayed. It usually followed the second addition of spasmogen(Figure 4,19). In most cases, the inhibition due to the second or third addition of the same concentration of reserpine was considerably greater than that due to the first. Once the initial "resistance" to reserpine had been overcome, the percentage inhibition did not vary greatly within fairly wide limits of dose.

The recovery time did not seem to bear any relationship to the degree of inhibition. The duration of the reserpine effect was generally less than that seen on guinea-pig ileum. In some experiments, recovery of the contractions to the spasmogen in use was incomplete. In these cases, however, recovery after the second addition of reserpine was usually complete (Figure 4,20).

The increase in sensitivity to the second dose of reserpine was also noted in guinea-pig ileum experiments (Section A, page 59). With the rat uterus, however, the quantitative difference between successive additions of reserpine was usually more marked.

Since the duration of effect did not seem to be

related to the maximum degree of inhibition, it was thought possible that the difference in magnitude may have been due to fatigue. Although complete recovery had ostensibly taken place (as judged by a return either to the control or to a constant height), it seemed possible that insufficient time was allowed between additions of reserpine. Two hours were therefore allowed to elapse between the addition of two doses of the same concentration of reserpine. The maximum inhibition after the second dose was still greater than after the first (Figure 4,20).

CHAPTER 4

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CHAPTER 5

CHAPTER 5

THE EFFECT OF RESERPINE ON THE BLOOD PRESSURE OF THE SPINAL CAT.

Reserpine has been shown to cause a gradual fall in the blood pressure of conscious and anaesthetized experimental animals[1,2]. This effect developed slowly after administration of the drug and was preceded by a latent period, the duration of which depended upon the dose and mode of administration of the drug. The hypotension developed more rapidly after larger doses. Even when given intravenously, a latent period of about fifteen to thirty minutes preceded the reduction of blood pressure.

When the experiments described in this chapter were carried out, only one report had appeared in the literature describing the effect of reserpine on the blood pressure of the spinal animal. On this preparation the drug was found to have no pressor or depressor action[5]. In view of the scarcity of information and also because of the work done on isolated components of the cardio-vascular system (pages 30, 39, 43) such a study was indicated. In addition it was felt that information obtained on spinal cats would be more easily interpreted

than that from animals in which the central nervous system was intact.

Bhargava and Borison[3] have since published their observations on the action of reserpine on the pressor response in spinal cats produced by elevation of the cerebrospinal fluid pressure.

Methods.

Healthy cats, within the weight range 2.25 kg. to 4.5 kg., were given atropine (1 mg. per kg.) by intraperitoneal injection, fifteen minutes before the induction of anaesthesia with ether. The common carotid arteries were dissected free from the accompanying vagosympathetic trunks and tied. The trachea was next freed from adjoining tissue and cannulated. The tracheal cannula was connected to a bottle containing ether. This bottle could be joined easily to an artificial respiration pump by means of rubber tubing. The cat was then turned over and the spinal cord exposed in the vicinity of the long spine of the second cervical vertebra. The bony covering of the spinal cord and finally the cord itself was cut using bone forceps. At this point artificial

respiration was started. Bleeding was arrested by means of cotton wool swabs soaked in hot normal saline. A probe was inserted through the foramen magnum and up to the brain. The cut end of the spinal canal was plugged with plasticine and the area swabbed clear. The skin over the back of the neck was closed with surgical clips and the animal turned on its back again. One of the carotid arteries was cannulated and connected to a pressure system filled with twenty-five per cent sodium thiosulphate solution as an anticoagulant. A mercury manometer carrying a writing flag on one arm was incorporated in this system for recording the blood pressure on a smoked surface. The femoral vein was cannulated and connected by rubber tubing to a burette filled with normal saline. Drug solutions were injected into the rubber connection between the cannula and burette. Each injection was followed by 3 ml. saline.

In all experiments the preparations were left for at least one hour after setting up. Before the administration of any drugs, the blood pressure had remained constant for 20 minutes.

During the work, the necessity arose for determining the approximate age of the animals used.

The criteria used for this purpose were as follows:

Cats from 1 to 3 years. All the front incisors were present and were not very worn (if at all). The teeth were not discoloured.

Cats from 3 to 8 years showed loss of incisors and the teeth were discoloured and striated. As the age of the animals increased, the incisors were gradually lost.

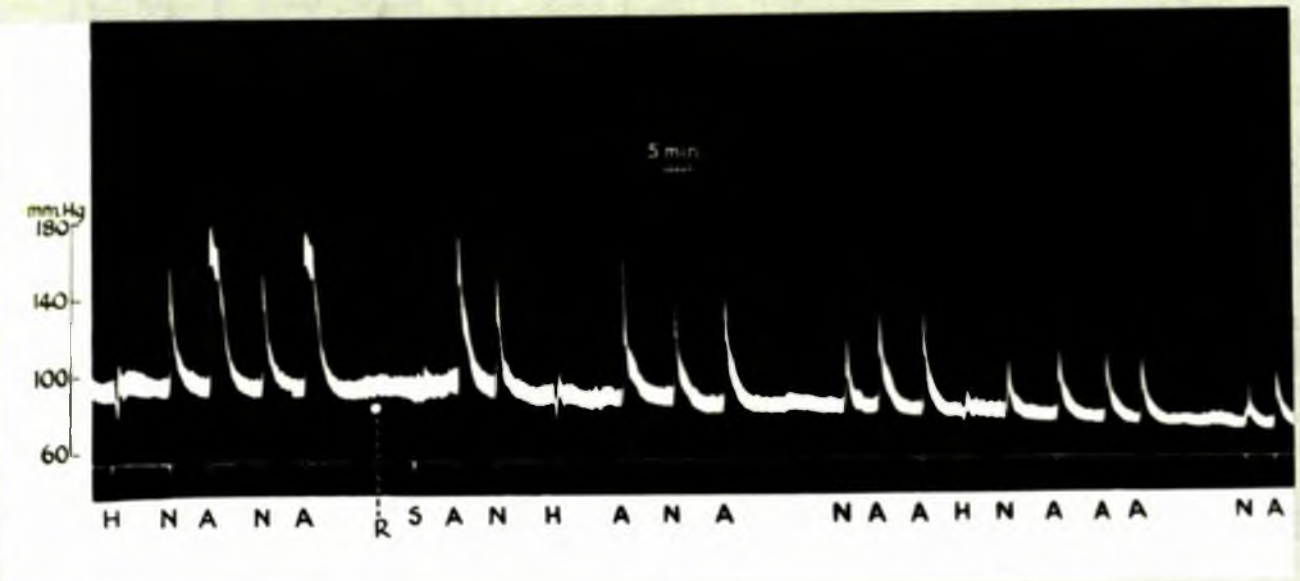
Cats older than 8 years and younger than 1 year were assessed directly. In this work, cats younger than 8 years were considered to be 'young'.

Although these criteria were not very satisfactory, they were used in the absence of a better method. Older cats were often heavier than younger ones but weight is an even less reliable index of age. In general, the initial blood pressure of the younger animals was lower than that of older cats.

Summary of results.

In 'young' cats, a gradually developing reduction of blood pressure was seen with 1 mg. per kg. reserpine.

Figure 5.1



The influence of reserpine upon the pressor response to adrenaline and nor-adrenaline in the spinal cat.

At H, administration of 5 µg. histamine + 3 ml. saline.
At S, administration of 3 ml. saline.
At A, administration of 5 µg. adrenaline + 3 ml. saline.
At N, administration of 1 µg. nor-adrenaline + 3 ml. saline.
At R, administration of reserpine 1 mg. per kg. + 3 ml. saline.

The responses to adrenaline and nor-adrenaline were progressively reduced. This effect was generally irreversible over an 8 hour observation period. The reduction of the pressor responses to adrenaline and nor-adrenaline were less marked in older animals, which did not show any fall in blood pressure following reserpine. At the point of maximal reserpine-hypotension, histamine produced no fall in blood pressure. While reserpine did not reduce the pressor response to posterior pituitary extract, the latter appeared in some experiments to antagonise the reserpine-induced reduction of the adrenaline response.

RESULTS.

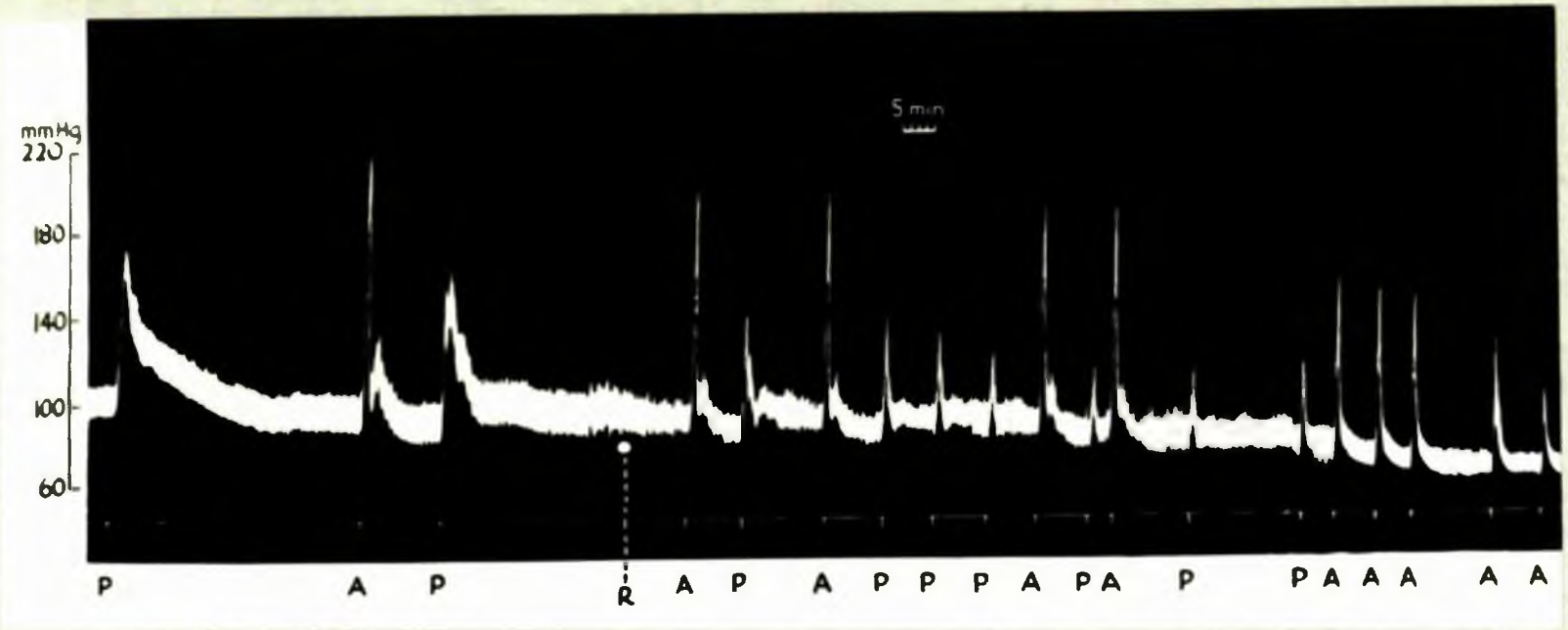
Reserpine in doses of 1 or 2 mg. per kg. produced a gradual reduction of the carotid artery blood pressure of the younger spinal cats. This reduction of blood pressure was preceded by a latent period of between 10 and 20 minutes after the injection of reserpine into the femoral vein (Figure 5,1). The initial blood pressure in the preparations used was between 100 and 140 mm. of mercury. In most cases, the injection of histamine (5 µg. to 10 µg.) at the

beginning of each experiment produced a fall in blood pressure. This depressor response, however, was gradually reduced after reserpine and at the point of maximal reserpine-induced-hypotension, the same dose of histamine no longer produced a fall in the blood pressure (Figure 5,1). When the vasodepressor response to histamine was very small or completely absent (in three experiments in which the initial pressure was between 60 and 80 mm. of mercury) no hypotensive effect was produced by reserpine.

The blood pressure did not return to its original value during the period of observation which was at least six hours after the administration of reserpine. The control solution had no observable action on the blood pressure of the preparations used. In older cats, the reduction of blood pressure following reserpine was not seen.

As the hypotension appeared, there was a gradual reduction in the response to both adrenaline, 5 µg. and nor-adrenaline, 1 µg. (Figure 5,1). This effect, also, was irreversible during the period of the experiment. The control solution had no effect on the adrenaline and nor-adrenaline pressor responses. Once again, this

Figure 5.2



The pressor response to posterior pituitary extract in the spinal cat: apparent inhibition by reserpine.

At P, administration of 1 i.u. posterior pituitary extract + 3 ml. saline.

At A, administration of 5 µg. adrenaline + 3 ml. saline.

At R, administration of 2 mg. per kg. reserpine + 3 ml. saline.

action of reserpine was less marked in older cats than in younger animals.

Experiments with isolated perfused blood vessels (Chapter 3, page 43) indicated that drug-induced vasoconstriction was depressed by reserpine whatever the drug used. It seemed possible, therefore, that in the spinal cat the action of peripherally acting vaso-constrictor substances might be depressed. Posterior pituitary extract is one such substance. When 1 i.u. of this drug was injected, the typical pressor response was seen. This consisted of a sharp rise in blood pressure followed by a very slow return to normal (Figure 5,2). 2 mg. per kg. reserpine apparently caused a marked reduction in the response to posterior pituitary extract, whilst the response to adrenaline in this case was reduced only after a latent period of over one hour (Figure 5,2). The control solution of ascorbic acid, equivalent to that normally containing 2 mg. per kg. reserpine, produced the same apparent reduction of the pressor action of posterior pituitary extract. This suggested that the solvent and not reserpine was responsible for the reduction of the effect of posterior pituitary extract. To test this hypothesis, three

experiments were carried out in which a constant dose of posterior pituitary extract was given at thirty minute intervals. In those cases exactly the same reduction of response was seen as when reserpine was given. It was concluded, therefore, that the reduction in response observed was due to a normal tachyphylaxis to posterior pituitary extract and not to reserpine or the control solution.

The experiments with posterior pituitary extract and adrenaline suggested that the former drug may have antagonised the inhibition by reserpine of the response to adrenaline. Some experiments, designed to test this suggestion, were carried out. Since the action of reserpine on the pressor response to adrenaline was irreversible, it was impossible to test more than one dose of the alkaloid in each experiment. In three out of four experiments carried out on young animals, the administration of 1 i.u. of posterior pituitary extract delayed the reduction of the response to adrenaline following the administration of reserpine. The effect seen was similar to that shown in Figure 5,2. The gradual fall in blood pressure described above (page 91) was seen after the usual delay of ten to twenty minutes.

The work of Bhargava and Borison[3] on the blood pressure of spinal cats was part of a general investigation of the effects of the alseroxylon fraction of the Rauwolfia alkaloids and reserpine on the vasoregulatory systems of the cat. They observed that the amplitude of the pressor response following elevation of the cerebrospinal fluid pressure in spinal cats was unaffected by 2.0 mg. per kg. reserpine. The record illustrated in their paper shows that the basic blood pressure of the spinal cat was only very slightly reduced by reserpine after one hour. The blood pressure at the start of the experiment, however, was about 60 mm. of mercury. From the work described in this chapter, it was apparent that the lower the initial blood pressure, the smaller was the fall of pressure produced by reserpine. Little reduction of blood pressure, if any, would be expected, therefore, when the initial pressure was only 60 mm. of mercury.

Neither Bhargava and Borison[3] nor Bein[5] gave any indication of the weight (or the approximate age) of the cats used in their work. It is difficult, therefore, to compare their work with the observations reported in this chapter.

The reduction of the response to adrenaline and

nor-adrenaline was not due to the atropine. Graham[4] has shown that, in the spinal cat, 1.0 mg. per kg. of atropine given intravenously potentiates, slightly, the pressor response to adrenaline and nor-adrenaline. It has also been shown[5] that atropine has no effect upon the reserpine-induced hypotension in the cat.

CHAPTER 5

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2). H.J. Bein, Pharmacol. Rev., (1956), 8, 435.

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5). H.J. Bein, Experientia, (1953), 9, 107.

CHAPTER 6

CHAPTER 6

DISCUSSION OF DATA PRESENTED IN PART I OF THE EXPERIMENTAL WORK.

It is perhaps appropriate at this point to summarize the experimental results presented and to examine them in some detail.

Depression of the activity of isolated cardiac muscle preparations was observed, preceded in some cases by slight stimulation. The amplitude of the contractions of the isolated kitten and rabbit hearts was decreased. This was accompanied by an increased outflow from the hearts; this may not, however, have been due entirely to coronary vasodilatation (Chapter 3, page 31). It seems clear, however, that it reflects either a general reduction in the vascular support of the coronary bed, or an increased aortic incompetence. In either case, the mechanism must involve muscular relaxation. Although little depression of the action of cardiotonic drugs on the isolated heart was seen, their duration of action in some cases, was reduced by reserpine.

Depression of the spontaneous activity of the isolated auricles and also of their responses to

stimulant drugs was seen.

In perfused blood vessel preparations, drug-induced vasoconstriction was reduced by reserpine.

The tests made with intestinal smooth muscle preparations indicated that reserpine reduced inherent tone when this was present. The spontaneous contractions of intestine from rabbit, kitten, guinea-pig, rat and monkey were reduced or inhibited completely. The response of these preparations to stimulant drugs, for example acetylcholine or histamine, was depressed.

In experiments with the isolated rat uterus, reserpine reduced the contractions due to potassium chloride, 5-hydroxytryptamine and acetylcholine.

Reserpine produced a slow contraction of the frog rectus-abdominis muscle. This effect was followed by a reduction of the acetylcholine-induced contractions of the latter muscle.

The preparations used differed widely in their histology and pharmacology. Certain characteristic features in the action of reserpine on isolated muscle, however, emerge from a consideration of the data.

Thus, reserpine appears to produce a depression of muscular activity preceded, in a few instances, by a brief stimulant action. In certain preparations, the depression is manifest as a reduced response to a constant concentration of a drug. The second generalisation which can be made is that reserpine seems to be entirely non-specific in its action on the preparation used. This conclusion is based on the fact that reserpine reduces muscular contraction following the addition of drugs with different sites of action within the muscle. The pharmacological nature of the agents used to cause contraction does not appear to be decisive in determining the antagonism of reserpine towards them. The suggested non-specificity of action may be supported by the very wide variation in the degree of inhibition produced by the same dose of reserpine in the same experiment. A third characteristic of the action of reserpine is the delay in the appearance of its maximum effect. The action of reserpine was prolonged, and it was reversible only in certain preparations. In other cases there was incomplete recovery of normal function.

No single mechanism of action can be suggested

to account for all the effects observed. Many of them may be explained, however, if it is assumed that reserpine acts by depressing the ability of smooth, cardiac and striped muscle to contract. This implies that a process common to muscular contraction, whether myogenic or drug-induced, is influenced by reserpine. The site or sites of this interference cannot, as yet, be defined.

It is well known that carbohydrate metabolism is closely linked with the production of energy for muscular contraction. It is not unlikely, therefore, that some point or points in this process may be affected. The observation, that citric acid appeared to reduce the reserpine inhibition of drug-induced contraction of guinea-pig ileum, is of interest in this respect.

If it is accepted that the ability of muscle to contract is depressed because of some interference with its energy production, then many of the observed actions of reserpine can be explained. Thus the pharmacological nature of the agent responsible for muscular contraction should not alter the depression caused by reserpine. The contractions of intestinal segments induced by all the drugs tested were, in fact, depressed by reserpine.

It is interesting to note that McQueen and his co-workers[1,2], found that reserpine caused dilatation in the perfused, innervated hind-quarters of the rabbit. In this preparation it is to be expected that a certain amount of neurogenic tone would be present in the vascular smooth muscle, whilst in the denervated preparation of the rat's hind-quarters, little, if any, tone would remain. Thus reserpine should have no effect on the normal outflow. This was observed (Chapter 3, page 48).

In the spinal cat, the peripheral resistance is low presumably because of a low basal tone in the vascular muscle. If reserpine decreases the ability of smooth muscle to maintain its inherent tone, then it would be expected that only a slight reduction of blood pressure would follow the administration of reserpine. This was observed, especially in the younger animals used (Chapter 5, page 91). It might also be expected that drug-induced tone would be depressed. This was confirmed by the progressive reduction seen in the action of both adrenaline and nor-adrenaline on blood pressure.

The experimental work described in Part 2 deals with attempts to investigate, in more detail, the

possibility of a metabolic site of action for reserpine. The suggestion made earlier (Chapter 2, page 23) that the potassium ion may be linked with the contractions of the frog rectus-abdominis muscle, was also investigated.

CHAPTER 6

REFERENCES.

1). E.G. McQueen, A.E. Doyle and F.H. Smirk, Nature, Lond., (1954), 174, 1015.

2). E.G. McQueen, A.E. Doyle and F.H. Smirk, Circulation, (1955), 11, 161.

EXPERIMENTAL WORK

PART 2.

CHAPTER 7

The concentrations of drugs mentioned in this chapter refer to the weight of the drug per millilitre of physiological saline in the isolated organ bath.

CHAPTER 7

AN INVESTIGATION OF POTENTIAL INHIBITORS OF THE ACTION OF RESERPINE ON SMOOTH, SKELETAL AND CARDIAC MUSCLE.

Citric acid was found (Chapter 4, page 70) to reduce the reserpine inhibition of drug-induced contractions of the guinea-pig ileum. It has been suggested (Chapter 6, page 98) that reserpine might interfere with carbohydrate metabolism and thus the production of energy for the contractile activity of smooth muscle. The activity of citric acid may therefore have been due to its metabolic involvement. This possibility was studied in detail. The tissue chosen for most of the work was guinea-pig ileum. The method of preparation has already been described (Chapter 4, page 52)

The investigation was extended to skeletal and cardiac muscle by using the isolated frog rectus-abdominis muscle and the isolated auricles of the guinea-pig (Chapter 2, page 17 and Chapter 3, page 39).

A series of known intermediates of carbohydrate, fat and protein metabolism was tested for possible antagonism to the action of reserpine in depressing

drug-induced contractions of isolated guinea-pig ileum. Solutions of the metabolites in Tyrode's solution were used either as the free acids or as their sodium salts. Some compounds were used in both forms. Each metabolite was tested in concentrations of 125 µg., 250 µg., 500 µg. and 1 mg. The dose most frequently used was 1 mg.

Acetylcholine (0.2 µg. to 0.5 µg.) was used at 3 minute intervals to stimulate the ileum in most of this work. Histamine (0.1 µg. to 0.5 µg.) was used in some experiments.

Reserpine was added to the bath 1 minute before acetylcholine or histamine and the metabolites, or other potential antagonists, 5 seconds before reserpine. In order to produce at least a 50 per cent inhibition of the contractions, reserpine was used at a concentration of 30 µg. Measurements of pH were made with a glass electrode on a 1 ml. sample of the bath fluid. In some later experiments, small volumes of 0.2 N hydrochloric acid, 5 per cent tartaric acid or 5 per cent sodium bicarbonate were added before either reserpine or a metabolite.

The metabolites were tested twice in each of

at least eight experiments. In many cases, the number of tests was larger than this. The mean percentage inhibition of the contractions, following the addition of reserpine alone and also that produced by reserpine together with a metabolite, was calculated. The number of values for the percentage inhibition after reserpine alone and after reserpine with a metabolite was the same in the tests made with each metabolite.

Summary of results.

Certain intermediates of the Krebs cycle, in concentrations of 125 μg. to 1 mg., were found to be effective in antagonising the depression by reserpine of drug-induced contractions of guinea-pig ileum. The bath pH in those cases was about 5. When used as solutions of their sodium salts, at pH 7.4, those compounds showed much less effect in antagonising the action of reserpine. Sufficient hydrochloric acid to reduce the bath pH to about 5 produced a reduction of the reserpine effect. In certain cases the degree of inhibition following hydrochloric acid was no greater than that seen following certain metabolites. Other intermediates of carbohydrate metabolism, previously

inactive, were rendered more active than hydrochloric acid by the reduction of the bath pH to about 5. Removal of calcium from the bath fluid by disodium dihydrogen versenate did not produce any antagonism to reserpine.

The possibility of release of histamine or acetylcholine by the metabolites was excluded by repeating the experiments using, where appropriate, Tyrode's solution containing either atropine (100 µg. per litre) or mepyramine maleate (200 µg. per litre).

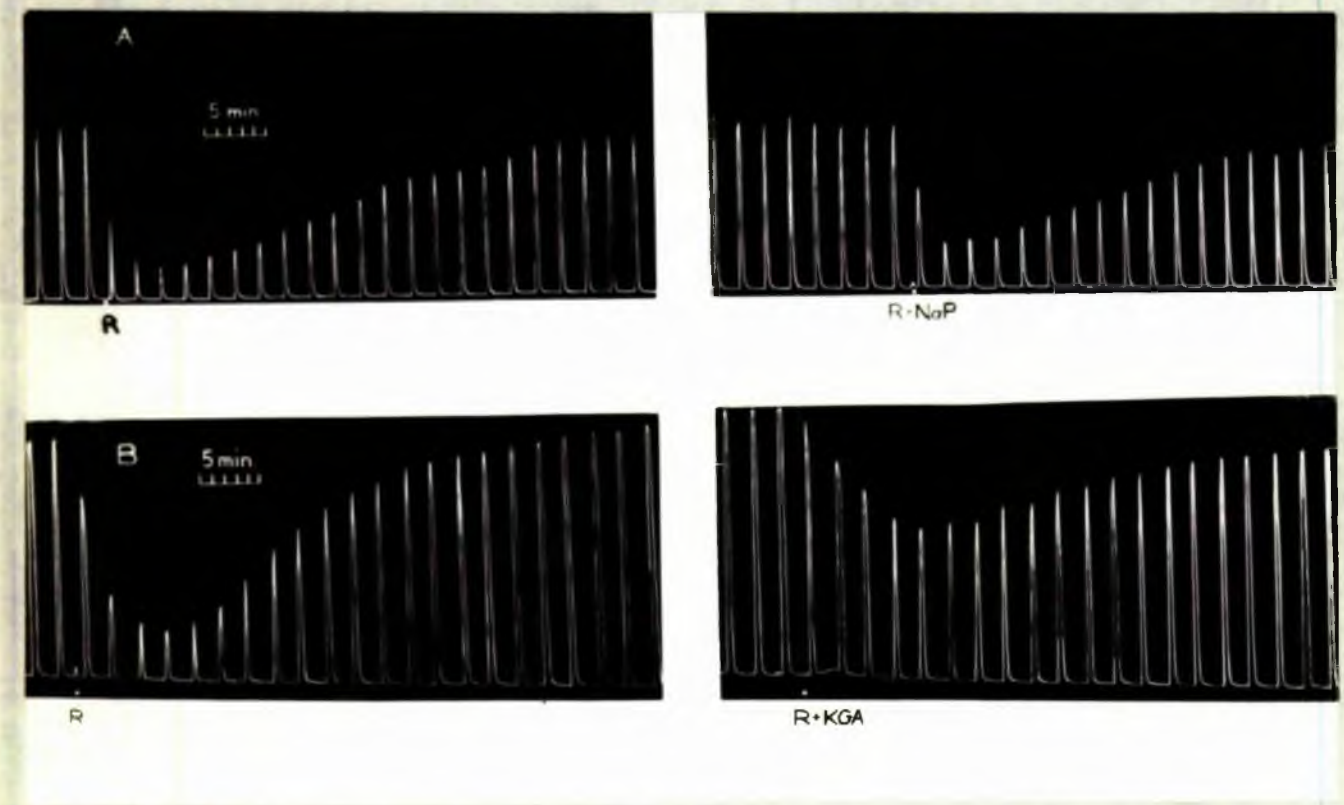
Experiments with the rectus-abdominis muscle of the frog and the isolated auricles of the guinea-pig were inconclusive. It could not be said that any definite antagonism to reserpine was shown by any of the metabolites tested.

RESULTS.

A) The isolated ileum of the guinea-pig.

The following substances were found to be active, at concentrations of 500 µg. and 1 mg., in reducing the inhibitory action of reserpine upon acetylcholine-induced

Figure 7.1



Modification of the effects of reserpine by certain intermediates of carbohydrate metabolism.

Isolated guinea-pig ileum.

A) All contractions produced by the addition to the bath of 0.25 µg. acetylcholine.

B) All contractions produced by the addition to the bath of 0.20 µg. histamine.

At R, 30 µg. reserpine added. At R + NaP, 1 mg. sodium pyruvate added, followed 5 seconds later by 30 µg. reserpine. At R + KGA, 1 mg. α-ketoglutaric acid added, followed 5 seconds later by 30 µg. reserpine.

contractions: citric acid, cis-aconitic acid anhydride, α-ketoglutaric acid, oxaloacetic acid, 3-phosphoglyceric acid, malic acid and maleic acid (Table 7,1). Even at a concentration of 1 mg., the active metabolites failed to abolish completely the effect of reserpine (Figure 7,1). When used in a concentration of 500 µg, the reduction of the action of reserpine was less marked than with 1 mg. Concentrations of the active metabolites below 500 µg. occasionally produced some depression, but this was never as marked as that seen following the use of 1 mg. It was noticed that those metabolites were most active in preparations of ileum on which reserpine also had a more pronounced effect.

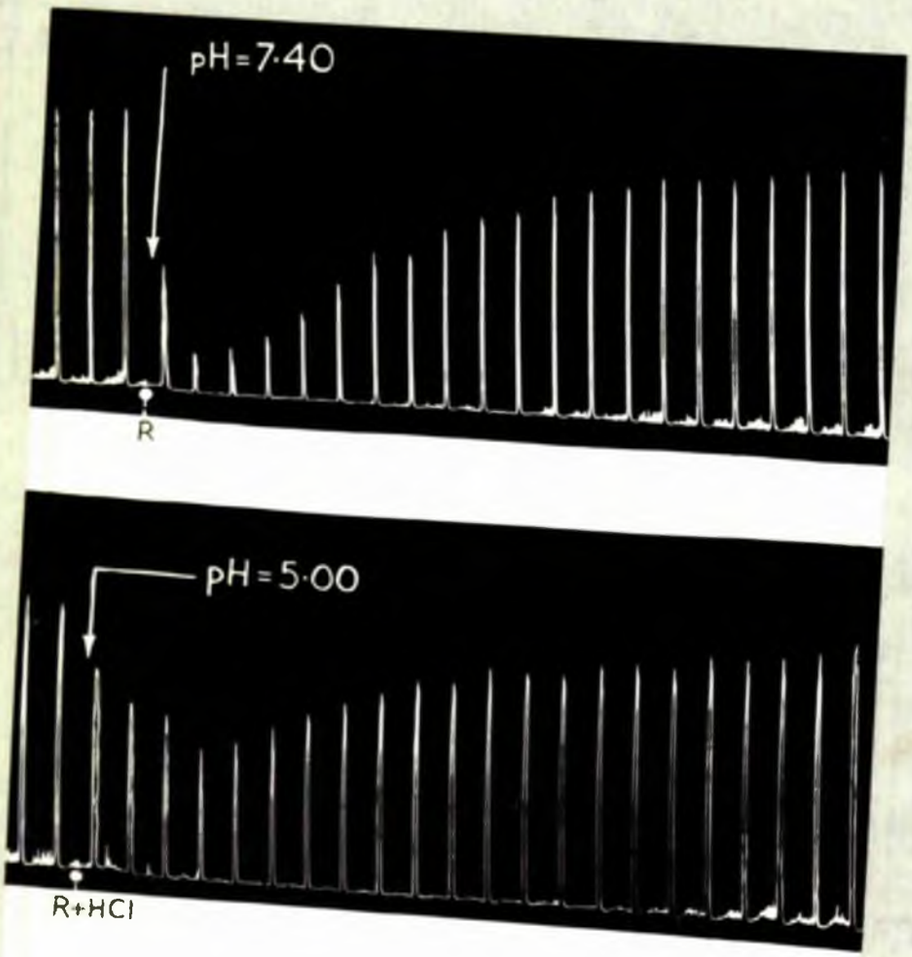
The following metabolites were ineffective as reserpine antagonists: glucose-1-phosphate, fructose-1, 6-diphosphate, fructose-6-phosphate, sodium pyruvate, sodium succinate, oxalosuccinic acid, iso-citric acid and adenosine triphosphate (ATP) (Table 7,1).

The metabolites themselves had either no effect or, in some experiments, produced a slight but transient depression of the contractions following acetylcholine. This occurred after 1 mg. but not after 500 µg. tested in the same experiment. It seems likely to have been

caused by too rapid a reduction of reaction of the bath fluid, since it was seen only when the metabolites were used as solutions of the free acids, which reduced the bath pH to 5.

It is well known that certain di- and tricarboxylic acids form undissociated complexes with calcium ion and therefore effectively remove these from solution. It seemed possible that some of the observed effects were due to this phenomenon. An amount of the disodium salt of ethylenediamine tetra-acetic acid, calculated to remove all the calcium ions in the Tyrode's solution, was added to the bath 5 seconds before reserpine. There was no alteration in the reserpine effect. The complex itself, when present in the bath for one minute, did not modify the response to acetylcholine or histamine. When calcium-free Tyrode's solution was substituted for the normal bath fluid, there was a gradual loss of sensitivity of the ileum to both histamine and acetylcholine. To exclude possible effects due to liberation of histamine or acetylcholine, the experiments were repeated using where appropriate, Tyrode's solution containing 100 µg. per litre of atropine or 200 µg. per litre of mepyramine maleate. Under these conditions, there was no alteration

Figure 7.2



The antagonism between hydrochloric acid and reserpine on the isolated guinea-pig ileum.

All contractions produced by the addition to the bath of 0.20 µg. acetylcholine.

At R, 30 µg. reserpine added.

At R + HCl, 0.05 ml. of 0.2N hydrochloric acid added followed 5 seconds later by 30 µg. reserpine.

in the inhibition of the reserpine effect produced by the active substances. It was concluded, therefore, that there was no evidence for either histamine or acetylcholine release.

It was difficult to explain why certain metabolites did not antagonise reserpine. If the active substances were effective because they were intermediates of carbohydrate metabolism, then it seemed reasonable to expect that all such metabolic intermediates should show some antagonism.

It was observed that the most active reserpine antagonists were those added to the bath as the free acids. These reduced the reaction of the bath fluid to between 4.8 and 5.4. This suggested that they may have been active only by virtue of their ability to reduce the bath pH. Small volumes of 0.2 N hydrochloric acid were therefore tested for reserpine antagonism. The volume used was generally 0.05 ml., which reduced the reaction of the bath fluid to about 5. Antagonism to the action of reserpine was seen in these experiments (Figure 7,2). Small amounts of 5 per cent tartaric acid solution showed a similar degree of antagonism to reserpine. In some experiments the reserpine and

hydrochloric acid produced an immediate maximum inhibition of the contractions. The bath pH was therefore an important aspect of the reserpine antagonism shown by the active metabolites. A further series of experiments were carried out in which the effect of hydrochloric acid was tested along with the active metabolites. A total of 40 teste using hydrochloric acid were made. These gave a mean value for the percentage inhibition of the acetylcholine-induced contractions following the addition of reserpine and hydrochloric acid of 48.4 ± 16.1 (S.D.). The mean values of the percentage inhibition following the use of the reserpine antagonists were calculated (Table 7,1). A Student's "t" test for significance was carried out using as pairs, the mean value of the percentage inhibition with each of the active metabolites and the mean value for hydrochloric acid (48.4 ± 16.1). The mean value for the hydrochloric acid antagonism was used since an insufficient number of additions (below 10) was made in the testing of each metabolite. The details of these analyses are given in Table 7,1.

TABLE 7.1

The antagonism between reserpine and certain intermediates of carbohydrate metabolism upon the acetylcholine-induced contractions of isolated guinea-pig ileum.

Metabolite	Percentage inhibition ± standard deviation.				Probability of difference between means (1).	pH of bath fluid after reserpine and metabolite.
	Reserpine alone.	Number of Additions.	Reserpine and Metabolite.	Number of additions.		
Adenosine triphosphate	73.4 ± 14.4	16	65.7 ± 15.8	16	< 0.1, > 0.05	7.40
Glucose-1-phosphate	59.8 ± 15.1	16	65.4 ± 14.8	16	< 0.3, > 0.2	7.35
Fructose-6-phosphate	60.7 ± 16.8	16	70.2 ± 17.3	16	< 0.2, > 0.1	7.40
Fructose-1, 6-diphosphate	64.5 ± 17.3	16	70.8 ± 20.4	16	< 0.4, > 0.3	7.40
3-phosphoglyceric acid	65.3 ± 18.3	24	40.1 ± 12.9	24	< 0.05, > 0.02 *	5.40
Sodium pyruvate	65.3 ± 17.1	30	70.1 ± 15.1	30	< 0.3, > 0.2	7.35
Citric acid	70.3 ± 18.4	40	33.8 ± 12.4	40	< 0.001 *	4.80
Cis-aconitic acid anhydride	64.9 ± 17.8	38	30.6 ± 12.1	40	< 0.001 *	4.90
Isocitric acid	63.7 ± 17.4	18	69.4 ± 16.1	18	< 0.4, > 0.3	7.35
Oxalosuccinic acid	62.3 ± 15.1	16	69.2 ± 16.9	16	< 0.3, > 0.2	7.30
α-ketoglutaric acid	65.0 ± 19.9	44	29.0 ± 12.3	44	< 0.001 *	4.80
Sodium succinate	69.3 ± 18.4	18	61.4 ± 16.3	18	< 0.2, > 0.1	7.40
Sodium fumarate	71.8 ± 15.9	18	60.9 ± 17.1	18	< 0.1, > 0.05	7.40
(-)-Malic acid	72.4 ± 16.9	22	37.9 ± 11.3	22	< 0.01, > 0.001 *	4.90
Oxaloacetic acid	73.8 ± 16.7	22	39.8 ± 11.3	22	< 0.05 > 0.02 *	5.95
Maleic acid	71.8 ± 19.3	34	38.4 ± 14.1	34	< 0.01 > 0.001 *	4.90

* Mean percentage inhibition following reserpine and metabolite compared with mean value for reserpine and hydrochloric acid (48.4 ± 16.1, n = 40). All others are comparisons of the percentage inhibition after reserpine with that following reserpine and the metabolite.

It can be seen from Table 7,1 that the percentage inhibition produced by the active metabolites with reserpine was significantly different ($P = 0.05$) from that due to hydrochloric acid and the alkaloid. It was concluded, therefore, that the activity of the metabolites concerned was not due entirely to the reduction of the pH of the bath fluid; this factor was, however, responsible for part of the antagonism shown.

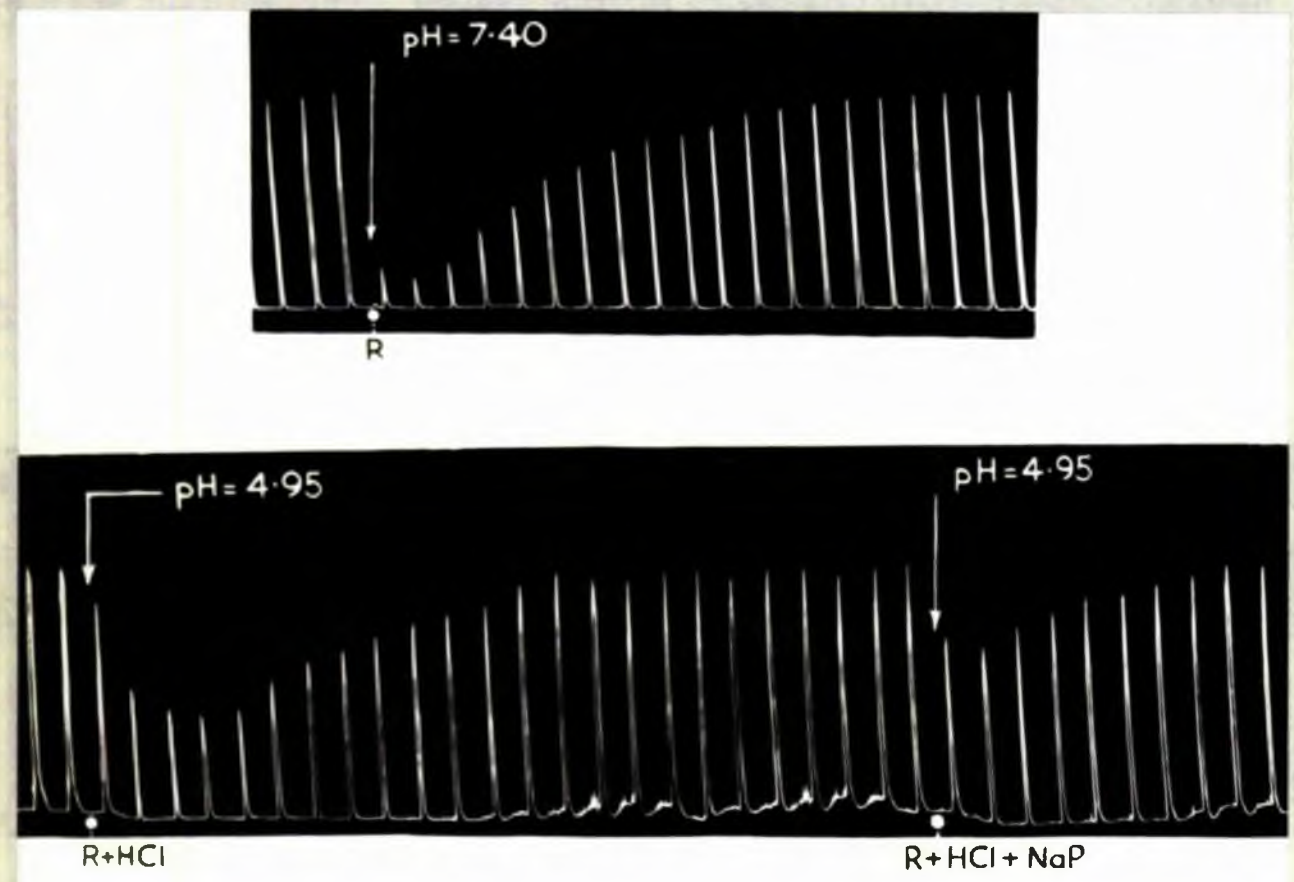
The fact that the active reserpine antagonists reduced the bath fluid pH to about 5, while the inactive substances did not alter the reaction of the medium, was of interest. It seemed possible that the reduction of pH may have been related to the availability of the metabolite for antagonising the action of reserpine.

At this point some mention must be made of the work of Furchgott and others[2-5]. These workers used isolated rabbit intestine suspended in glucose-free Krebs-Henseleit solution. The spontaneous contractions of the intestinal segments decreased and reached a constant value of approximately 20 per cent of the original amplitude. Partial or complete restoration of amplitude could be brought about by the addition of certain substances. At pH 7.4, these included glucose,

acetate, oxaloacetate, pyruvate and some fatty acids and intermediates of fat metabolism[2]. When the pH of the bath fluid was reduced to 6.1, however, succinate, α-ketoglutarate, fumarate, malate and citrate were utilized for the production of energy for contraction. It was considered, therefore, that "the Krebs citric acid cycle plays a major role in the intermediary metabolism of intestinal smooth muscle"[4]. They suggested further that the increased effectiveness of, for example, succinate, α-ketoglutarate and fumarate at low pH values of the medium, was directly related to the increase in the relative concentration of the singly ionized forms of the dicarboxylic acids. Their data indicated that the membranes of smooth muscle cells were highly impermeable to the completely ionized molecule of these dicarboxylic acids, but were much more permeable to the singly ionized molecule. The concentration of the latter moeity in the medium therefore determined the rate of penetration of the added substance into the muscle cells and hence its availability for the production of energy[4].

These considerations suggested a possible explanation for the absence of antagonism to reserpine in certain intermediates of carbohydrate metabolism.

Figure 7.3



The antagonism to reserpine by sodium pyruvate at pH 4.95.

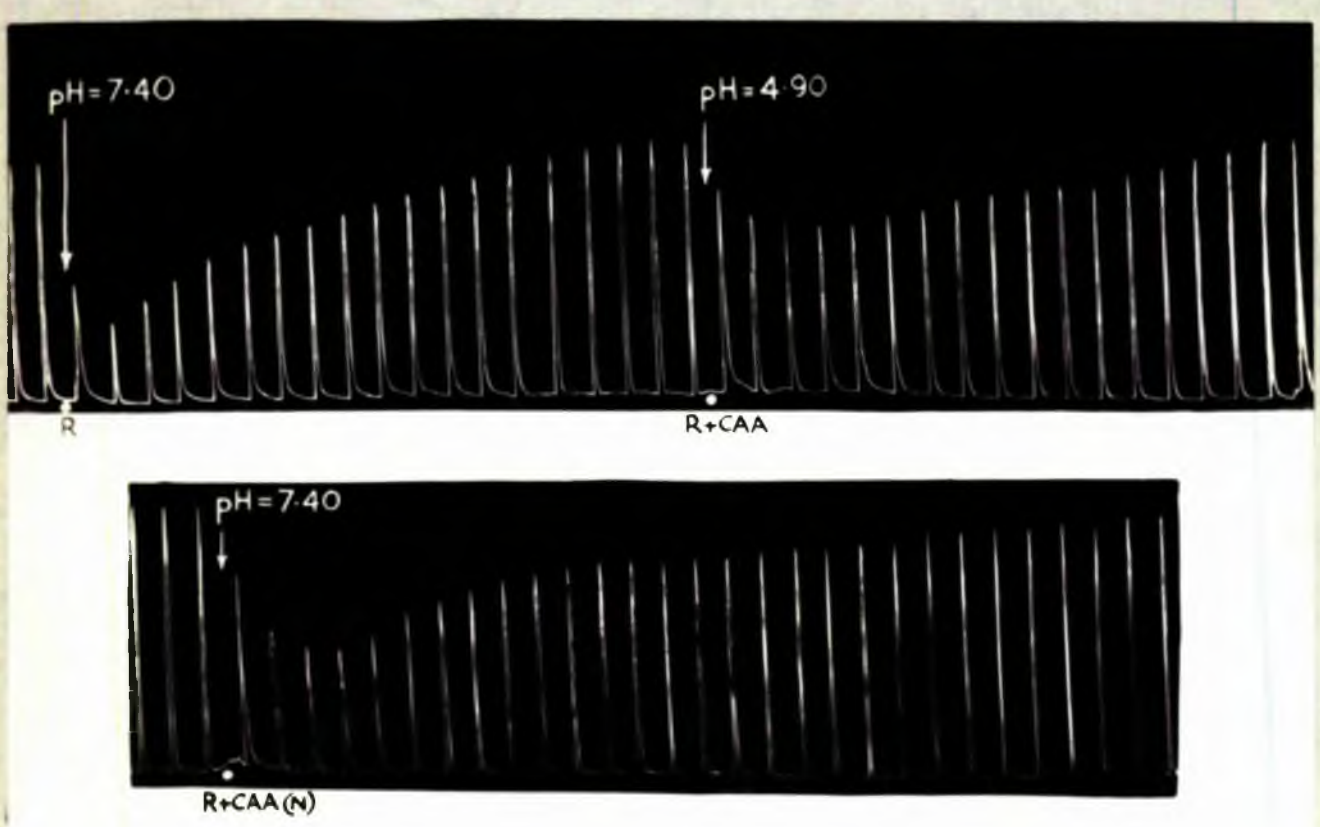
All contractions produced by the addition to the bath of 0.2 µg. acetylcholine.

At R, 30 µg. reserpine added.

At R + HCl, 0.06 ml. of 0.2N hydrochloric acid added to the bath, followed 5 seconds later by 30 µg. reserpine.

At R + HCl + NaP, 0.06 ml. of 0.2N hydrochloric acid and 1 mg. sodium pyruvate added, followed 5 seconds later by 30 µg. reserpine.

Figure 7.4



The reduced reserpine antagonism with cis-aconitic acid anhydride at pH 7.4.

All contractions produced by the addition to the bath of 0.2 µg. acetylcholine.

At R, 30 µg. reserpine added.

At R + CAA, 1 mg. cis-aconitic acid anhydride added, followed 5 seconds later by 30 µg. reserpine.

At R + CAA(N), 1 mg. cis-aconitic acid anhydride (pH of solution = 7.4) added, followed 5 seconds later by 30 µg. reserpine.

Thus the concentration of their singly ionized forms at pH 7.4 may have been below that required to penetrate the muscle cell and produce antagonism to reserpine. If this were true, reduction of the bath pH to 5 should increase the concentration of what may be termed the "active moeity" of the metabolites. Alternatively, increasing the bath pH to about 7 should have reduced the activity of substances previously found to be effective. Experiments designed to test this hypothesis were carried out. Previously inactive metabolites were added to the bath with sufficient hydrochloric acid or tartaric acid to reduce the pH of the medium to 5. Under these conditions sodium pyruvate, sodium succinate and isocitric acid were observed to be more effective reserpine antagonists than hydrochloric acid alone, which reduced the pH of the bath fluid to between 4.9 and 5.0 (Figure 7,3). Oxalosuccinic acid and sodium fumarate, however, were no more effective at the reduced pH than at pH 7.3. On the other hand, when solutions of active metabolites which had been adjusted previously to pH 7.4 were used, less reserpine antagonism was seen (Figure 7,4).

It was found that hydrochloric acid, which reduced the pH of the bath fluid to 6 or 6.5, did not

influence the degree of inhibition produced by reserpine. Within this range of pH, however, the active metabolites still showed reserpine antagonism, but this was less than at pH 5.

It seemed likely, therefore, that the reason for the absence of effect, noted previously in certain constituents of carbohydrate metabolism, was due to insufficient penetration, by the active ionic species, of the muscle cell membrane. It is a well known principle of physical chemistry that in the dissociation of a univalent ion,

$$H_2A^- \rightleftharpoons HA^{--} + H^+,$$

any decrease in the concentration of the hydrogen ion in solution must involve an increase in the concentration of the divalent ion. This is true regardless of the proceeding or subsequent equilibria. The amount of undissociated di- or tri-carboxylic acid can be neglected at pH 7.4 and will be small at pH 5.

Several intermediates of fat and protein metabolism were tested for possible reserpine antagonism. The results have been presented in Table 7,2.

TABLE 7.2

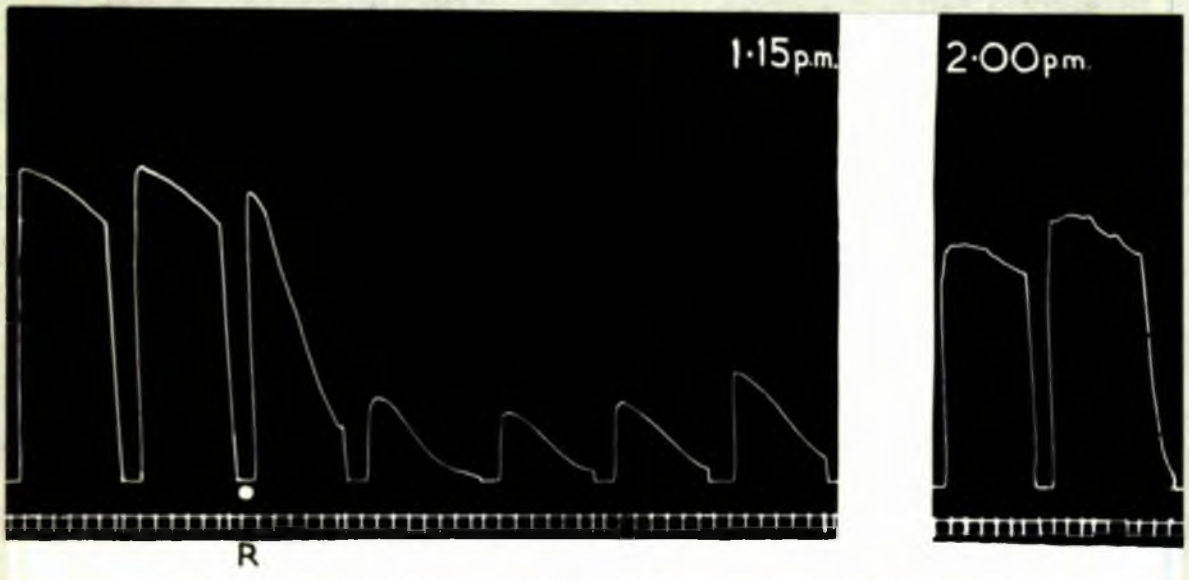
The effect of certain intermediates of fat and protein metabolism upon the depression by reserpine of acetylcholine-induced contractions of isolated guinea-pig ileum.

Metabolite	Percentage inhibition ± standard deviation.				Probability of difference between means (1)	pH of bath fluid after reserpine and metabolite.
	Reserpine alone.	Number of Additions	Reserpine and Metabolite.	Number of Additions.		
(±)-leucine	66.8 ± 19.8	22	54.1 ± 15.4	22	< 0.2, > 0.1*	5.25
(±)-α-alanine	63.4 ± 22.0	16	69.1 ± 18.6	16	< 0.5, > 0.4	7.35
(-)-glutamic acid	70.3 ± 17.1	16	62.4 ± 19.1	16	< 0.3, > 0.2	7.00
Sodium propionate	57.5 ± 18.5	18	64.8 ± 19.3	18	< 0.3, > 0.2	6.90
β-hydroxy-butyric acid	64.8 ± 16.3	16	59.4 ± 14.1	16	< 0.4, > 0.3	6.85

* Mean percentage inhibition following reserpine and (±)-leucine compared with mean value for reserpine and hydrochloric acid (48.4 ± 16.1, n = 40).

All others are comparisons of the percentage inhibition after reserpine with that following reserpine and the metabolite.

Figure 7.5



The decreased ability of intestinal smooth muscle to maintain drug-induced tone after reserpine.

All contractions produced by the addition to the bath of 0.3 µg. acetylcholine.

At R, 30 µg. reserpine added.

Time = 5 seconds.

It can be seen from Table 7,2 that none of the substances tested showed any significant (P = 0.05) reserpine antagonism. The only metabolite to reduce the reaction of the bath fluid below 6.85 was (±)-leucine, which is seen to be no more effective as a reserpine antagonist than hydrochloric acid alone (Table 7,2). The effects of 1 mg. of each of the substances tested in this group were not influenced by lowering the pH. The lack of activity in the non-carbohydrate intermediates tested supports the contention that the action of reserpine is related to energy production *via* carbohydrate metabolism.

A characteristic feature of the inhibition seen following reserpine was the inability of the preparation to maintain drug-induced tone. In Figure 7,5 is illustrated part of the record from an experiment in which this effect was recorded by increasing the speed of the kymograph. The addition of atropine in these experiments, which produced approximately the same degree of maximum inhibition of the contractions, did not cause any alteration of the ability to maintain acetylcholine-induced tone. *Cis*-aconitic acid anhydride and α-ketoglutaric acid prevented completely the inability to maintain tone after reserpine. The other active metabolites, namely 3-phospho-glyceric acid, citric acid, (-)-malic acid, oxaloacetic acid

and maleic acid possessed this activity but to a lesser degree. In three out of the eight tests carried out with hydrochloric acid, there was a similar antagonism to the action of reserpine on acetylcholine-induced tone.

B) The rectus-abdominis muscle of the frog.

The experiments described in this section were carried out during July and August.

No consistent effect upon the reserpine-induced contractions could be demonstrated with the following:- oxaloacetic acid, 200 µg., cis-aconitic acid anhydride, 1 mg., α-ketoglutaric acid, 200 µg., sodium pyruvate, 200 µg., maleic acid, 1 mg., β-hydroxybutyric acid, 200 µg., sodium fumarate, 1 mg., glucose-1-phosphate, 1 mg., fructose-1, 6-diphosphate, 1 mg.

200 µg. of α-ketoglutaric acid and 1 mg. of oxaloacetic acid or maleic acid caused the rectus muscle to contract.

C) /

C) The isolated auricles of the guinea-pig.

The metabolites which had been found to exhibit reserpine antagonism on the ileum of the guinea-pig were also tested for possible antagonism to the action of the alkaloid on the isolated auricles of this animal.

The results were extremely varied, but in general gave no evidence for or against any antagonism to the depressant effect of reserpine on this tissue. They were added to the bath to give final concentrations of from 100 µg. to 1 mg., 2 minutes before 1 µg. and 10 µg. reserpine. None of the metabolites tested (3-phosphoglyceric acid, citric acid, cis-aconitic acid anhydride, α-ketoglutaric acid, (-)-malic acid, oxaloacetic acid and maleic acid) showed any definite effect when added to the bath in solution at pH 7.4. In some experiments, the addition of α-ketoglutaric acid or cis-aconitic acid anhydride appeared to cause a decreased rate and amplitude of the beat. Subsequent addition of reserpine further reduced the rate and amplitude.

It was found impossible to add the metabolites as the free acids since this involved a reduction of the

bath fluid pH which adversely affected the activity of the auricles. The beat became most irregular and the action of reserpine and the metabolite could not therefore be compared confidently with the effects following addition of the alkaloid alone.

CHAPTER 7

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CHAPTER 8

CHAPTER 8

THE ACTION OF RESERPINE UPON THE METABOLIC ACTIVITY OF ISOLATED RABBIT DUODENUM.

As discussed previously (Chapter 6, page 98) it seemed possible that reserpine had an effect upon the metabolic processes which supplied energy for the contraction of muscle. If this were so, it seemed reasonable to expect that this activity would be reflected in a decreased ability of the muscle to utilize glucose. Under aerobic conditions, a decreased oxygen uptake might be expected, whilst a decreased production of lactic acid might be seen under anaerobic conditions. The action of reserpine on the oxygen uptake and glycolytic activity of the smooth muscle of isolated rabbit intestine was studied using the Warburg "Direct" Manometric technique[1].

Methods.

Preparations of duodenum and ileum from young rabbits weighing between 1.45 and 2.1 kg. were used. The animals were between 4 and 9 months old. The method of preparation was the same for both oxygen uptake and glycolysis determinations. Eight animals

were used for respiration and ten for glycolysis studies. Rabbits were killed by a sharp blow on the back of the neck. The throats were cut and the blood allowed to drain out. About 60 cm. of the small intestine proximal to the stomach was rapidly removed, placed immediately upon a dissecting board and moistened with the ice-cold saline appropriate to the experiment (see below). The segment was then opened along the line of the mesenteric attachment, stretched gently and, with the mucosa upwards, pinned at its four corners on the dissecting board. The mucosa was removed as completely as possible by scraping gently with a scalpel blade held at an acute angle to the longitudinal axis of the intestinal segment. When the preparation had a translucent appearance, indicating almost complete removal of the mucosa, two incisions were made about 2 mm. from and parallel to both outer edges of the segment. The remaining portion was cut into segments weighing approximately 100 mg. Each thin strip of muscle was placed in chilled saline and stored at $0^{\circ}C$ until use.

A modified Krebs-Henseleit solution (Appendix I) was used for oxygen consumption determinations. It was found that the addition of 12.5 mM glucose did not

influence the $Q_{O_2}^{O_2}$ values over the first, second or third hours of observation. This presumably indicated that the tissue had sufficient endogenous glucose to support aerobic metabolism, as measured in the experiment. Glucose was therefore omitted from the physiological solution in this series of determinations. The experiments were carried out in an atmosphere of oxygen. Carbon dioxide produced during the aerobic metabolism was removed by means of 0.2 ml. of a 20 per cent potassium hydroxide solution in the centre well of the Warburg flask. A small piece of fluted alkali resistant Whatman filter paper was seated in the centre well after the addition of the potassium hydroxide solution. This served to increase the surface area of the solution, thus ensuring an efficient removal of carbon dioxide from the atmosphere within the flask.

Krebs-bicarbonate solution[1] (Appendix I) was used for glycolysis determinations. 12.5 mM glucose was added to the solution in this series of experiments.

The production of acid under anaerobic conditions is usually spoken of as glycolysis[1]. In animal tissues, where the acid concerned is mainly lactic acid[1], this seems a fair assumption to make.

The metabolically produced lactic acid liberated carbon dioxide from the bicarbonate in solution; the volume of the gas so produced was measured manometrically and gave a measure of glycolysis. An atmosphere of 95 per cent nitrogen / 5 per cent carbon dioxide was used for these determinations. As soon as possible after preparation, the segments of intestine were dried gently between two sheets of filter paper, weighed on a torsion balance, and placed in the Warburg flasks. The solutions required for the measurement of either glycolysis or respiration had previously been pipetted into each flask. The reaction mixtures for both determinations are given in Tables 8,1(A) and 8,1(B).

TABLE 8.1(A)

The reaction mixture used for the determination of respiration in isolated rabbit intestine.

	Flask number	1	2	3	4	5	6	THERMOBAROMETER (containing 2 ml. water)	8	9	10	11	12	13	THERMOBAROMETER (containing 3 ml. water)
Modified Krebs-Henseleit solution (ml.)	a) in pot	2.2	2.2	2.2	2.2	2.2	2.2		2.2	2.2	2.2	2.2	2.2	2.2	
	b) in side arm	0.5	0.4	0.4	0.4	0.4	0.4		0.5	0.4	0.4	0.4	0.4	0.4	
20 per cent potassium hydroxide solution (ml.)	\	0.2	0.2	0.2	0.2	0.2	0.2		0.2	0.2	0.2	0.2	0.2	0.2	
Reserpine solution (ml.) giving a final flask concentration of,	a) 50 µg. per ml.	-	0.1	-	-	-	0.1		-	0.1	-	-	-	-	
	b) 10 µg. per ml.	-	-	0.1	-	-	-		-	-	0.1	-	-	-	
Control solution (ml.) equivalent to,	a) 50 µg. per ml. reserpine	-	-	-	0.1	-	-		-	-	-	0.1	-	0.1	
	b) 10 µg. per ml. reserpine	-	-	-	-	0.1	-		-	-	-	-	0.1	-	

TABLE 8.1(B)

The reaction mixture used for the determination of glycolysis in isolated rabbit intestine.

	Flask number	1	2	3	4	5	6	THERMOBAROMETER (containing 3 ml. water)	8	9	10	11	12	13	THERMOBAROMETER (containing 3 ml. water)
Krebs-bicarbonate solution (ml.)	a) in pot	2.4	2.4	2.4	2.4	2.4	2.4		2.4	2.4	2.4	2.4	2.4	2.4	
	b) in side arm	0.5	0.4	0.4	0.4	0.4	0.5		0.5	0.4	0.4	0.4	0.4	0.5	
Reserpine solution (ml.) giving a final flask concentration of,	a) 50 µg. per ml.	-	0.1	-	-	-	-		-	-	-	0.1	-	-	
	b) 10 µg. per ml.	-	-	0.1	-	-	-		-	-	0.1	-	-	-	
Control solution (ml.) of tartaric acid equivalent to,	a) 50 µg. per ml.	-	-	-	-	0.1	-		-	-	-	-	0.1	-	
	b) 10 µg. per ml. reserpine	-	-	-	0.1	-	-		-	-	-	-	-	0.1	

Reserpine was dissolved in a 5 per cent aqueous solution of tartaric acid. It was added from the side arm to give a final flask concentration of 10 µg. and 50 µg. per ml. of physiological saline. Reserpine was added to the side arm in a volume of 0.1 ml. (see Tables 8,1(A) and 8,1(B) above). A tartaric acid control solution (Appendix I) equivalent to 10 µg. and 50 µg. per ml. of reserpine, was tested in each experiment. The addition of saline/reserpine or saline/control mixtures from the side arm would normally have resulted in a slight change of the final salt concentration in the main compartment of the flasks. This could conceivably have altered the metabolic activity of the tissue. Therefore, when either reserpine or tartaric acid control solutions were added from the side arms, the composition of the physiological saline in the side arm was adjusted to avoid changing the final salt concentrations in the reaction compartment of the flasks.

The total volume of the reaction mixture and approximately 100 mg. of tissue was taken to be 3 ml. The flask constants were therefore calculated using this value. The manometer fluid used was that described by Krebs[2].

Two thermobarometers, each containing 3 ml. of water, were incorporated in each experiment.

Summary of results.

50 µg. per ml. reserpine reduced significantly the oxygen uptake of the isolated duodenum. At the same concentration the tartaric acid control solution had no effect. 10 µg. per ml. reserpine did not affect respiration.

At concentrations of 50 µg. and 10 µg. per ml., reserpine had no significant effect upon glycolysis in isolated rabbit duodenum.

RESULTS.

The results of the series of experiments on respiration are given in Table 8,2.

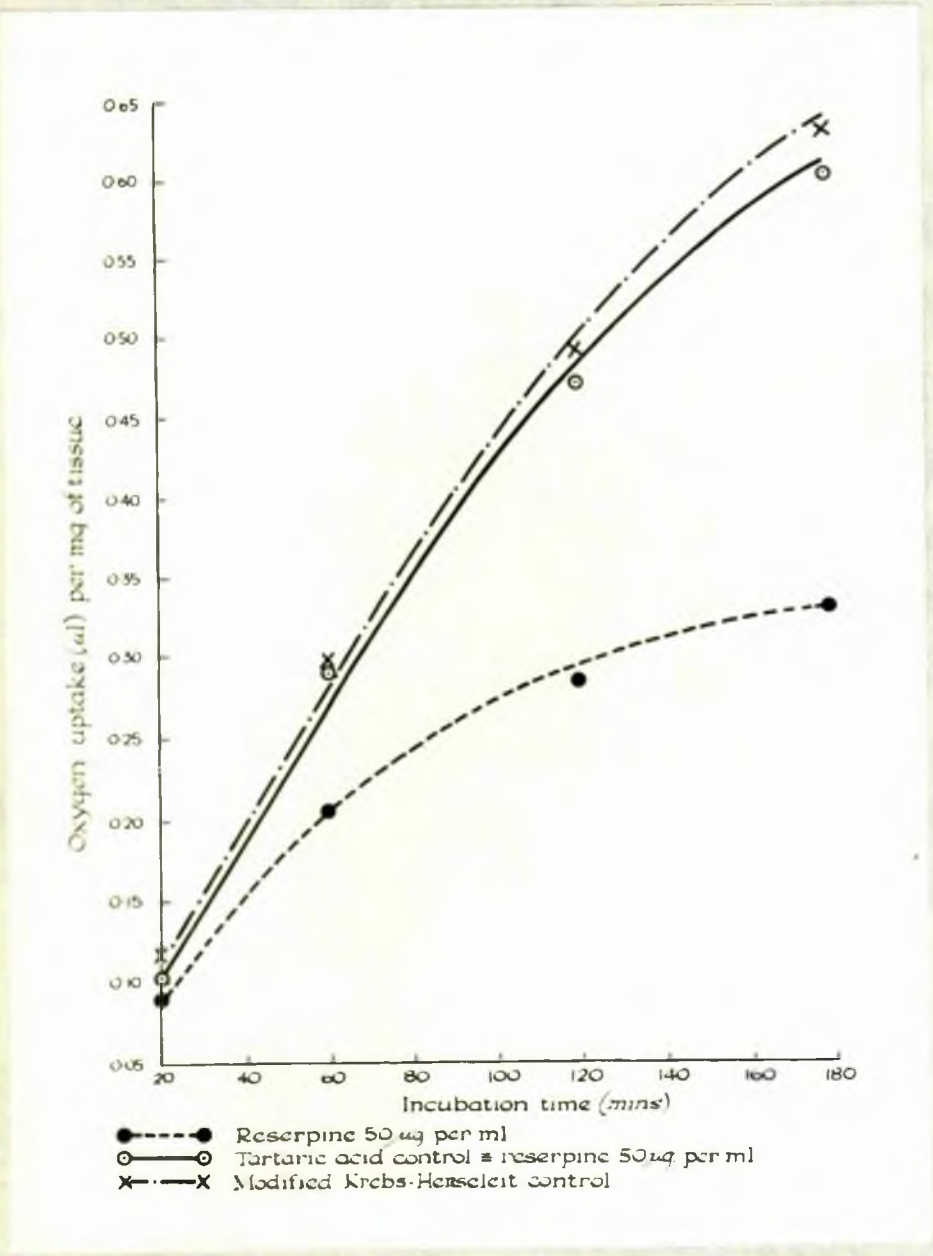
TABLE 8.2

The effect of reserpine upon respiration in isolated rabbit intestinal muscle.

Oxygen uptake (µl. ± Standard Deviation) per mg. of tissue.

Incubation time (minutes)	20	60	120	180
Modified Krebs-Henseleit solution.	0.116 ± 0.062	0.296 ± 0.114	0.491 ± 0.145	0.629 ± 0.219
Reserpine 50 µg. per ml.	0.090 ± 0.021	0.206 ± 0.086	0.283 ± 0.129	0.330 ± 0.143
Reserpine 10 µg. per ml.	0.114 ± 0.055	0.264 ± 0.140	0.460 ± 0.278	0.550 ± 0.291
Tartaric acid solution equivalent to, a) 50 µg. per ml. reserpine	0.105 ± 0.004	0.292 ± 0.108	0.469 ± 0.158	0.600 ± 0.231
b) 10 µg. per ml. reserpine	0.097 ± 0.045	0.306 ± 0.120	0.511 ± 0.217	0.618 ± 0.218

Figure 8.1



The effect of reserpine (50 µg. per ml.) and tartaric acid control solution upon the normal respiration of isolated rabbit intestinal muscle.

For the sake of clarity the curves for reserpine (10 µg. per ml.) and the corresponding tartaric acid control solution have been omitted.

Using a Student's "t" test, the mean values for oxygen uptake in the presence of 10 µg. and 50 µg. per ml. reserpine and the equivalent tartaric acid solutions were compared with the corresponding values for modified Krebs-Henseleit solution. The analyses showed that reserpine significantly (P = 0.05) depressed respiration only at a concentration of 50 µg. per ml. At this dose level, the value after 20 minutes incubation was not significantly different from that of the Krebs-Henseleit solution. It was concluded, therefore, that the depression caused by reserpine was slight and appeared only after a considerable latent period (longer than 20 minutes). The depression of respiration produced by reserpine is shown graphically in Figure 8,1.

10 µg. per ml. reserpine and the control solutions of tartaric acid equivalent to 50 µg. and 10 µg. per ml. reserpine had no significant (P = 0.05) effect upon respiration.

During preliminary experiments in the series measuring glycolysis, tests were made of the effect of glucose. This was prompted by the finding that the addition of glucose during observations of respiration

did not seem to influence the uptake of oxygen over the period of observation. Experiments were carried out in which 12.5 mM glucose was added to one half of the flasks in the experiment. The others did not contain glucose. The mean volumes of carbon dioxide released in both groups (Table 8,3) were compared by means of a Student's "t" test. The presence of glucose was found to increase significantly ($P = 0.01$) the production of carbon dioxide. In all tests with reserpine, therefore, 12.5 mM glucose was added to the reaction mixture.

Reserpine (10 µg. or 50 µg. per ml.) had no significant effect ($P = 0.05$) upon the glycolytic activity of rabbit intestinal muscle.

TABLE 8.3

The effect of reserpine upon glycolysis in isolated rabbit intestinal muscle.

Volume of carbon dioxide produced (µl. ± Standard Deviation) per mg. of tissue

Incubation time (minutes)	60	120	180
Krebs-bicarbonate solution with 12.5 mM glucose	0.177 ± 0.056	0.217 ± 0.070	0.275 ± 0.068
Krebs-bicarbonate solution without glucose	0.118 ± 0.051	0.149 ± 0.061	0.217 ± 0.082
Reserpine 50 µg. per ml.	0.152 ± 0.051	0.212 ± 0.064	0.261 ± 0.077
Reserpine 10 µg. per ml.	0.146 ± 0.054	0.199 ± 0.147	0.256 ± 0.064
Tartaric acid control solution equivalent to, a) 50 µg. per ml. reserpine	0.159 ± 0.070	0.204 ± 0.090	0.273 ± 0.070
b) 10 µg. per ml. reserpine	0.156 ± 0.068	0.184 ± 0.050	0.220 ± 0.036

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CHAPTER 9

CHAPTER 9

THE INFLUENCE OF RESERPINE UPON THE EXTRACELLULAR POTASSIUM CONCENTRATION OF ISOLATED FROG SKELETAL AND ISOLATED RABBIT INTESTINAL MUSCLE.

Evidence has been presented (Chapter 7, page 105) that reserpine may interfere with carbohydrate metabolism in isolated intestinal muscle. Similar considerations may also apply to frog skeletal muscle. It is well known that the potassium ion participates in the activation of certain important enzyme systems taking part in carbohydrate metabolism[1]. Alteration of normal metabolic activity by reserpine might therefore have been associated with a change in the intra- and extracellular potassium relationships in muscle. Isolated frog rectus-abdominis muscle was found to contract following the addition of reserpine (Chapter 2, page 20). The contraction was delayed in onset and prolonged and might well have been associated with some change in the ionic balance of the muscle cells.

For the reasons outlined above, an attempt was made in the work described in this chapter to demonstrate, qualitatively, an increased potassium concentration in the fluid bathing isolated frog skeletal and rabbit

intestinal muscle following the administration of reserpine.

Methods.

a) Frog muscle.

The sartorius muscle was used in most of the work; some experiments were also carried out with the gastrocnemius muscle.

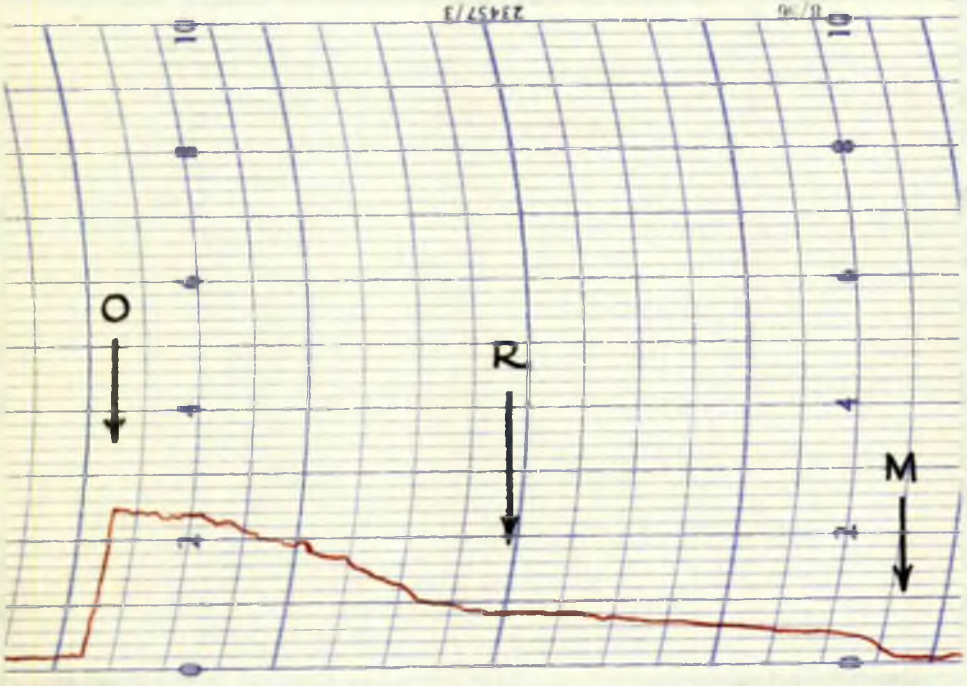
The determinations were made using ^{42}K. The radioactive material was obtained as a 1.15 per cent w/v solution of ^{42}KCl from the Atomic Energy Research Establishment, Harwell. Frogs received 1 ml. of this solution by injection into the dorsal lymph sac. After a two hour equilibration period, the frogs were decapitated and pithed and both sartorius or both gastrocnemius muscles were removed. One muscle served as a test object for reserpine activity, the other being used to observe the action of the control solution.

In a preliminary series of experiments, the muscles were suspended in test tubes containing 10 ml. of frog Ringer's solution (Appendix I), through which oxygen

was bubbled. The two series of tubes contained either 50 μg. per ml. reserpine or an equivalent volume of control solution (Appendix I). Nine tubes were used for reserpine and control testing. The muscles were stretched vertically by fixing to a support at the top of the test tubes and suspending a 2 g. weight at their lower ends. At twenty minute intervals, each muscle was removed to the next test tube in its series. After removing the muscle, the ^{42}K in the bathing fluid was measured by means of a Geiger-Muller liquid counter (type M6). The results, in counts per minute, were corrected for background and lost counts. Allowance was made for decay and the final value for the activity, so corrected, was converted to parts per million of ^{42}K. By referring all values back to the time of receiving the sample in the laboratory, the results of experiments at different times could be compared.

In another method, which was used for most of this work, the bathing fluid was circulated continuously from a small organ bath (which held the muscle) by means of a pump to an M6 flow counter and thence back to the bath (Figure 9,2). The pump tended to warm slightly the circulating fluid, which was therefore passed through

Figure 9.1



Part of a typical record obtained using a "Labgear" recording ratemeter.

Sartorius muscle of the frog. Integrated record of the ^{42}K content of the bathing fluid in counts per second.

At M, the muscle was placed in the bath and removed at O, after which the system was flushed with inactive Frog Ringer's solution.

At R, 50 µg. per ml. reserpine added.

The squared paper moved at 2" per hour. Full scale deflection of galvonometer = 100 counts per second. Time constant = 10" and paralysis time = 300 µs.

Figure 9.2

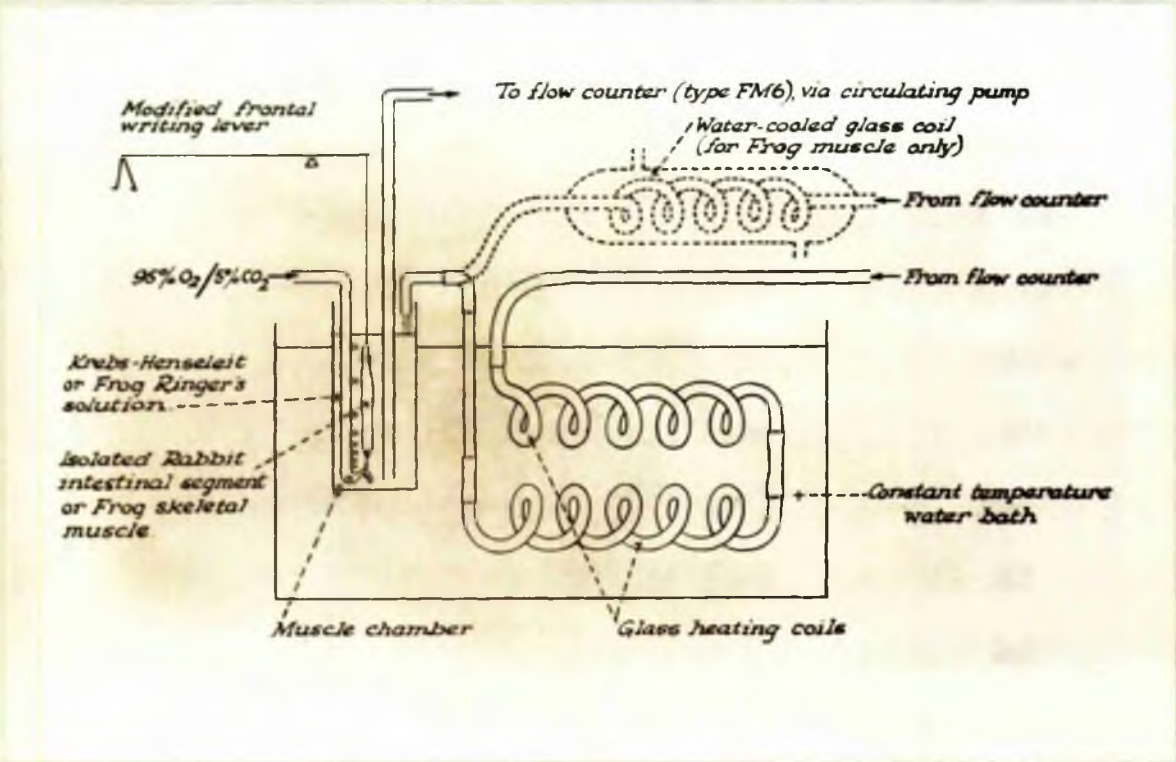


Diagram of the apparatus used for continuous measurement of radioactivity in the fluid bathing rabbit intestinal segments or frog sartorius and gastrocnemius muscles. When frog tissue was used, a cooling coil (shown by means of a broken line) was inserted in the system. In this case the constant temperature water bath was dispensed with and only oxygen was bubbled through the solution in the muscle chamber.

a glass cooling coil before returning to the bath. The temperature of the fluid in all experiments was between 16° and 18°C. The flow counter was completely encased in a lead castle. Under these circumstances, the background activity was very low. A continuous, integrated record of the ^{42}K in the bathing fluid ($^{42}K_b$) was obtained with a "Labgear" type recording count ratemeter. A typical record obtained with this experimental arrangement is shown in Figure 9,1. Any drug-induced increase in $^{42}K_b$ was recorded as an increase in the gradient of the ratemeter tracing. The tone of the muscle was recorded simultaneously with a modified frontal point writing lever which gave a magnification of 1 in 6. The total volume of the circulating fluid was 110 ml. and doses of reserpine and control solution were calculated on this basis.

This method had the advantage that the tissue was maintained in a constant environment throughout the experiment.

b) Rabbit muscle./

b) Rabbit muscle.

Segments of rabbit duodenum about 5 cm. in length were removed and placed in a solution of Krebs-Henseleit solution at 37°C, containing ^{42}KCl instead of the potassium chloride normally used. Born and Bülbring reported[2] that the taenia coli muscle of guinea-pigs took up ^{42}K from a radioactive solution rapidly at first but the rate decreased gradually until the total taken up had reached a maximum in 3 hours. The same period of exposure was therefore used for rabbit intestine. Born and Bülbring also found that a 5 minute wash with inactive solution before measuring radioactivity reduced the high initial count usually seen. They suggested this was due to extracellular ^{42}K and that it bore no relationship to the movement of potassium across the cell membrane. Accordingly, the muscle segments in this work were washed with normal Krebs-Henseleit solution before being set up in the recording apparatus.

The apparatus was similar, basically, to the continuous circulation apparatus used for frog muscle and described above. The muscle chamber was placed in a constant temperature water bath. Krebs-Henseleit

solution, flowing into the muscle chamber, passed through two glass heating coils immersed in the water bath containing water at 37°C. The pH of the bathing fluid after the addition of reserpine or of the control solutions in experiments with both frog and rabbit muscle was between 7.0 and 7.4.

Summary of results.

Reserpine in doses of 20 µg. and 50 µg. per ml., in all experiments, caused a marked increase in the $^{42}K_b$ when frog muscle was used. A slow increase in the tone of the muscle accompanied this phenomenon. The contraction produced by reserpine was preceded by a latent period of between 4 and 11 minutes after its addition. The increased tone was maintained only so long as reserpine remained in contact with the muscle. The same delay occurred in the appearance of the increased $^{42}K_b$. The length of the delay before the appearance of the contraction was markedly increased by 25 µg. per ml. tubocurarine, being in one case 22 minutes. On the other hand, it was reduced or abolished by 20 µg. per ml. decamethonium. The magnitude of the contraction was unaffected by both drugs. Neither agent affected the

increased $^{42}K_b$ following the addition of reserpine. The appearance of an increased $^{42}K_b$, however, was either delayed or hastened to the same extent as was the contraction of the muscle. Neither drug had any effect upon an established contraction following reserpine.

0.2 mM. dinitrophenol did not produce an increased $^{42}K_b$ when ordinary frog Ringer's solution was used. This phenomenon could only be demonstrated when potassium free Ringer's solution was used.

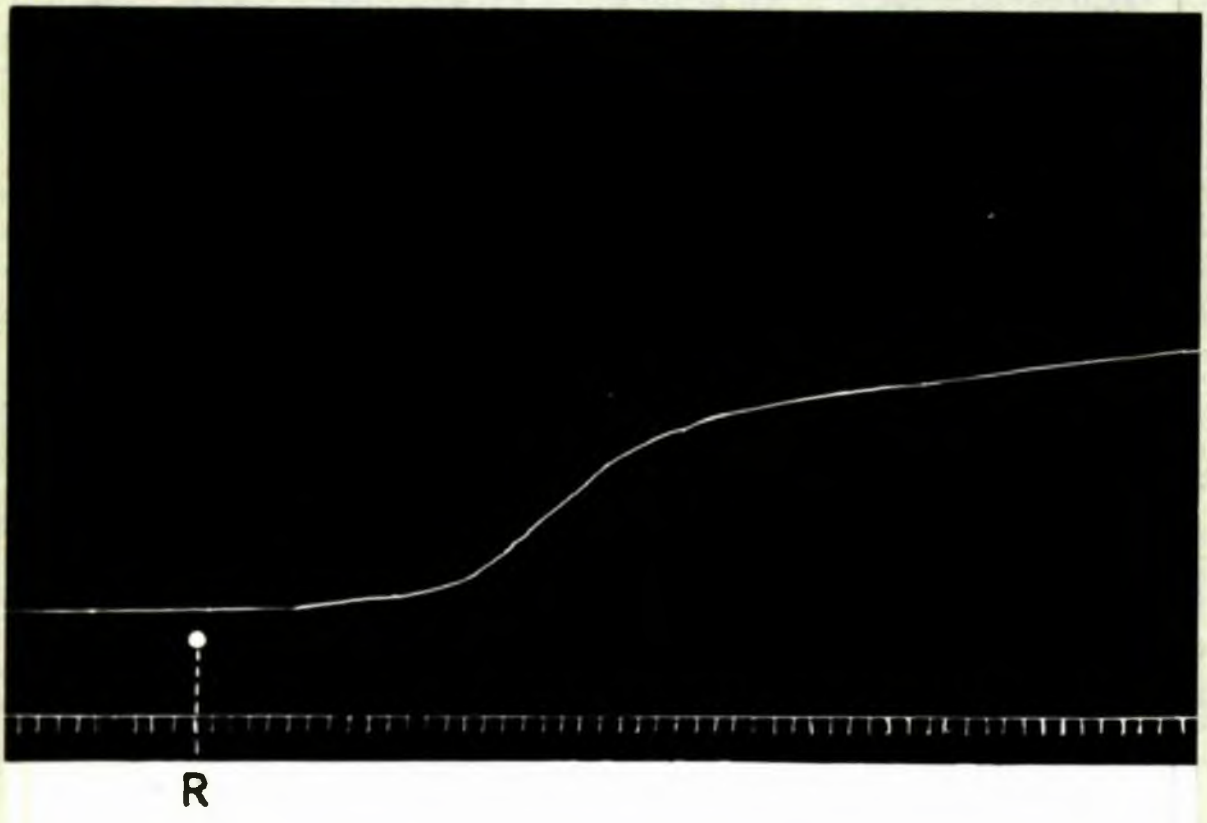
The frog gastrocnemius muscle contracted following the addition to the bath of 20 µg. or 50 µg. per ml. reserpine. The contraction was accompanied by an increased $^{42}K_b$.

No increase in the $^{42}K_b$ could be demonstrated with rabbit intestinal segments.

RESULTS.

Reserpine, in doses of 20 µg. or 50 µg. per ml., caused a contraction of the isolated frog sartorius muscle

Figure 9.3



The stimulant action of reserpine upon the frog sartorius muscle

At R, reserpine added to give a final bath concentration of 50 µg. per ml.

Time = 60 seconds.

(Figure 9,3). The contraction was slow and was preceded by a latent period which was usually about 6 minutes but, in some experiments, was as long as 10 or 11 minutes. Doses below 20 μg. per ml. did not consistently have any direct stimulant action on the muscle. The increased tone was maintained for the duration of the experiment when the continuous circulation of bathing fluid was employed. This procedure had the disadvantage that reserpine could not be washed out without decreasing, almost to zero, the record of $^{42}K_b$ obtained on the ratemeter. In three experiments, however, the bathing fluid was replaced when the reserpine contractions had reached a maximum. The tone of the muscle fell to its original value when this was done. The control solution produced a slight contraction in two of ten experiments carried out. In these cases, there was no increase in the gradient of the ratemeter recording.

An increase in the $^{42}K_b$ was associated with the stimulant action of reserpine (Figure 9,1, page 134). This showed approximately the same delay in appearance as did the contraction following reserpine.

Although the experiments gave only a qualitative estimate of the movement of the potassium ion, it was

felt that some graphic representation should be produced. This was complicated by the fact that the length of time between setting up the preparation and adding reserpine to the muscle chamber varied slightly from experiment to experiment. In all experiments, reserpine was added between the twentieth and fortieth minute after placing the muscle in the bath. The following procedure was therefore used. The $^{42}K_b$ at a point 20 minutes before the addition of reserpine was calculated in parts per million of potassium and was subtracted from the values at points 15, 25, 30, 45, 60 and 75 minutes after this. Thus a series of values were obtained from time = 0 minutes to time = 75 minutes with the addition of reserpine at time = 20 minutes. Using this procedure, values of $^{42}K_b$ were obtained for the 75 minute period following the addition of 50 µg. per ml. of reserpine or the corresponding volume of control solution. These are given in Table 9,1.

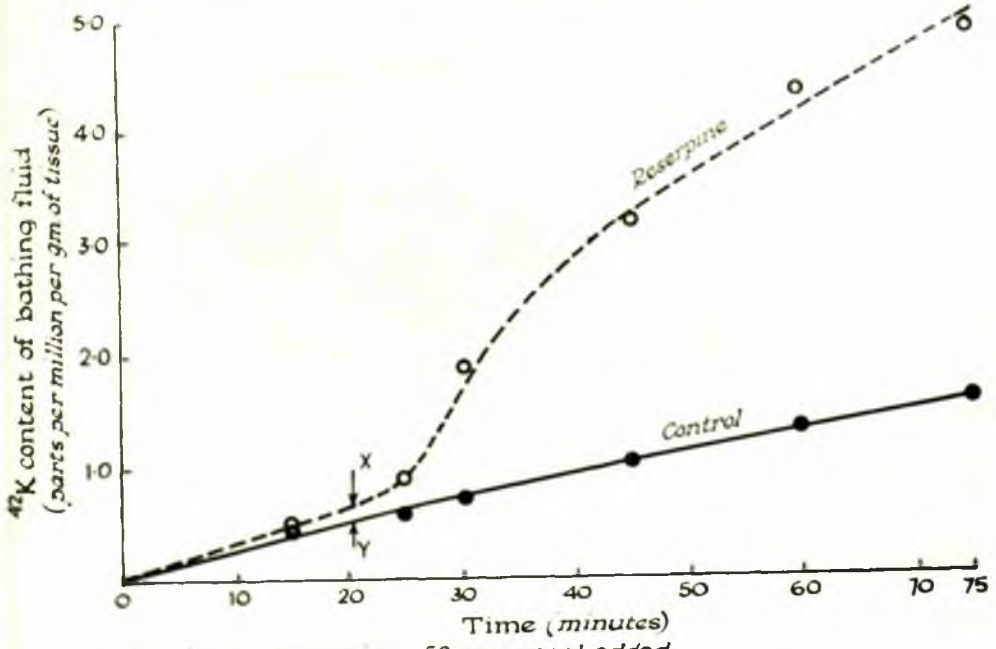
TABLE 9.1

^{42}K content of fluid bathing frog sartorius muscle when reserpine or control solution was added.

All values are in parts per million and were calculated from tracings obtained with a "Labgear" recording count ratemeter.

	Reserpine 50 µg. per ml.						Control=reserpine 50 µg. per ml.					
Time in minutes	15	25	30	45	60	75	15	25	30	45	60	75
Values obtained in individual experiments	0.55	1.58	3.29	4.59	5.97	6.48	0.40	0.55	0.64	1.01	1.60	2.10
	0.89	1.23	2.38	3.92	5.99	6.69	0.36	0.38	0.40	0.48	0.57	0.72
	0.32	0.86	1.23	2.49	3.89	4.40	0.62	0.73	0.97	1.52	1.99	2.40
	0.19	0.32	0.61	1.80	2.32	2.75	0.55	0.70	0.94	1.03	1.52	1.89
	0.20	0.56	1.49	2.09	2.47	2.74	0.18	0.19	0.21	0.41	0.54	0.65
	0.87	1.16	3.69	6.67	7.71	8.17	0.15	0.20	0.24	0.34	0.67	0.73
	0.42	0.67	0.90	2.02	2.68	3.38	0.30	0.40	0.50	0.96	1.19	1.51
	0.48	1.02	1.75	3.31	4.57	5.72	0.45	0.71	0.86	1.10	1.46	1.72
	0.46	0.73	0.98	1.36	2.89	3.55	0.67	1.13	1.30	1.79	1.80	1.98
	-	-	-	-	-	-	0.38	0.87	0.93	1.38	1.57	1.78
Mean value	0.49	0.90	1.81	3.14	4.28	4.88	0.41	0.59	0.70	1.00	1.29	1.55
± standard deviation.	± 0.25	± 0.39	± 1.09	± 1.69	± 1.92	±1.96	± 0.17	± 0.30	± 0.36	± 0.48	± 0.53	± 0.71

Figure 9.4



At point X, reserpine, 50 µg. per ml. added.
At point Y, control solution ≡ reserpine 50 µg. per ml. added.

The effect of reserpine upon the ^{42}K concentration of the fluid bathing isolated frog sartorius muscle.

Figure 9,4 shows graphically the effect of 50 µg. per ml. of reserpine on the $^{42}K_b$. The lines were constructed using the mean values shown in Table 9,1.

The contraction of frog muscle, following the addition of reserpine, was maintained only so long as reserpine was present in the system (page 138). It seemed important, therefore, to determine whether the increased $^{42}K_b$ also required the presence of reserpine for its maintenance. For the reasons mentioned above (page 138) the ratemeter was unsuitable for this work. Two series of 16 test tubes were therefore used, each containing 10 ml. of oxygenated frog Ringer's solution. The sartorius muscles were moved from one tube to the next at 20 minute intervals and the activity of the bathing fluid measured as before (page 132). Tubes 3, 4 and 5 contained 50 µg. per ml. reserpine. The same concentration of reserpine was contained in tubes 11, 12 and 13. The corresponding tubes of the other series contained the control solution equivalent to 50 µg. per ml. of reserpine.

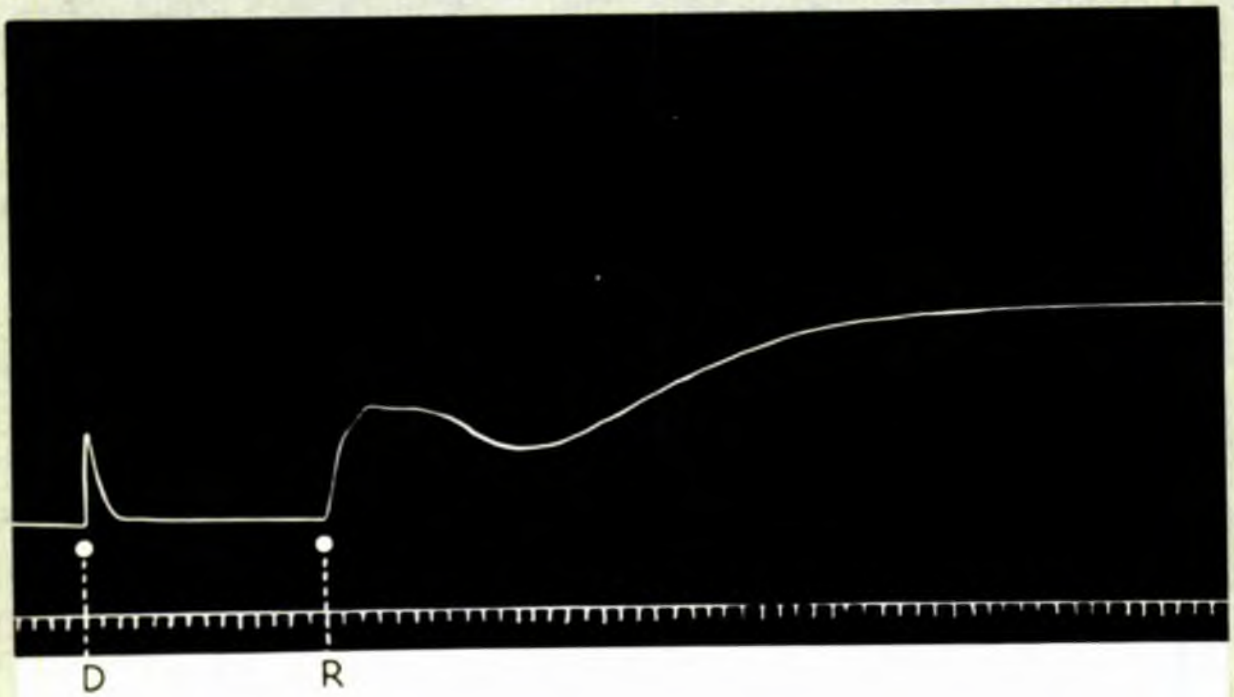
It was found that the increased $^{42}K_b$ following reserpine tended to fall to control values in those tubes

which did not contain the drug. Moving the muscle from one tube to another must markedly have altered the potassium balance of the muscle cells: since, however, control tests showed no increased $^{42}K_b$, it was concluded that the latter effect was in fact caused by reserpine.

It seemed important to show that a substance known to release potassium from muscle would in fact cause an increased $^{42}K_b$ in the apparatus used. Such a substance is 2:4 dinitrophenol (DNP)[3]. No increase could be demonstrated when ordinary frog solution containing 0.2 mM. DNP was used. When the bathing fluid used was potassium free frog Ringer's solution, the $^{42}K_b$ was increased in the presence of 0.2 mM. DNP. This was observed as a steeper gradient of the ratemeter record, when compared with the normal control tracings.

The addition, 15 minutes before reserpine, of 25 µg. per ml. tubocurarine consistently increased the latent period before contraction of the muscle. It did not appear to block the increased $^{42}K_b$ which also appeared after an increased latent period. 20 µg. per ml. of decamethonium, however, usually completely abolished the latent period and frequently caused a

Figure 9.5



The action of reserpine upon the isolated frog sartorius muscle in the presence of decamethonium bromide.

At D, 20 µg. per ml. decamethonium bromide added.

At R, 50 µg. per ml. reserpine added.

Time = 60 seconds.

sharp twitch-like response after its addition. In some cases a biphasic response followed the subsequent addition of reserpine (Figure 9,5). Decamethonium (20 μg. per ml.) did not affect the increased $^{42}K_b$ following reserpine.

Neither tubocurarine nor decamethonium altered the magnitude of the reserpine-induced contraction and had no effect upon an established contraction.

The gastrocnemius muscle of the frog was used in only three experiments. A contraction which had similar characteristics to that seen with the sartorius muscle was produced by 50 μg. per ml. reserpine in each case. The $^{42}K_b$ was increased, but in all cases, the magnitude of the increase seemed smaller than with the sartorius muscle.

No increased $^{42}K_b$ was observed in four experiments with rabbit intestinal segments.

CHAPTER 9

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CHAPTER 10

CHAPTER 10

DISCUSSION.

Reserpine has been shown to depress both spontaneous and drug-induced activity in isolated skeletal, visceral and cardiac muscle. In some cases, a direct stimulant action precedes the depression.

In a preliminary discussion (Chapter 6, page 98) it was suggested that reserpine might interfere with the energy production of isolated muscle. Many of the observed effects can be explained on this basis. A more detailed analysis of this suggestion must now be made.

Szent-Gyorgyi[1] described the contraction of muscle as a reaction of actomyosin, adenosine triphosphate (ATP) and certain inorganic ions. This process utilizes endogenously produced energy. The final source of this energy is the terminal high energy phosphate bond of ATP which is produced by the oxidative reactions of carbohydrate metabolism[1]. Whilst more work on these relationships has been done using skeletal muscle, the same considerations probably apply to the

chemistry of smooth muscle[2]. The enzymes which control the larger part of tissue oxidation form an integral part of the Krebs tricarboxylic acid cycle[3], which is operative in rabbit intestinal smooth muscle[4].

The evidence presented in Chapter 7 (page 105) points to some relationship between reserpine and carbohydrate metabolism in the smooth muscle of guinea-pig ileum. Under suitable conditions of bath pH, most intermediates of carbohydrate metabolism antagonised the depression by reserpine of drug-induced contractions, whilst intermediates of fat and protein metabolism were ineffective. This work also confirms the observations of Furchgott and Wales[4] that the cell membrane of the smooth muscle is more permeable to the singly ionized than to the completely ionized forms of dicarboxylic acids. The large doses of the acid carbohydrate intermediates, which were used in this work, caused the production of a relatively small concentration of the singly ionized forms in solution. It is assumed that only these could penetrate the cell membrane in sufficient concentration to produce antagonism to reserpine. The fact that ATP itself and the phosphorylated glucose and fructose compounds were ineffective

as reserpine antagonists may be a reflection of the inability of phosphorylated compounds to penetrate the cell membrane as such[5,6].

Anoxia has been shown to cause either a reduction in, or complete inhibition of, spontaneous tone and activity in isolated rabbit intestinal muscle[5] and the taenia coli muscle of the guinea-pig[7]. At the same time the ability of the tissues to maintain electrically[7] or drug-induced tone[5] was completely abolished. These effects were accompanied by a decreased ATP content in the taenia coli muscle[7]. Anoxia has also been shown to cause a reduction in the ATP content of intestinal muscle[8]. The effects of reserpine upon spontaneous and drug-induced tone were similar in many respects to those of experimentally produced anoxia. It is possible, therefore, that in effect, reserpine renders intestinal smooth muscle anoxic, by virtue of its ability to interfere with the energy-producing reactions of carbohydrate metabolism.

The spontaneous activity of isolated smooth muscle must require the steady production of energy from some readily available source. The addition of a stimulant drug can be considered to cause an increase

in the production of energy. Under aerobic conditions the metabolism can cope with the extra requirement, but anoxia inactivates the more efficient[9] energy source, namely oxidative metabolism. West and his colleagues[5] considered anaerobic energy to be only a small fraction of that available during aerobic activity. As a result of the loss of its most efficient energy-producing reaction, the total available energy is depressed during anoxia or, as is postulated, following reserpine. It has been shown[10] that 2:4, dinitrophenol (DNP) which decreased oxygen uptake and the production of ATP[11], decreased the ability of isolated guinea-pig ileum to maintain histamine-induced contractions. The immediate component of the contraction (spike phase) was unaffected. Born extended this work and suggested[7] that two mechanisms provided energy for the response to histamine;

a) that responsible for the immediate response to drugs or electrical stimuli, which was unaffected by DNP, and,

b) that responsible for both the sustained contraction and spontaneous activity, which was presumably the oxidative mechanism since it was abolished by anoxia or DNP.

Thus the energy for muscular contraction is probably derived from aerobic and anaerobic processes, the former contributing the larger amount of energy. Reserpine may depress more effectively the aerobic oxidative mechanism than the anaerobic reactions. It has, in fact, been observed that both spontaneous movements and the ability of guinea-pig ileum to maintain drug-induced tone were very markedly affected by reserpine. According to Born[7] both types of activity are supported largely by oxidative metabolism. The reserpine antagonists reduced the actions of reserpine upon both functions of the muscle. The inability of the drug to inhibit completely the drug-induced contractions of gut may reflect the existence of two systems for the production of energy; it can be envisaged that the contraction at the point of maximum inhibition may be almost entirely supported by the less affected anaerobic mechanism. The degree of inhibition produced by reserpine would therefore be inversely related to the dependence of the muscle upon anaerobic energy. Alternatively, it may be that reserpine does not completely inhibit oxidative energy production.

The "anoxia like" condition which is apparently produced by reserpine is not incompatible with the observations on gut and uterus. The latency in the appearance of the maximum inhibition of drug-induced contractions can be explained by means of this hypothesis. When the production of ATP is temporarily interfered with, the small store of ATP known to be present in smooth muscle[9,10] would presumably be expended rapidly. Until it is exhausted, however, one would expect to see a gradual reduction in the height of the response to drugs. When this stage is reached, such contraction as occurred would perhaps be supported largely by the energy produced by anaerobic means. It is interesting to note that the adverse effects of anoxia upon muscle tone also develop slowly[5,7].

The increased effect of reserpine upon drug-induced contractions, which is seen with the passage of time, may be due to fatigue of the mechanism responsible for the production of ATP.

The wide variation in the action of reserpine from tissue to tissue is not unexpected since the degree of dependance of intestinal muscle upon oxidative mechanisms must vary widely. It is perhaps significant

that the reserpine antagonists showed a greater effect in those tissues in which the drug itself had the greatest antagonistic effect to drug-induced contractions. In those cases, aerobic mechanisms may have contributed considerably more than anaerobic ones to the production of energy for contraction. It is possible that the effective reserpine antagonists enable the oxidative energy-producing mechanism of the tissue to overcome a block produced by reserpine.

The first addition to the bath of reserpine rarely produced a marked reduction in the drug-induced contractions of the rat uterus. It is possible that the initial store of ATP in this preparation is high, whilst the action of reserpine may be partially irreversible. It is possible to explain on this basis the observed fact that the second addition of reserpine produced a greater degree of inhibition than the first.

The results of experiments with reserpine which were carried out on the spinal cat may also be explained on the basis of a direct peripheral mode of action. The depressor action of reserpine upon the arterial blood pressure level of this preparation was seen only when young animals were used. That this

action was related to existing tone was supported by the finding that histamine failed to produce any depressor response when the reserpine-induced hypotension was maximal. Vasodilatation is seen following histamine only if some vascular tone exists[12,13]. The mechanism responsible for maintaining basal tone in blood vessels is not fully understood. Certainly, in the spinal cat, central influences may be discounted, but the possibility of some spinal regulating mechanism still exists. Yet in pithed animals, in which this was no longer possible, reserpine still reduced any tone present. It has been suggested that basal vascular tone is a result of specific constrictor agents circulating in the blood[14]. Granaat[15] has suggested that such agents may be released from the spleen. In addition, it seems to be widely assumed that the suprarenal medulla makes a considerable contribution to the maintenance of basal vascular tone.

Lofving and Mellander[16] could find no evidence for a circulating vasoconstrictor agent in cats which had been sympathectomised. They suggested[16] that basal tone was the result of "smooth muscle automaticity",

which they thought was probably to be regarded as a characteristic property both of heart muscle and many types of smooth muscle cells. Whatever the cause of basal tone, it seems clear that it must involve, to a greater or lesser degree, contraction of vascular smooth muecle and this in turn involves an increased energy turnover. Thus if reserpine affects the energy production of the muscle cell, then a reduction of inherent tone would be expected. One manifestation of this would be the observed fall in blood pressure. It is perhaps significant that reserpine had a depressor action only when some basal tone (as indicated by the depressor action of histamine) existed. In older cats, in which basal tone was low, or completely absent, the alkaloid had no effect. Bein stated[17] that "as a rule in man and experimental animals, the higher the level prior to the administration of reserpine, the greater is the subsequent reduction in blood pressure". It must be pointed out that many other considerations are, of course, involved in the analysis of the action of reserpine in intact experimental animals and in man. Nevertheless, a contribution of direct peripheral effects to the depression of blood pressure caused by reserpine cannot be ruled out.

The pressor response in the spinal cat to adrenaline and nor-adrenaline must also involve an increased production of energy, thereby placing an added "strain" upon the energy-yielding reactions. It would therefore be expected that the processes involved would be sensitive to reserpine. This was seen experimentally as a gradual reduction in the height of the pressor response to both drugs. Once again, the effect was particularly evident in younger animals. It may be that in older animals vascular smooth muscle is dependent to a greater degree than it is in younger animals upon anaerobic mechanisms for its energy supply. As outlined earlier, anaerobic metabolism may be much less affected by reserpine than oxidative metabolism.

The delay which is caused by posterior pituitary extract in the onset of the reserpine effects on the blood pressure of the spinal cat is of interest. It is widely held that posterior pituitary extract stimulates smooth muscle cells "directly". This presumably indicates that the drug ultimately increases in some way the energy available for muscular contraction. It may be that stimulation of the vascular smooth muscle

of the spinal cat is due to increased production of ATP. The effects of reserpine on the muscle may then be delayed until the elevated ATP concentration falls below some critical value, when both the reserpine-induced hypotensive effect and depression of the responses to adrenaline and nor-adrenaline are seen.

It follows from the arguments presented above that in the isolated, denervated hind-quarters of the rat and the rabbit's ear, which are assumed to be toneless, reserpine would be expected to have no dilator effect. This has been demonstrated and confirms the observations, made on other preparations, of Tripod and Meier[18]. The vasoconstrictor actions of adrenaline, nor-adrenaline and histamine would similarly be depressed by reserpine because of the decreased ability of the cellular metabolism to produce sufficient energy to support a drug-induced contraction of the muscle. On the other hand, reserpine has also been shown to depress either neurogenic or drug-induced vascular tone in isolated systems. McQueen and his colleagues[19] have found that reserpine caused vaso-dilatation (which clearly involved some reduction of basal vascular tone) in the innervated hind-quarters of

the rabbit. Increased vascular tone in the isolated hind-quarters of the rat, produced by the infusion of either nor-adrenaline, pitressin or barium chloride, was antagonised by reserpine. In completely isolated, perfused blood vessels, the possibility of any action other than a direct one upon the vascular smooth muscle is difficult to conceive.

Support for a metabolic site of action in vascular muscle is provided by Kirpekar and Lewis[20]. Using isolated strips of horse carotid artery, they have shown that reserpine markedly depressed the contractions of this preparation which were produced by acetylcholine, histamine, adrenaline, nor-adrenaline, 5-hydroxytryptamine, potassium chloride and barium chloride. Preliminary investigations have revealed that sodium pyruvate was very effective in antagonising the action of reserpine.

Whilst the action of reserpine upon the drug-induced contractions of intestinal muscle was generally reversible, it was not so on the isolated heart and auricles. Possibly the more vigorous metabolism of those tissues is more sensitive to reserpine. It is also possible that some other mechanism of action

is operative in vascular muscle as well as an interference with oxidative energy producing reactions since the latter cannot, in all cases, explain the irreversible effects of reserpine. It does not explain, for example, the irreversibility of the action of reserpine in isolated perfused blood vessels or in the isolated uterus. Both types of smooth muscle might be expected, on purely empirical grounds, to have a less reserpine-sensitive metabolism than heart muscle and should therefore not be affected by reserpine to the same extent as the latter.

Few studies on the action of reserpine on metabolism have been carried out. The alkaloid has been found to inhibit the effect of the thyroid hormone on oxygen consumption in rats[21] and guinea-pigs[22]. An <u>in vitro</u> antithyroid action of reserpine has been reported by Mayer and his colleagues[23], Other authors[25] have been unable to confirm any direct effect on the thyroid gland since they did not observe any reduction in the uptake of radioiodine in human hypertensives or rats. Kuschke and Frantz[24] reported that reserpine caused hyperglycaemia in rabbits and dogs which was blocked by the hydrogenated ergot alkaloids. They

suggested this was due to stimulation of hypothalamic "substrates" and subsequent suprarenal stimulation. Hyperglycaemia was still seen when splanchnicotomised rabbits were used, but they assumed that section of the splanchnic nerves in the rabbit did not guarantee complete suprarenal denervation. The purpose of such a negative experiment is not at all clear. It is difficult and probably quite wrong to draw any definite conclusions from this work.

Reserpine inhibits ATP-ase[26], but only at concentrations of 100 µg. per ml. or above. Oxidative phosphorylation in rat brain is only slightly depressed by 25 µg. and 50 µg. per ml. reserpine[27]. Up to 50 µg. per ml., however, has no effect upon enzymatic ATP katabolism.

In Chapter 8 (page 122) it was reported that the oxygen uptake of rabbit intestinal muscle was depressed, after a latent period of more than twenty minutes, by high doses (50 µg.) of reserpine. It seems likely that only an active energy producing system is sensitive to reserpine. If it were possible to study the effect of reserpine upon the oxygen uptake of intestinal smooth muscle under tension, a more marked

depression would probably be revealed. Recently a method has been reported[61] for studying the oxygen consumption of electrically stimulated rat ventricle preparations using the Warburg manometric technique. A modification of this method could be used to study the effect of reserpine on the oxygen uptake of intestinal musole stimulated electrically or by means of drugs. If a depression of respiration was observed, then the effect of the reserpine antagonists on this action could be studied. In this way a pointer to the exact site of the metabolic interference caused by reserpine might be obtained.

The more marked effect of reserpine upon tissues from younger animals seems, again on purely empirical grounds, to be the result of some alteration in cellular metabolism with increasing age. Published data on this problem is scarce. Barrows[3] has reviewed the available literature and concludes that overall metabolic activity (total oxygen uptake and anaerobic glycolysis) decreased with advancing age. It cannot yet be said definitely whether the decreased activity is due to a loss of cells or cellular elements or whether the metabolic activity of the individual cells

decreases with age[3]. Since it seemed probable that the more active the metabolism, the greater would be the effect of reserpine, the increased effect observed in segments of ileum from younger guinea-pigs was probably to be expected. The same phenomenon was seen in younger spinal cats. It is of interest in this respect to note that reserpine had little effect upon the segments of human gut, both of which came from old patients. Since only two experiments were carried out with human tissue, the results must naturally be viewed with caution. It is impossible, however, to generalise on this point, since the age factor was not taken into account in all the experimental tissues used. Many drugs, however, have a greater effect on younger animals with a more active metabolism and it is probable that reserpine conforms to this pattern. Before reaching any definite conclusions, a carefully controlled study of the effect of reserpine upon a wide range of tissues from animals of different ages must be carried out.

In this context, some studies by other workers[28,29,30] are of particular interest. Holzbauer and Vogt[28] found that reserpine more seriously affected

motility and posture and caused a greater release of nor-adrenaline from the hypothalamus of younger than of older cats. Isaacs[29] and Esselier and his co-workers[30] occasionally observed orthostatic hypotension in young normotensives. This was very rarely seen in older subjects. Isaacs[29] also found a marked increase in hand blood flow (measured plethysmographically) in young normotensives, which was delayed in onset but prolonged. This may have been due, at least in part, to a direct peripheral action.

At this point, it is appropriate to consider the observed release of potassium from frog skeletal muscle. The effect of pH upon the ^{42}potassium concentration in the bathing fluid($^{42}K_b$) can be neglected. Whilst it is known[31] that a decreased pH will cause an increased K_b, this factor can be discounted in the work carried out since the pH change involved in the addition of reserpine was small. In addition, the control solution, at the same pH rarely had any effect on $^{42}K_b$.

The possible nature of the action responsible for the increased $^{42}K_b$, following reserpine must now

be discussed. It is very probable that the first step in the initiation of muscular contraction is a depolarization[32-34], which is manifest as a decreased muscle action potential. No report of a depolarising action of reserpine has been found by the author. This is a point which should be investigated. Nevertheless, it seems probable that depolarization is involved at some stage in the reserpine-induced contraction of skeletal muscle and in the increased $^{42}K_b$. It is well known that decamethonium (C10) depolarizes the motor end plate of both normal and denervated voluntary muscle[35,37]. On the other hand, tubocurarine (dTC) stabilizes the end plate by raising the threshold for acetylcholine-induced depolarization[36]. The experimental observations reported (Chapter 9, page 141) indicate that dTC delays the appearance of the reserpine-induced contraction of frog sartorius muscle, whilst the previous addition to the bath of C10 abolishes the delay. It may therefore be said that whilst neither drug has any effect upon the magnitude of the response to reserpine, the mechanism probably involves depolarization, which is increased by C10 and antagonised by dTC. This conclusion is supported

by the work of Barret and his co-workers[38] who suggested on the basis of indirect evidence, that reserpine might be acting on the frog rectus-abdominis muscle as a depolarising agent with a selective action similar to that of nicotine.

It is well established that contraction of smooth[39] cardiac[40] and skeletal[41,42,43] muscle is associated with an increased K_b. This seems likely to have been produced by an increased outward movement rather than a decreased inward movement of potassium, since adrenaline-induced relaxation in intestinal smooth muscle is accompanied by an increased inward movement of potassium[39]. Although no potassium uptake studies were carried out by the author, there seems little doubt that the increased $^{42}K_b$ following reserpine was caused by an increased outward movement of potassium, since it was accompanied by contraction of the muscle. It is probable also that similar considerations apply to the rectus-abdominis and gastrocnemius muscles of the frog. The suggestion that potassium release from muscle may have been a general phenomenon associated with the action of reserpine prompted the experiments which were made to

study whether there was a loss of potassium from rabbit intestinal muscle. No increased loss was found (Chapter 9, page 142) although the usual contraction in Krebs-Henseleit solution was seen. The explanation of this negative finding may be that the contraction of rabbit muscle after reserpine was followed by a marked relaxation. This secondary relaxation phase may have been accompanied by an increased inward movement of potassium. The magnitude of this influx may have masked the hypothetical, transient outward movement during the reserpine-induced contraction. Such a situation would not have been revealed when output was studied. The necessity for studying the effect of reserpine upon the uptake by intestinal smooth muscle of potassium seems clear.

It must now be decided whether the release of potassium from frog muscle is compatible with a metabolic site of action for reserpine. The inactivity of intermediates of carbohydrate metabolism on reserpine-induced depression of the frog rectus-abdominis muscle may have been due to an insufficient penetration of the cell membrane. The absence of activity may not, however, invalidate the possibility

of a metabolic site of action in frog skeletal muscle. The actions of reserpine on this tissue bear certain similarities to those seen on intestinal smooth muscle. It was observed, for example, that a latent period preceded the depression of acetylcholine-induced contractions, whilst on no occasion was complete inhibition of the contractions seen.

The potassium ion is known to play an important part in many of the enzyme systems controlling the energy production of cells[44-50]. It is possible, therefore, that a release of potassium following depolarization may have decreased the efficiency of enzyme systems requiring the potassium ion for optimal function.

On the other hand, Krebs and his associates[51] have shown that guinea-pig brain and kidney cortex retained potassium only in the presence of (-)-glutamic acid and α-ketoglutaric acid, to which the former could easily be converted. It is possible, therefore, that in the presence of a substance which interferes with oxidative and energy-producing reactions, potassium may be released because it cannot be utilized. This

seems a more likely interpretation than that postulating a decreased enzyme activity due to a fall in the intracellular potassium level, since it also explains the delay in onset of the reserpine-induced increase in $^{42}K_b$. Thus one can envisage the delay as being a reflection of the time required to deplete existing stores of ATP. When this happens, as a result of some interference with the oxidative metabolism, potassium ions are released by cells which are no longer able to retain or use them.

It must finally be considered what light is shed upon the mode of action of the drug in normotensive and hypertensive human subjects by experiments upon isolated tissues and organs. This is a difficult problem. In general, animal experiments give some indication of the effects which may be produced in human subjects, but this is by no means a general rule and care must be exercised in the interpretation of results. It can be argued that the doses used in some experiments were far in excess of those which produce a clinically useful reduction of blood pressure. Whilst this criticism is undoubtedly valid for some preparations, it is of

only equivocal value in the case of the isolated heart and perfused blood vessels in which perfusion with 1 µg. per ml. reserpine produced demonstrable effects. In some experiments, 0.1 µg. per ml. produced effects similar to those seen in man. These were bradycardia and peripheral vasodilatation. A dose of 0.1 µg. per ml. is comparable with that used clinically in the treatment of hypertension. This has been estimated[18] to be 0.1 µg. per ml. of blood. The dose of reserpine used in the experiments on spinal cats was not therefore excessive. It may be, as Tripod and Meier[18] point out, that the isolated tissue is less sensitive than the intact experimental animal or man and that the doses used do in fact correspond. The characteristics of the action of reserpine on isolated tissues are in many cases similar to those seen in intact experimental animals and man. The latency and gradual development of its action, together with the prolonged duration of effect, once established, may be cited as examples.

Studies of the action of reserpine upon metabolic function in man are inconclusive. Hafkenscheid and his colleagues[52] were unable to show any alteration in glucose or oxygen uptake in man with doses of reserpine

which reduced the blood pressure by 20 per cent when compared with control levels. Cerebral vascular resistance was decreased, but was unaccompanied by any metabolic disturbance in the brain. This finding seems to be supported by the *in vitro* observations[26,27] on brain metabolism mode in animal tissue and mentioned earlier (page 157). In a second paper, Hafkenschiel and his co-workers[53] reported that the cerebral glucose utilization, but not cerebral oxygen uptake, was depressed in 5 hypertensive patients. They suggested[53] that the ability of the brain to utilize glucose might be impaired by reserpine. Their observations on cerebral glucose and oxygen uptake were made only one hour after the administration of reserpine. This was probably too short a time since it seems very possible that the reserpine action would not yet have reached its maximum.

Burrel[54,55] found that the mental depression (a frequent and troublesome side effect of reserpine) was abolished by oral administration of sodium succinate, which he considered may have been active by virtue of its ability to restore the metabolism of brain cells to its optimal value. He pointed out that disorders

of carbohydrate metabolism were known to occur in functional psychoses.

These conflicting reports do not rule out the possibility of some reserpine interference with metabolism. The failure of *in vitro* studies[26,27] to yield a conclusive answer to the problem of the action of reserpine in brain metabolism may well be due to a decreased overall metabolism in brain tissues. A depressed metabolism might be less sensitive to reserpine than a more active *in vivo* brain metabolism.

The release by reserpine of 5-hydroxytryptamine and *nor*-adrenaline from a number of tissues (Chapter 1, pages 7 and 9) may be due to some interference with cellular metabolism. There have been suggestions recently that ATP may be associated with histamine and 5-hydroxytryptamine in blood platelets[62] and with catechol amines in the suprarenal medulla[63].

Bein has suggested[64] that the release of catechol amines may be "an indication that the pharmacologically active grouping of the reserpine molecule intervenes in the cellular processes in some as yet unknown manner".

Peripheral vasodilatation, supposedly mediated by the central nervous system[17], is a feature of the hypotensive or antihypertensive action of reserpine [29,30,56,57,58]. McQueen, Doyle and Smirk[19] considered on the basis of experiments with the perfused, innervated hind-quarters of the rat and the rabbit, that "the hypotensive effect cannot be solely ascribed to this (i.e. depression of sympathetic predominance) action". The doses of reserpine which they used were in excess of those effective in man, but the considerations regarding a decreased sensitivity of tissue to reserpine in vitro (page 166) may also be applicable to these experiments.

There is no evidence of any release of potassium in man, since the concentration of sodium and potassium in the plasma of human hypertensives or normotensive dogs[59,60] was unaltered by reserpine. If a direct peripheral action is assumed to be operative in man, this would probably take the form of a relaxation of vascular smooth muscle. This might be expected to result in a decreased rather than an increased blood level of potassium, since muscular relaxation is accompanied by an increased inward movement of the ion[39].

Bein[17] has said that "there does not seem to be any significant difference in the potency of its (reserpine) vasodepressor action, whether the experimental hypertension be of nervous, renal or endocrine origin". He also stated that clinical studies have indicated no difference between its actions in "essential" and "nephrogenic" hypertension. Thus the actions of reserpine in both normotensive and hypertensive man, whatever the cause, are probably due to a similar effect. This is supported by the fact that the same side effects were noted in normotensives as in hypertensives [29,30,56,58].

Whatever its precise etiology, clinical or experimentally produced hypertension must involve an increase in vascular tone. The evidence seems to indicate one point of reserpine attack common to all types of experimental and possibly clinical hypertension.

In conclusion, it may be said that available evidence does not preclude the possibility of a peripheral site of attack for reserpine. The drug has pronounced effects upon isolated tissues and organs, which, in the opinion of the author, point to a non-specific attack on metabolism, probably that supplying energy for

muscular contraction. Thus, the ability of vascular smooth muscle to respond to normal regulatory or to abnormal vasoconstrictor impulses may be decreased.

The drug undoubtedly has central effects which seem to be located in or above the level of the hypothalamus. Until evidence is produced which proves convincingly the contrary, it is considered probable that the mode of action of reserpine in these areas may also involve some direct disturbance in cellular metabolism.

CHAPTER 10

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SUMMARY AND CONCLUSIONS.

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1). Reserpine is used in the treatment of hypertension and certain psychiatric disorders in man. In the introductory chapter of this thesis, current hypotheses on its mode of action as an anti-hypertensive agent are described. Reserpine is thought to depress central sympathetic predominance, although the exact means by which this is achieved is not clear. Evidence also exists pointing to peripheral effects, which may play a greater or lesser part in the alleviation of human hypertension. The work described in the thesis was undertaken to elucidate the mode of action of the drug at cellular level.

2). In Chapters 2, 3 and 4, experimental studies using certain preparations of isolated cardiac, smooth and skeletal muscle have been described. These showed that reserpine reduced inherent muscular tone and, after a latent period, depressed drug-induced contractions of the muscle. In some cases, the depression of activity was preceded by a transitory stimulant action. It was observed that

the action of the drug was greater on guinea-pig ileum from younger than from older animals. The slow contraction of the isolated frog rectus-abdominis muscle produced by reserpine, suggested the possibility of some alteration in the potassium balance between intra- and extra-cellular fluids.

3). The action of reserpine upon the blood pressure of the spinal cat was studied. In Chapter 5, the results of these experiments have been presented. They indicated that any inherent vascular tone was reduced to a point at which histamine failed to produce a depressor response. The pressor responses to adrenaline and nor-adrenaline were simultaneously depressed. Again, a latent period preceded these effects and they were more marked when younger animals were used. Prior administration of posterior pituitary extract delayed still further the onset of the reserpine effect.

4). In Chapter 6, the effects of the drug upon isolated muscle have been discussed. It is pointed out that many of these effects can be explained if it is assumed that reserpine reduces the ability of muscle to respond to drug-induced or myogenic stimuli. This

may involve some interference with certain metabolic processes supplying the energy necessary for muscular contraction.

5). Chapter 7 contains the results of experiments which implicate carbohydrate metabolism in the action of reserpine upon isolated guinea-pig ileum. Intermediates of carbohydrate metabolism antagonised the reserpine depression of drug-induced contractions of this tissue. Intermediates of fat and protein metabolism were ineffective in this respect. No conclusive evidence was obtained for the effectiveness of intermediates of carbohydrate metabolism in antagonising the depressant action of reserpine upon the isolated frog rectus-abdominis muscle and the isolated guinea-pig auricles. The relative impermeability of the cell membrane to these intermediary metabolites and the deleterious effect on the preparation of a reduction of the bath fluid pH may have been responsible for the inconclusive findings.

6). Manometric studies of respiration and glycolysis in isolated rabbit intestinal muscle segments were

carried out using the Warburg "Direct" method. The results of these experiments, presented in Chapter 8, showed that reserpine reduced significantly the oxygen uptake of this tissue, after a latent period. The reduction of respiration was seen only when high doses of reserpine were used. Glycolysis was unaffected.

7). In Chapter 9, experiments have been described in which the action of reserpine upon cellular potassium balance in frog skeletal muscle was studied using an isotopic tracer technique. These showed that the alkaloid increased the ^{42}K content of the fluid bathing the muscles. This effect was associated with a contraction of the muscles, which was probably linked with depolarization of the motor nerve end plates.

8). In the discussion, it is argued that interference with carbohydrate metabolism would result in a decreased availability of energy for muscular contraction. It is postulated that reserpine produces an "anoxia-like" condition in smooth muscle. Such an effect may explain the reduction

of inherent tone in vascular smooth muscle. A reduction in the efficiency of the enzymes controlling carbohydrate metabolism may also reduce the ability of frog skeletal muscle to retain potassium, resulting in a release of the potassium ion.

The possibility of a peripheral component in the effect of reserpine in human hypertension is discussed. It is concluded that whilst the drug has definite actions upon the central nervous system, which probably contribute largely to its antihypertensive effect in man, peripherally-induced relaxation of vascular smooth muscle may also play a part. The means by which the central actions of reserpine are produced may also involve carbohydrate metabolism, since the evidence to the contrary is, in the opinion of the author, inconclusive. Such an interference with metabolism may explain the observations of others, that reserpine causes a release of 5-hydroxytryptamine and catechol amines from the brain and other tissues of experimental animals.

APPENDIX I

APPENDIX I

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

TABLE I.A

Formulae of Physiological Saline solutions used.

Salts (g. for 1 litre)	Frog Ringer's Solution	Tyrode's Solution	Locke's Solution	de Jalon's Solution	* Krebs-Henseleit Solution	Krebs-bicarbonate Solution	Page and Green's Solution
Sodium chloride	6.50	8.00	9.00	9.00	6.92	6.92	8.20
Potassium chloride	0.14	0.20	0.42	0.42	0.35	0.35	0.84
Calcium chloride (anhydrous)	0.12	0.20	0.24	0.06	0.29	0.29	-
Calcium chloride (dihydrate)	-	-	-	-	-	-	0.04
Sodium dihydrogen phosphate (dihydrate)	-	0.05	-	-	-	-	-
Potassium dihydrogen phosphate	-	-	-	-	0.16	0.16	-
Sodium bicarbonate	0.20	1.00	0.50	0.50	1.19	2.10	0.40
Magnesium sulphate (heptahydrate)	-	-	-	-	0.29	0.29	-
Magnesium chloride	-	0.01	-	-	-	-	0.06
Glucose	2.00	1.00	1.00	0.50	2.00	2.00	1.00

* Modified Krebs-Henseleit solution (used for the determination of respiration in isolated rabbit intestinal muscle, Chapter 8, page 122), contained neither sodium bicarbonate nor glucose. The pH was adjusted before use to 7.4 with a phosphate buffer.

Reserpine was dissolved in an aqueous solution of 10 per cent ascorbic acid to give a concentration of 2 mg. per ml. This solution had a pH of about 2.5. Immediately before use, the pH was adjusted to 4.5 by the addition of small amounts of 5 per cent sodium bicarbonate solution. The mixture was then diluted with the physiological saline in use to give a final reserpine concentration of 1 mg. per ml. The addition of sodium bicarbonate was carried out immediately before use, since reserpine tended to precipitate from the final solution. The control solution was prepared by treating the 10 per cent ascorbic acid solution in exactly the same fashion.

In some experiments (Chapter 4, page 52) 0.2 per cent citric acid solution was used as a solvent. The procedure in this case was the same as described above for ascorbic acid.

For the determination of respiration and glycolysis in isolated rabbit intestinal muscle (Chapter 8, page 122), a 5 mg. per ml. reserpine solution was prepared in a 5 per cent aqueous solution of tartaric acid. The pH of a 2 ml. aliquot was adjusted to 4.5 with sodium bicarbonate and the mixture

diluted to 7.4 ml. A further 1 in 5 dilution of this solution was made. 0.1 ml. of these solutions then contained sufficient reserpine to give a final flask concentration of 50 µg. or 10 µg. per ml. respectively.

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

APPENDIX II

APPENDIX II

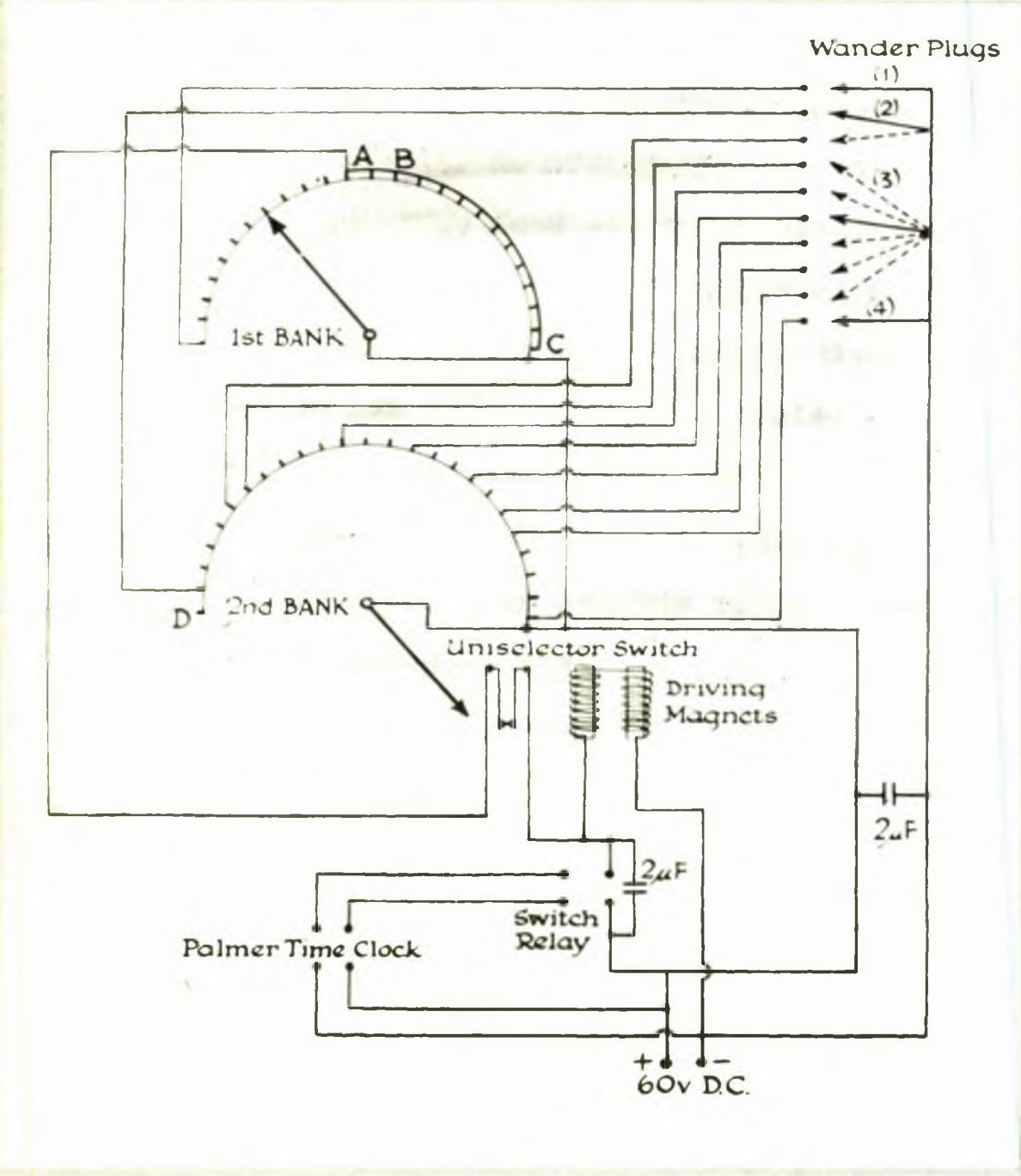
THE ELECTRICAL CONTROLLING MECHANISM OF THE SEMI-AUTOMATIC ISOLATED ORGAN BATH.

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

The flow of solutions into the isolated organ bath was regulated by two modified electro-magnetic relay switches (Figure 4,1, page 54). Solutions flowed only when these were activated. The remainder of the equipment, which is illustrated diagramatically in Figure II,A served to control the activation of the relay switches in a predetermined time cycle.

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

Figure II A



Circuit diagram of the electrical equipment used to control the semi-automatic isolated organ bath.

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

Certain contacts on both banks were connected to wander plugs. Negative current was led to the two modified relay switches and two indicator lights. Each

was fitted with a connection to the wander plug board. By placing these plugs into the appropriate sockets (which were fed with negative current *via* the contacts of the uniselector), the events in the three minute cycle could be pre-set. The plugs were connected as follows:

Wander plug number 1 was connected to an indicator light which lit 5 seconds before the inflow of a stimulant drug solution. This was used only during the preliminary part of an experiment, when a suitable concentration of the stimulant drug was being determined by hand.

Wander plug number 2 was connected to a light indicating the time for addition of an antagonist (60 or 90 seconds before the inflow of a stimulant drug solution).

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

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

APPENDIX II

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