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BIOLOGICALLY ACTIVE THIONAPHTHEN DERIVATIVES

AND

AN INVESTIGATION OF THE EFFECTS OF SOME
VOLATILE ANAESTHETICS ON THE MUSCLE
RELAXANT ACTIONS OF TUBOCURARINE

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

Doctor of Philosophy

in the

Faculty of Medicine

by

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COMMUNICATION

Certain aspects of the work described in this thesis has been communicated jointly with J. J. Lewis, M. Martin-Smith and S. T. Reid, at the following meeting:-

British Pharmacological Society in Edinburgh, in July 1961.

Pharmacological activity in some thionaphthenylamines.

PAPERS IN PREPARATION

1. Preparation and pharmacological properties of some simpler thionaphthen bases.
2. An investigation of the effects of some volatile anaesthetics on the muscle relaxant action of tubocurarine.

C O N T E N T S

	<u>Page</u>
Acknowledgments	i
Communication	iii
Papers in preparation	iii

CHAPTER I

Introduction	1
Distribution of 5-hydroxytryptamine	5
Metabolism of 5-hydroxytryptamine	6
Identification of 5-hydroxytryptamine	9
Pharmacological properties of 5-hydroxytryptamine.	
Isolated tissues and organs.	
The gastro-intestinal tract	11
Cardiovascular actions of 5-hydroxytryptamine	13
Effects of 5-hydroxytryptamine on respiration	15
The stimulant actions of 5-hydroxytryptamine	
upon chemoreceptors	16
The antidiuretic effect of 5-hydroxytryptamine	17
The role of 5-hydroxytryptamine in	
the central nervous system	18
Receptor theory	21

	<u>Page</u>
5-Hydroxytryptamine receptors	27
Stimulant analogues of 5-hydroxytryptamine	31
Pharmacological antagonists of 5-hydroxytryptamine	35
Specificity of action of 5-hydroxytryptamine antagonists	49
The object of research	56

CHAPTER II

A. Materials	59
B. Experimental methods	60
I. Experiments on isolated tissue preparations	60
(a) Experiments on the isolated rat uterus	60
(b) Experiments on the isolated rat fundus strip	68
(c) Experiments on the isolated guinea pig ileum	72
(d) Experiments on the perfused isolated rat hindquarters	74
II. Experiments on intact animals	77
(a) Experiments on the blood pressure of the anaesthetised cat	77
(b) Experiments on the blood pressure of the anaesthetised rat	80
(c) Toxic effects on mice	85

	<u>Page</u>
III. Experiments on isolated smooth muscle preparations using Ariens' technique	85
(a) (i) The isolated guinea pig jejunum	86
(ii) Horse carotid artery strips	86
(b) Experiments on the isolated rat jejunum	90
<u>CHAPTER III. Results</u>	93
I. (a) Effect of the thionaphthen compounds on the contractions produced by 5-hydroxytryptamine on the isolated rat uterus	93
(b) The direct stimulant effect of the thionaphthen compounds on the isolated rat fundus strip and the effect on the contraction produced by 5-hydroxytryptamine	103
(c) Isolated guinea pig ileum	118
(i) The direct stimulant effect of the thionaphthen compounds tested	118
(ii) The effects of the lysergic acid diethylamide, mepyramine and atropine on the contractions produced by the thionaphthen compounds	118
(iii) Effects of the thionaphthen compounds on the contractions produced by 5-hydroxytryptamine	128
(iv) Effects of the thionaphthen compounds on the contraction produced by histamine ...	141
(v) Effects of the thionaphthen compounds on the contraction produced by acetylcholine	153
(d) Effects of the thionaphthen compounds on the isolated, perfused rat hindquarters	165

	<u>Page</u>
II. Effects of the thionaphthen compounds in intact animals	177
(a) Effects on the blood pressure of the anaesthetized cat	177
(b) Effects on the blood pressure of the anaesthetized rat	177
(c) Toxic effects on mice	190
III. Results of experiments with the thionaphthen compounds on the isolated rat jejunum using Ariens' technique	198
 <u>CHAPTER IV</u>	
Discussion	214
Summary	243
Appendix I	246
References	247
Appendix II. The muscle relaxant actions of certain volatile anaesthetics, tetrahydrofuran, ethylene glycol dimethyl ether and nitrous oxide in the spinal cat	290
Introduction	290
Materials	305
Method	306
Results	316
Conclusion	328
References	333

C H A P T E R I

Pages 1 to 58

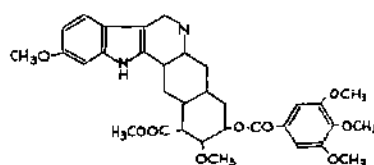
INTRODUCTION

The indole nucleus can be identified as a structural component of a large number of pharmacologically interesting compounds. These compounds show a great divergence in chemical complexity ranging from indole (Fig. 1, page 2) itself to the extremely complex alkaloids of the reserpine type (Fig. 1, page 2). The alkaloids of calabash curare, e.g., calabash curarine (Fig. 1, page 2) and strychnine (Fig. 1, page 2) can also be regarded as indole derivatives.

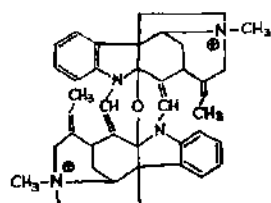
The pharmacology of the simpler indolic compounds such as indole, skatole (Fig. 1, page 2) and β -indolylthylamine (Fig. 1, page 2) which arise in nature from the bacterial decomposition of the essential amino acid tryptophan, has seen considerable investigation, but the results are somewhat contradictory. Thus Guggenheim and Löffler (1916) reported that a number of proteinogenic amines and related compounds including indole stimulated the isolated guinea pig intestine, but Garcia-Blanco, del Castillo and Rodeles (1941) reported that the motility of the isolated rabbit intestine was inhibited by indole. Waddell (1927) who studied the action /



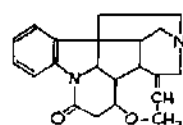
Indole



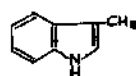
Reserpine



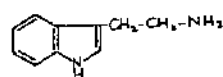
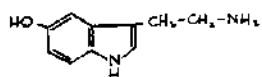
Calabash curarine



Strychnine



Skatole

 β -indolyethylamine
(tryptamine)

5-hydroxytryptamine

Fig. 1.

action of indole and skatole on the excised hearts of several warm and cold blooded animal species, noted that they diminished cardiac activity. Yanai (1935) and Ets and Feinberg (1942) have demonstrated convulsions in frogs and dogs respectively following the administration of indole. In confirmation of the work of Torda and Wolff (1945), Izquierdo and Stoppani (1950, 1953), found that indole increased the sensitivity of frog striated muscle to acetylcholine, and indole and skatole enhanced the effects of potassium on the same tissue. But on smooth muscle, indole and skatole diminished the contractions produced by acetylcholine and potassium. The spontaneous contractions of the rabbit duodenum and rat uterus were also diminished by these substances (Izquierdo and Stoppani, 1953).

One of the most interesting and indeed by far the most studied derivatives of indole is 5-hydroxytryptamine (Fig. 1, page 2). Owing to its widespread occurrence, its interesting pharmacological properties, and to the need to ascertain its precise physiological role in the organism, 5-hydroxytryptamine has attracted the attention of many workers. There are several important reviews giving detailed information on this substance among which can be mentioned those by Erspamer, 1954; Page, 1954, 1958; G.P. Lewis, 1958; and Maupin, 1961.

Although /

Although the presence of a circulating substance with the properties of 5-hydroxytryptamine had been known since 1918, this compound was not identified until 1949. Before 5-hydroxytryptamine was identified, various workers had independently investigated the properties of what were believed to be two different, naturally occurring, physiologically active substances. It was in 1918 that Janeway, Richardson and Park first showed that the platelets contained an extractable vasoconstrictor substance which was not present to any great extent in the rest of the blood. Vialli and Erspamer (1933) in Italy, extracted a substance from the rabbit gastric mucosa which stimulated the isolated intestine and uterus and named it enteramine. They believed it to be a diphenolic or polyphenolic amine. It gave a colour reaction with the diazonium salt of p-nitroaniline. They thought that this substance originated from the enterochromaffin tissue of the gastrointestinal mucosa of mammals. Later, in 1952, Erspamer and Asero found this colour-producing and pharmacologically active substance to be identical with synthetic 5-hydroxytryptamine. Other workers, including Rapport and his colleagues in the United States, and Reid and Rand in Australia, attempted to isolate and identify this principle, which had been known for a number of years under various names such as: vasoconstrictine, vasoconstrictor principle, spätgift, thrombo-cylin /

thromboeytin and serotonin (Janeway and his associates, 1918; Borgert and Keitel, 1926; Simon, 1940; Freund, 1920, 1921; Rand and Reid, 1952; Reid and Rand, 1951, 1952; Rapport, Green and Page, 1948 a,b,c).

Rapport (1949) finally demonstrated the structure of this vasoconstrictor substance as 5-hydroxytryptamine (Fig. 1, page 2) in a molecular complex with creatinine and sulphuric acid. Hamlin and Fischer (1951) were the first to synthesise 5-hydroxytryptamine and Bacq (1952) suggested that the names of serotonin and enteramine should be dropped in favour of 5-hydroxytryptamine. This suggestion will be adopted in this thesis.

Distribution of 5-hydroxytryptamine

The distribution of 5-hydroxytryptamine has been reviewed by Erspamer (1954). It is present in all tissues which contain cells belonging to enterochromaffin system. It was also shown to occur in dog, rat and rabbit brain by Twarog and Page (1953). Amin, Crawford and Gaddum (1954) made the first comprehensive study of its distribution in the dog brain and showed that there was a variable distribution of 5-hydroxytryptamine in the different parts of the central nervous system. 5-Hydroxytryptamine is not present in areas which consist mainly or solely, of medullated nerve fibres.

It /

It appears to be confined to regions of the grey matter where it is present in varying concentrations. The highest concentration is found in tissues associated with the central representation of the autonomic nervous system and in the area postrema. Bogdanski and Udenfriend (1956) found high concentrations of this substance in the hypothalamic region of the brain of cats and dogs. Humphrey and Jaques (1954) observed that 5-hydroxytryptamine found in the blood of most species was confined to the platelets. Weissbach, Waalkes and Udenfriend (1957) found 5-hydroxytryptamine in the lungs of mice, rats and rabbits in greater amounts than in the lungs of guinea pigs. Its presence in the mast cells of rats has been shown by many workers (Benditt, Wong, Arase and Roeper, 1955; Bhattacharya and Lewis, 1956; and Parratt and West, 1956). Sjoerdsma, Waalkes and Weissbach (1957) have found 5-hydroxytryptamine in mast cell tumours of mice. Florey and Florey (1954) found 5-hydroxytryptamine in the cerebral and stellar ganglia and peripheral nerves of Sepia. It occurs in carcinoid tumours (Lembeck, 1953). Sachs (1957) has detected 5-hydroxytryptamine in the spinal fluids of patients with head injuries, brain tumours and meningitis and in dogs and cats with head injuries.

The metabolism of 5-hydroxytryptamine

Fig. 2, page 7, shows the metabolism of 5-hydroxytryptamine as suggested /

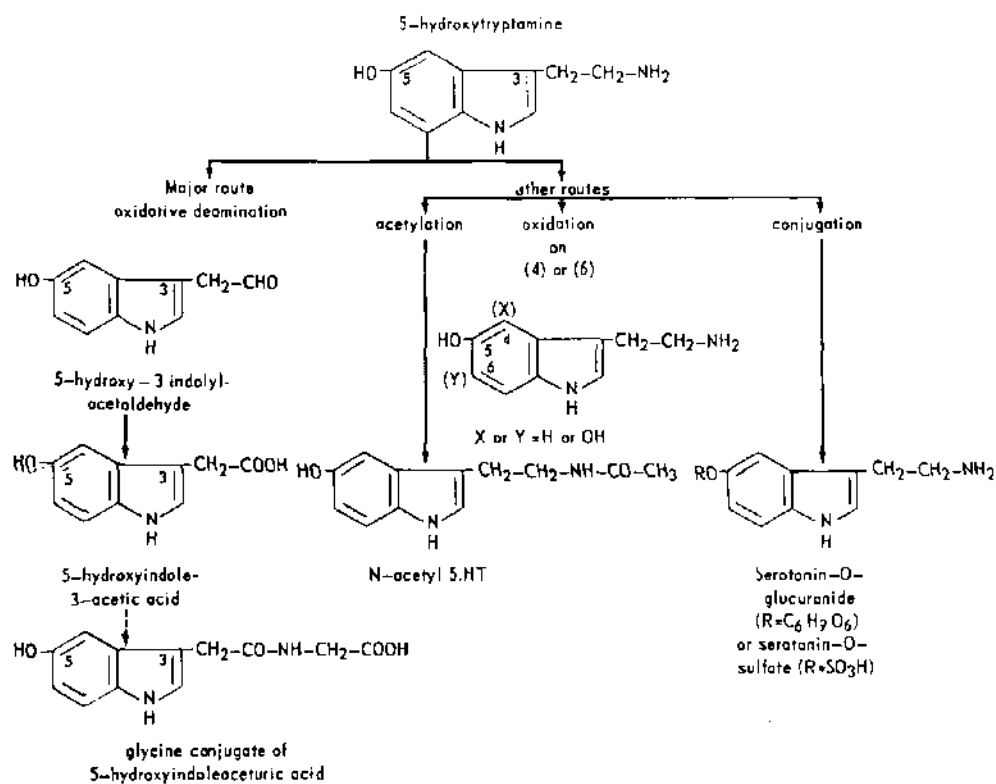


Fig. 2.

Diagram showing pathways of the metabolism of 5-hydroxytryptamine. (After Maupin, 1961)

suggested by Maupin (1961). The metabolism of 5-hydroxytryptamine has been reviewed in detail (Udenfriend, 1957). The conversion of tryptophan to 5-hydroxytryptophan has been clearly demonstrated in Chromobacterium violaceum (Mitoma, Weissbach and Udenfriend, 1955). Experiments with radio-active tryptophan indicate that such conversion does take place in vivo in the venom gland of the toad (Udenfriend, Titus, Weissbach and Peterson, 1956). When 5-hydroxytryptophan is fed to animals, there is a large rise in the 5-hydroxytryptamine content of the brain and when it is given to human beings, there is a large rise in the excretion of 5-hydroxyindole acetic acid (Davidson, Sjoerdsma, Loomis and Udenfriend, 1957). Udenfriend, Weissbach and Bogdanski (1957) have shown that when 5-hydroxytryptophan is administered to animals, it is rapidly taken up by the tissues and wherever 5-hydroxytryptophan decarboxylase is present, is converted to 5-hydroxytryptamine. 5-Hydroxytryptophan decarboxylase occurs in large quantities in the kidney, liver and stomach. Gaddum and Giarman (1956) have found it in sympathetic ganglia and in brain. Its distribution is reported to be similar in dogs and cats to that of 5-hydroxytryptamine (Bogdanski, Weissbach and Udenfriend, 1957). Evidence for the requirement of pyridoxal phosphate for the activity of the decarboxylase /

decarboxylase has been presented by many workers. Weissbach, Bogdanski, Redfield and Udenfriend (1957) have demonstrated that the tissues of pyridoxine-deficient chickens are deficient in 5-hydroxytryptamine and they also showed a diminished ability to convert 5-hydroxytryptophan to 5-hydroxytryptamine. Blaschko (1952) demonstrated that 5-hydroxytryptamine was inactivated by amine oxidase which is widely distributed in the animal kingdom. He has mentioned in detail (1957) its presence in other vertebrates and invertebrates. It is also present in the fundus of the rat stomach (Vane, 1957). Udenfriend (1957) has reported that the conversion of 5-hydroxytryptamine to 5-hydroxyindole acetic acid is the major route of metabolism in man and in other species, urinary output of 5-hydroxyindole acetic acid is small. Blaschko (1957) has discussed in detail the various possible pathways of inactivation of 5-hydroxytryptamine other than oxidative deamination.

Identification of 5-hydroxytryptamine

Various isolated tissues have been used for the identification and estimation of 5-hydroxytryptamine. The carotid artery ring was the first tissue to be used for the assay of the serum vasoconstrictor substance by Janeway and Park (1912) and later by Woolley and Shaw (1952 a). Erspamer (1940, a,b,c) and Amin and his associates (1954) have /

have used the isolated rat uterus for the assay of 5-hydroxytryptamine. The perfused vessels of the rabbit ear were used by Page (1942). Dalglish, Toh and Work (1953); and Feldberg and Toh (1953), have used the rat colon for the assay of 5-hydroxytryptamine. The use of the heart of Venus mercenaria for the assay of 5-hydroxytryptamine has been described by Twarog and Page (1953). Gaddum and Paasonen (1955) were able to show that the heart of Spisula solida was also very useful for the assay of 5-hydroxytryptamine. The superfused isolated anterior byssus retractor muscle of Mytilus edulis was used by Cambridge and Holgate (1955). Chick amnion has been used by Ferguson (1957) and Vane (1957) has been successful in using the fundus of the rat stomach.

Physico-chemical methods have now been introduced for the isolation and identification of 5-hydroxytryptamine. These methods often give quick and reliable results and chromatography has played an important part in the extraction of 5-hydroxytryptamine from the tissues. Udenfriend, Weissbach and Clark (1955) have described the first chemical method of estimating 5-hydroxytryptamine. Shepherd, West and Erspamer (1953) used the fluorescence reaction as a simple means of detecting small quantities of 5-hydroxytryptamine in tissue extracts on paper chromatograms. Jepson and Stevens /

Stevens (1953) modified this method to increase the sensitivity. Gluckman and Abrams (1959) have developed a new method involving the use of ion exchange chromatography which gives 100 per cent recovery of 5-hydroxytryptamine from tissue extracts.

Pharmacological Properties of 5-Hydroxytryptamine

Isolated tissues and organs. The gastrointestinal tract.

Feldberg and Toh (1953) and Dalgliesh, Toh and Work (1953) have shown that 5-hydroxytryptamine stimulated the isolated, atropinised rat colon. According to Page (1954) it has been known for a long time that 5-hydroxytryptamine causes evacuation of the bowels even under deep anaesthesia. 5-Hydroxytryptamine stimulates the smooth muscle of some parts of the gastrointestinal tract in certain species, and the sensitivity varies greatly (Erspamer, 1954; Vane, 1957). Gaddum (1953a) has shown that prolonged contact of the isolated intestine with large doses of 5-hydroxytryptamine inhibits the further stimulant action of added 5-hydroxytryptamine. Rocha e Silva, Valle and Picarelli (1953) concluded that the action of 5-hydroxytryptamine on the guinea pig ileum was cholinergic in nature because it was blocked by atropine. This effect was not blocked by hexamethonium, so presumably it was not at the intramural ganglia. /

ganglia. The provisional conclusion drawn was that 5-hydroxytryptamine acted on the post-ganglionic cholinergic fibres of the intramural nervous system of the guinea pig ileum. Gaddum and Picarelli (1957) divided the 5-hydroxytryptamine and tryptamine receptors in the guinea pig ileum into two types.

- (1) M-receptors which were blocked by morphine, and
- (2) D-receptors which were blocked by dibenzylene.

The morphine-sensitive receptors were probably in the nervous tissues and the dibenzylene-sensitive receptors were probably in the muscle. Gaddum (1957a) compared the action of 5-hydroxytryptamine with that of acetylcholine. Both drugs act on at least two different kinds of receptors which are blocked by different groups of antagonists. Just as atropine blocks the effect of acetylcholine on plain muscle, revealing effects upon ganglia, so dibenzylene or lysergic acid diethylamide, block the effect of 5-hydroxytryptamine on plain muscle so revealing ganglionic effects. Hexamethonium specifically blocks the ganglionic effects of acetylcholine. Morphine appears to block the ganglionic effects of 5-hydroxytryptamine but this block is less specific since the actions of nicotine were also antagonised, although less effectively than those of 5-hydroxytryptamine.

Cardiovascular actions of 5-hydroxytryptamine

The response of the cardiovascular system to 5-hydroxytryptamine is very variable. Page (1952) has investigated the effect of 5-hydroxytryptamine on the blood pressure of anaesthetised dogs and cats. He has shown that in dogs there is an initial fall in blood pressure with slowing of the heart rate and this is followed by a rise and finally by a prolonged fall in the blood pressure. The response in cats usually differs from that in dogs. It consists of a sharp rather prolonged fall in pressure with slowing of the heart rate. The initial fall is believed to be due to a von Bezold-like reflex since it was to a large extent eliminated by vagotomy or atropine (Page, 1952). But Reid (1952) reported that intravenous administration of 5-hydroxytryptamine in doses ranging from 10 to 200 μ g. caused an initial fall in the systemic arterial pressure in the chloroelosed cat, followed by a rise and then by a more prolonged fall. This effect was seen irrespective of vagotomy or previous administration of atropine. Salmoiraghi, Page and McCubbin (1956) have shown that the response of the arterial blood pressure in rats is more like that observed in cats than that found in dogs. Page and McCubbin (1953) from experiments on anaesthetised cats, dogs and rabbits, have suggested that the arterial response to 5-hydroxytryptamine is the resultant of several variables of which four /

four are of major importance:

- (1) A direct vasoconstrictor action.
- (2) A von Bezold-like reflex.
- (3) Transient autonomic ganglion blockade.
- (4) Peripheral inhibition of neurogenic vasoconstriction.

They gave the name "amphibarcic" to the type of response which 5-hydroxytryptamine produces.

MacCanon and Horvath (1954) have shown a rise in the cardiac output in dogs following the injection of 5-hydroxytryptamine and Page (1957) has reported an increase in the cardiac output in human beings. Hollander, Michelson and Wilkins (1957) have shown a variable effect on the arterial blood pressure and an increase in the pulmonary ventilation following the intravenous administration of 5-hydroxytryptamine to human beings.

According to Ginzel and Kottegoda (1953) the vasoconstrictor power of 5-hydroxytryptamine in the lungs of the dog and the cat was greater than that of adrenaline or noradrenaline. The constriction of the pulmonary blood vessels and vasodilation of peripheral vessels in dogs by 5-hydroxytryptamine has been demonstrated by Rudolph and Paul (1957). An increased venous, right arterial and pulmonary /

pulmonary arterial pressure and increased heart rate and stroke volume were demonstrated in human beings following intravenous administration of 5-hydroxytryptamine (Baldrighi and Ferrari, 1955).

The vasoconstriction followed by vasodilatation of the blood vessels of the isolated hind leg of the cat following injections of 5-hydroxytryptamine was demonstrated by Reid and Rand (1951) and Reid (1952). Page (1954) has reported that 5-hydroxytryptamine is less potent than adrenaline on the blood vessels of the rabbit ear. Gaddum and Hameed (1954) have used this preparation routinely in their studies on drug antagonists to 5-hydroxytryptamine. Haddy, Fleishman and Emanuel (1957) have shown that adrenaline and noradrenaline caused constriction of the small vessels of the foreleg of the dog while 5-hydroxytryptamine caused dilatation. However, 5-hydroxytryptamine caused constriction of the large arteries and veins.

Effects of 5-hydroxytryptamine on respiration

The effect of 5-hydroxytryptamine on respiration varies from one species to another. Reid and Rand (1952) and Reid (1952) found that intravenous injection of 5-hydroxytryptamine in the anaesthetised cat with intact vagi caused a brief apnoea lasting for up to 30 seconds, followed by tachypnoea and a simultaneous bronchoconstriction. /

constriction. Ginzel and Kottegoda (1954) from their experiments on cats, believed that the stimulation of respiration was due to stimulation of chemoreceptors and the resulting apnoea was of a central origin. In dogs and cats, the injection of 5-hydroxytryptamine caused a transitory stimulation of respiration followed in half of the animals by a period of apnoea (Douglas and Toh, 1952, 1953; Heymans and Heymans, 1953; Mott and Paintal, 1953; Page, 1952; and Schneider and Yonkman, 1954). There was however no agreement between the above workers with regard to the explanation of the causes of these phenomena.

The stimulant actions of 5-hydroxytryptamine upon chemoreceptors

Direct evidence for a stimulant action of 5-hydroxytryptamine upon the chemoreceptors has been presented by McCubbin, Green, Salmoiraghi and Page (1956). When given by injection in quantities of 12 μ g. into the common carotid artery of the pentobarbitone-anaesthetised dog, 5-hydroxytryptamine caused a pronounced increase in the frequency of chemoreceptor impulses in the carotid artery sinus nerve. This effect does not only account for the hyperpnoea but also for the pressor effect of 5-hydroxytryptamine. Small doses of 5-hydroxytryptamine fail to stimulate respiration unless the drug reaches /

reaches the chemoreceptors. After section of the vagus nerve, much larger doses (60 to 120 μ g.) of 5-hydroxytryptamine caused reappearance of the respiratory response which was assumed to depend on a central mechanism. Recently Braun and Stern (1961) have reported that in the anaesthetised "open chest" dog, large doses of 5-hydroxytryptamine (200 μ g. per kg.) administered into the femoral vein, right heart, pulmonary artery, left heart, ascending aorta or common carotid arteries, caused a marked pressor response in the systemic circulation. They suggested that the systemic pressor response and the rise of both pulmonary arterial and venous pressure was due to chemoreceptor stimulation.

The antidiuretic effect of 5-hydroxytryptamine

Erspamer and Ottolenghi (1950, 1951, 1952, a,b,c) investigated in detail the effect of 5-hydroxytryptamine on the kidney. Erspamer (1953) concluded that the antidiuretic effect of 5-hydroxytryptamine was due to preferential vasoconstriction of the afferent glomerular arterioles and considered 5-hydroxytryptamine to be a hormone designed for the physiological regulation of renal function. Pickford (1957), reporting on her work with Abrahams (1956), stated that in conscious dogs there was an antidiuretic effect /

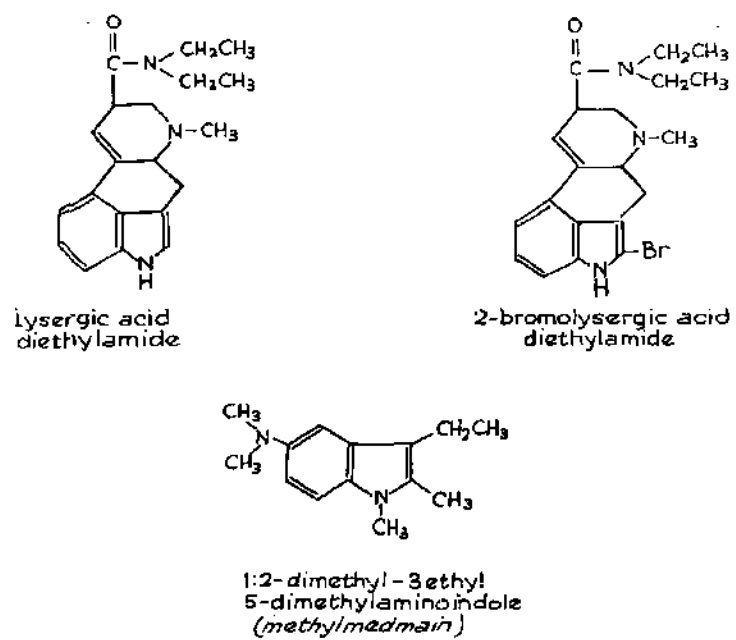
effect following administration of 5-hydroxytryptamine. Recently, Goran Boj's (1961) reported that 5-hydroxytryptamine infusion in normal human beings caused antidiuresis and decreased excretion of sodium in the urine.

The role of 5-hydroxytryptamine in the central nervous system

At the present time, the precise role of 5-hydroxytryptamine in the central nervous system is by no means clear, and a number of complicated and often conflicting theories have been advanced. The idea that 5-hydroxytryptamine was involved in the control of normal mental function seems to have arisen from three arguments. Firstly, the fact that 5-hydroxytryptamine is present in the brain as was shown by Twarog and Page (1953) gave rise to the assumption that it played a vital role there; secondly, on the basis that compounds known to act peripherally as antagonists to 5-hydroxytryptamine, showed central actions (Woolley and Shaw, 1954a,b; Gaddum, 1953b), and thirdly, Marrazzi and Hart's (1955) finding that substances with psychotomimetic activity seemed to have the common property of impeding ganglionic transmission. Whether 5-hydroxytryptamine has any function in the central nervous system or not is still in dispute, but experimental evidence strongly suggests that it may have.

Gaddum /

Gaddum (1953b) observed that lysergic acid diethylamide was a highly potent and specific 5-hydroxytryptamine antagonist on the isolated rat uterus and he suggested that the hallucinogenic action of lysergic acid diethylamide might be due to interference with the action of 5-hydroxytryptamine in the brain. He pointed out (1957b) that it was difficult to find definite evidence for or against this theory. Woolley and Shaw (1954a) have suggested that substances which antagonized 5-hydroxytryptamine caused "mental aberrations". Gaddum and Vogt (1956) found that the depressant action of 5-hydroxytryptamine in the brain of cats was antagonized by lysergic acid diethylamide (Fig. 3, page 20), ergometrine, morphine, methadone and amphetamine, but 2-bromo-lysergic acid diethylamide (Fig. 3, page 20), 5-benzyloxygramine or 1,2-dimethyl-3-ethyl-5-dimethylamino indole (methyl medmain, Fig. 3, page 20) were without such an effect although they showed strong antagonism in peripheral tissues. They therefore concluded that the central actions were probably unrelated to the specific antagonism of 5-hydroxytryptamine by lysergic acid diethylamide on peripheral tissues. Vogt (1957) has postulated that the antagonistic effect of 5-hydroxytryptamine and lysergic acid diethylamide on behaviour depends on the selective sensitisation or inhibition of a characteristic group of centres by each drug and not on simple interaction /

Fig. 3.

interaction by competition for the same receptors within the brain. Marrazzi and Hart (1955) from the study of cerebral synaptic transmission using the transcallosal preparation of the optic cortex of the cat, found that 5-hydroxytryptamine, like adrenaline and nor-adrenaline, blocked synaptic transmission. It was actually the most active of the synaptic inhibitors tested. Gluckman, Hart and Marrazzi (1957) reported that the cerebral synaptic inhibitory activity of 5-hydroxytryptamine was more powerful than that of adrenaline.

Receptor Theory

The relationship between chemical structure and biological activity has always been a topic of much interest and has long been the subject of extensive chemical and pharmacological investigation.

Compounds exhibiting biological activity may conveniently be divided into two classes, the structurally specific and the structurally non-specific (Beckett and Casy, 1955; Ing, 1959), but there is no hard and fast line of demarcation between these groups - one group merging into the other by way of compounds exhibiting intermediary properties (Martin-Smith and Reid, 1959). It is clear that certain drugs owe their pharmacological properties to the /

the possession of chemical groupings which enable them to react with receptors. The idea that drugs exert their action by interacting with certain receptors in the tissues has been widely accepted.

The receptor theory in its original form is due to Ehrlich.

Ehrlich's theory of the presence of reactive chemical groupings in cells and of similar reactive groupings in the molecules of drugs was a great advance and much of the present-day receptor theory is based upon a modified form of it. Ehrlich and Morgenroth (1910) defined a receptor as; "that combining of the protoplasmic molecule to which a foreign group, when introduced, attaches itself".

Ehrlich postulated that receptors were small, chemically defined areas which gave a biological response upon uniting with certain chemically complementary areas of natural or foreign molecules.

Histamine and antihistamine antagonism and the antagonism between acetylcholine and atropine are good examples of specific drug effects. But the actions of volatile anaesthetics such as ether and chloroform are not due to the possession of specific chemical groupings; they are due to their power of penetration into the central nervous system, and they produce their anaesthetic effects when a certain threshold concentration is reached. The ability which structurally non-specific substances possess of accumulating in certain cells is due to the possession of certain physicochemical properties. /

properties. The theory of Overton and Meyor (1901) of the mode of action of general anaesthetics may be regarded as the first reasonably substantiated physicochemical theory of drug action. The Overton and Meyor concept of narcotic activity was based upon the observation that many narcotics were more soluble in oils and lipids than in water. It states that there is a direct parallelism between the affinity of an anaesthetic for lipid and its depressant action upon the central nervous system. This concept has been formulated as a ratio known as the oil-water partition coefficient. Traube (1904) correlated narcotic potency with the ability to lower surface tension at an air-water interface. This property of anaesthetics may account for the accumulation of drugs on cell surfaces, but does not explain their depressant action. In many of these investigations, the concentrations were measured either in solution or in the surrounding atmosphere, so that they were in no case measured in the centres directly affected. In 1939, Ferguson introduced a method of comparison which eliminated entirely the disturbing effect of phase distribution. Instead of measuring concentrations, the chemical potentials of the substance in a phase which is in equilibrium with that phase which is the seat of the pharmacological action, are calculated. According to Ferguson, if an equilibrium exists, the chemical potential of the toxic substance must be the same in all the phases partaking in the equilibrium. It seems that /

that compounds which are completely non-specific exert their biological activity by virtue of favourable physical properties according to Ferguson's principle and their biological activity is independent of their functional groups and stereochemistry.

Compounds acting by structurally specific mechanisms are considered to interact with hypothetical receptors forming a drug-receptor complex. Very little is known concerning the intimate nature of drug receptors and indeed they may not be physically discrete entities. However, a number of attempts have been made to deduce their shape and electrical charge distribution in the belief that these characteristics exist. Van Rossum and Ariëns (1957) consider drug-receptor interaction to involve a general interaction of fields of force in which electrostatic and van der Waal's forces play a dominant role. Nothing is said, however, of the nature of the receptor. Other workers regard the receptor as a volume in space which is defined by the surface of enzymes, co-enzymes and metallic ions and so they think that drugs exert their actions primarily on enzyme systems (Martin-Smith and Reid, 1959). Although it is well-established that many drugs act by interfering with enzymatic process (e.g., anticholinesterases, monoamine oxidase inhibitors, etc.), it is dangerous to create the generalisation that all drugs do so. For instance, Katz (1956) believes that /

that neuromuscular blocking agents exert their actions by a non-enzymatic process although Welsh (1949) and Drill (1958) suggest that an enzyme is involved. Another school of thought pictures the receptor as a physical unit possessing shape and electric charges complementary to those of the most active drug molecule. Examples of this approach are afforded by the work of Lands (1951); Long, Luduena, Tullar and Lands (1956); Beckett (1956); Beckett, Casy, Harper and Phillips, (1956); Beckett, Casy and Harper (1956); and Beckett, Harper, Clitherow and Lesser (1961) on the muscarinic receptor and on the morphine receptors.

Another point of view is the "induced fit" theory in which it is postulated that the receptor changes its characteristics at the demand of a drug molecule (Koshland, 1958). Recently, Waser (1960) has suggested that the receptor sites have a definite three dimensional structure and described a model of the cholinergic receptor.

The picture is somewhat complicated, however, by the fact that there are at least three sites at which the metabolite may be involved and the analogue could conceivably act at only one /

one of these or at any combination of them. Firstly, the analogue could act by releasing the metabolite from its bound inactive form. Secondly, the metabolite may exert its action at the receptor site proper. Thirdly, there is the site at which the metabolite is destroyed. When there is more than one site of action, it is possible that the receptors involved in each case may be somewhat different in character. For example, Gaddum and Hameed, 1954, and Gaddum and Picarelli, 1957, postulated that there were two receptors for tryptamine in the guinea pig ileum. Such variations in the nature of receptors could create different values for the intrinsic activity and the affinity of the antimetabolite at each site. In such cases, the antimetabolite might mimic its natural analogue at one site, inhibit it at another and act in a totally different way at a third. A new compound designed as an analogue of a specific metabolite might exert a completely unexpected form of biological activity due to its ability to act upon a totally different set of receptors in the organism.

The intentional design of drug molecules capable of acting as antimetabolites is now a well established procedure and one of the most successful approaches so far, is the preparation of compounds /

compounds bearing an isosteric relationship to the metabolite in question. This requires substitution of one atom or group of atoms in the parent compound for another with a similar electronic and steric configuration. Such isosteres will have similar steric and electronic properties and possess molecular weights of the same order of magnitude and so can be expected to have very similar solubilities. Since 1919, when Langmuir first introduced the term isosterism, the concept has been developed and expanded by the work of many others as well as by the formulation of new ideas of structure and reactivity. Erlenmeyer and Berger (1932) and Erlenmeyer, Berger and Leo (1933) demonstrated the serological similarities of various isosteric atoms. They showed that the isosteres, 4-aminodiphenylamine and 4-aminodiphenyl ether had similar antigenic activity. It is from this early work that the concept of bioisosterism has arisen. The idea that isosteres should possess similar biological properties to those of their natural analogues is inherent in the term "bioisosterism" introduced by Friedman (1951). This term covers the case where an isostere opposes the action of a metabolite as well as that where it mimics or intensifies it, depending upon the intrinsic activity and the affinity of the isosteres for the receptors.

5-Hydroxytryptamine receptors

Rocha/

Rocha e Silva, Valle and Picarelli (1953) studied the actions of 5-hydroxytryptamine on the isolated guinea pig ileum and compared them with those of adrenaline, pilocarpine, hexamethonium, tubocurarine, histamine and acetylcholine. They showed that 5-hydroxytryptamine acted on receptors which were distinct from those for histamine, acetylcholine and pilocarpine. They suggested that 5-hydroxytryptamine acted upon the post-ganglionic cholinergic fibres of the intramural nervous system of the guinea pig ileum. These findings provide a basis for the possible existence of pharmacologically different 5-hydroxytryptamine receptor sites in different organs.

5-hydroxytryptamine receptors, like other tissue receptors, can be investigated either by using a variety of antagonists or by employing stimulant drugs structurally related to the agonist. Knowledge of the properties of 5-hydroxytryptamine receptors and their blockade is based upon the study of simple isolated tissue preparations such as the rat uterus, guinea pig ileum, rabbit ear vessels and the rat fundus strip. Gaddum and Hameed (1954) suggested the existence of two kinds of receptors; one in the plain muscle of the rat uterus and the rabbit ear vessels, which was easily blocked by lysergic acid diethylamide, gramine or dihydroergotamine and another in the intestine of the guinea pig which was not easily blocked by these drugs. Gaddum and Picarelli (1957) continued /

continued this investigation and demonstrated that there were two kinds of tryptamine receptors in the guinea pig ileum, namely the M-receptors which could be blocked with morphine, atropine, cocaine and methadone and the D-receptors which could be blocked with dibenzylamine, lysergic acid diethylamide, dihydroergotamine and 5-benzyloxytryptamine. These workers suggested that the M-receptors were probably in the nervous tissues and the D-receptors were probably in the muscular tissues of the ileum. Trendelenburg (1957) has presented evidence for the presence of 5-hydroxytryptamine receptors in the superior cervical ganglion of the cat. Trendelenburg (1960) has also studied the effects of 5-hydroxytryptamine and histamine on the isolated atria of the cat, rabbit and guinea pig in order to investigate the presence of 5-hydroxytryptamine receptor sites. He found that lysergic acid diethylamide blocked the direct stimulant response to 5-hydroxytryptamine on the isolated cat atria and suggested that the atrial receptors for 5-hydroxytryptamine were similar to the D-receptors of the guinea pig ileum. Hertzler (1961) from experiments upon rat sympathetic ganglia, has demonstrated that low concentrations of 5-hydroxytryptamine ($1.3 \times 10^{-6} M$) reduced the threshold of the post-ganglionic response to pre-ganglionic stimulation and caused an increase in the amplitude of the post-ganglionic response evoked /

evoked by submaximal preganglionic stimulation. 5-hydroxytryptamine had no effect on direct (non-synaptic) nerve fibre transmission. Hertzler (1961) suggested that 5-hydroxytryptamine facilitated synaptic transmission in the sympathetic ganglia of the rat. Bindler and Gyermek (1961) studied the effects of various antagonists of 5-hydroxytryptamine on the inferior mesenteric ganglion of the anaesthetised cat. Although the results of these experiments indicated the existence of specific 5-hydroxytryptamine receptor sites in the sympathetic ganglia, these receptors did not in this case seem to be the same as the M-receptors of the guinea pig ileum.

The nature of the 5-hydroxytryptamine receptors can also be analysed by using structural analogues of 5-hydroxytryptamine. Barlow and Khan (1959c) found that the actions of analogues of 5-hydroxytryptamine on the dibenzylene-sensitive receptors of the guinea pig ileum resembled their actions on the rat uterus and the actions on the morphine-sensitive receptors slightly resembled those on the rat fundus strip. Vane (1959) reported that monoamine oxidase inhibitors, e.g., iproniazid, potentiated the actions of tryptamine and many of its analogues on the isolated rat fundus preparation, but not the action of 5-hydroxytryptamine. This observation /

observation suggested that the tryptamine entered the cells and the 5-hydroxytryptamine did not.

Stimulant analogues of 5-hydroxytryptamine

Tryptamine and 5-hydroxytryptamine have been shown to have similar pharmacological properties except that tryptamine failed to release adrenaline from the adrenal medulla when injected into the arterial supply of the suprarenal gland. Both these substances caused contraction of the isolated guinea pig intestine and rat uterus and caused a rise in the systemic arterial blood pressure in the cat which was reduced by yohimbine (Reid, 1951; Reid and Rand, 1951). This effect of yohimbine in reducing the pressor response to tryptamine was observed as early as 1941 by Raymond-Hamet. Because tryptamine resembles 5-hydroxytryptamine in several pharmacological tests (Gaddum, 1953a; and Page, 1952) the view arose that the two chemically related compounds acted on the same receptors. But Woolley and Shaw (1953a) showed that in the sheep carotid artery ring test, the tryptamine receptors could be destroyed while the 5-hydroxytryptamine receptors remained. In experiments on the blood pressure of anaesthetised cats, Woolley and Shaw (1957b) obtained further evidence that 5-hydroxytryptamine and tryptamine acted on separate receptors. Two substances, 1-benzyl-2, 5-dimethyl- /

5-dimethyl-5-hydroxytryptamine (BAS) and 1-benzyl-2-methyl-5-hydroxytryptamine (BAS-phenol), blocked the pressor effects of 5-hydroxytryptamine in the anaesthetised dog without affecting the response to tryptamine. Vane (1959) has reported that mono-amine oxidase inhibitors potentiated the actions of tryptamine and many of its analogues on the isolated rat fundus strip preparation but had no effect on the actions of 5-hydroxytryptamine or other hydroxytryptamines and suggested that in isolated organs the mono-amine oxidase was unable to inactivate 5-hydroxytryptamine but could inactivate tryptamine, 5-methoxytryptamine and many other related compounds. He supposed that tryptamine entered the cell whereas 5-hydroxytryptamine and the other compounds could not do so because of the presence of a polar hydroxyl group in the molecule.

Chen and Chen (1933) have shown that N-trimethyl tryptamine was very active in raising the blood pressure of the decerebrate cat and N-methyl tryptamine was less active.

N-dimethyl-5-hydroxytryptamine (bufotenine) has been studied by various workers. According to Erspamer (1954), the stimulant activity of bufotenine is less than that of 5-hydroxytryptamine on the rat uterus. Gaddum and his associates (1955) have compared the activity of bufotenine and cinobufotenine with that of 5-hydroxytryptamine /

5-hydroxytryptamine on the rat uterus and on the guinea pig ileum and found that they were less active than 5-hydroxytryptamine. Raymond-Hamet (1943) has shown that the pressor effect of cinobufotenine in dogs was blocked by yohimbine. It has been reported (Fabing and Hawkins, 1956) that hallucinations were caused in man by bufotenine. Evarts, Landau, Freygang and Marshall (1955) have reported that in the monkey, intravenous injection of bufotenine and lysergic acid diethylamide produced a syndrome characterised by a decrease or absence of the response to visual stimuli without any gross effect upon muscular power. Szara (1956, 1957) has shown that N,N-dimethyl tryptamine and N,N-diethyltryptamine produce psychosis-like effects in man somewhat similar to those caused by mescaline and lysergic acid diethylamide. The most outstanding difference was in the onset of symptoms. In the case of the tryptamine derivatives, the onset was quicker than when the other two substances were used. Both the derivatives of tryptamine caused choreiform athetoid movements. The psychosis-like effect of the tryptamine derivatives supports the idea that schizophrenia may be the result of abnormal metabolism of indolic compounds in the body.

Very recently, Bertaccini and Zamboni (1961), who studied 78 analogues of tryptamine and 5-hydroxytryptamine, have reported that 5-hydroxy-N-methyltryptamine was a very potent stimulant compound but was /

was weaker than 5-hydroxytryptamine on the isolated guinea pig ileum and rat uterus.

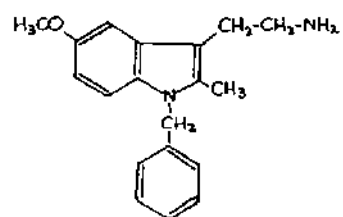
The simplest true indole alkaloid is gramine. In 1932 and 1933, Euler and Hellström["] discovered the presence of an indole alkaloid during an investigation of albino mutants of barley and isolated it in a crystalline form. Later, however, it was detected in normal barley and named gramine by Euler, Hellström and Löfgren (1935). This compound was first synthesized by Wieland and Hsing (1936) who established that it was 3-dimethyl aminoindole. The pharmacological properties of gramine were studied by Supniewski and Serafinowna (1937) who reported that in small doses it excited the central nervous system (causing clonic convulsions and stimulation of the respiratory centre); large doses caused paralysis in mammals with death apparently due to respiratory failure. There was a lowering of the blood pressure due to a depressant action on the heart. The depressant effect on the isolated frog heart was antagonised by atropine. Very dilute solutions (1 in 20,000) produced contractions of the isolated uterus which were suppressed by atropine. Raymond-Hamet (1937) has demonstrated variable responses to gramine on the blood pressure of the anaesthetised cat. Powell and Chen (1945) showed that small doses of gramine raised the blood pressure in the anaesthetised cat but /

but larger doses (30 to 40 mg./kg.) lowered it, causing, however, a secondary rise. In general, it stimulated smooth muscle, but the tonus and movements of the rabbit intestine were reduced and inhibited. It also reduced the effect of adrenaline on the blood pressure, on intestinal movements and on contractions of the isolated uterus. Apart from the synthetic derivatives of gramine which behave as 5-hydroxytryptamine antagonists, others, including 3-(piperidyl-(N)-methyl)-indole and related compounds have proved to be oxytocics (De Jongh and Proosdij-Hartzema, 1952). It has been shown that some of the gramine derivatives possess local anaesthetic properties (Erdtman and Löfgren, 1937).

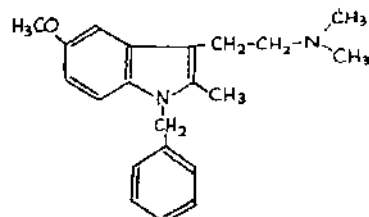
Pharmacological antagonists of 5-hydroxytryptamine

In attempts to understand the precise role of 5-hydroxytryptamine, a large number of 5-hydroxytryptamine antagonists have been synthesized during the past few years. However, none of the antagonists so far available has been of value in the treatment of patients with malignant 5-hydroxytryptamine-producing carcinoids, although such compounds are active on isolated tissue preparations where they show a high specificity against 5-hydroxytryptamine. Of the potential antimetabolites of 5-hydroxytryptamine many have been simple synthetic indole derivatives, usually derivatives of 5-hydroxytryptamine, /

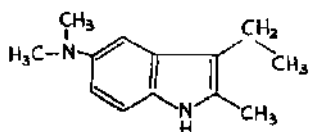
5-hydroxytryptamine, tryptamine or gramine. It is of considerable theoretical interest that these synthetic derivatives may show different types of activity on different preparations or on the same preparation when administered at different concentrations. The first synthetic antimetabolite was prepared by Woolley and Shaw (1952a) who reported that 2-methyl-3-ethyl-5-amino-indole was an antagonist of 5-hydroxytryptamine on isolated sections of the carotid arteries of sheep. Woolley and Shaw (1952b) showed that this compound, when it was fed to dogs in 500 mg. daily doses for four days, prevented the pressor response to injected 5-hydroxytryptamine but Spies and Stone (1952) and Page and McCubbin (1953) failed to demonstrate any change in the blood pressure of hypotensive patients with this compound. Woolley and Shaw (1953a) reported that 3-(β -dimethylaminoethyl)-5-aminoindole, like 2-methyl-3-ethyl-5-dimethylaminoindole (medmain, Fig. 4, page 37) had both 5-hydroxytryptamine-like and anti-5-hydroxytryptaminic activity on the isolated rat uterus. Shaw and Woolley (1954) showed that 2-methyl-3-ethyl-5-dimethylaminoindole (medmain) was a very active antagonist to 5-hydroxytryptamine on the carotid artery ring and isolated rat uterus. On the rat uterus at higher concentrations than those required to block the action of 5-hydroxytryptamine, medmain itself caused stimulation and the stimulant action usually took /



1-benzyl-2,5-dimethyl-
serotonin (BAS)



1-benzyl-2,5-dimethyl
bufotenine (BAB)



2-methyl-3-ethyl-5-dimethyl-
amino indole (medmain)

Fig. 4.

took the form of repeated contractions which persisted over a period even after repeated washings. Medmain, although highly active on these isolated tissue preparations, was found to be incapable of protecting normal dogs from the pressor effect of 5-hydroxytryptamine. Medmain also caused convulsions in mice when injected intraperitoneally. It was suggested that these convulsions might be due to 5-hydroxytryptamine-like effects in the central nervous system. Neither 5-hydroxytryptamine nor its methyl ether were found to be capable of preventing this convulsant action. The 5-hydroxytryptamine-like action of medmain on the uterus was antagonised by other anti-5-hydroxytryptamines such as 2-methyl-3-ethyl-5-aminoindole and 2-methyl-3-ethyl-5-hydroxyindole. Methyl medmain did not have any stimulating action on the rat uterus. Shaw and Woolley (1956a) reported that 2,5-dimethyl-5-hydroxytryptamine was a potent antagonist to the action of 5-hydroxytryptamine on the isolated rat uterus, artery ring and on the blood pressure of the anaesthetised dog.

Substitution of methyl groups in the 2 and 5 positions in 5-hydroxytryptamine yielded antagonists to 5-hydroxytryptamine, but substitution of methyl groups in the 1 and 5 positions gave 5-hydroxytryptamine-like substances. Thus, whereas 1,5-dimethyl-5-hydroxytryptamine showed /

showed a considerable amount of 5-hydroxytryptamine-like activity on the rat uterus and the benzyl compound exerted an irreversible antagonism on this tissue, 1-benzyl-2, 5-dimethyl-5-hydroxytryptamine (BAS, Fig. 4, page 37) was highly active against the pressor action of 5-hydroxytryptamine when fed to dogs. Wilkins (1956) reported that in hypertensive patients, the effects of 1-benzyl-2, 5-dimethyl-5-hydroxytryptamine were similar to those of reserpine. Wilkins and Hollander (1957) found that this substance alone, or in combination with other drugs, reduced to some extent the blood pressure of hypertensive patients.

Shaw and Woolley (1956b) have shown that the benzyl analogue of bufotenin, 1-benzyl-2, 5-dimethylbufotenin (BAB, Fig. 4, page 37), was a potent antagonist of 5-hydroxytryptamine on the isolated rat uterus and on the pressor effect of 5-hydroxytryptamine in the dog, even when given by the oral route. Quadbeck and Röhm (1954), have reported that there was a marked increase in the antagonistic potency when a di-methylamino group was introduced into the 3-position and an even greater one when it was introduced into the 5-position. The most active compound was 2-methyl-5-chloro-gramine. Other simple, synthetic antagonists of 5-hydroxytryptamine were tested by Erspamer (1955) who reported that the anti-5-hydroxytryptamine activity of some gramine derivatives appeared to be very conspicuous /

conspicuous when tested in vitro on the rat uterus preparation, but the same activity was negligible when tested in vivo, using the 5-hydroxytryptamine antidiuresis test in hydrated rats. He suggested that these drugs were rapidly destroyed in the body. Erspamer (1954a) and Gaddum and Hameed (1954) have shown that gramine itself was a specific antagonist to 5-hydroxytryptamine. Gaddum, Hameed, Hathway and Stephens (1955) studied a number of synthetic indole derivatives for their anti-5-hydroxytryptamine action on the isolated rat uterus. The most active compounds were 5- and 6-benzyloxygramine. The antagonistic effect of these compounds to 5-hydroxytryptamine developed slowly and became irreversible and the blockade could not be overcome by high doses of 5-hydroxytryptamine. They termed this type of antagonism "unsurmountable". The other drugs, for example the gramine derivatives with methyl groups in positions 1, 4, 5 or 6 and 2-methyl-3-ethyl-5-amino indole were less active. The blockade which they caused, developed more rapidly and was reversible and surmountable. It was also reported that indole derivatives with methyl groups in positions 1, 4, 5, 6 or 7, some methyl-3-indolylacetonitriles and certain carbazole derivatives were feeble antagonists to 5-hydroxytryptamine. Barlow and Khan (1959a) have reported that other tryptamine derivatives showed in low concentrations, antagonism to the 5-hydroxytryptamine-induced stimulation of the rat uterus and rat fundus strip. In higher concentrations these compounds themselves stimulated the tissue. In continuation of the search for substances /

substances which antagonised 5-hydroxytryptamine and 5-hydroxytryptamine-like compounds, Barlow and Khan (1959b) prepared and studied the actions of a number of analogues of 5-hydroxytryptamine and observed that 3-(2-aminopropyl)-5-benzyloxyindole and 5-benzyloxy-3-(2-dimethylaminoethyl) indole were the most active antagonists on the isolated rat uterus and rat fundus respectively. Another analogue, however, 3-(2-aminopropyl)-5-hydroxyindole showed a powerful stimulant action on both of these tissues. Woolley and Shaw (1957b) showed that dimethylaminomethyl tetrahydrocarbazole and tetrahydrocarbazole-N-phenylcarboxamidine when given intravenously to dogs, were quite effective in preventing the pressor response to 5-hydroxytryptamine. Woolley and Shaw (1957b) and Barlow and Khan (1959a,b) found substances which antagonised the effects of 5-hydroxytryptamine more than tryptamine, and this led them to suggest that there may be separate tryptamine and 5-hydroxytryptamine receptors. However, from experiments on the guinea pig ileum, Barlow and Khan (1959c) have confirmed that on this tissue, tryptamine and 5-hydroxytryptamine act on the same receptors.

Gyermek (1955) and Gyermek, Lázár and Csák (1956) found that the anti-5-hydroxytryptamine properties of chlorpromazine, phenergan and diparcol ran in parallel with their sedative effects. Berger, Campbell, Hendley, Ludwig and Lynes (1957) have reported that chlorpromazine, /

chlorpromazine, reserpine and benactyzine powerfully antagonised contractions produced by 5-hydroxytryptamine or acetylcholine on the isolated rat colon. Phillippot and Dallemagne (1956) have shown that 5-hydroxytryptamine re-establishes neuromuscular transmission in cats when this had been blocked by tubocurarine and suggested that tubocurarine might be an antagonist to 5-hydroxytryptamine.

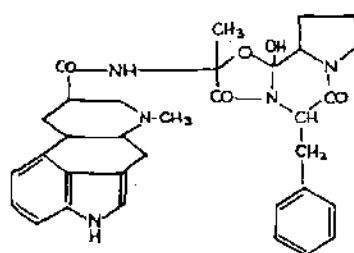
In addition, several of the more complex indole derivatives have been postulated to act in part at least, by interference with the metabolism of 5-hydroxytryptamine. Thus Woolley and Shaw (1954a) and Gaddum (1953b) have suggested that the central actions of lysergic acid diethylamide might be due to interference with the functioning of 5-hydroxytryptamine in the brain and could result from similarities in the chemical structure of the two compounds. In support of this contention, Gaddum and Hameed (1954) and Sollero, Page and Salmoiraghi (1956) were able to show that lysergic acid diethylamide specifically antagonised the actions of 5-hydroxytryptamine on the rabbit ear and on the rat uterus. On the guinea pig ileum, however, lysergic acid diethylamide in both high and low concentrations reduced the response to 5-hydroxytryptamine by only 50 per cent. This finding led them to suggest the existence of two sets /

sets of receptors for 5-hydroxytryptamine in the guinea pig ileum. Savini (1956) has shown that in low concentrations (1 μ g. per ml.), lysergic acid diethylamide, 2-bromo lysergic acid diethylamide and ergometrine antagonised the vasoconstrictor action of 5-hydroxytryptamine and in higher concentrations these drugs (except 2-bromo-lysergic acid diethylamide) caused direct vasoconstriction of the blood vessels of the perfused rabbit ear. Cerletti and Konzett (1956) have reported that among the derivatives of lysergic acid diethylamide, acetyl and bromo-lysergic acid diethylamide were the most active compounds. Recently Fanchamps, Doepfner, Weidmann and Cerletti (1960) have shown that the 1-methyl derivative of d-lysergic acid butanolamide (Deseril) was four times more potent than lysergic acid diethylamide in antagonizing the contractions produced by 5-hydroxytryptamine on the isolated rat uterus. Its antagonism to 5-hydroxytryptamine has also been demonstrated in studies of the cardiovascular system. Fanchamps and his associates regarded the antagonism as highly specific.

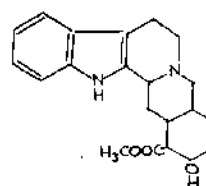
Rothlin (1957) has reviewed in detail the role of lysergic acid diethylamide and its derivatives in the production of mental disturbances. Considerable doubt as to the validity of the theories of lysergic acid diethylamide interference with brain 5-hydroxytryptamine /

5-hydroxytryptamine has recently been voiced and the view has been put forward that the psychological effects of lysergic acid diethylamide do not result from a simple antagonism to 5-hydroxytryptamine (Zanowiak and Rodman, 1959). It has been concluded that other derivatives of lysergic acid, including certain ergot alkaloids, have the ability to antagonise certain actions of 5-hydroxytryptamine. As early as 1932, before the successful isolation and identification of 5-hydroxytryptamine, Heymans, Bouckaert and Moraes found that ergotamine antagonised the vasoconstrictor action of defibrinated blood, an observation which can now be interpreted as being due to antagonism to 5-hydroxytryptamine.

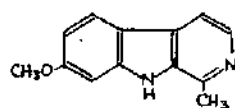
Shaw and Woolley (1953b) found that yohimbine (Fig. 5, page 45), ergotamine (Fig. 5, page 45), and ergotoxine antagonised the effect of 5-hydroxytryptamine on carotid artery rings. Savini (1956) reported that ergotamine antagonised the constrictor effect of 5-hydroxytryptamine on the isolated rabbit ear preparation. Rothlin (1957) maintained that ergometrine possessed anti-5-hydroxytryptamine-like properties because when given by the intraventricular route, it antagonised the depressant effect of 5-hydroxytryptamine in the same way as lysergic acid diethylamide. This simple comparison, however, cannot be the full explanation because /



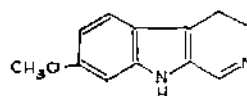
ergotamine



Yohimbine



Harmine



Harmaline

because ergometrine is reported to have no psychogenic properties at all.

Erspaner (1953) and Fingl and Gaddum (1953) have demonstrated the anti-5-hydroxytryptamine action of dibenamine on the rat uterus and on the antidiuretic effect of 5-hydroxytryptamine in hydrated rats. Gaddum and Hameed (1954) found that dibenamine blocked the action of 5-hydroxytryptamine on the isolated rat uterus much more effectively than on the isolated rabbit ear and guinea pig ileum. Furchgott (1954) from his studies on strips of rabbit aorta, suggested that dibenamine exerted its blocking action by reacting, in an essentially irreversible manner, with the free receptors involved in the action of the stimulant drugs, 5-hydroxytryptamine, tryptamine, histamine and acetylcholine. Another anti-adrenaline drug, dibenzylene, has been shown by Gaddum and Picarelli (1957) to block one set of 5-hydroxytryptamine receptors in the guinea pig ileum more or less permanently.

The alkaloids harmaline (Fig. 5, page 45) and harmine (Fig. 5, page 45) also possess the power of antagonising the actions of 5-hydroxytryptamine (Woolley and Shaw, 1957a).

The structural resemblance between histamine, tryptamine and 5-hydroxytryptamine /

5-hydroxytryptamine led Rapport and Koelle (1952) to investigate the blockade of the action of 5-hydroxytryptamine by using antihistaminic agents. Since then a number of workers have investigated the anti-5-hydroxytryptaminic activity of many antihistaminic drugs. For example, Stone, Wenger, Ludden, Stavorski and Ross (1961) recently have reported that 1-methyl-4-5-(dibenzo-(a, e) cycloheptatrienyli-
dene)-piperidine hydrochloride (cyproheptadine) antagonised the pressor effect of 5-hydroxytryptamine in the anaesthetised "ganglion blocked" (0.4 mg. per kg. of 4,5,6,7-tetrachloro-2-(2-dimethylaminoethyl)-indole bimethochloride) dog. The stimulation caused by 5-hydroxytryptamine on the isolated rat uterus was also antagonised by cyproheptadine. It is interesting to note that little or no inhibition occurred when the antagonist was present along with 5-hydroxytryptamine in the organ bath. After its removal by washing, the inhibitory activity gradually appeared and became more intensified with each subsequent response to 5-hydroxytryptamine and this suggested that the substance may interfere with the contractile mechanism in a non-specific manner.

The antagonistic effect of atropine on the contraction produced by 5-hydroxytryptamine on the guinea pig ileum has been reported by many workers (Robertson, 1953; Rocha e Silva and his associates, 1953; /

1953; Cambridge and Holgate, 1955; and Rapport and Koelle, 1952). On the isolated guinea pig lung, atropine was found to be a very feeble antagonist to 5-hydroxytryptamine (Bhattacharya, 1955). In the anaesthetised guinea pig and cat, atropine diminishes the bronchoconstrictor effect of 5-hydroxytryptamine. Although on the guinea pig ileum atropine in its strong inhibitory actions shows similarities to cocaine and morphine, on the superior cervical and inferior mesenteric ganglia of the cat and in the pelvic nerve bladder preparations of the dog, atropine was found to be inactive against the stimulant action of 5-hydroxytryptamine (Trendelenburg, 1956; Gyermek, 1960; and Bindler and Gyermek, 1961).

Two other complex indole alkaloids found in various species of Rauwolfia, reserpine and rescinnamine, are of interest in that their tranquillising properties have been postulated to arise from the displacement of 5-hydroxytryptamine in the brain (Shore, Pletscher, Tomich, Carlsson, Kuntzman and Brodie, 1957). But Carlsson, Rosengren, Bertler, and Nilsson (1957) and Shore and Brodie (1957) have given evidence that reserpine also causes a long-lasting depletion of noradrenaline from the brain. Carlsson suggested that the lack of free 5-hydroxytryptamine and noradrenaline had to be /

be considered as the cause of some of the pharmacological effects of reserpine.

Specificity of action of 5-hydroxytryptamine antagonists

The use of isolated organs for determining the specificity of 5-hydroxytryptamine antagonists is limited by the fact that only a few stimulant drugs can be tested on the same piece of tissue. From studies on the isolated rat uterus, Gaddum and Hameed (1954), Gaddum and his associates (1955) and Barlow and Khan (1959a) considered that the anti-5-hydroxytryptamine action was specific, if no blockade of the stimulant response of the tissue to acetylcholine or carbachol occurred. In experiments on the isolated guinea pig ileum, histamine was used as the control substance (Gaddum and his associates, 1955). Most of the indole compounds tested have shown either lack of specificity or have produced, besides antagonism to 5-hydroxytryptamine, contraction of the uterus itself. Cerletti and Doepfner (1958) have studied the degree of specificity of different ergot alkaloids on the isolated rat uterus by using 5-hydroxytryptamine and acetylcholine as stimulant drugs. Many of the ergot alkaloids investigated were found to be specific antagonists of 5-hydroxytryptamine. Although acetylcholine was found to be one of the most potent of the compounds which /

which antagonised the vasoconstriction of the perfused hind legs of the rabbit produced by 5-hydroxytryptamine, its effect was non-specific (Meier, Tripod and Wirz, 1957). Acetylcholine antagonised the vasoconstriction produced by adrenaline, noradrenaline, barium chloride and histamine, thus showing no specificity towards 5-hydroxytryptamine. Chlorpromazine, which was a potent antagonist of 5-hydroxytryptamine was postulated to be non-specific, since it also had a strong blocking action against histamine and a less marked action against noradrenaline.

Lysergic acid diethylamide was, however, more specific than chlorpromazine since it had only slight adrenergic blocking and antihistaminic actions.

Trendelenburg (1957) and Bindler and Gyermek (1961) reported that morphine and cocaine showed considerable specificity towards the action of 5-hydroxytryptamine on the superior cervical and inferior mesenteric ganglia of the cat. Trendelenburg (1957) observed that contraction of the smooth muscle of the nictitating membrane of the cat produced by 5-hydroxytryptamine was not inhibited by morphine. Similarly, Gaddum (1957a) noticed that the vasoconstrictor effect on the perfused rabbit ear and the stimulant response of the isolated rat uterus produced by 5-hydroxytryptamine were not inhibited by morphine. Morphine, however, antagonised the effect of 5-hydroxytryptamine on the M-receptors of the guinea pig ileum.

Gyermek /

Gyermek (1961) has reported that "in contrast to lysergic acid diethylamide, which seems to be a relatively poor and non-specific 5-hydroxytryptamine antagonist at autonomic ganglia, some indole compounds were extremely potent and selective inhibitors of 5-hydroxytryptamine receptors in nervous tissue; on isolated smooth muscles these compounds showed only very low potency".

These examples show the different degrees of specificity of 5-hydroxytryptamine antagonists and indicate that the receptors for 5-hydroxytryptamine vary from organ to organ.

An antimetabolite may show strong antagonistic effects when tested at one range of concentrations but may act in place of the metabolite at a higher range of concentrations. This situation is seen even though the testing is done using the same organism or tissue preparation and hence it is not to be explained as being due to a variation among species. Woolley (1946) has reported for example that at different concentrations, the para-nitrobenzyl ether of N-acetyl-diiodotyrosine, a substance related chemically and pharmacologically to thyroxine, showed both thyroxine-like and anti-thyroxine activity on the metamorphosis of the tadpole.

Woolley and Shaw (1953a) have reported that 3-(β -dimethyl-aminoethyl)- /

aminoethyl)-5-amino indole showed both pro- and anti-5-hydroxytryptamine activity on the isolated sheep carotid artery ring preparation. This compound showed an anti-5-hydroxytryptamine activity at relatively high concentrations and 5-hydroxytryptamine-like activity at somewhat lower concentrations. The exhibition of both kinds of action (pro-metabolite and anti-metabolite) by a single substance has been previously observed on bacteria with compounds related to pantothenic acid (Pollack, 1943). There are a number of interesting examples of this kind of dualistic action in connection with antimetabolites of 5-hydroxytryptamine. Shaw and Woolley (1954) have reported that at lower concentrations, 2-methyl-3-ethyl-5-(N-pyrrolidyl)-indole showed 5-hydroxytryptamine-like activity on the isolated carotid artery ring and at higher concentrations, this compound inhibited the stimulant response to 5-hydroxytryptamine. At concentrations considerably higher than those required to prevent 5-hydroxytryptamine-induced contractions of the isolated rat and guinea pig uteri, medmain (2-methyl-3-ethyl-5-dimethylaminoindole) caused a contraction. In contrast to this, on the isolated sheep carotid artery, medmain showed purely anti-5-hydroxytryptamine activity and did not cause any contraction of the tissue. The exposure of the rat uterus to relatively large amounts /

amounts of medmain made the tissue insensitive to 5-hydroxytryptamine. These facts indicated that medmain was bound to the tissue in such a way that it was not displaced readily. Gaddum and his associates (1955) reported that on the rat uterus, low concentrations of 2-methylgramine and gramine methosulphate increased the sensitivity of the tissue to 5-hydroxytryptamine; higher concentrations of these two drugs caused a contraction of the tissue. After this high dose, the tissue showed prolonged insensitivity to added 5-hydroxytryptamine. Tetrahydroharman and 2-aminocarbazole and 3-aminocarbazole, however, in concentrations of 10 μ g. per ml. caused stimulation which was not followed by insensitivity of the tissue to 5-hydroxytryptamine; higher concentrations (25 μ g. per ml.) caused no stimulation, but the tissue exhibited a marked insensitivity to 5-hydroxytryptamine. Woolley and Shaw (1957a) reported that 3-ethyl-2-methyl-5-nitroindole opposed the pressor action of 5-hydroxytryptamine on the anaesthetised dog, but mimicked the stimulant action of 5-hydroxytryptamine on the heart of the clam (Venus mercenaria). Many analogues of tryptamine and 5-hydroxytryptamine, when tested at low concentrations on the isolated rat uterus and rat fundus strip (Barlow and Khan, 1959b) showed antagonism to the stimulant response to 5-hydroxytryptamine. At higher concentrations they showed 5-hydroxytryptamine-like activity.

After /

After stimulation with these compounds, the preparations became insensitive to 5-hydroxytryptamine. Barlow and Khan (1959b) have reported that 3-(2-aminopropyl)-1-methylindole, an analogue of 5-hydroxytryptamine, exhibited very variable activity on the isolated rat uterus. At similar concentrations it antagonised the stimulant effect of 5-hydroxytryptamine in one experiment; in another it had a synergistic action with 5-hydroxytryptamine, and in the third it caused a direct contraction of the tissue itself. This is an example of the heterogeneous activity of a single analogue of 5-hydroxytryptamine.

From the examples previously mentioned, it is clear that the results of many pharmacological investigations using 5-hydroxytryptamine and its antagonists have been characterised by the lack of a qualitatively uniform response, both between different species and within the same species.

The observation that certain substituted para-aminobenzoic acid derivatives possessed both growth promoting and growth inhibiting properties in certain bacteria, the dualistic actions of certain muscle paralysing substances and the ability of certain phenylethylamines possessing both hypotensive and hypertensive properties led Ariëns (1954) to introduce the concepts of affinity and intrinsic activity. /

activity. He defined the affinity as the ability of a drug to combine with the receptor to form a drug-receptor complex and the intrinsic activity, as the power of the drug to produce a biological response once the drug-receptor complex has been formed. Ariëns (1954) showed by a mathematical treatment of drug-receptor interaction, how these properties of a drug could be represented by two rational constants. Drug-receptor interaction was regarded as being mediated by a general interaction of fields of force originating both in the drug molecule and in the tissue. Of these forces, the electrostatic and van der Waal's forces played predominant roles, and the affinity of a drug was visualised as the product of a general interaction of fields of force while the intrinsic activity was believed to be induced by the interaction of specific centres of charge intensity within the general pattern of drug-receptor interaction. Using the concepts of affinity and intrinsic activity, Van Rossum and Ariëns (1957) and Van Rossum, Ariëns and Linssen (1958) reclassified different types of pharmacological agents including neuromuscular blocking substances and parasympathomimetics and parasympatholytic drugs.

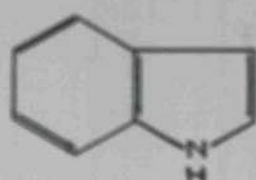
There is, however, no published information to indicate that this approach has been extended to cover drugs competing for 5-hydroxytryptamine receptors. One considerable advantage offered by /

by an approach of this type is that it gives a means of making a direct comparison of the pharmacological properties of both chemically similar and dissimilar molecules. Moreover as certain compounds both mimic and oppose the actions of 5-hydroxytryptamine depending on the concentration used, the application of the Ariëns approach to 5-hydroxytryptamine receptors would seem logical. It seemed worthwhile therefore to ascertain whether the concepts of Ariëns could be applied to the study of 5-hydroxytryptamine antagonists. This necessitated securing a suitable isolated smooth muscle preparation. Preliminary studies using the isolated guinea pig jejunum and the isolated horse carotid artery strip, indicated their lack of suitability but it was found that the rat jejunum was a suitable if not ideal preparation. Consequently qualitative experiments were carried out on this preparation using Ariëns experimental procedure as described by Van Rossum and Ariëns (1959) for the study of parasympathetic drugs.

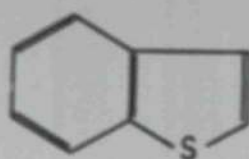
The object of research

The work described in this part of the thesis was undertaken in order to investigate the pharmacological properties of a number of biologically active thionaphthen compounds which were related to indole by replacement of the NH group of the latter by a sulphur /

sulphur atom (Fig. 6, page 58). In particular, the object was to find whether the thionaphthen isosteres were more or less specific in their actions than the corresponding indole compounds as this information might be used to obtain further information as to the existence, or otherwise, of specific receptors for the indole derivatives. Such a project seemed all the more desirable in view of the fact that the existence of at least two distinct types of 5-hydroxytryptamine receptors has been postulated. The situation is analogous to that pertaining to the adrenergic receptors where alpha, beta, gamma and delta types have been postulated (Ahlquist, 1948; McCutcheon and Ahlquist, 1959; and Furchott, 1959).



Indole



Thionaphthen

Fig. 6.

CHAPTER II

Pages 59 to 92

C H A P T E R I I

A. M A T E R I A L S

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

- | | | | |
|----|--|---------------------|--------------------------------|
| 1. | Acetylcholine chloride, | is described as | acetylcholine. |
| 2. | Atropine sulphate | " " " | atropine. |
| 3. | (-)-Adrenaline
hydrochloride | " " " | adrenaline. |
| 4. | (-)-Noradrenaline
bitartrate | " " " | noradrenaline. |
| 5. | Histamine acid phosphate | " " " | histamine. |
| 6. | 5-Hydroxytryptamine
creatinine sulphate | " " " | 5-hydroxytryptamine. |
| 7. | Mepyramine maleate | " " " | mepyramine. |
| 8. | D-lysergic acid diethyl-
amide tartrate | " " " | lysergic acid
diethylamide. |

The thionaphthen isosteres investigated in this section of the thesis together with their structural formulae and reference numbers, are shown in Figs. 7 to 10, pages 61 to 64. The compounds are divided into four main groups.

The composition and methods of preparation of all saline solutions used, are to be found in Appendix I, page 246.

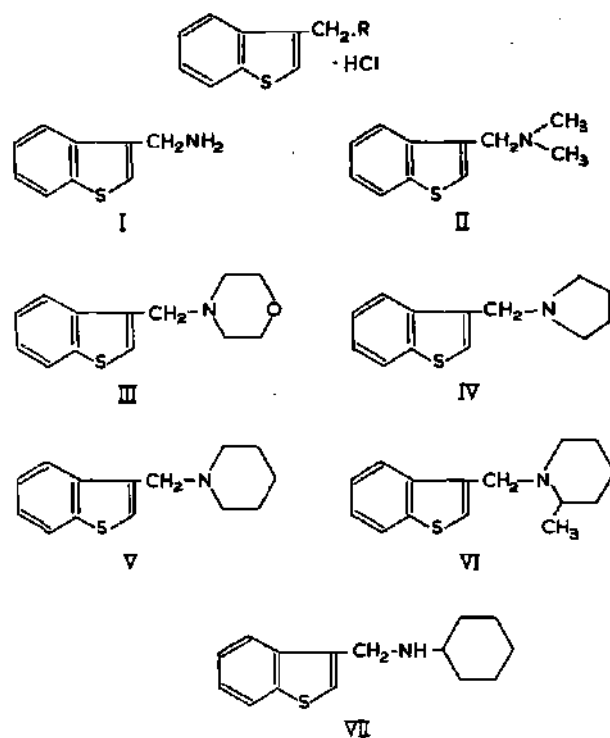
The conventional abbreviations of the metric system for volumes and weights are used throughout this thesis.

B. EXPERIMENTAL METHODS

I. Experiments on isolated tissue preparations

(a) Experiments on the isolated rat uterus

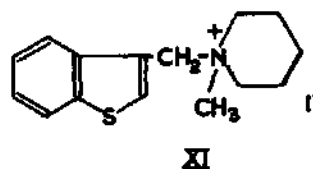
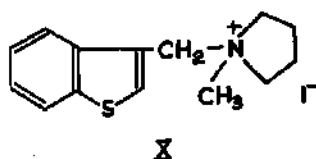
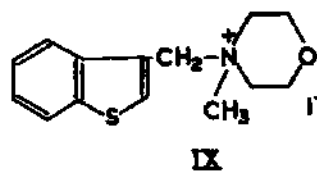
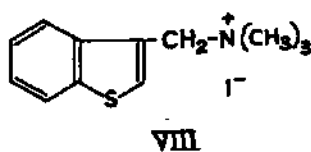
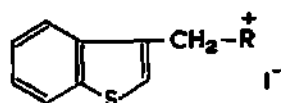
The rat uterus was first used for the assay of 5-hydroxy-tryptamine in tissue extracts by Erspamer (1940a,b,c). In order to increase the sensitivity, Erspamer (1942, 1952) subsequently used ovariectomized rats which were brought into oestrus by injection of oestradiol propionate. Amin, Crawford and Gaddum (1954) simplified this technique by using normal, virgin, female rats weighing between 160 and 200 g. in which the uterus had been brought into oestrus /

Group A. Gramine-like isosteres

- I. 3-(aminomethyl) thionaphthen.
 II. 3-(dimethylaminomethyl) thionaphthen.
 III. 3-(morpholinomethyl) thionaphthen.
 IV. 3-(pyrrolidinomethyl) thionaphthen.
 V. 3-(piperidinomethyl) thionaphthen.
 VI. 3-(2'-methylpiperidinomethyl) thionaphthen.
 VII. 3-(cyclohexylaminomethyl) thionaphthen.

Fig. 7.

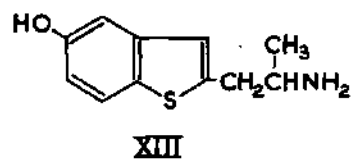
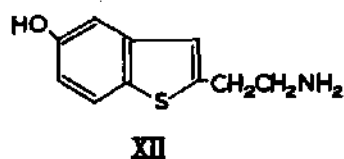
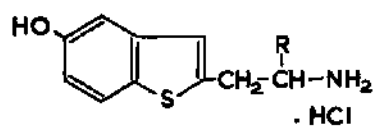
Group B. Quaternary Salts of
Gramine-like isosteres



- VIII. 3-(dimethylaminomethyl) thionaphthen methiodide.
IX. 3-(morpholinomethyl) thionaphthen methiodide.
X. 3-(pyrrolidinomethyl) thionaphthen methiodide.
XI. 3-(piperidinomethyl) thionaphthen methiodide.

Fig. 8.

Group C. Isosteres of 5-hydroxy-
isotryptamine

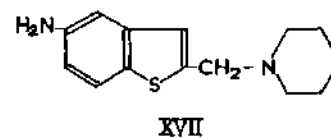
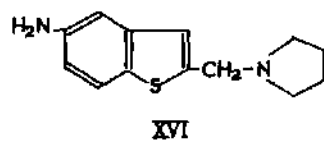
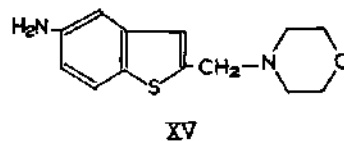
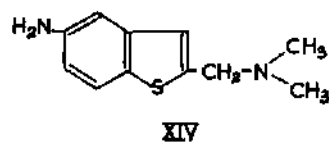
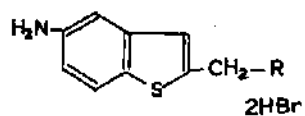


XII. 2-(2'-aminoethyl)-5-hydroxythionaphthen.

XIII. 2-(2'-aminopropyl)-5-hydroxythionaphthen.

Fig. 9.

Group D. 5-amino-isogramine-like
isosteres



- XIV. 5-amino-2-(dimethylaminomethyl) thionaphthen.
 XV. 5-amino-2-(morpholinomethyl) thionaphthen.
 XVI. 5-amino-2-(pyrrolidinomethyl) thionaphthen.
 XVII. 5-amino-2-(piperidinomethyl) thionaphthen.

Fig. 10.

oestrus by the subcutaneous injection of stilboestrol (0.1 mg. per kg. in arachis oil) 24 hours prior to use. The procedure adopted in the following experiments was based on that of Amin, Crawford and Gaddum (1954).

Virgin female albino rats weighing between 160 and 200 g. were used as the experimental animals. They were brought into oestrus by the subcutaneous injection of stilboestrol (0.1 mg. per kg. in arachis oil) given 24 hours prior to use. The next day the rat was killed by a blow on the back of the neck and bled out from the carotid arteries. The abdomen was opened, both the horns and the body of the uterus were removed and placed in a petri dish containing de Jalon's solution (Appendix I, page 246). All fatty and other extraneous tissues were carefully removed using fine scissors. A piece of one horn of the uterus about 2.5 cm. long was taken and a loop of cotton thread tied on to each end. It was then suspended in a 2 ml. organ bath containing de Jalon's solution. One end of the cotton thread was attached to a frontal point writing lever and the other to a hook fixed into the base of the bath. The fluid in the bath was oxygenated by passing oxygen through a hypodermic needle fixed into the base. The de Jalon's solution was passed from a reservoir into the organ bath through a spiral glass coil which was kept in an outer heated water bath and the flow /

flow was controlled by means of a spring clip. Fig. 11, page 67, is a diagram of the apparatus used in these experiments. The temperature was maintained thermostatically at $29 \pm 0.5^{\circ}\text{C}$. 5-hydroxytryptamine solution was added to the bath at intervals of three minutes by means of a 1 ml. tuberculin syringe and the contraction produced by the uterus was recorded on a moving smoked surface. This regular time cycle was strictly adhered to throughout the experiment. The 5-hydroxytryptamine was allowed to act for 30 seconds and then washed out with de Jalon's solution by the overflow method for about 10 to 15 seconds. From 5 to 10 reproducible submaximal contractions were recorded with 5-hydroxytryptamine before adding the drug under investigation. After obtaining reproducible submaximal contractions, the drug solution under investigation was added to the bath 30 seconds before the next addition of 5-hydroxytryptamine. The contractions were allowed to return to a constant level before a further addition of the drug solution. The total volume of 5-hydroxytryptamine and of the drug solution added to the bath was not more than 0.2 ml. in any one case.

In the case of experiments carried out to obtain the dose ratio, the method followed was essentially the same as that of Gaddum, Hameed, Hathway and Stephens (1955). In these experiments /

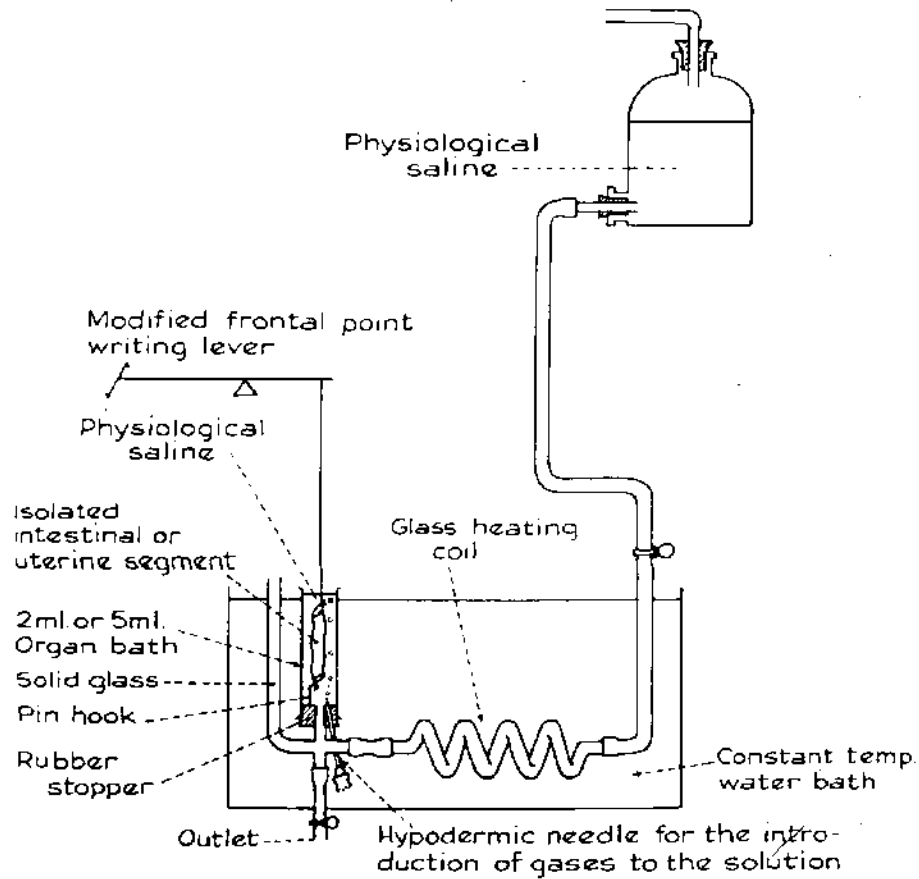


Fig. 11.

Diagram of the apparatus used for experiments on isolated tissues.

experiments the inlet tube at the lower end of the organ bath was connected through glass heating coils to two reservoirs, one of which contained de Jalon's solution, and the other a solution of the antagonist in the same solution. A dose of from 20 to 30 ng. of 5-hydroxytryptamine generally caused a suitable contraction of the rat uterus. A dose within this range was added at regular intervals until a reproducible submaximal response was obtained. The dose was then altered, and a dose response curve was obtained. The antagonist was added to one reservoir at a concentration of 1 mg. per litre and this antagonist-containing de Jalon's solution was used to wash out the bath after the contraction induced by 5-hydroxytryptamine. When the effect of the 5-hydroxytryptamine was diminished, the dose of 5-hydroxytryptamine was increased, until the response was 50 per cent of the maximum response at the beginning of the experiment, or until it became apparent that this result could not be achieved.

(b) Experiments on the isolated rat fundus strip

The rat fundus strip was first introduced by Vane (1957) for the direct assay of low concentrations of 5-hydroxytryptamine in mixtures of 5-hydroxytryptamine, acetylcholine and histamine in the presence of hyoscine. The method described in these experiments /

experiments is essentially the same as that of Vane (1957).

Rats of either sex, weighing between 200 and 400 g. were used as experimental animals. A rat was killed by a blow on the back of the neck, the throat was cut and the animal was bled from the carotid arteries. The abdomen was opened, the stomach dissected free from the surrounding abdominal viscera and removed to a petri dish containing Tyrode's solution (Appendix I, page 246). The translucent fundus of the stomach was identified (the pyloric portion was thicker and redder) and dissected away, leaving a small band of pyloric tissue attached to act as a marker. The fundus was then opened by cutting along the lesser curvature, washed in Tyrode's solution, and cut into the form of a strip in the manner described by Vane (1957). Fig. 12, page 70, shows the rat stomach, the fundus and the preparation of the fundal strip. A piece of cotton thread was tied to each end and the strip was gently stretched as shown in Fig. 12, page 70, and any protrusions and fringes of pyloric tissue trimmed away to give a long, clean, thin strip of tissue. This strip was suspended in an organ bath (which had an internal diameter of 0.85 cm. and was 15 cm. long). The bath contained oxygenated Tyrode's solution. One end of the cotton thread was attached to a hook fixed at the base of the bath and the other to a frontal-point writing lever. Fig. 11, page 67, is /

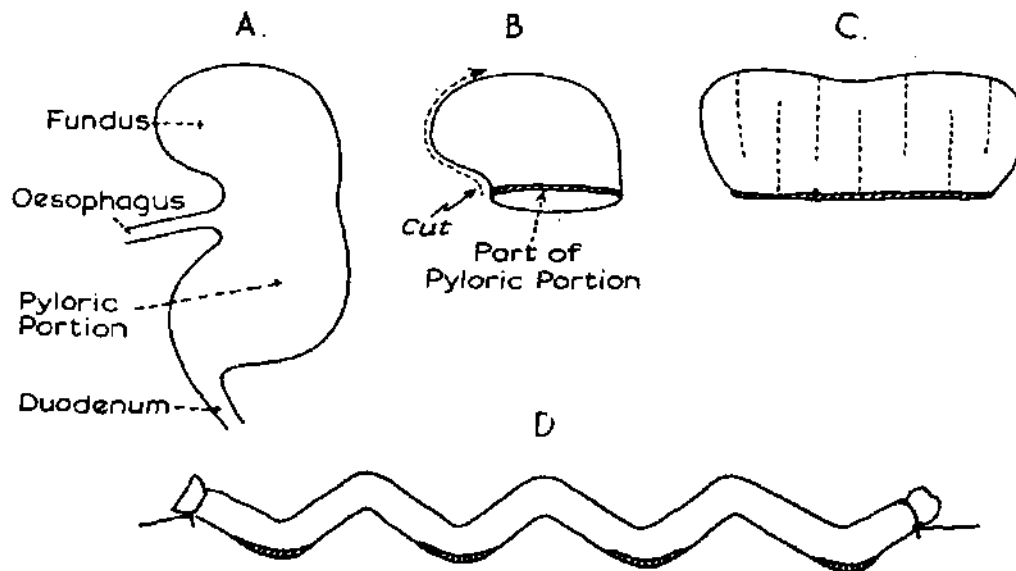


Fig. 12.

Diagram showing the preparation of the fundus strip of rat's stomach (After Vane, 1957).

- A - Whole stomach.
- B - Fundus cut away from the pyloric region, but with a small band of pyloric tissue left attached and then cut as shown around the lesser curvature.
- C - The fundus opened into a plate of tissue.
- D - The fundus strip pulled out by cotton thread attached to each end. The pyloric tissue and pieces of extraneous tissue then removed.

is a diagram of the apparatus used in these experiments. The fluid in the bath was oxygenated by passing the gas through a hypodermic needle fixed into the base of the bath. The Tyrode's solution was passed from a reservoir into the organ bath through a spiral glass coil which was kept in an outer heated water bath and the flow was controlled by a spring clip. The temperature was maintained thermostatically at $37 \pm 0.5^{\circ}\text{C}$. Normally, there was a slow steady flow of Tyrode's solution through the bath, overflowing at the top. Before the drug was added, this flow was stopped and when the contraction of the tissue was completed, the flow was restarted at a rapid rate and maintained for from 15 to 20 seconds in order to wash the drug out of the bath. The flow was then reduced to the original rate. The tissue was allowed to relax for about one hour before the start of the experiment. The drug solution was added to the bath by means of a 1 ml. tuberculin syringe in a volume not exceeding 0.2 ml. The movements of the muscle were recorded on a moving smoked surface and the tissue was stretched after each contraction for a period of 60 seconds by bringing down the lever to a fixed level just below the normal base line (a load of 0.5 g. weight was used to stretch the muscle). Hyoscine hydrobromide was added routinely to the Tyrode's solution in a concentration of 0.1 μg . per ml. This drug reduced or abolished any acetylcholine-like effects and also diminished any irregularities /

irregularities of the base line. The contractions induced by 5-hydroxytryptamine were completed in about 90 seconds, the drug was then washed out for about 20 seconds and at the same time the tissue was stretched for about 60 seconds. A further $2\frac{1}{2}$ minutes were allowed for the tissue to relax and a complete time cycle took 5 minutes. The drug solution under investigation was added to the bath by means of a 1 ml. tuberculin syringe 30 seconds before the next addition of 5-hydroxytryptamine and the effect was recorded. The total volume of 5-hydroxytryptamine solution and drug solution was not more than 0.3 ml. in any one case.

(c) Experiments on the isolated guinea pig ileum

The guinea pig ileum was used as a test preparation for histamine and certain other substances by Guggenheim and Löffler in 1916. The action of 5-hydroxytryptamine on this tissue has been studied by Robertson (1953), Rocha e Silva, Valle and Picarelli (1953) and Gaddum (1953a). The technique used in these experiments was based on that of Guggenheim and Löffler (1916).

Guinea pigs of either sex, weighing between 200 and 400 g. were used as experimental animals. A guinea pig was fasted overnight then killed by a blow on the back of the neck, the throat was cut and the animal bled from the carotid arteries. The abdomen /

abdomen was opened and the ileocaecal junction found. A piece of ileum about 3 cm. long was removed from this region about 3 cm. proximal to the ileocaecal junction and placed in a petri dish containing cold Tyrode's solution (Appendix I, page 246). It was then freed from the extraneous tissues and the contents washed out by inserting a pipette into the end away from the ileocaecal junction and the gut perfused with Tyrode's solution. The mesentery was removed from the gut using fine scissors, care being taken not to injure the ileum itself. A small loop of cotton thread was sewn to one end of the piece of ileum and a long piece of cotton thread sewn into the other end. The cotton thread was attached to the wall of the ileum away from the side to which the mesentery was attached. The gut was suspended in a 2 ml. organ bath containing oxygenated Tyrode's solution. The long piece of cotton thread was attached to a frontal-point writing lever and the other to a hook fixed into the base of the bath. Fig. 11, page 67, is a diagram of the apparatus used in these experiments. The fluid in the bath was oxygenated by passing oxygen through a hypodermic needle fixed into the base of the bath. The Tyrode's solution was passed from a reservoir into the organ bath through a spiral glass coil which was kept in an outer heated water bath and the flow was controlled by means of a spring clip. The temperature /

temperature was maintained thermostatically at $37 \pm 0.5^{\circ}\text{C}$. Spasmogens such as 5-hydroxytryptamine, acetylcholine or histamine were added to the bath in solution by means of a 1 ml. tuberculin syringe at three minute intervals and the contractions produced by the gut were recorded on a moving smoked surface. A regular time cycle of three minutes was strictly adhered to throughout the experiment. The spasmogen was allowed to act for 30 seconds and was then washed out by overflow of Tyrode's solution for from 10 to 15 seconds. From 5 to 10 reproducible submaximal contractions were recorded with the spasmogen before adding the drug under investigation. The drug solution was then added to the bath 30 seconds before the next addition of spasmogen. The contractions were allowed to return to a constant level before the next addition of the drug solution. The total volume of the spasmogen and the drug solution was not more than 0.2 ml. in any one case.

(d) Experiments on the perfused isolated rat hindquarters

The method adopted was based on that of Burn (1952). In these experiments the pressure at which the physiological fluid passed through the blood vessels was kept constant and the alterations in the rate of outflow of the perfusion fluid which were produced by the drug, were recorded by means of Thorp's impulse counter. /

counter. The vessels were perfused with oxygenated Locke's solution (Appendix I, page 246) at room temperature.

Albino rats of either sex weighing between 150 and 200 g. were used as experimental animals. The rat was killed by a blow on the back of the neck, the throat was cut and the animal bled out from the carotid arteries. The abdominal cavity was opened, the rectum and the inferior and superior mesenteric arteries were divided between ligatures. The intestines were pushed upwards in order to bring into view the abdominal aorta and the accompanying vein. The aorta was freed from the vein and surrounding fatty tissue; a piece of cotton thread was then passed around the aorta and a small transverse cut made in it by means of sharp-pointed scissors; finally a polythene cannula was inserted through the cut into the artery and tied into place. The body wall and vertebral column were transected above the point of cannulation and the hinder part of the rat was laid upon a circle of muslin attached to a wire loop which rested on a glass funnel. The reservoir containing oxygenated Locke's solution was connected to the polythene cannula. Fig. 13, page 76, shows the apparatus used in these experiments. The rate of flow of the Locke's solution was controlled by means of an adjustable screw clip. The outflow was led through a filter funnel and connected across the /

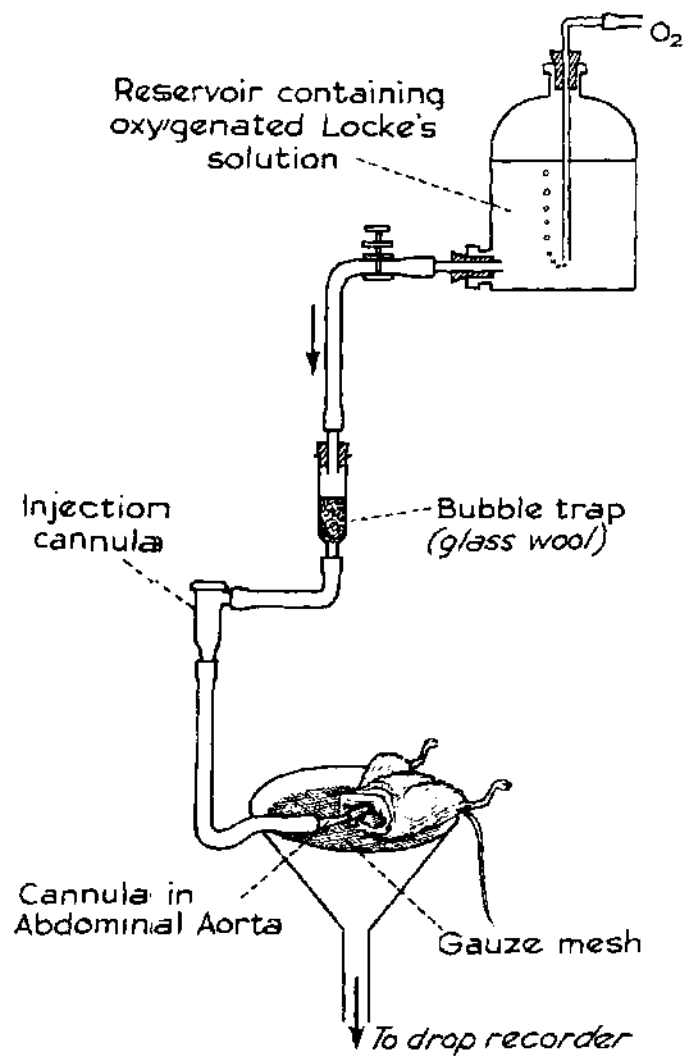


Fig. 13.

Diagram of apparatus used for perfusion
of the isolated rat's hindquarters,

the points of a drop recording assembly to a Thorp's impulse counter.

After setting up the preparation, a uniform outflow was obtained for at least 15 minutes before drugs were injected. Solutions of 5-hydroxytryptamine, adrenaline or noradrenaline, were injected into the rubber tubing by means of a 1 ml. tuberculin syringe and the altered rate of flow was recorded on a moving smoked surface. A constant dose of 5-hydroxytryptamine, adrenaline or noradrenaline was injected until a reproducible response was obtained. Then the drug solution under investigation was injected 30 seconds before the next injection of 5-hydroxytryptamine, adrenaline or noradrenaline and the effect on the rate of outflow was recorded.

II. Experiments on intact animals

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

Cats of either sex, weighing between 2 and 3.5 kg. were used as experimental animals. Anaesthesia was induced by means of the intraperitoneal injection of sodium pentobarbitone and a dose of 50 to 60 mg. per kg. was usually sufficient to produce surgical anaesthesia in about 10 to 15 minutes.

The /

The anaesthetised cat was placed on its back upon a warm operating table, the legs were secured to the table by means of strings and the head extended. The fur covering the ventral aspect of the neck was cut away and a longitudinal midline incision extending from the sternum below, to the point of the jaw above, was made by means of a scalpel. The fascia and the muscles covering the trachea were separated by blunt dissection and the latter was freed from the surrounding tissue. A piece of cotton thread was passed around the trachea and a transverse incision made in it by means of scissors. The cut edge was held by means of forceps and a tracheal cannula was inserted through the cut and tied into place. This was done as a precautionary measure to keep the cat alive under artificial respiration if this became necessary. The amount of air entering and leaving the cannula could be altered by means of an adjustable sleeve.

The skin on the anterolateral side of the neck was carefully stretched by means of a blunt dissector, one of the external jugular veins was identified and freed from the surrounding tissues. A bulldog clip was applied to the vein on the cardiac end and the former was closed on the cephalic end by means of a cotton tie. A piece of cotton thread was passed around the vein and /

and a small transverse cut made in the dilated vein by means of sharp-pointed iris scissors. A glass vein cannula about 6 cm. long was filled with a solution of heparin, inserted through the incision into the vein and tied into place. The cannula was connected by means of rubber tubing to a graduated 50 ml. burette containing normal saline, all the air having previously been removed from the system. The flow of the saline was controlled by means of a spring clip. The bulldog clip was taken away and the observation that the saline in the burette ran freely into the vein indicated that the cannula had been correctly inserted. The rubber tube in between the cannula and the burette was used for injection of the drugs into the vein. About 1 to 2 ml. of a solution of heparin was injected into the vein to prevent intravascular clotting.

The carotid arteries were identified and one of them freed from the accompanying nerves and other surrounding tissues. The artery was first tied off as near to the head as possible, a bulldog clip was applied to it about 3 cm. distal to the ligature, and a piece of cotton thread passed around it midway between the ligature and bulldog clip. A small transverse cut was made in the artery by means of sharp-pointed scissors and an artery cannula /

cannula was inserted through the cut and tied into place. The cannula was connected to the mercury manometer, the space between the mercury and the artery being filled with heparinised normal saline. Before making the connection, extreme care was taken to displace all the air bubbles from the system. The pressure was set to about 100 mm. of mercury and the bulldog clip was taken away. The writing flag on one arm of the mercury manometer recorded the variations in blood pressure on a moving smoked surface.

Drugs in aqueous solution were administered to the animal by means of a 1 ml. tuberculin syringe, injecting into the rubber tubing between the vein cannula and the burette. After each injection, 3 ml. of normal saline were allowed to flow into the vein to wash the drug into the system.

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

This technique was originally introduced by Landgrebe, Macauley and Waring in 1946 for the assay of vasopression. A modified version of the preparation was introduced by Dekanski in 1952 and the method used in these experiments was based upon that.

Albino /

Albino rats of either sex weighing between 300 and 400 g. were used as experimental animals. Anaesthesia was induced by means of a subcutaneous injection of urethane. A 25 per cent $\frac{W}{V}$ solution of urethane in distilled water was made, and a dose of 175 to 200 mg. per 100 g. body weight was usually sufficient to produce surgical anaesthesia in about one hour.

The anaesthetised animal was placed on its back upon a warmed rat operating table; the legs were secured to the table by means of strings and the neck was extended. The skin covering the ventral aspect of the neck was cut away, the fascia and the muscles covering the trachea separated and the latter was freed from the surrounding tissues. A piece of cotton thread was passed around the trachea and a transverse incision made in it by means of a pair of iris scissors. The cut edge was held by means of forceps and a polythene cannula was inserted and tied into place. This was done as a precautionary measure to keep the rat alive under artificial respiration if this became necessary.

The skin on the anterolateral side of the neck was carefully stretched by means of a blunt dissector, and one of the external jugular veins was identified and freed from the surrounding fascia. A bulldog clip was applied to the vein on the cardiac end and the vein /

vein was closed on the cephalic end by means of a piece of cotton thread. Another piece of cotton thread was passed around the vein and a small transverse cut was made in the dilated vein by means of sharp-pointed iris scissors. A thin cannula made of hard polythene tubing was filled with a solution of heparin and inserted through the cut, into the vein and tied into place with the pointed end towards the heart. The cannula was connected by means of rubber tubing to a graduated 5 ml. burette containing normal saline, all the air bubbles had been previously removed from the system. The flow of saline was controlled by a spring clip. The bulldog clip was removed from the vein and the observation that the saline in the burette ran freely into the vein, indicated that the cannula had been correctly inserted. The rubber tube in between the cannula and the burette was used for injection of the drugs. Injections were made by means of a 1 ml. tuberculin syringe. About 0.25 to 0.5 ml. of a solution of heparin was routinely injected into the vein at the beginning of each experiment to prevent intravascular clotting.

The carotid arteries were identified and one of them freed from the accompanying nerves and surrounding tissue. The artery was first tied off as near to the head as possible, a bulldog clip was /

was then applied to the artery about 2 cm. distal to the ligature and a piece of cotton thread passed around the artery midway between the ligature and the bulldog clip. A small transverse cut was made in the artery by means of sharp pointed iris scissors and a polythene cannula filled with a solution of heparin was inserted through the cut into the artery with the pointed end towards the heart and tied into place. The cannula was connected to a manometer of the type designed by Condon (1953), the space between the artery and the mercury being filled with normal saline. Fig. 14, page 84, is a diagram of the manometer used in these experiments. Before making the connection, extreme care was taken to exclude all the air bubbles from the system. The bulldog clip which was applied to the artery was taken off and a writing flag on one arm of the mercury manometer recorded the blood pressure on a moving smoked surface.

Drugs in aqueous solution were administered to the animal by means of injection into the rubber tubing between the vein cannula and burette using a 1 ml. tuberculin syringe. Each injection was followed by infusion of 0.2 ml. of saline from the burette. The volume of drug solution injected was not more than 0.2 ml. in any one case.

(c) /

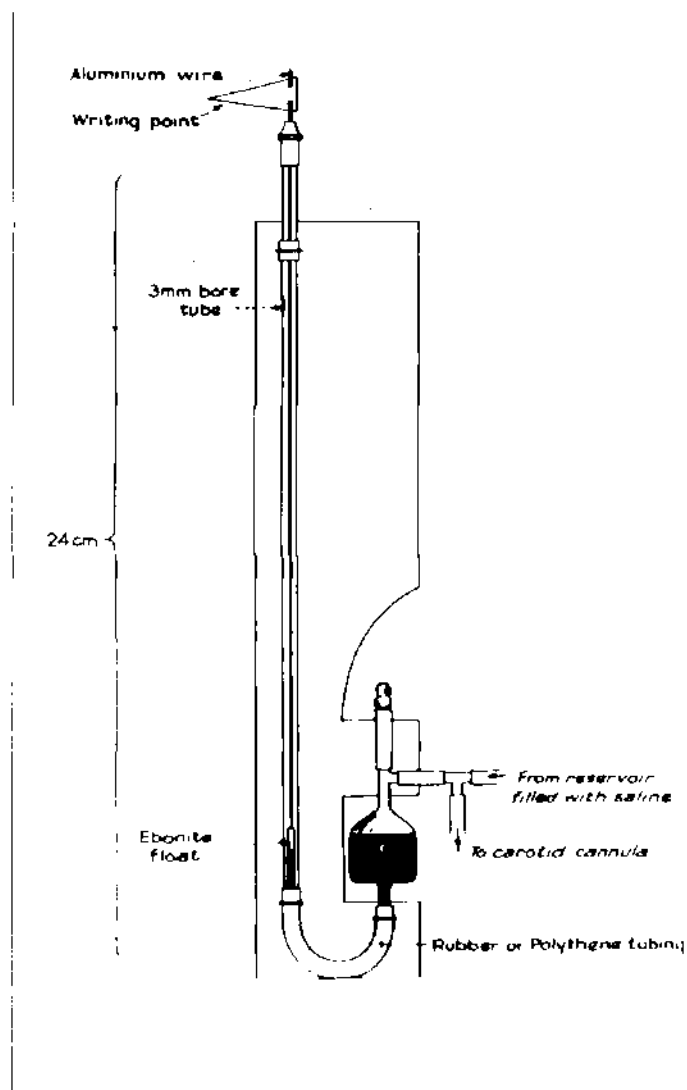


Fig. 14.

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

(c) Toxic effects on mice

Albino mice of either sex weighing 20 ± 1.0 g., and which had not previously been used for experimental purposes, were employed. Groups of five mice were selected. The members of one group of mice were injected intraperitoneally with 0.75 ml. of normal saline and used as controls. The other groups of mice were injected with the drug solutions at various dose levels (from 25 to 150 mg. per kg.). The total quantity of drug solution was not more than 0.75 ml. in any one case. The control group and the drug-treated groups of mice were kept in separate cages and the effects were noted for about eight hours.

III. Experiments on isolated smooth muscle preparations using the Ariens technique

In order to carry out experiments using the Ariens technique, a suitable smooth muscle preparation was necessary which would give a reproducible cumulative log. concentration response to 5-hydroxytryptamine. A search was therefore made for a suitable smooth muscle preparation during which the following preparations were tried. The isolated guinea pig jejunum, the isolated horse carotid artery strip and the isolated rat jejunum. The first two /

two preparations were not suitable for reasons which will become clear later.

(a) (i) The isolated guinea pig jejunum.

A piece of jejunum from 2 to 3 cm. in length was taken from a freshly killed guinea pig. This was mounted in a 2 ml. organ bath in the manner described in pages 72 and 73. A diagram of the apparatus used for this experiment is shown in Fig. 11, page 67. To obtain a suitable cumulative log. concentration response curve for 5-hydroxytryptamine, the tissue had to be left for a long time in contact with the cumulative log. concentration of 5-hydroxytryptamine in the bath. From this preparation reproducible cumulative concentration response curves to 5-hydroxytryptamine could not be obtained. This was because prolonged contact with high doses of 5-hydroxytryptamine made the tissue insensitive to further additions of 5-hydroxytryptamine. Fig. 15, page 87, shows a typical experimental tracing. Gaddum (1953a) and Rocha e Silva, Valle and Picarelli (1953) have also observed this effect in the isolated guinea pig ileum. For the reasons stated above, the guinea pig jejunum was found not to be suitable for this type of work.

(a) (ii) Horse carotid artery strips.

Lengths of carotid artery were removed from freshly killed horses /

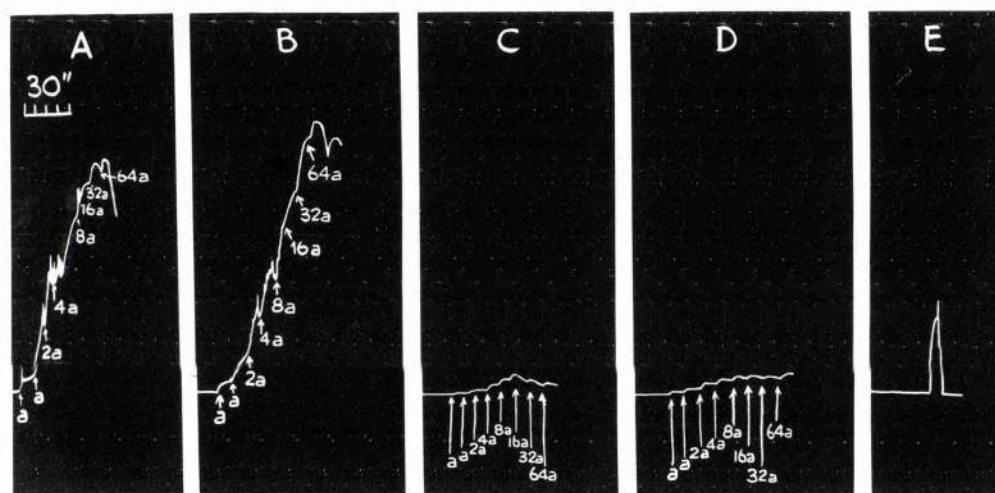


Fig. 15.

The isolated guinea pig jejunum.

Experimental, cumulative log-dose response curves, based on the Ariens procedure.

At A, B, C and D, cumulative log-dose response curve for 5-hydroxytryptamine.

Dose a = $0.00568 \mu \text{ Mol/L.}$

Other doses are multiples of a as shown on the tracings.

At E, a single dose of $0.35632 \mu \text{ Mol/L.}$ of 5-hydroxytryptamine.

horses at the slaughter house. A portion of the artery was freed from fascia and a strip of about 4 cm. in length and 2 mm. wide was made by cutting the artery into a spiral by means of a pair of iris scissors. Threads were tied to both ends of the segment and the strip of artery set up in a 40 ml. organ bath containing oxygenated Tyrode's solution (Appendix I, page 246) at $37 \pm 0.5^{\circ}\text{C}$. The thread at one end of the artery strip was fixed to the base of the organ bath and the thread at the other end was attached to a frontal-point writing lever. The strip was stretched for approximately one hour by means of a 10 g. weight. Before the experiment was started, the additional weight was removed and the lever readjusted. The contractions were recorded on a moving smoked surface.

Even after leaving the tissue for more than one and a half hours and reducing the magnification of the lever, it was found that the tissue itself contracted slowly without the addition of any stimulant drug. An example is shown in Fig. 16, page 89. In order to avoid this slow contraction, the weight upon the lever was increased and it was found that the response of the tissue to added 5-hydroxytryptamine was now very sluggish and the contraction produced was not great enough to be measurable.

Reproducible /

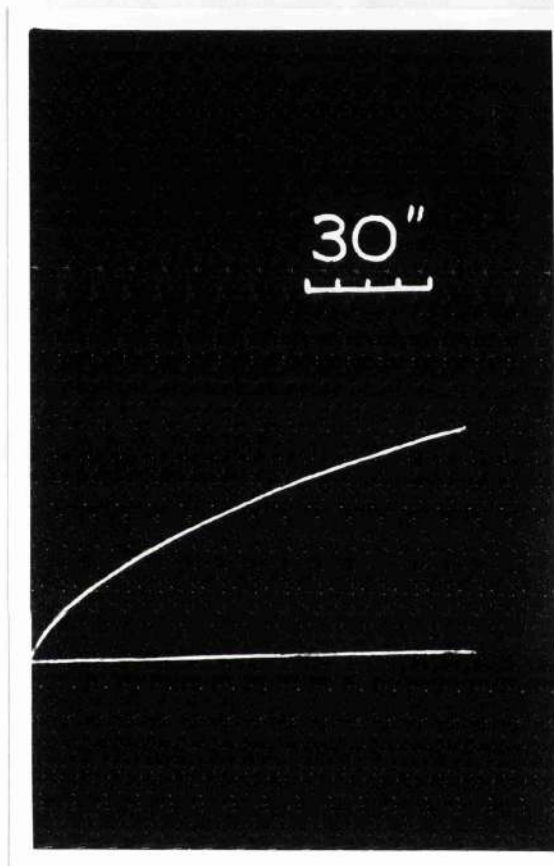


Fig. 16.

The isolated horse carotid artery strip.
Experimental record showing spontaneous
slow contraction of the tissue.

Reproducible cumulative concentration response curves for 5-hydroxytryptamine could not be obtained. For these reasons this tissue was obviously not suitable and was therefore discarded.

Strips of rabbit and cat thoracic aortae were not tried because many of the strips were found to be refractory to stimulant drugs including acetylcholine, 5-hydroxytryptamine and histamine (Kirpekar, 1959).

These difficulties were overcome by using the rat jejunum. Reproducible cumulative concentration-response curves to 5-hydroxytryptamine could be obtained from this tissue.

(b) Experiments on the isolated rat jejunum.

Method. The method described is based mainly on that of Van Rossum and Ariëns (1959).

Albino rats of either sex, weighing between 150 and 200 g. were used as experimental animals. A rat was stunned by a blow on the head, the throat cut and the animal bled out. The abdominal cavity was opened and the duodenum was identified. The first 6 to 8 cm. of small intestine which originated from the pyloric end of the stomach was discarded and 2 to 3 cm. of jejunum taken. The contents were washed out by means of a stream /

stream of Tyrode's solution (Appendix I, page 246). The two ends of the jejunum were sewn with cotton thread and the tissue was mounted in a 20 ml. organ bath through which oxygen was bubbled while the temperature was kept constant at $35 \pm 0.5^{\circ}\text{C}$. (Fig. 11, page 67, is a diagram of the apparatus used). The tissue was left for about one half to one hour in the bath before starting the experiment. The movements of the jejunum were recorded on a moving smoked surface. Cumulative log. concentration-response curves were obtained by adding amounts of 5-hydroxytryptamine in the following sequence; a; a; 2a; etc. A given concentration of 5-hydroxytryptamine was left in contact with the jejunum for one minute. This appeared to be sufficient to give a steady state of contraction after which the concentration was doubled by adding the subsequent dose. When the contraction had reached its maximum, the addition of 5-hydroxytryptamine was stopped and the drug was washed out for about five to ten minutes. The tissue was then allowed to rest for another twenty minutes. This time cycle was strictly adhered to throughout the experiment. After obtaining reproducible cumulative log. concentration-response curves to 5-hydroxytryptamine, the drug solution under investigation was added to the bath one minute before the next addition of the appropriate cumulative log. concentration dose of 5-hydroxytryptamine.

If /

If the height of the contraction was not definitely reduced, higher concentrations of the drug solutions were tried until such time as the tissue failed to give a further contraction to added 5-hydroxytryptamine. Cumulative log.dose response curves were plotted for 5-hydroxytryptamine alone and for 5-hydroxytryptamine in the presence of various concentrations of the drug under investigation.

C H A P T E R I I I

Pages 93 to 213

C H A P T E R I I I

RESULTS

I. (a) Effect of the thionaphthen compounds on the contractions produced by 5-hydroxytryptamine on the isolated rat uterus.

With the exception of compound VI, none of the gramine-like isosteres when tested at dose levels of from 25 to 50 μ g. per ml., had any direct stimulant effect on the isolated rat uterus. Compound VI exhibited variable activity. In two experiments, similar concentrations (50 and 100 μ g. per ml.) of compound VI potentiated the stimulant response to 5-hydroxytryptamine and induced spontaneous activity in the tissue. This could still be observed after the drug was washed out of the bath (Fig. 17, page 94). In another two experiments using similar dose levels it incompletely inhibited the stimulant response to 5-hydroxytryptamine and still induced spontaneous activity (Fig. 18, page 95). At dose levels of from 2.5 to 50 μ g. per ml., the other six compounds in this series antagonized the stimulant effect of from 0.01 to 0.05 μ g. per ml. of 5-hydroxytryptamine. Tracings obtained from five different experiments are shown in Figs. 19 to 21, pages

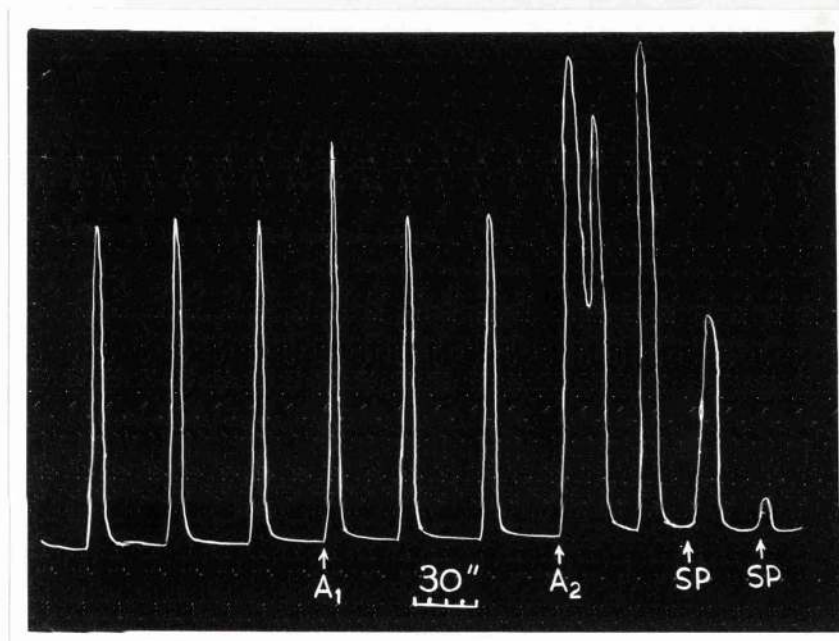


Fig. 17.

The isolated rat uterus.

All unlabelled contractions are due to 0.025 μ g. per ml. of 5-hydroxytryptamine.

Addition of 5-hydroxytryptamine was preceded 30 seconds earlier by:

At A_1 - 5 μ g. per ml. 3-(2-methylpiperidinomethyl) thionaphthen (VI).

At A_2 - 50 μ g. per ml. " " " " " "

At SP - Spontaneous movements of the tissue.

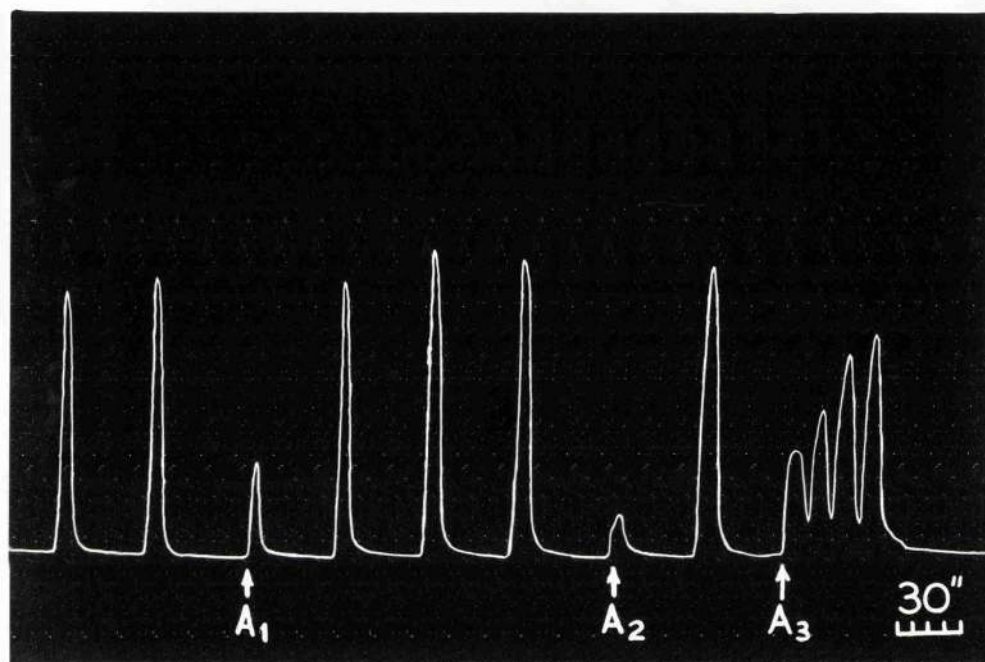


Fig. 18.

The isolated rat uterus.

All unlabelled contractions are due to 0.05 μ g. per ml. of 5-hydroxytryptamine.

Addition of 5-hydroxytryptamine was preceded 30 seconds earlier by:

At A_1 - 10 μ g. per ml. 3-(2'-methylpiperidinomethyl) thionaphthen (VI)

At A_2 - 50 μ g. " " " " " " "

At A_3 - 100 μ g. " " " " " " "

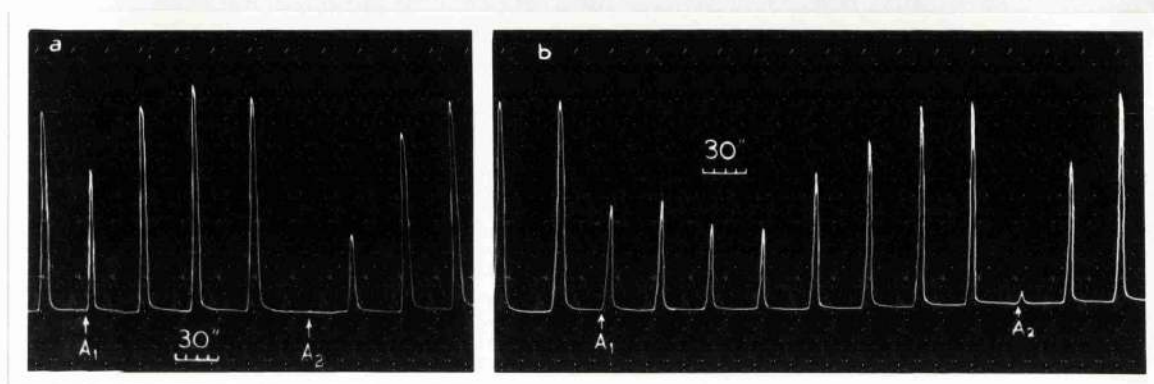


Fig. 19.

The isolated rat uterus.

- (a) All unlabelled contractions are due to 0.01 μ g. per ml. of 5-hydroxytryptamine.

Addition of 5-hydroxytryptamine was preceded 30 seconds earlier by:-

At A_1 - 1 μ g. per ml. 3-(aminomethyl) thionaphthen (I).

At A_2 - 10 μ g. " " " " "

- (b) All unlabelled contractions are due to 0.05 μ g. per ml. of 5-hydroxytryptamine.

Addition of 5-hydroxytryptamine was preceded 30 seconds earlier by:-

At A_1 - 0.5 μ g. per ml. 3-(morpholinomethyl)thionaphthen (III)

At A_2 - 5 μ g. per ml. " " " " "

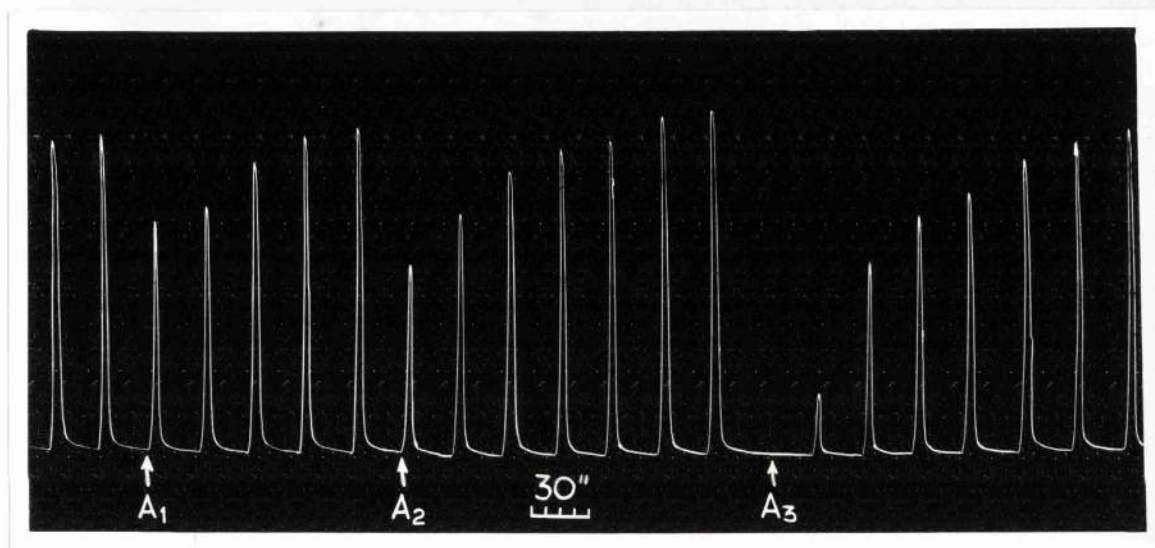


Fig. 20.

The isolated rat uterus.

All unlabelled contractions are due to 0.025 μ g. per ml. of 5-hydroxytryptamine.

Addition of 5-hydroxytryptamine was preceded 30 seconds earlier by:

At A_1	-	0.025 μ g. per ml.	3-(pyrrolidinomethyl) thionaphthen (IV)
At A_2	-	0.5 μ g.	" " " " " "
At A_3	-	2.5 μ g.	" " " " " "

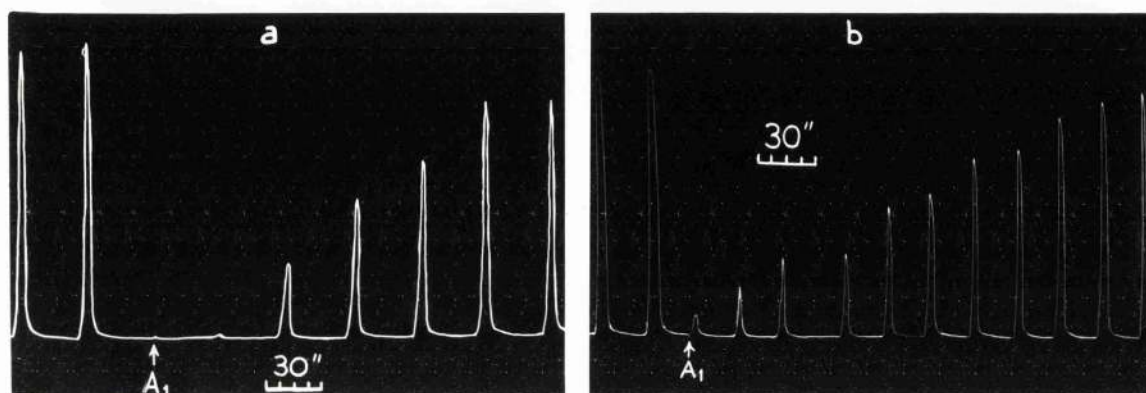


Fig. 21.

The isolated rat uterus.

- (a) All unlabelled contractions are due to 0.05 $\mu\text{g. per ml.}$ of 5-hydroxytryptamine.
Addition of 5-hydroxytryptamine was preceded 30 seconds earlier by:
At A_1 - 2.5 $\mu\text{g. per ml.}$ 3-(piperidinomethyl) thionaphthen (V).
- (b) All unlabelled contractions are due to 0.025 $\mu\text{g. per ml.}$ of 5-hydroxytryptamine.
Addition of 5-hydroxytryptamine was preceded 30 seconds earlier by:-
At A_1 - 50 $\mu\text{g. per ml.}$ 3-(cyclohexylaminomethyl) thionaphthen (VII).

96 to 98.

At dose levels of from 5 to 100 μ g. per ml., none of the quaternary salts of the gramine-like isosteres, exerted any direct stimulant action on the isolated rat uterus but at these dose levels, all the compounds antagonized the stimulant actions of 5-hydroxytryptamine (0.01 to 0.05 μ g. per ml.). Among the members of this group, compound VIII was the most potent 5-hydroxytryptamine antagonist and was effective at dose levels of from 5 to 20 μ g. per ml. (Fig. 22, page 100); compound X was next in order of potency (25 to 50 μ g. per ml.). A typical tracing obtained from an experiment is shown in Fig. 23a, page 101. Compounds IX and XI were active at dose levels ranging from 25 to 100 μ g. per ml. (Fig. 23b, page 101).

At dose levels of from 50 to 200 μ g. per ml. compounds XII and XIII showed no direct stimulant effects on the isolated rat uterus preparation, but at the same dose levels they antagonized the stimulant actions of 5-hydroxytryptamine (0.02 to 0.04 μ g. per ml.). A typical tracing is shown in Fig. 24, page 102.

At dose levels ranging from 25 to 500 μ g. per ml., none of the 5-amino-isogramine-like isosteres had any direct stimulant action on the isolated rat uterus. All antagonized the response to /

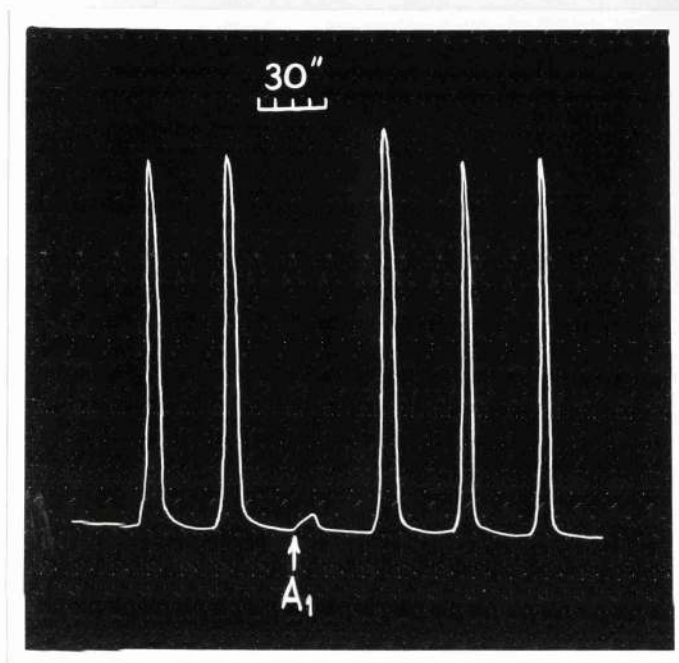


Fig. 22.

The isolated rat uterus.

All contractions are due to 0.01 μ g. per ml. of 5-hydroxytryptamine.

Addition of 5-hydroxytryptamine was preceded 30 seconds earlier by:-

At A_1 - 5 μ g. per ml. 3-(dimethylaminomethyl) thionaphthen
methiodide (VIII).

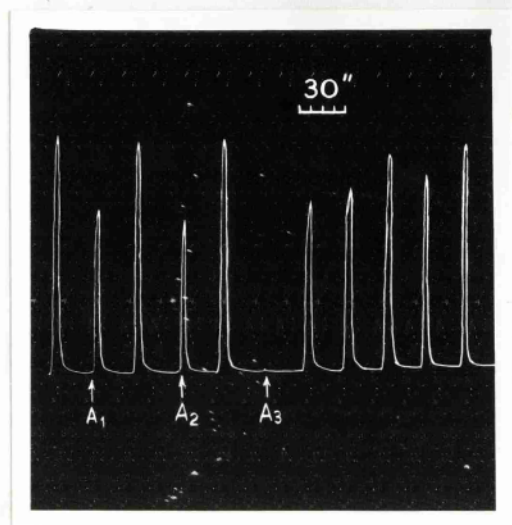


Fig. 23a.

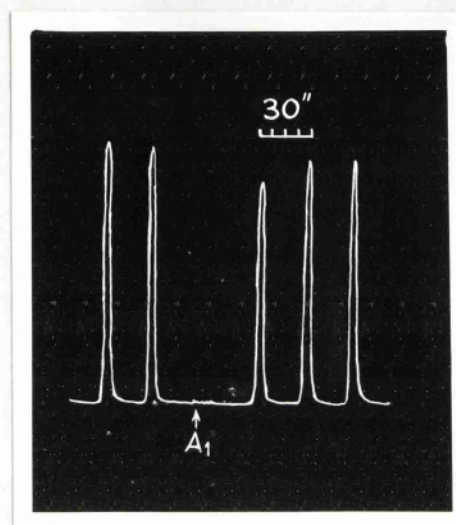


Fig. 23b.

The isolated rat uterus.

- (a) All unlabelled contractions are due to 0.01 $\mu\text{g. per ml.}$ of 5-hydroxytryptamine.

Addition of 5-hydroxytryptamine was preceded 30 seconds earlier by:-

At A_1 - 5 $\mu\text{g. per ml.}$ 3-(pyrrolidinomethyl) thionaphthen methiodide (X).

At A_2 - 10 $\mu\text{g.}$ " " " "

At A_3 - 30 $\mu\text{g.}$ " " " "

- (b) All unlabelled contractions are due to 0.05 $\mu\text{g. per ml.}$ of 5-hydroxytryptamine.

Addition of 5-hydroxytryptamine was preceded 30 seconds earlier by:-

At A_1 - 25 $\mu\text{g. per ml.}$ 3-(piperidinomethyl) thionaphthen methiodide (XI).

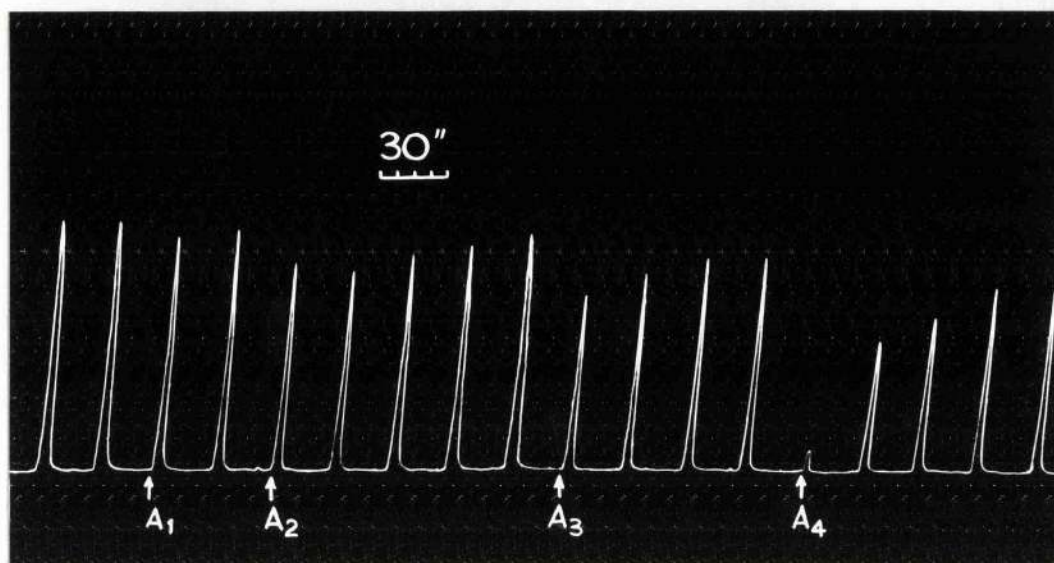


Fig. 24.

The isolated rat uterus.

All contractions are due to 0.02 μ g. per ml. of 5-hydroxytryptamine.

Addition of 5-hydroxytryptamine was preceded 30 seconds earlier by:

At A_1 - 7.5 μ g. per ml. 2-(2'-aminopropyl)-5-hydroxythionaphthen
(XIII).

At A_2 - 15 μ g. " " " " " " "

At A_3 - 25 μ g. " " " " " " "

At A_4 - 50 μ g. " " " " " " "

to 5-hydroxytryptamine (0.01 to 0.05 μ g. per ml.). Compound XIV was the most potent among this series and was effective at dose levels of from 10 to 25 μ g. per ml. (Fig. 25, page 104). At dose levels of from 50 to 100 μ g. per ml. the other compounds were antagonistic to 5-hydroxytryptamine. A typical experimental record is shown in Fig. 26, page 105.

Dose Ratio

Except for compound VI, the dose ratios of the gramine-like isosteres for 5-hydroxytryptamine were calculated. The dose ratio was not constant from experiment to experiment. For example, the dose ratio of compound V was 14 in one experiment and in another it was 10.

(b) The direct stimulant effect of the thionaphthen compounds on the isolated rat fundus strip and the effect on the contractions produced by 5-hydroxytryptamine.

At dose levels of from 1 to 2 μ g. per ml., but with the exception of compound II, all of the gramine-like isosteres potentiated the contractions due to 5-hydroxytryptamine on the isolated rat fundus strip. Compound II exhibited variable activity. At dose levels of from 2 to 5 μ g. per ml., it incompletely inhibited the stimulant response to 5-hydroxytryptamine in /

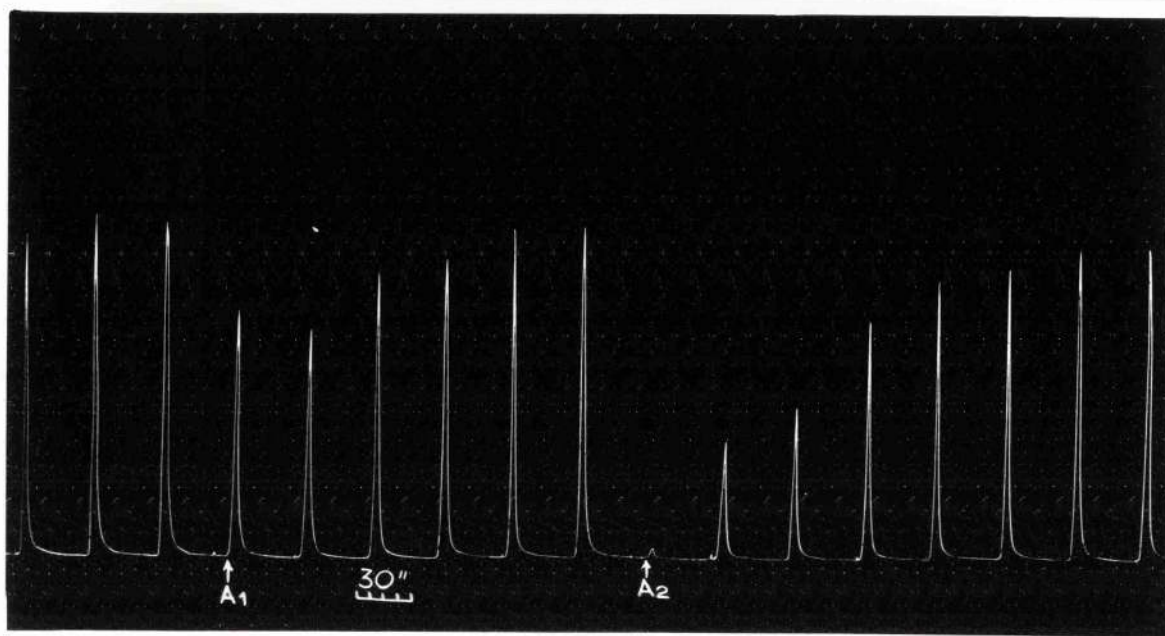


Fig. 25.

The isolated rat uterus.

All contractions are due to 0.05 μ g. per ml. of 5-hydroxytryptamine.

Addition of 5-hydroxytryptamine was preceded 30 seconds earlier by:-

At A_1 - 10 μ g. per ml. 5-amino-2-(dimethylaminomethyl)
thionaphthen. (XIV).

At A_2 - 25 μ g. " " " "

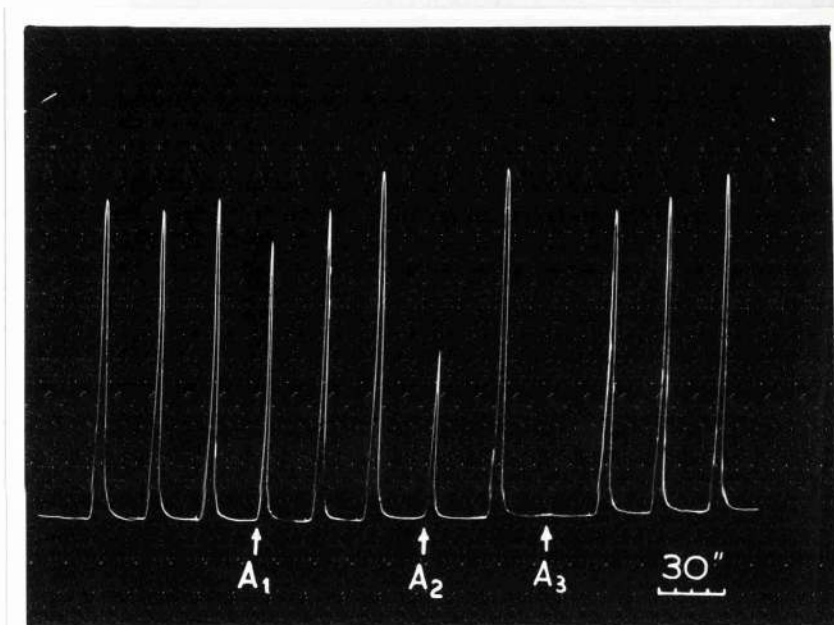


Fig. 26.

The isolated rat uterus.

All contractions are due to 0.01 μ g. per ml. of 5-hydroxytryptamine.

Addition of 5-hydroxytryptamine was preceded 30 seconds earlier by:-

At A_1 - 25 μ g. per ml. 5-amino-2-(morpholinomethyl) thionaphthen (XV)

At A_2 - 50 μ g. " " " " "

At A_3 - 75 μ g. " " " " "

in two experiments. At a dose level of from 5 to 20 $\mu\text{g. per ml.}$, this compound exhibited a direct stimulant action in a third experiment (Fig. 27, page 107). However, in a fourth experiment at a dose level of 1 to 2 $\mu\text{g. per ml.}$, it potentiated the stimulant action of 5-hydroxytryptamine. At dose levels of from 1 to 5 $\mu\text{g. per ml.}$ compounds III, IV, V and VI exhibited a direct stimulant action on the isolated rat fundus strip (Fig. 28, page 108). Compound I was a very weak stimulant at this dose level but potentiated the stimulant response to 5-hydroxytryptamine (Fig. 29, page 109) and at the same dose level, compound VII did not cause any stimulation but potentiated the effect of 5-hydroxytryptamine. A typical tracing is shown in Fig. 30, page 110.

At dose levels of from 10 to 100 $\mu\text{g. per ml.}$ the compounds of Group B exhibited a direct stimulant action upon the isolated rat fundus strip. The most potent compounds were VIII and X (at dose levels of from 5 to 200 $\mu\text{g. per ml.}$). A typical tracing is shown in Fig. 31, page 111. Next in order of potency was compound XI, which was effective at dose levels of from 40 to 80 $\mu\text{g. per ml.}$ (Fig. 32, page 112). Compound IX was very weak, causing stimulation of the tissue at dose levels of from 100 to 200 $\mu\text{g. per ml.}$ All the compounds in this group potentiated the stimulant response to /

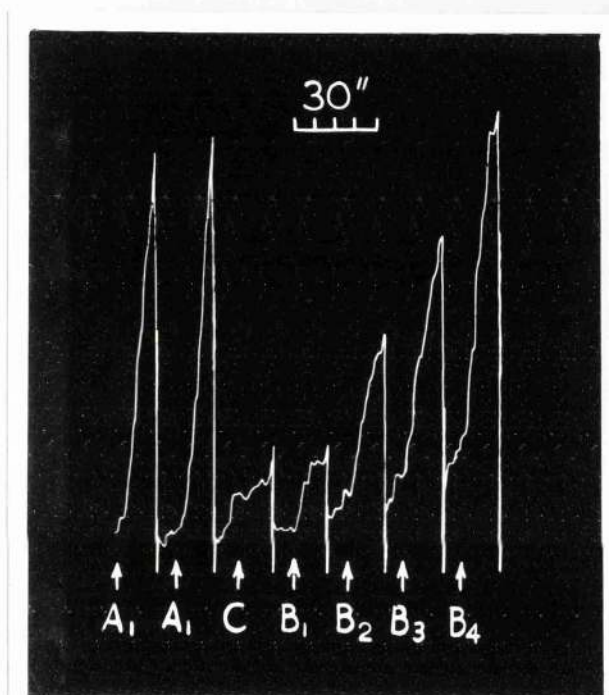


Fig. 27.

The isolated rat fundus strip.

At A_1 - Effect of 0.2 ng. per ml. 5-hydroxytryptamine.

At C - Control.

At B_1 - Effect of 5 μ g. per ml. 3-(dimethylaminomethyl)
thionaphthen (II).

At B_2 - " 10 μ g. " " "

At B_3 - " 15 μ g. " " "

At B_4 - " 20 μ g. " " "

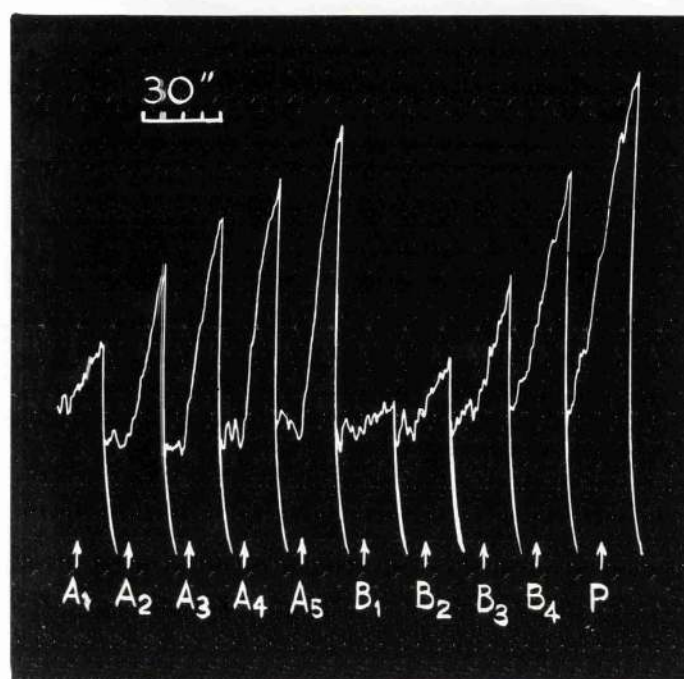


Fig. 28.

The isolated rat fundus strip.

At A₁ - Effect of 0.1 ng. per ml. 5-hydroxytryptamine.

At A₂ - " 0.2 ng. " "

At A₃ - " 0.3 ng. " "

At A₄ - " 0.4 ng. " "

At A₅ - " 0.5 ng. " "

At B₁ - " 1 µg. " 3-(piperidinomethyl) thionaphthen (V).

At B₂ - " 1.5 µg. " " "

At B₃ - " 2.5 µg. " " "

At B₄ - " 3 µg. " " "

Addition of 5-hydroxytryptamine (0.1 ng. per ml.) was preceded 30 seconds earlier by:-

At P - 1.5 µg. per ml. 3-(piperidinomethyl) thionaphthen.

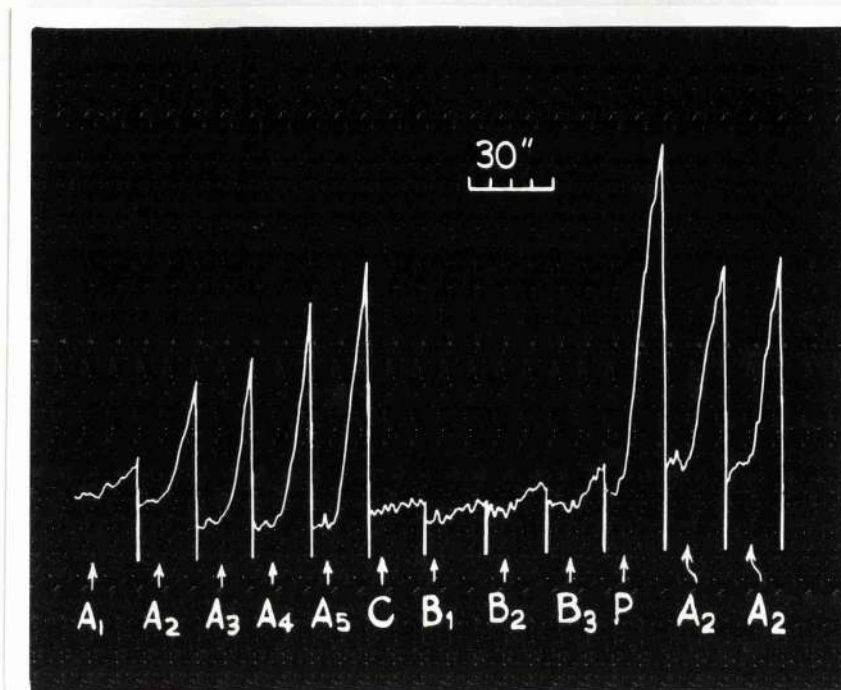


Fig. 29.

Isolated rat fundus strip.

At A_1 - Effect of 0.02 ng. per ml. 5-hydroxytryptamine.

At A_2 - " 0.03 ng. " "

At A_3 - " 0.04 ng. " "

At A_4 - " 0.05 ng. " "

At A_5 - " 0.06 ng. " "

At C - Control.

At B_1 - Effect of 1 μ g. per ml. 3-(aminomethyl) thionaphthen (I).

At B_2 - " 2 μ g. " " "

At B_3 - " 4 μ g. " " "

Addition of 5-hydroxytryptamine (0.03 ng. per ml.) was preceded

30 seconds earlier by:-

At P - 1 μ g. per ml. 3-(aminomethyl) thionaphthen.

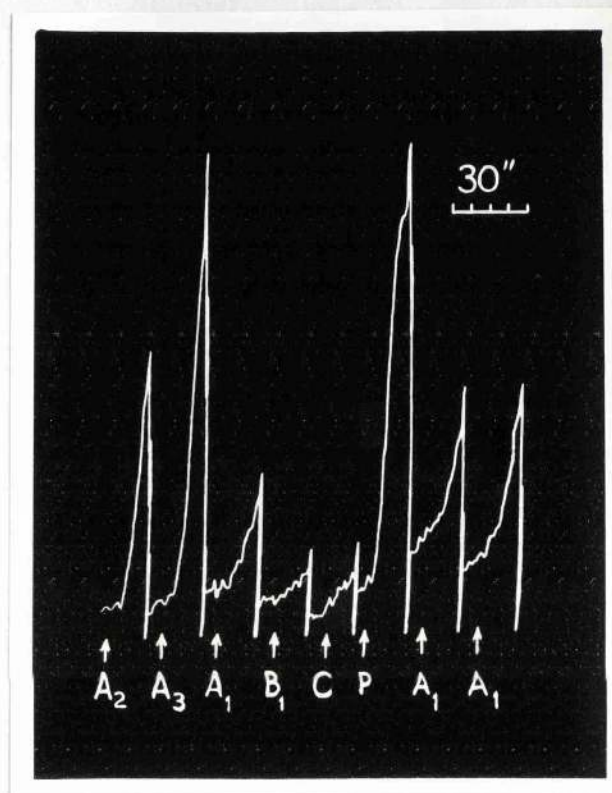


Fig. 30.

The isolated rat fundus strip.

At A ₁	-	effect of 0.02 ng. per ml. 5-hydroxytryptamine.
At A ₂	-	" 0.04 ng. " "
At A ₃	-	" 0.06 ng. " "
At B ₁	-	" 1 µg. " 3-(cyclohexylaminomethyl) thionaphthen (VII).

At C - Control.

Addition of 5-hydroxytryptamine (0.02 ng. per ml.) was preceded 30 seconds earlier by:-

At P - 1 µg. per ml. 3-(cyclohexylaminomethyl) thionaphthen.

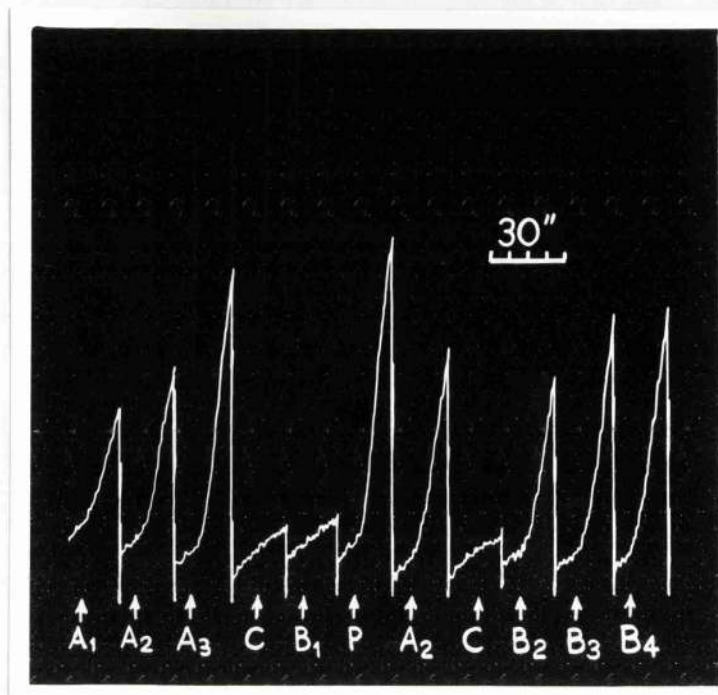


Fig. 31.

The isolated rat fundus strip.

At A₁ - effect of 0.02 ng. per ml. 5-hydroxytryptamine.

At A₂ - " 0.04 ng. " "

At A₃ - " 0.06 ng. " "

At C - Control.

At B₁ - effect of 1 µg. per ml. 3-(pyrrolidinomethyl) thionaphthen
methiodide (X).

At B₂ - " 5 µg. " " "

At B₃ - " 7 µg. " " "

At B₄ - " 9 µg. " " "

Addition of 5-hydroxytryptamine (0.04 ng. per ml.) was preceded
30 seconds earlier by:-

At P - 1 µg. per ml. 3-(pyrrolidinomethyl) thionaphthen methiodide.

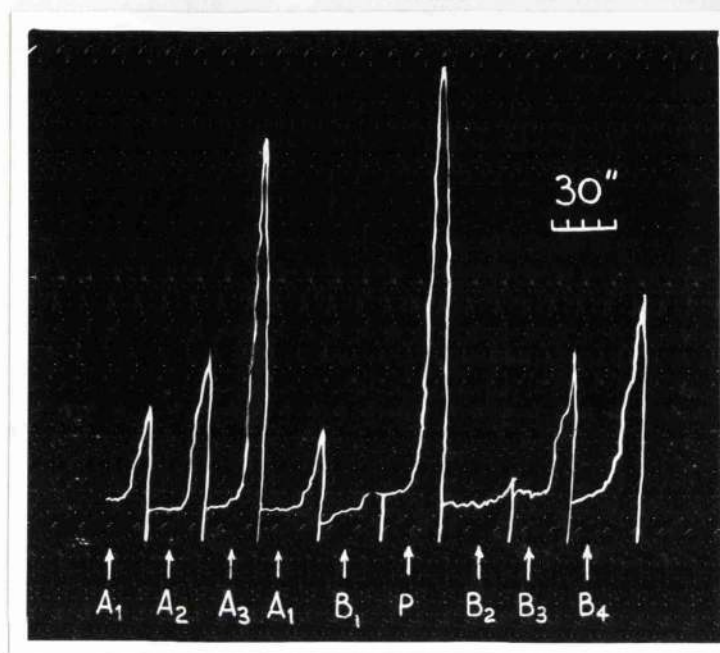


Fig. 32.

The isolated rat fundus strip.

At A₁ - Effect of 0.2 ng. per ml. 5-hydroxytryptamine.

At A₂ - " 0.4 ng. " "

At A₃ - " 0.8 ng. " "

At B₁ - " 4 µg. " 3-(piperidinomethyl) thionaphthen
methiodide (XI).

At B₂ - " 10 µg. " " "

At B₃ - " 20 µg. " " "

At B₄ - " 40 µg. " " "

Addition of 5-hydroxytryptamine (0.2 ng. per ml.) was preceded

30 seconds earlier by:-

At P - 4 µg. per ml. 3-(piperidinomethyl) thionaphthen methiodide

5-hydroxytryptamine. Compounds X and XI were effective at dose levels of from 1 to 4 $\mu\text{g. per ml.}$ (Figs. 31, 32, pages 111, 112); and at the same dose levels, compounds VIII and IX caused a very slight potentiation of the response to 5-hydroxytryptamine (Fig. 33, page 114).

At dose levels of from 40 to 50 $\mu\text{g. per ml.}$, compound XII had no direct stimulant effect on the isolated rat fundus strip. At the same dose level this compound inhibited the response to 5-hydroxytryptamine (0.2 ng. per ml.). A typical tracing of one such experiment is shown in Fig. 34, page 115. At dose levels of from 0.1 to 20 $\mu\text{g. per ml.}$, compound XIII caused powerful contractions of the tissue and greatly increased the muscle tone. The tissue did not relax to the original length even after it had been left in the bath for more than $1\frac{1}{2}$ hours and washed with Tyrode's solution at intervals of 5 minutes (Fig. 35, page 116).

With the exception of compound XV, all of the compounds of Group D, at dose levels of from 20 to 100 $\mu\text{g. per ml.}$, caused direct stimulation of the rat fundus strip. At dose levels of from 1 to 2 $\mu\text{g. per ml.}$, compounds XIV, XVI and XVII potentiated the stimulant response to 5-hydroxytryptamine (Fig. 36, page 117). At doses of up to 80 $\mu\text{g. per ml.}$, compound XV showed no stimulant effect /

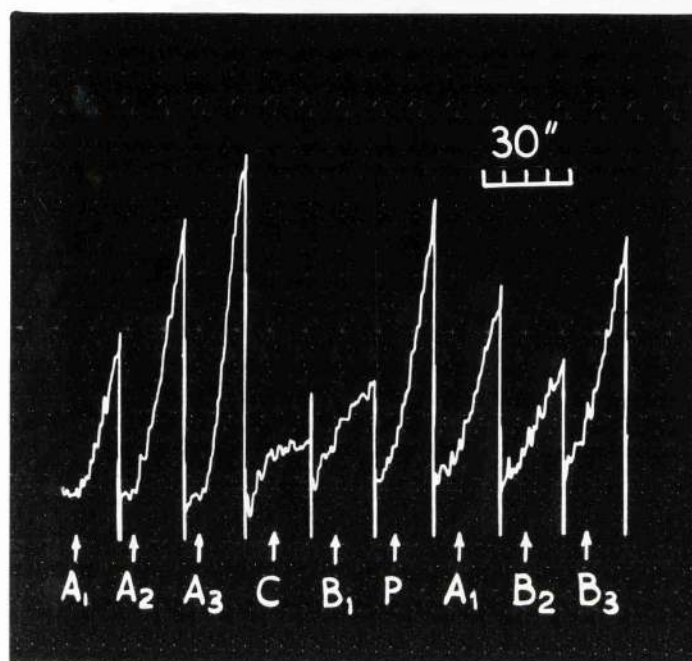


Fig. 33.

The isolated rat fundus strip.

At A_1 - Effect of 0.02 ng. per ml. 5-hydroxytryptamine.

At A_2 - " 0.04 ng. " "

At A_3 - " 0.06 ng. " "

At C - Control.

At B_1 - Effect of 1 μ g. per ml. 3-(morpholinomethyl) thionaphthen
methiodide (IX).

At B_2 - " 40 μ g. " " "

At B_3 - " 100 μ g. " " "

Addition of 5-hydroxytryptamine (0.02 ng. per ml.) was preceded
30 seconds earlier by:-

At P - 1 μ g. per ml. 3-(morpholinomethyl) thionaphthen methiodide.

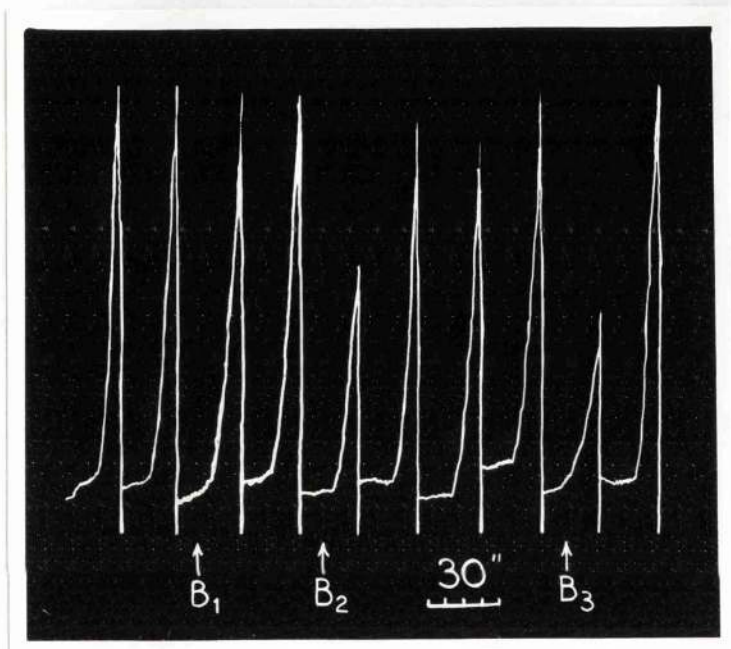


Fig. 34.

The isolated rat fundus strip.

All unlabelled contractions are due to 0.2 ng. per ml. of 5-hydroxytryptamine.

Addition of 5-hydroxytryptamine was preceded 30 seconds earlier by:

At B_1 - 10 μ g. per ml. 2-(2'-aminoethyl)-5-hydroxythionaphthen (XII)

At B_2 - 20 μ g. " " " "

At B_3 - 40 μ g. " " " "

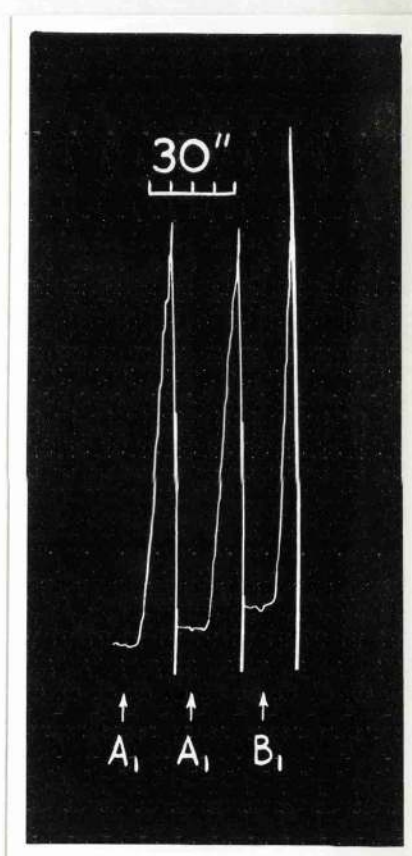


Fig. 35.

The isolated rat fundus strip

At A₁ - Effect of 1 ng. per ml. 5-hydroxytryptamine.

At B₁ - " 0.1 μg. per ml. 2-(2'-aminopropyl)-5-hydroxy-
thionaphthen (XIII).

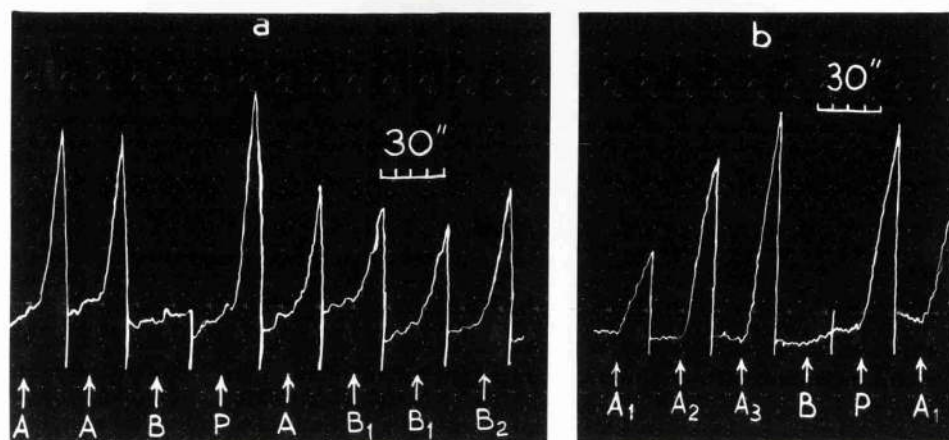


Fig. 36.

The isolated rat fundus strip.

(a) At A - Effect of 0.2 ng. per ml. 5-hydroxytryptamine.

At B - " 1 μ g. per ml. 5-amino-2-(pyrrolidinomethyl)thionaphthen (XVI).

At B₁ - " 20 μ g. " " "

At B₂ - " 100 μ g. " " "

Addition of 5-hydroxytryptamine (0.2 ng. per ml.) was preceded 30 seconds earlier by:-

At P - 1 μ g. per ml. 5-amino-2-(pyrrolidinomethyl)thionaphthen.

(b) At A₁ - Effect of 0.2 ng. per ml. 5-hydroxytryptamine.

At A₂ - Effect of 0.4 ng. " "

At A₃ - " 0.6 ng. " "

At B - " 2 μ g. per ml. 5-amino-2-(dimethylaminomethyl)thionaphthen (XIV).

Addition of 5-hydroxytryptamine (0.2 ng. per ml.) was preceded 30 seconds earlier by:-

At P - 2 μ g. per ml. 5-amino-2-(dimethylaminomethyl)thionaphthen.

effect on the rat fundus strip but at this dose level it inhibited the contraction produced by 5-hydroxytryptamine (1 μ g. per ml.). A typical tracing is shown in Fig. 37, page 119.

(c) Isolated guinea pig ileum

- (i) The direct stimulant effect of the thionaphthen compounds tested, and
- (ii) The effects of lysergic acid diethylamide, mepyramine, and atropine on the contractions produced by the thionaphthen compounds.

Among the gramine-like isosteres, compounds I and III, at dose levels of from 100 to 500 μ g. per ml., had no direct stimulant effects on the isolated guinea pig ileum. The other compounds in this group had a direct stimulant effect at dose levels of from 25 to 200 μ g. per ml. and caused contractions of varying degrees of intensity. In many cases, measurable, and reproducible responses could not be obtained and frequently the response to a second and higher dose of the drug was considerably less than that to the first (Fig. 38, page 120). When contractions of reasonable and measurable height were obtained, the effects of lysergic acid diethylamide, mepyramine and atropine were tried on the contractions /

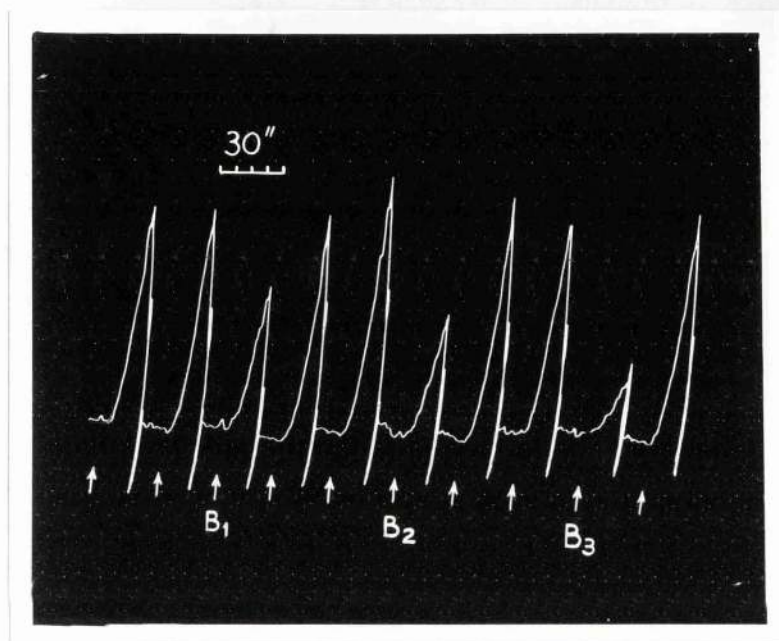


Fig. 37.

The isolated rat fundus strip.

All contractions are due to 1 μ g. per ml. of 5-hydroxytryptamine.

Addition of 5-hydroxytryptamine was preceded 30 seconds earlier by:-

At B_1 - 20 μ g. per ml. 5-amino-2-(morpholinomethyl) thionaphthen
(XV).

At B_2 - 40 μ g. " " " "

At B_3 - 80 μ g. " " " "

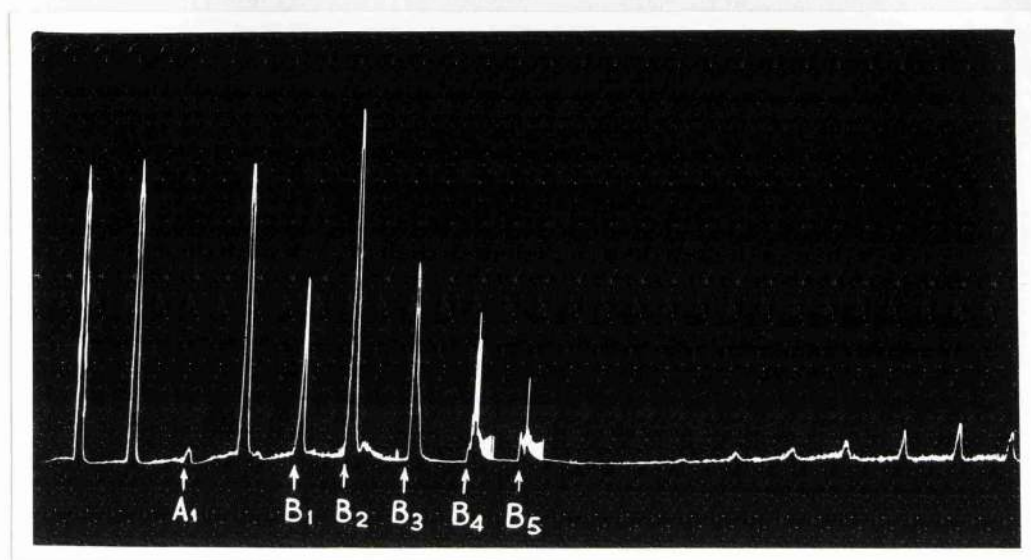


Fig. 38.

The isolated guinea pig
ileum.

All unlabelled contractions are due to 0.01 μ g. per ml. of histamine.

Addition of histamine was preceded 30 seconds earlier by:-

At A_1 - 0.5 μ g. per ml. 3-(dimethylaminomethyl) thionaphthen (II).

At B_1 - Effect of 25 μ g. per ml. 3-(dimethylaminomethyl)
thionaphthen.

At B_2 -	"	50 μ g.	"	"
At B_3 -	"	100 μ g.	"	"
At B_4 -	"	200 μ g.	"	"
At B_5 -	"	300 μ g.	"	"

contractions produced by the thionaphthen compounds. The contractions produced by compound II (5 to 25 $\mu\text{g. per ml.}$) were antagonized by 0.5 $\mu\text{g. per ml.}$ of atropine and 0.5 $\mu\text{g. per ml.}$ of mepyramine (Fig. 39, a and b, page 122). The contractions produced by compound V (10 to 50 $\mu\text{g. per ml.}$) were inhibited by 50 $\mu\text{g. per ml.}$ of mepyramine. Atropine and lysergic acid diethylamide did not inhibit the contractions produced by this compound. Higher concentrations of lysergic acid diethylamide (2.5 to 5 $\mu\text{g. per ml.}$) had a direct stimulant effect on the isolated guinea pig ileum (Figs. 40, 41, pages 123, 124).

The compounds in Group B had a direct stimulant action on the isolated guinea pig ileum. Compounds VIII and X were the most potent, causing contractions at dose levels of from 1 to 4 $\mu\text{g. per ml.}$ Next in order of potency came compounds IX and XI which were effective at dose levels of from 10 to 50 $\mu\text{g. per ml.}$ The contractions produced by compounds in this group were antagonized completely by atropine (0.5 to 50 $\mu\text{g. per ml.}$) and by mepyramine (25 to 50 $\mu\text{g. per ml.}$). Tracings obtained from three different experiments are shown in Figs. 42 to 44a, pages 125 to 127. At dose levels ranging between 0.001 and 0.5 $\mu\text{g. per ml.}$ lysergic acid diethylamide did not antagonize, or incompletely /

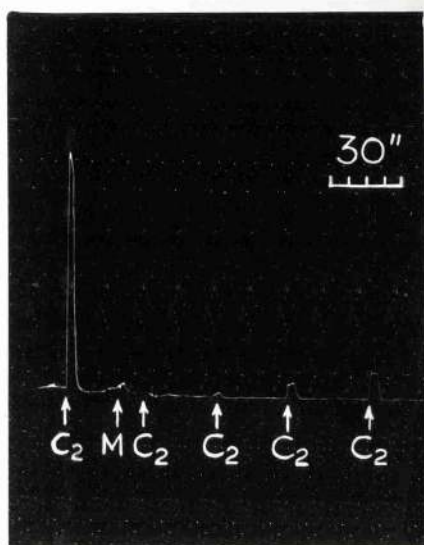


Fig. 39a.

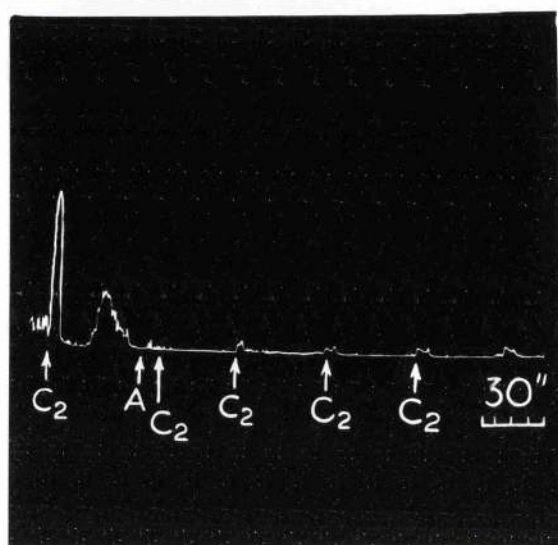


Fig. 39b.

The isolated guinea pig ileum.

(a) At C_2 - Effect of 5 μ g. per ml. 3-(dimethylaminomethyl) thionaphthen (II).

At M - Effect of 0.5 μ g. per ml. mepyramine followed 30 seconds later by 5 μ g. per ml. 3-(dimethylaminomethyl) thionaphthen.

(b) At C_2 - Effect of 25 μ g. per ml. 3-(dimethylaminomethyl) thionaphthen.

At A - Effect of 0.5 μ g. per ml. atropine followed 30 seconds later by 25 μ g. per ml. 3-(dimethylaminomethyl) thionaphthen.

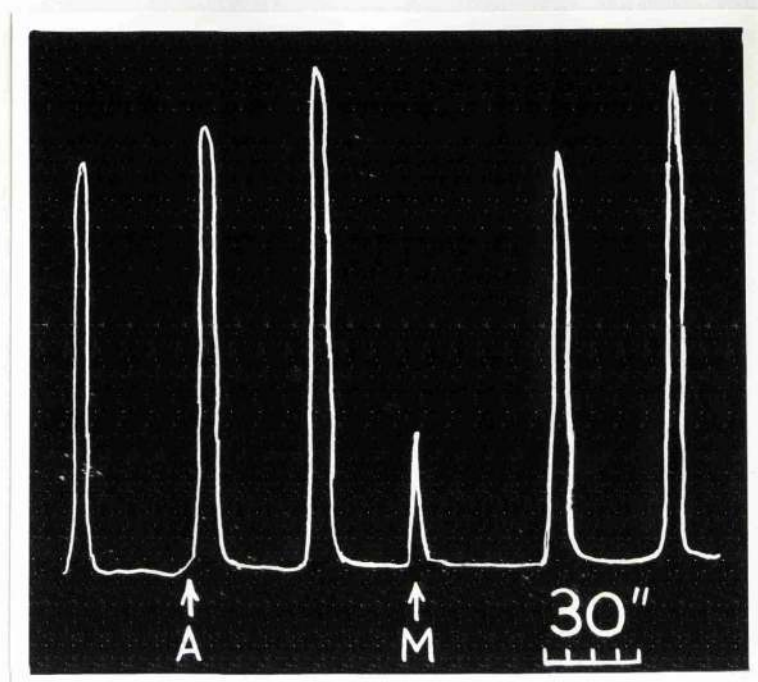


Fig. 40.

The isolated guinea pig ileum.

All unlabelled contractions are due to 50 μ g. per ml. 3-(piperidinomethyl) thionaphthen (V).

Addition of 3-(piperidinomethyl) thionaphthen was preceded 30 seconds earlier by:-

At A - 50 μ g. per ml. atropine.

At M - 50 μ g. per ml. mepyramine.

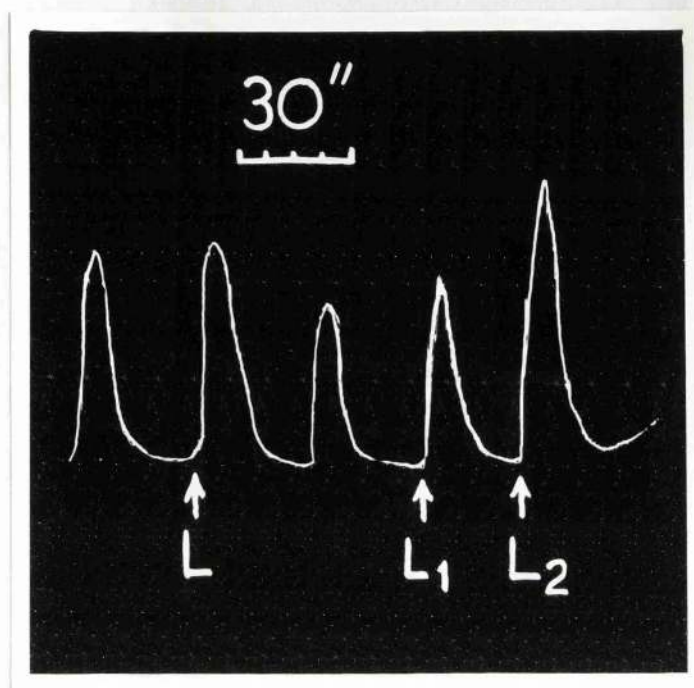


Fig. 41.

The isolated guinea pig ileum.

All unlabelled contractions are due to 10 μ g. per ml. of 3-(piperidinomethyl) thionaphthen (V).

Addition of 3-(piperidinomethyl) thionaphthen was preceded 30 seconds earlier by:-

At L - 0.05 μ g. per ml. lysergic acid diethylamide.

At L₁ - effect of 2.5 μ g. per ml. lysergic acid diethylamide.

At L₂ - " 5 μ g. " " " "

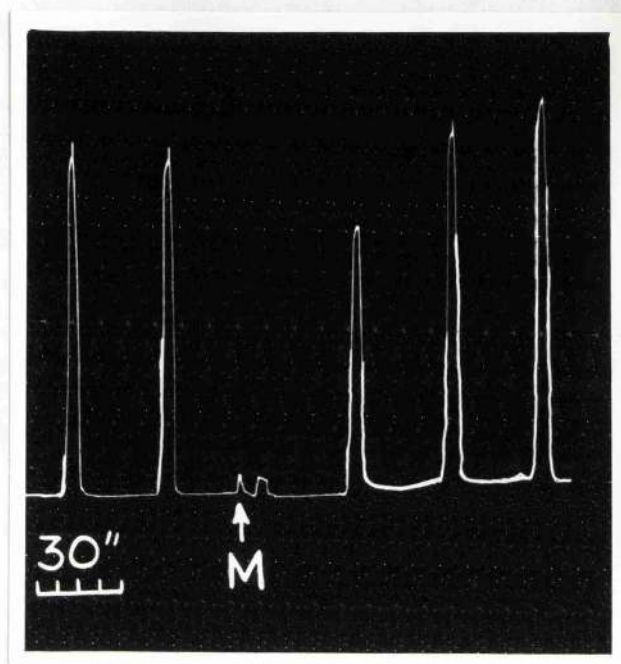


Fig. 42.

The isolated guinea pig ileum.

All unlabelled contractions are due to 2.5 μ g. per ml. of 3-(dimethylaminomethyl) thionaphthen methiodide (VIII).

Addition of this compound was preceded 30 seconds earlier by:-

At M - 25 μ g. per ml. of mepyramine.

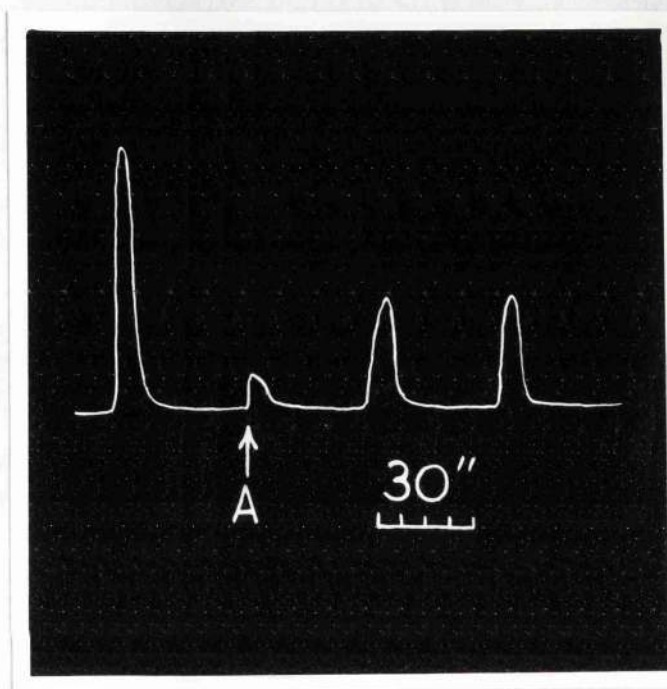


Fig. 43.

The isolated guinea pig ileum.

All contractions are due to 25 μ g. per ml. of 3-(morpholinomethyl) thionaphthen methiodide (IX).

Addition of 3-(morpholinomethyl) thionaphthen methiodide was preceded 30 seconds earlier by:-

At A - 25 μ g. per ml. atropine

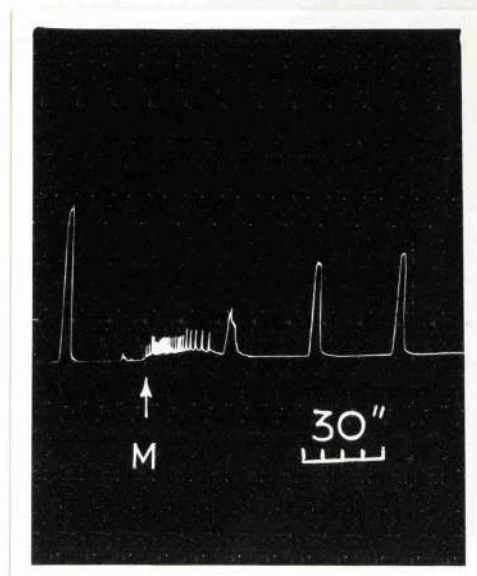


Fig. 44a.

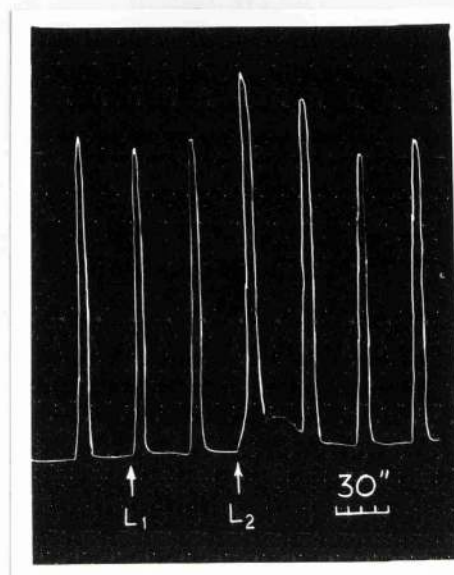


Fig. 44b.

The isolated guinea pig ileum.

- (a) All unlabelled contractions are due to 5 μ g. per ml. of 3-(pyrrolidinomethyl) thionaphthen methiodide (X)

At M - Effect of 25 μ g. per ml. mepyramine, followed 30 seconds later by 5 μ g. per ml. 3-(pyrrolidinomethyl) thionaphthen methiodide.

- (b) All contractions are due to 5 μ g. per ml. of 3-(pyrrolidinomethyl) thionaphthen methiodide (X).

Addition of this compound was preceded 30 seconds earlier by:-

At L_1 - 0.5 μ g. per ml. lysergic acid diethylamide.

At L_2 - 5 μ g. " " " "

incompletely antagonized, the contractions produced by these compounds (Figs. 44b, 45, pages 127, 129). At higher concentrations (5 to 10 μ g. per ml.) lysergic acid diethylamide itself caused a contraction of the tissue and did not induce a further inhibition.

At dose levels of from 10 to 20 μ g. per ml., compound XIII had a slight direct stimulant effect on the isolated guinea pig ileum. In some experiments, compound XII, at a dose level of 50 μ g. per ml., exerted a slight stimulant effect. In others at dose levels of up to 100 μ g. per ml., it had no stimulant effects. The contractions were not reproducible and not measurable and so antagonism by lysergic acid diethylamide, mepyramine and atropine was not investigated.

Among the 5-amino-isogramine-like isosteres, compound XV had no direct stimulant effect on the guinea pig ileum. At dose levels of from 200 to 500 μ g. per ml., compounds XIV, XVI and XVII had very slight stimulant effects on this tissue. The contractions produced were not measurable and not reproducible and therefore the effects of lysergic acid diethylamide, mepyramine and atropine upon the contractions produced by these compounds were not investigated.

(iii) Effect of the thionaphthen compounds on the
contractions /

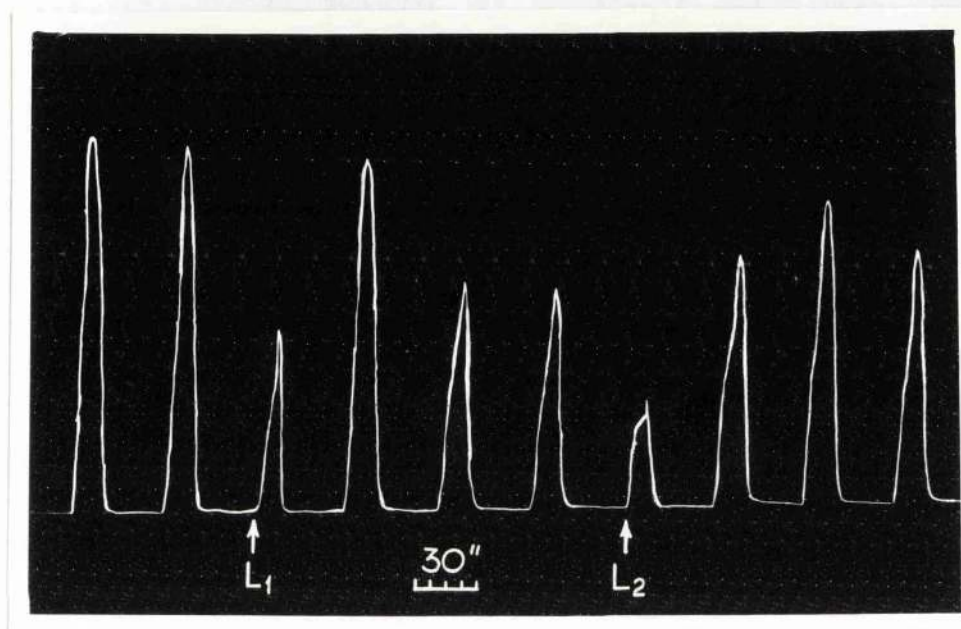


Fig. 45.

The isolated guinea pig ileum.

All unlabelled contractions are due to 25 μ g. per ml. of 3-(piperidinomethyl) thionaphthen methiodide (XI).

Addition of this compound was preceded 30 seconds earlier by:-

At L_1 - 0.05 μ g. per ml. lysergic acid diethylamide.

At L_2 - 0.5 μ g. per ml. " " "

contractions produced by 5-hydroxytryptamine.

Except in the case of compound VI, all the compounds in Group A inhibited the stimulant effects of 5-hydroxytryptamine on the isolated guinea pig ileum. The most potent were compounds I and III which, at dose levels of from 2 to 5 $\mu\text{g. per ml.}$, completely inhibited the stimulant response to 5-hydroxytryptamine (0.01 to 0.5 $\mu\text{g. per ml.}$). Typical tracings from two experiments are shown in Figs. 46, 49a, pages 131, 134. At dose levels ranging from 1 to 4 $\mu\text{g. per ml.}$ compounds II and V incompletely inhibited the stimulant response to 5-hydroxytryptamine. At higher concentrations than this, there was no further inhibition but a direct stimulant effect was seen (Fig. 47, page 132). At dose levels of from 1 to 2 $\mu\text{g. per ml.}$ compound IV caused an incomplete (10 to 20 per cent) inhibition of the stimulant effect of 5-hydroxytryptamine and at higher concentrations (5 to 50 $\mu\text{g. per ml.}$) it caused the tissue to contract. The contractions were not reproducible and the stimulant response to a second and higher dose of the drug was considerably less than that to the first (Fig. 48, page 133). In one experiment, however, at a dose level of 50 $\mu\text{g. per ml.}$ no direct stimulant effect was produced by this compound on the isolated guinea pig ileum. At the same dose /

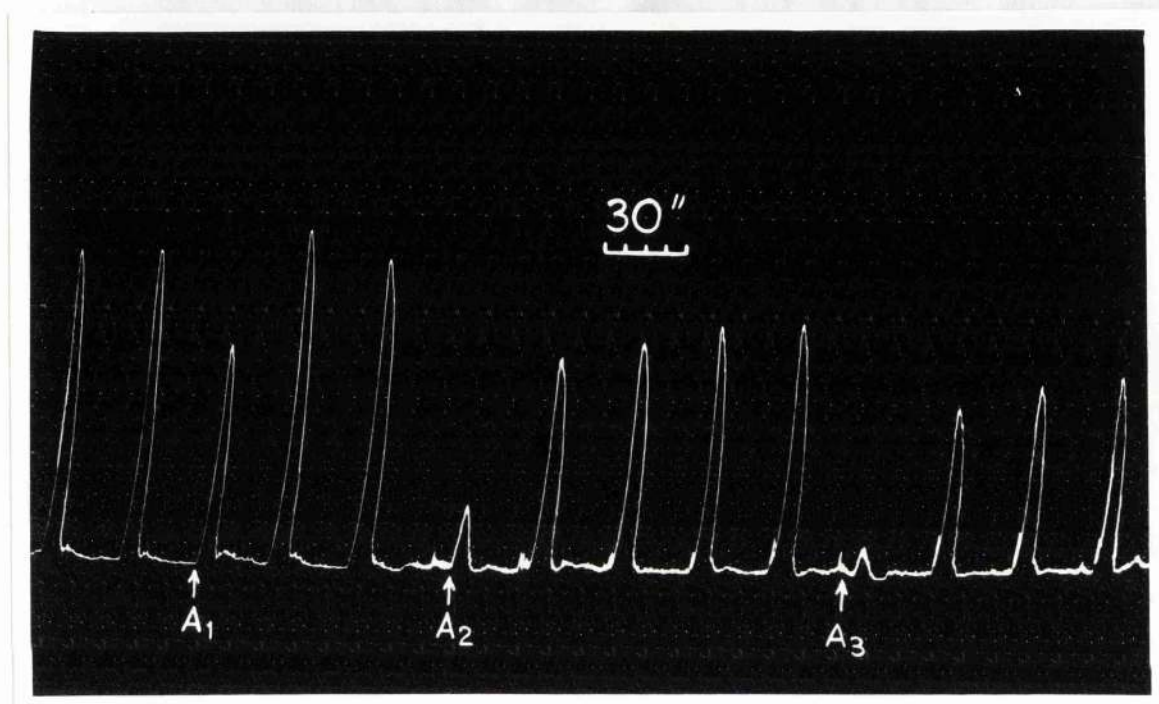


Fig. 46.

The isolated guinea pig ileum.

All contractions are due to 0.05 μ g. per ml. of 5-hydroxytryptamine.

Addition of 5-hydroxytryptamine was preceded 30 seconds earlier by:-

At A_1 - 0.3 μ g. per ml. 3-(morpholinomethyl) thionaphthen (III).

At A_2 - 0.6 μ g. " " "

At A_3 - 1 μ g. " " "

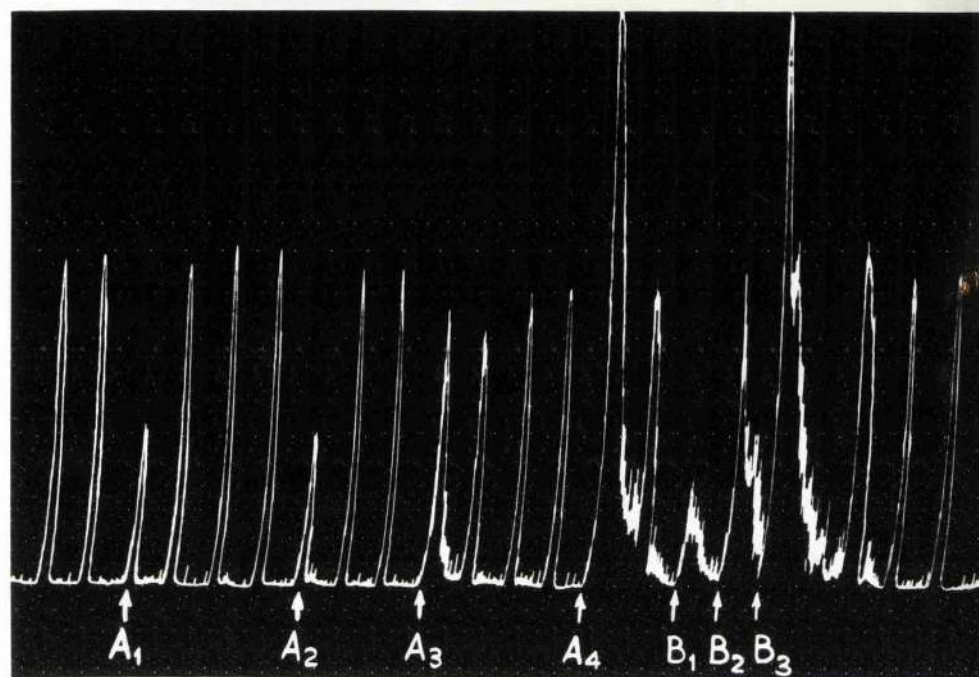


Fig. 47

The isolated guinea pig ileum.

All unlabelled contractions are due to 0.02 μ g. per ml. of 5-hydroxytryptamine.

Addition of 5-hydroxytryptamine was preceded 30 seconds earlier by:

At A ₁	- 0.1 μ g. per ml.	3-(piperidinomethyl) thionaphthen (V)
At A ₂	- 0.2 μ g. per ml.	" " "
At A ₃	- 1 μ g. per ml.	" " "
At A ₄	- 2 μ g. per ml.	" " "
At B ₁	- Effect of 1 μ g. per ml. 3-(piperidinomethyl) thionaphthen.	
At B ₂	- " 2 μ g. per ml.	" " "
At B ₃	- " 3 μ g. per ml.	" " "

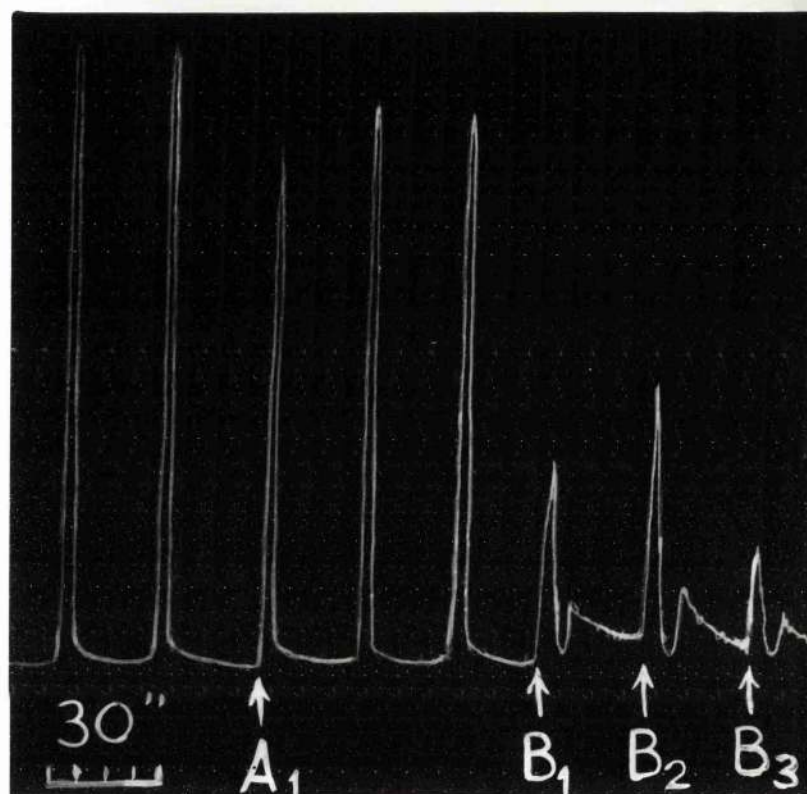


Fig. 48

The isolated guinea pig ileum.

All unlabelled contractions are due to $0.025 \mu\text{g. per ml.}$ of 5-hydroxytryptamine.

Addition of 5-hydroxytryptamine was preceded 30 seconds earlier by:

At A_1 - $1 \mu\text{g. per ml.}$ 3-(pyrrolidinomethyl) thionaphthen (IV).

At B_1 - Effect of $5 \mu\text{g. per ml.}$ 3-(pyrrolidinomethyl) thionaphthen.

At B_2 - " $10 \mu\text{g.}$ " " " "

At B_3 - " $50 \mu\text{g.}$ " " " "

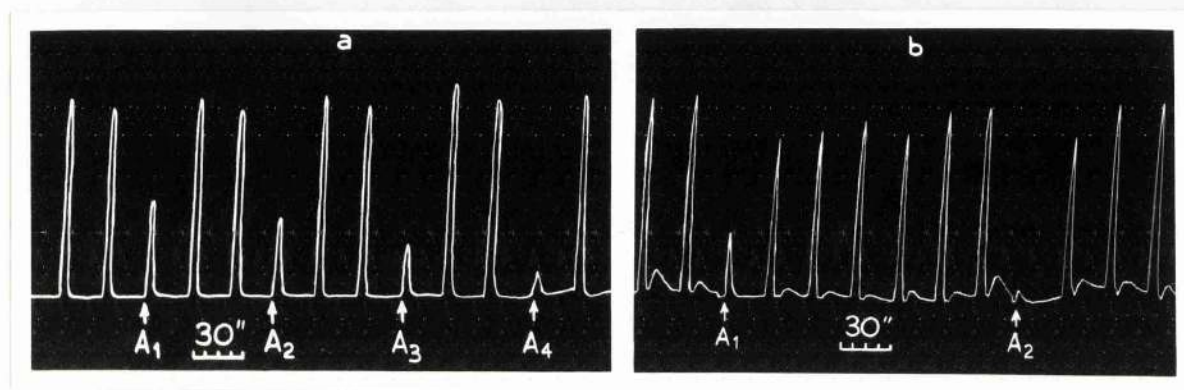


Fig. 49.

The isolated guinea pig ileum.

- (a) All unlabelled contractions are due to 0.1 μ g. per ml. 5-hydroxytryptamine.

Addition of 5-hydroxytryptamine was preceded 30 seconds earlier by:-

At A_1 - 1 μ g. per ml. 3-(aminomethyl) thionaphthen (I).

At A_2 - 1.5 μ g. " " "

At A_3 - 2.5 μ g. " " "

At A_4 - 5 μ g. " " "

- (b) All contractions are due to 0.5 μ g. per ml. 5-hydroxytryptamine.

Addition of 5-hydroxytryptamine was preceded 30 seconds earlier by:-

At A_1 - 10 μ g. per ml. 3-(pyrrolidinomethyl) thionaphthen (IV).

At A_2 - 50 μ g. " " "

dose level it completely inhibited the stimulant response to 0.5 $\mu\text{g. per ml.}$ of 5-hydroxytryptamine (Fig. 49b, page 134). Compound VII was the weakest antagonist to 5-hydroxytryptamine and at dose levels of from 10 to 20 $\mu\text{g. per ml.}$, it incompletely inhibited the stimulant response to 5-hydroxytryptamine. At dose levels of 1 to 2 $\mu\text{g. per ml.}$, compound VI did not inhibit the stimulant effects of 5-hydroxytryptamine (0.01 to 0.5 $\mu\text{g. per ml.}$) but increased the effect of 5-hydroxytryptamine and the tone of the muscle was also increased. This increased tone was very persistent and remained even after the tissue was washed several times with Tyrode's solution (Fig. 50, page 136).

At dose levels of from 0.1 to 4 $\mu\text{g. per ml.}$, compounds IX and X did not inhibit the contractions produced by 5-hydroxytryptamine (0.01 to 0.5 $\mu\text{g. per ml.}$) and at higher concentrations, they exerted a direct stimulant effect upon the isolated guinea pig ileum (Fig. 51, page 137). At dose levels of from 0.1 to 4 $\mu\text{g. per ml.}$, however, compounds VIII and XI partially inhibited the stimulant response produced by 0.05 to 0.1 $\mu\text{g. per ml.}$ of 5-hydroxytryptamine (Figs. 52, 52a, pages 138, 139).

At dose levels of from 20 to 40 $\mu\text{g. per ml.}$, compound XII inhibited the contractions produced by 5-hydroxytryptamine (Fig. 53, /

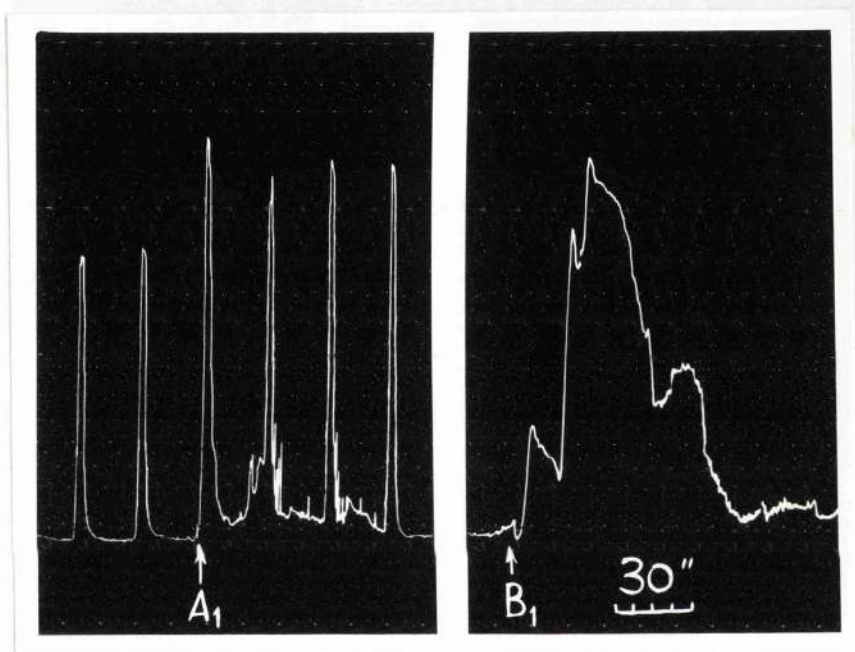


Fig. 50.

The isolated guinea pig ileum.

All unlabelled contractions are due to 0.1 $\mu\text{g. per ml.}$ of 5-hydroxytryptamine.

Addition of 5-hydroxytryptamine was preceded 30 seconds earlier by:

At A_1 - 1 $\mu\text{g. per ml.}$ 3-(2'-methylpiperidinomethyl) thionaphthen
(VI).

At B_1 - Effect of 50 $\mu\text{g. per ml.}$ 3-(2'-methylpiperidinomethyl)
thionaphthen.

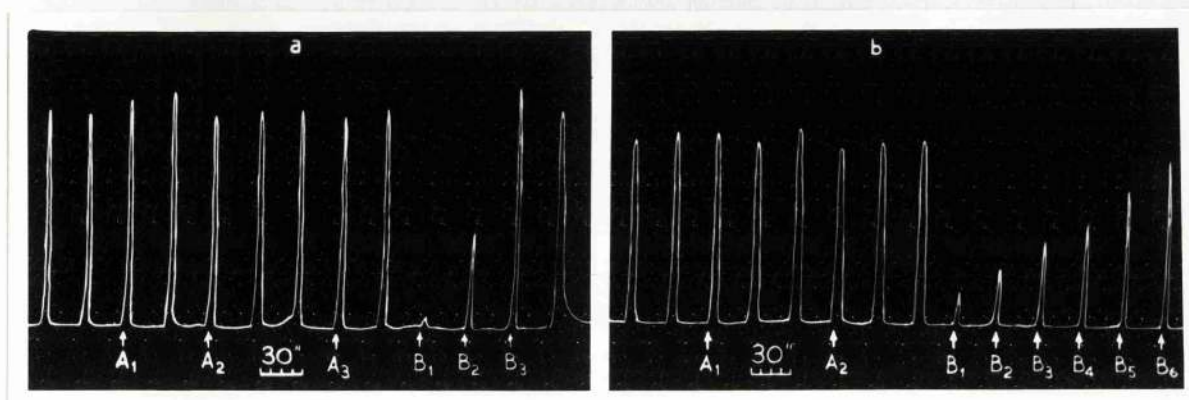


Fig. 51.

The isolated guinea pig ileum.

- (a) All unlabelled contractions are due to 0.5 μ g. per ml. of 5-hydroxytryptamine.

Addition of 5-hydroxytryptamine was preceded 30 seconds earlier by:-

At A_1 - 1 μ g. per ml. 3-(morpholinomethyl) thionaphthen methiodide (IX).

At A_2 - 2.5 μ g. " " "

At A_3 - 5 μ g. " " "

At B_1 - Effect of 20 μ g. per ml. 3-(morpholinomethyl) thionaphthen methiodide.

At B_2 - " 30 μ g. " "

At B_3 - " 40 μ g. " "

- (b) All unlabelled contractions are due to 0.1 μ g. per ml. of 5-hydroxytryptamine.

Addition of 5-hydroxytryptamine was preceded 30 seconds earlier by:-

At A_1 - 0.1 μ g. per ml. 3-(pyrrolidinomethyl) thionaphthen methiodide (X).

At A_2 - 1 μ g. " " "

At B_1 - Effect of 2 μ g. per ml. 3-(pyrrolidinomethyl) thionaphthen methiodide.

At B_2 - " 2.5 μ g. " "

At B_3 - " 3 μ g. " "

At B_4 - " 3.5 μ g. " "

At B_5 - " 4 μ g. " "

At B_6 - " 4.5 μ g. " "

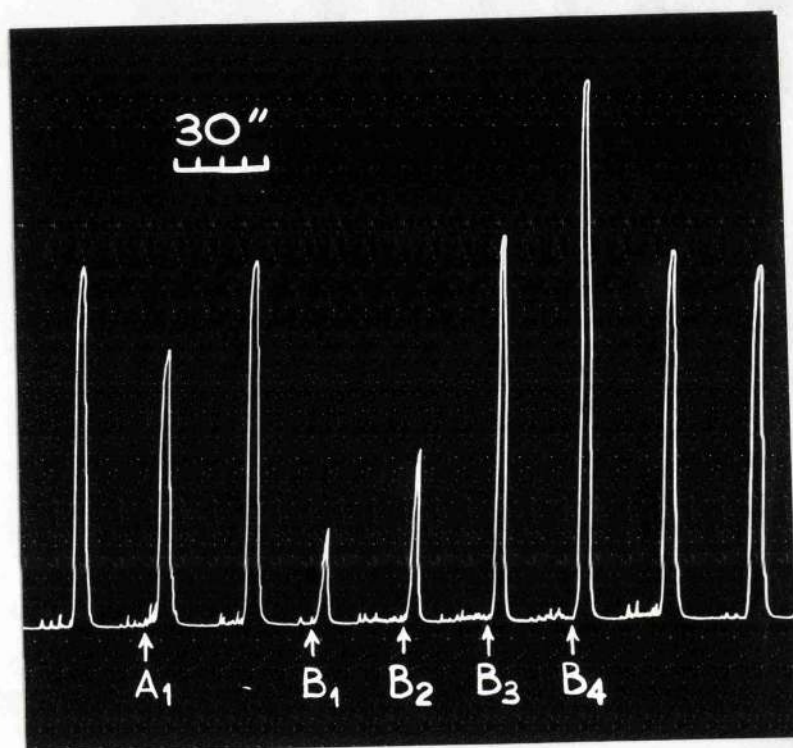


Fig. 52.

The isolated guinea pig ileum.

All unlabelled contractions are due to 0.05 $\mu\text{g.}$ per ml. of 5-hydroxytryptamine.

Addition of 5-hydroxytryptamine was preceded 30 seconds earlier by:-

At A_1 - 1 $\mu\text{g.}$ per ml. 3-(dimethylaminomethyl) thionaphthen
methiodide (VIII).

At B_1 - Effect of 2 $\mu\text{g.}$ per ml. 3-(dimethylaminomethyl)
thionaphthen methiodide.

At B_2 - " 3 $\mu\text{g.}$ " " "

At B_3 - " 3.5 $\mu\text{g.}$ " " "

At B_4 - " 4 $\mu\text{g.}$ " " "

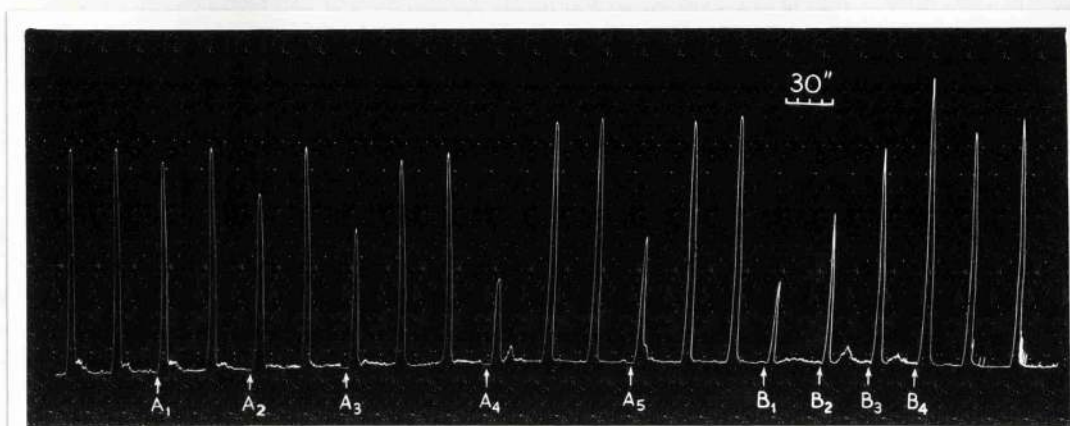


Fig. 52a.

The isolated guinea pig ileum.

All unlabelled contractions are due to 0.05 μ g. per ml. of 5-hydroxytryptamine.

Addition of 5-hydroxytryptamine was preceded 30 seconds earlier by:-

At A_1 - 0.5 μ g. per ml. 3-(piperidinomethyl) thionaphthen methiodide (XI).

At A_2 - 1 μ g. " " " "

At A_3 - 2 μ g. " " " "

At A_4 - 4 μ g. " " " "

At A_5 - 15 μ g. " " " "

At B_1 - Effect of 30 μ g. per ml. 3-(piperidinomethyl) thionaphthen methiodide.

At B_2 - " 40 μ g. " " "

At B_3 - " 50 μ g. " " "

At B_4 - " 60 μ g. " " "

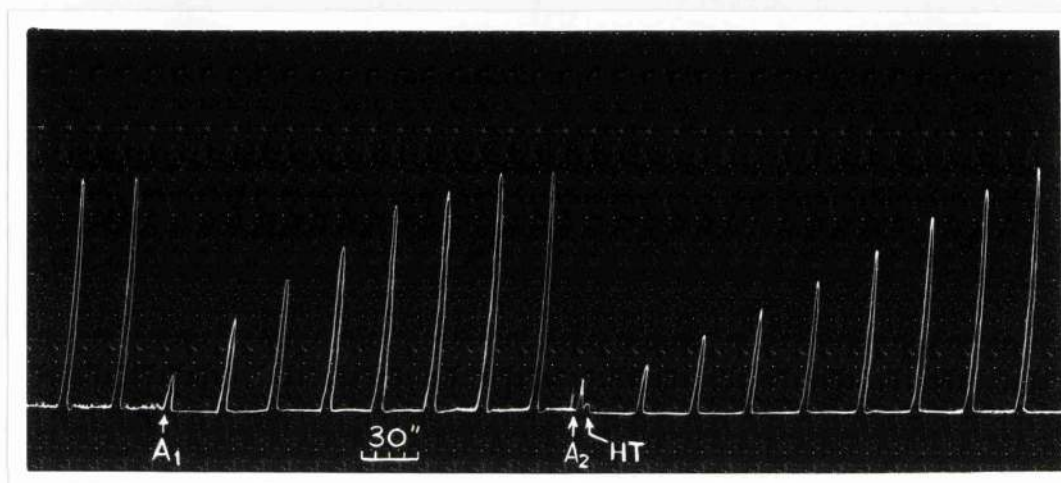


Fig. 53.

The isolated guinea pig ileum.

All unlabelled contractions are due to 0.015 μ g. per ml. of 5-hydroxytryptamine.

Addition of 5-hydroxytryptamine was preceded 30 seconds earlier by:

At A_1 - 40 μ g. per ml. 2-(2'-aminoethyl) 5-hydroxythionaphthen (XII)

At A_2 - 80 μ g. per ml. " "

followed 30 seconds later by 0.015 μ g. per ml. 5-hydroxytryptamine(HT).

53, page 140). At a dose level of 1 μ g. per ml., compound XIII did not inhibit the stimulant response to 5-hydroxytryptamine. Higher concentrations (10 to 50 μ g. per ml.) exerted a slight, direct stimulant effect. After the drug was washed out from the bath, the response to the next dose of 5-hydroxytryptamine was less than the control one (Fig. 54, page 142).

At dose levels ranging from 100 to 250 μ g. per ml., all the compounds in Group D, completely or incompletely inhibited the stimulant response to 5-hydroxytryptamine (0.01 to 0.5 μ g. per ml.). At 500 μ g. per ml., they did not cause any further inhibition of the stimulant response to 5-hydroxytryptamine but exerted a slight, direct stimulant effect on the isolated guinea pig ileum (Figs. 55 to 57, pages 143 to 145).

(iv) Effects of thionaphthen compounds on the contraction produced by histamine.

All the compounds in Group A completely or incompletely inhibited the response of the isolated guinea pig ileum to histamine (0.001 to 0.1 μ g. per ml.). The most potent was compound IV which was effective at dose levels of from 0.03 to 1 μ g. per ml. (Fig. 58, page 146). Compounds II and V were active at dose levels of from 0.1 to 1 μ g. per ml. (Fig. 59, page 147). Next
in /

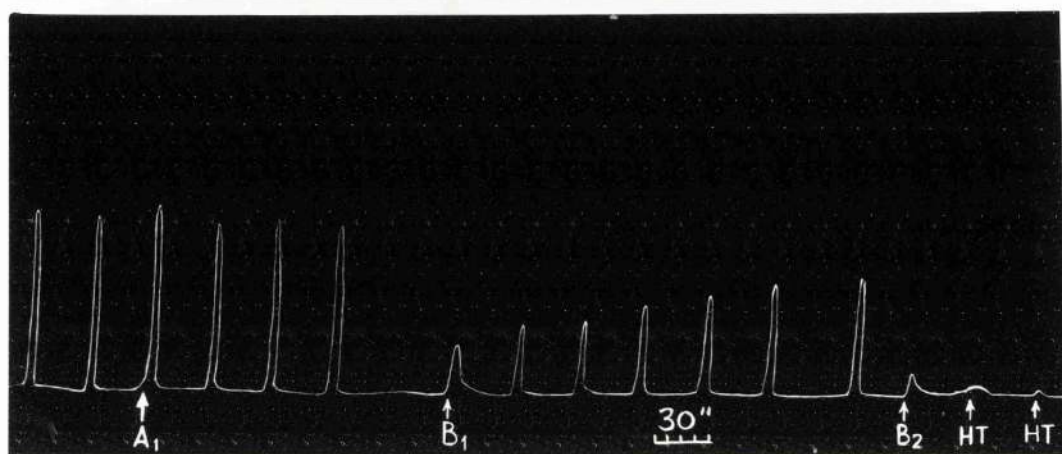


Fig. 54.

The isolated guinea pig ileum.

All unlabelled contractions are due to 0.02 μ g. per ml. of 5-hydroxytryptamine.

Addition of 5-hydroxytryptamine was preceded 30 seconds earlier by:

At A_1 - 0.2 μ g. per ml. 2-(2'-aminopropyl)-5-hydroxythionaphthen
(XIII).

At B_1 - Effect of 10 μ g. per ml. 2-(2'-aminopropyl)-5-hydroxy-
thionaphthen.

At B_2 - " 50 μ g. " " "

At HT - " 0.02 μ g. per ml. 5-hydroxytryptamine.

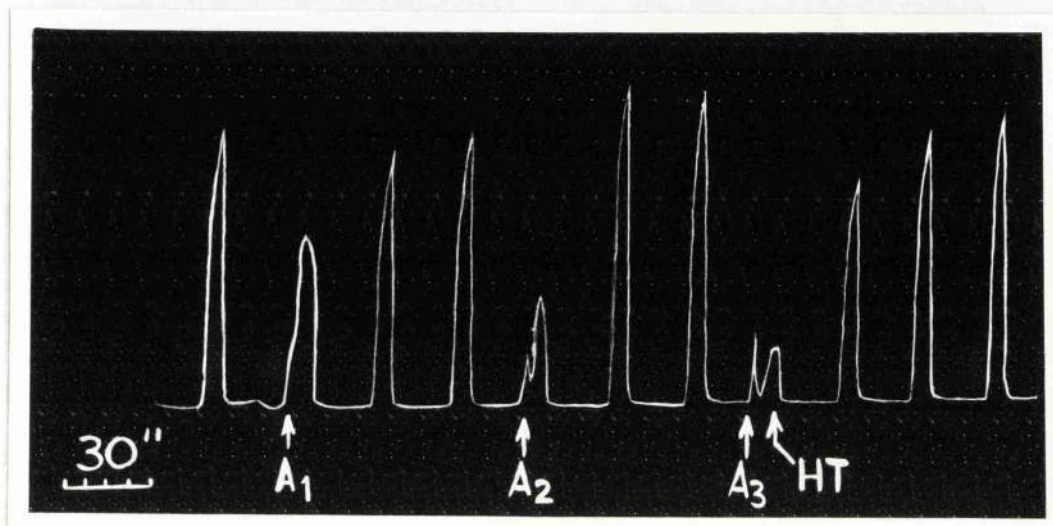


Fig. 55.

The isolated guinea pig ileum.

All unlabelled contractions are due to 0.05 μ g. per ml. of 5-hydroxytryptamine.

Addition of 5-hydroxytryptamine was preceded 30 seconds earlier by:

At A_1 - 100 μ g. per ml. 5-amino-2-(dimethylaminomethyl)
thionaphthen (XIV).

At A_2 - 200 μ g. " " " "

At A_3 - Effect of 500 μ g. per ml. 5-amino-2-(dimethylaminomethyl)
thionaphthen.

followed 30 seconds later by 0.05 μ g. per ml. 5-hydroxytryptamine (HT),

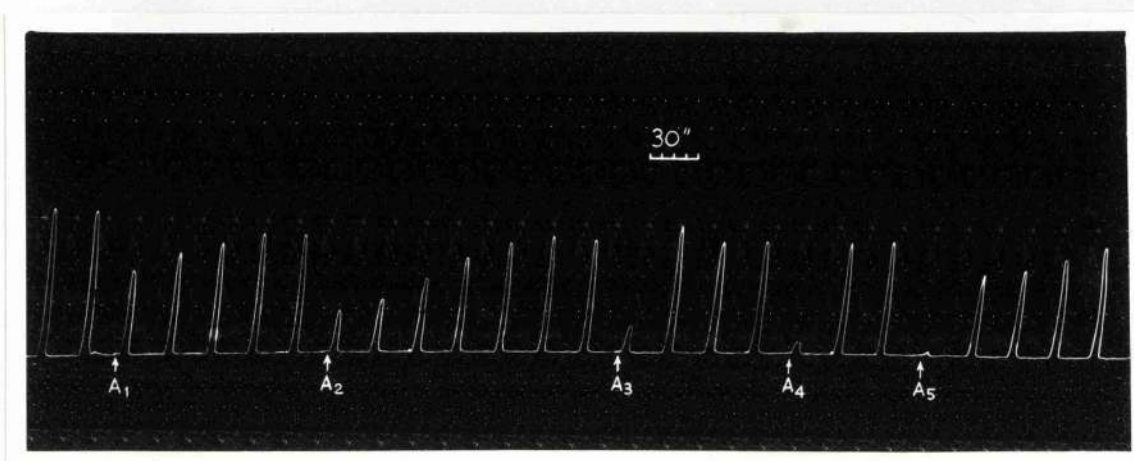


Fig. 56.

The isolated guinea pig ileum.

All contractions are due to 0.075 μ g. per ml. of 5-hydroxytryptamine.

Addition of 5-hydroxytryptamine was preceded 30 seconds earlier by:-

At A_1 - 5 μ g. per ml. 5-amino-2-(morpholinomethyl) thionaphthen
(XV).

At A_2 - 10 μ g. " " " "

At A_3 - 50 μ g. " " " "

At A_4 - 100 μ g. " " " "

At A_5 - 200 μ g. " " " "

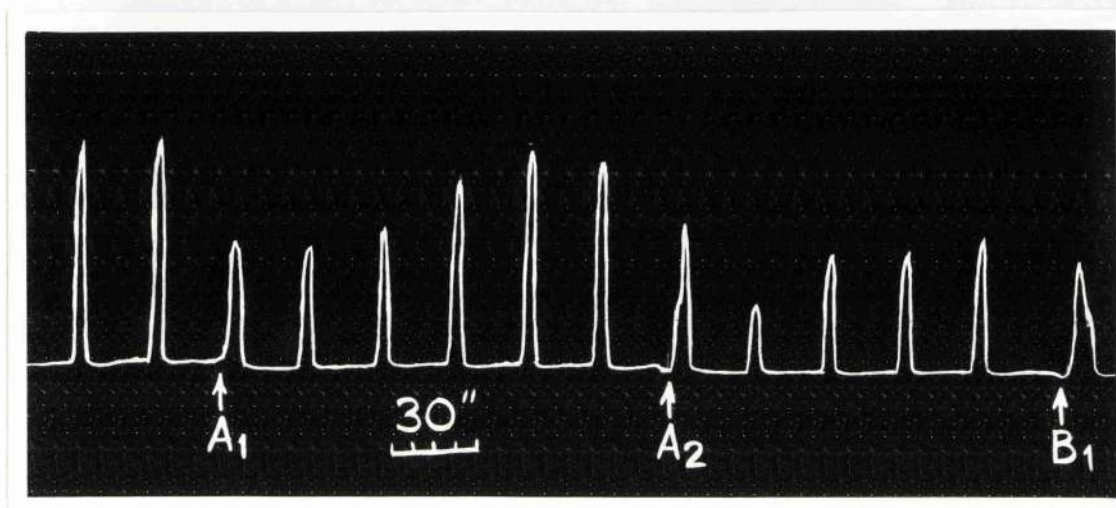


Fig. 57.

The isolated guinea pig ileum.

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

Addition of 5-hydroxytryptamine was preceded 30 seconds earlier by:

At A_1 - 100 μ g. per ml. 5-amino-2-(pyrrolidinomethyl) thionaphthen
(XVI)

At A_2 - 250 μ g. " " " " "

At B_1 - Effect of 1 mg. per ml. 5-amino-2-(pyrrolidinomethyl)
thionaphthen.

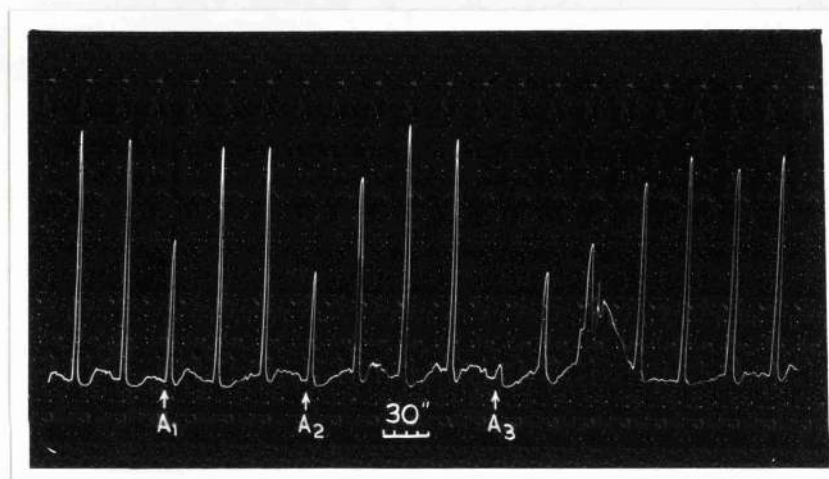


Fig. 58.

The isolated guinea pig ileum.

All contractions are due to 0.01 μ g. per ml. of histamine.

Addition of histamine was preceded 30 seconds earlier by:-

At A_1 - 0.01 μ g. per ml. 3-(pyrrolidinomethyl) thionaphthen (IV)

At A_2 - 0.02 μ g. " " "

At A_3 - 0.04 μ g. " " "

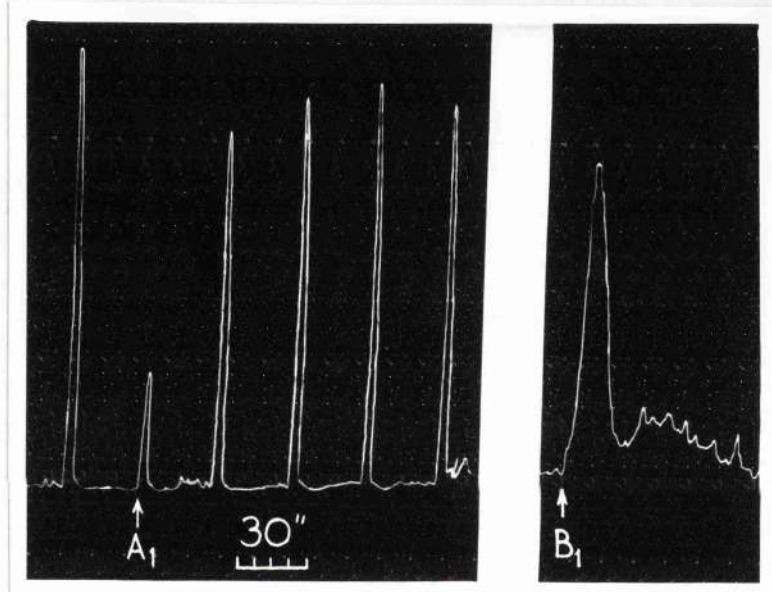


Fig. 59.

The isolated guinea pig ileum.

All unlabelled contractions are due to 0.01 μ g. per ml. of histamine.

Addition of histamine was preceded 30 seconds earlier by:-

At A₁ - 0.1 μ g. per ml. 3-(piperidinomethyl) thionaphthen (V).

At B₁ - Effect of 20 μ g. per ml. 3-(piperidinomethyl) thionaphthen.

in order of potency was compound VI (at 1 to 2 $\mu\text{g. per ml.}$); compound III at dose levels of from 5 to 20 $\mu\text{g. per ml.}$ also inhibited the stimulant response to histamine (Fig. 60, page 149). At dose levels of from 5 to 20 $\mu\text{g. per ml.}$, compound VII incompletely inhibited the stimulant response to histamine and at higher concentrations, it exerted a direct stimulant effect on the tissue. Compound I was a very weak antagonist (at dose levels of from 50 to 100 $\mu\text{g. per ml.}$) of the stimulant response to histamine.

The quaternary salts of the gramine-like isosteres completely or incompletely inhibited the stimulant response to histamine (0.001 to 0.1 $\mu\text{g. per ml.}$) on the isolated guinea pig ileum. Compound X (at dose levels of from 0.03 to 1 $\mu\text{g. per ml.}$) was the most potent (Fig. 61, page 150); next in order of potency were compounds VIII and XI (0.01 to 0.5 $\mu\text{g. per ml.}$)(Fig. 62, page 151); and compound IX was effective at dose levels of from 1 to 4 $\mu\text{g. per ml.}$ At higher concentrations than this, there was no further inhibition of the stimulant response to histamine but instead a direct stimulant effect was observed (Fig. 63, page 152).

At a dose level of 10 $\mu\text{g. per ml.}$, compound XII incompletely or completely inhibited the stimulant response of the isolated guinea /

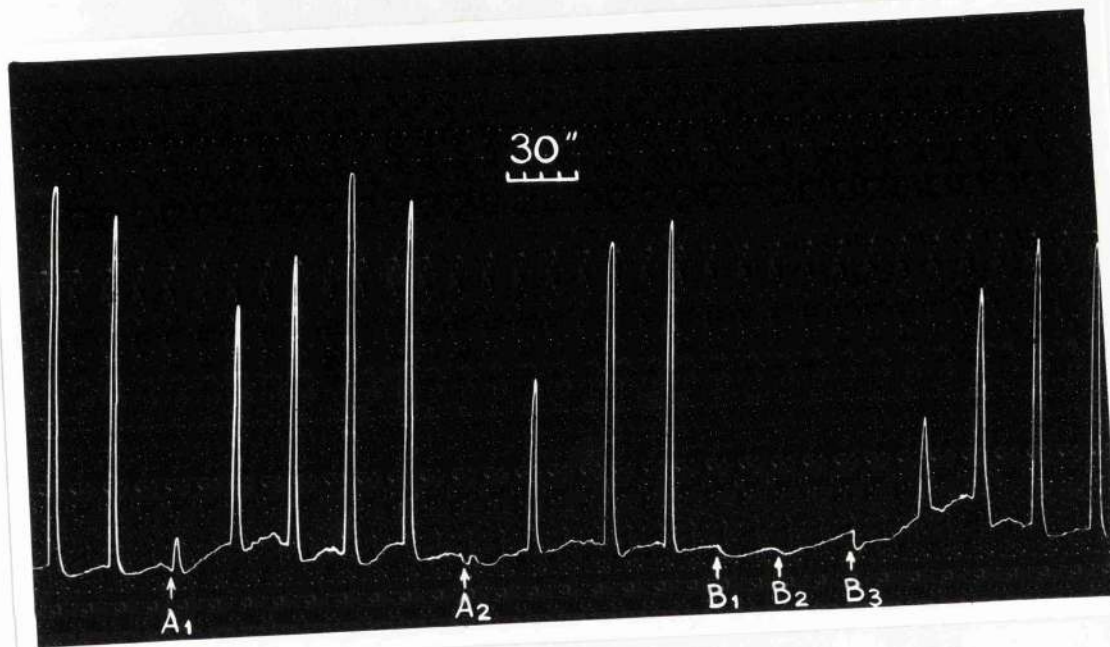


Fig. 60.

The isolated guinea pig ileum.

All unlabelled contractions are due to 0.01 μ g. per ml. histamine.

Addition of histamine was preceded 30 seconds earlier by:-

At A₁ - 2.5 µg. per ml. 3-(morpholinomethyl) thionaphthen (III).

At A₂ - 5 µg. per ml.

At B₁ - Effect of 25 µg. per ml. 3-(morpholinomethyl)thionaphthen.

At B₂ - " 100 µg. per ml. "

At B₃ - " 300 µg. per ml. "

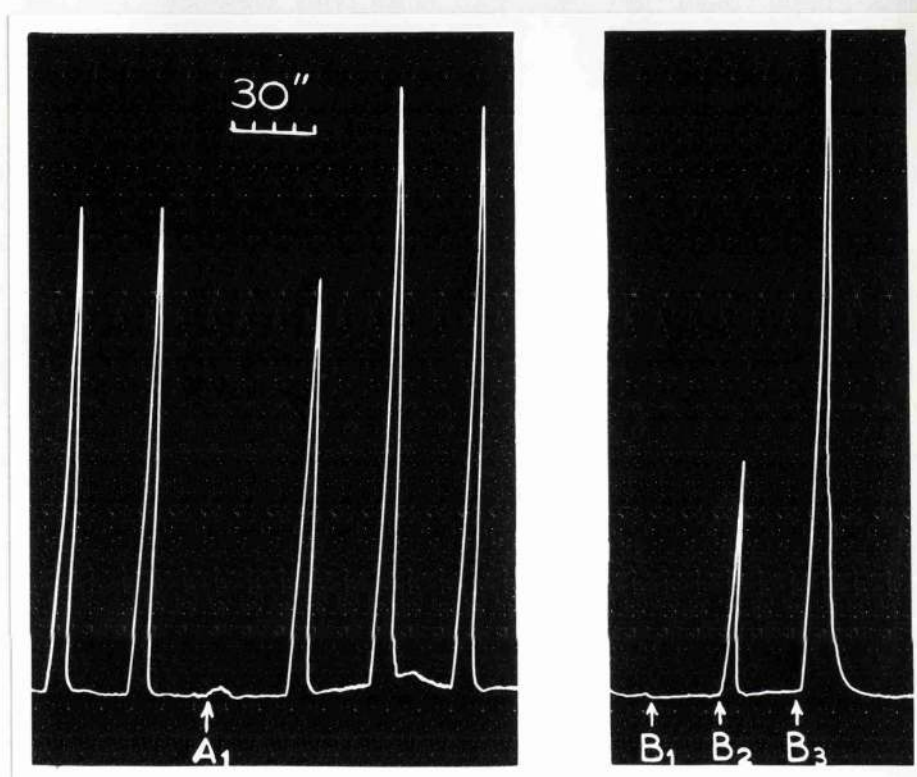


Fig. 61.

The isolated guinea pig ileum.

All unlabelled contractions are due to 0.05 μ g. per ml. of histamine.

Addition of histamine was preceded 30 seconds earlier by:-

At A_1 - 0.5 μ g. per ml. of 3-(pyrrolidinomethyl) thionaphthen
methiodide (X).

At B_1 - Effect of 1 μ g. per ml. of 3-(pyrrolidinomethyl)
thionaphthen methiodide.

At B_2 - " 1.5 μ g. " "

At B_3 - " 2 μ g. " "

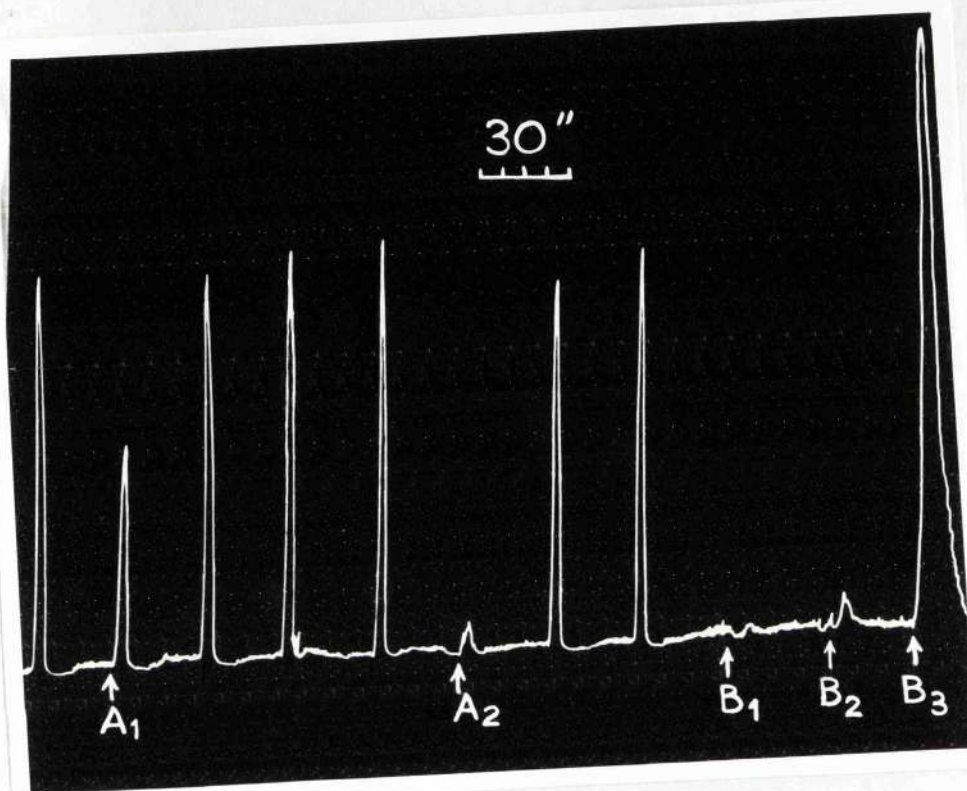


Fig. 62.

The isolated guinea pig ileum.

All unlabelled contractions are due to 0.03 μ g. per ml. of histamine.

Addition of histamine was preceded 30 seconds earlier by:-

At A_1 - 0.1 μ g. per ml. 3-(piperidinomethyl) thionaphthen methiodide (XI).

At A_2 - 0.5 μ g. " " " "

At B_1 - Effect of 1 μ g. per ml. 3-(piperidinomethyl) thionaphthen methiodide.

At B_2 - " 10 μ g. " " "

At B_3 - " 25 μ g. " " "

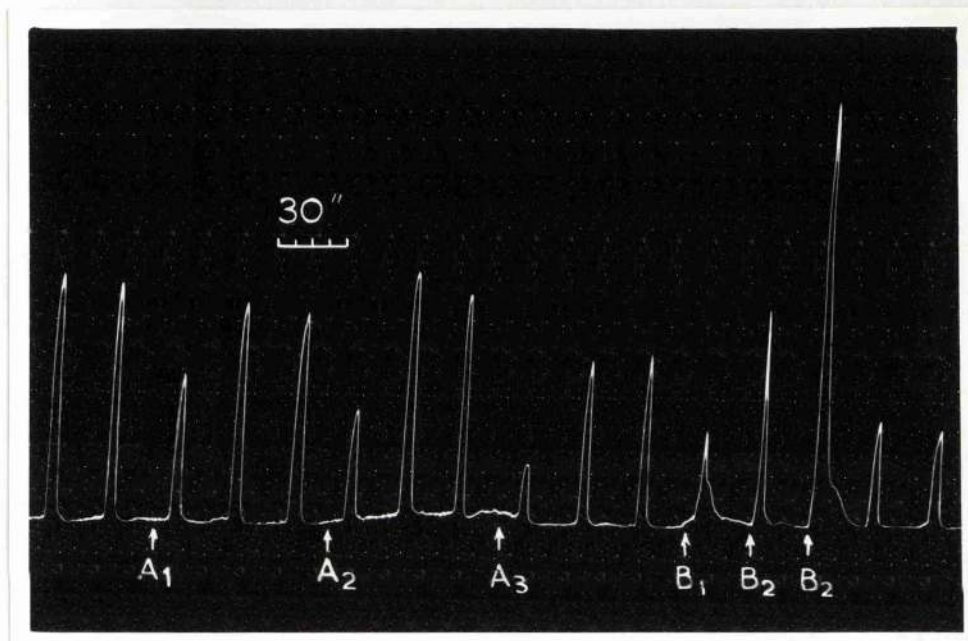


Fig. 63.

The isolated guinea pig ileum.

All unlabelled contractions are due to 0.15 μ g. per ml. histamine.

Addition of histamine was preceded 30 seconds earlier by:-

At A_1 - 0.2 μ g. per ml. 3-(morpholinomethyl) thionaphthen
methiodide (IX).

At A_2 - 0.4 μ g. " " "

At A_3 - 1 μ g. " " "

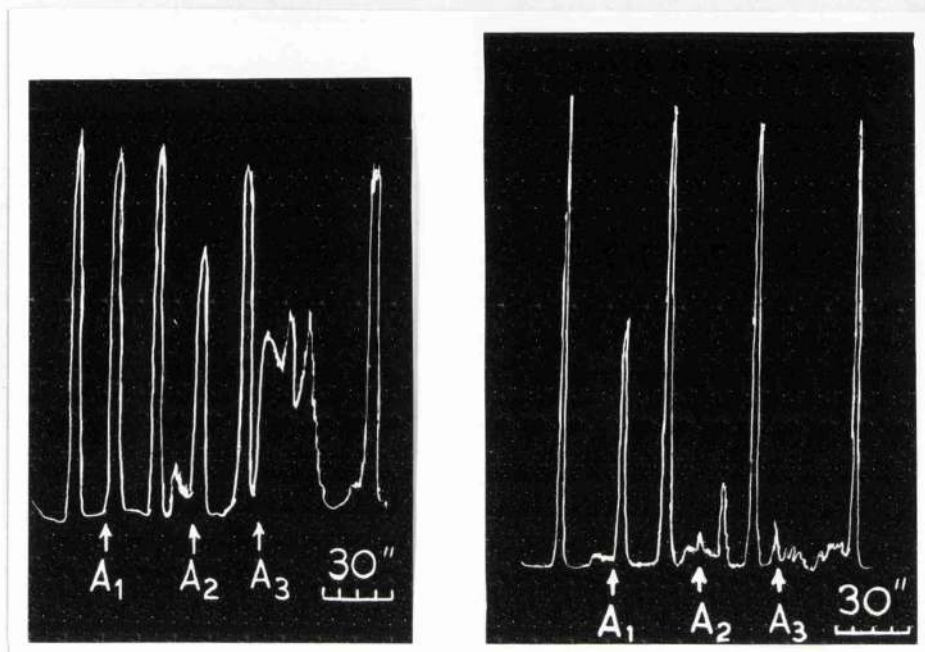
At B_1 - Effect of 10 μ g. per ml. 3-(morpholinomethyl) thionaphthen
methiodide.

At B_2 - " 15 μ g. " " "

guinea pig ileum to 0.001 μ g. per ml. of histamine. At a higher concentration (50 μ g. per ml.) it caused a direct contraction of the tissue and at this level, histamine had no stimulant effect (Fig. 64a, page 154). Compound XIII at dose levels of from 2 to 4 μ g. per ml. incompletely inhibited the stimulant response to 0.01 μ g. per ml. of histamine and at 10 μ g. per ml. caused a slight, direct stimulant effect. The contractions were not reproducible and the stimulant response to a second higher dose of the drug was considerably less than to the first (Fig. 65, page 155).

The compounds of Group D inhibited the stimulant response of the isolated guinea pig ileum to histamine (0.001 to 0.1 μ g. per ml.). Compound XIV was the most potent, acting at dose levels of from 20 to 50 μ g. per ml; compounds XV and XVII were active at doses of 100 μ g. per ml. and at higher concentrations (500 μ g. per ml.) they caused a slight contraction of the tissue (Fig. 66, page 156). At dose levels of from 100 to 500 μ g. per ml., compound XVI inhibited the stimulant response to 0.01 μ g. per ml. of histamine.

(v) Effects of the thionaphthen compounds on the contraction produced by acetylcholine.

Fig. 64a.Fig. 64b.

The isolated guinea pig ileum,

- (a) All unlabelled contractions are due to 0.003 μ g. per ml. of histamine.

Addition of histamine was preceded 30 seconds earlier by:-

At A_1 - 2 μ g. per ml. 2-(2'-aminoethyl)-5-hydroxythionaphthen
(XII).

At A_2 - 10 μ g. " " "

At A_3 - 50 μ g. " " "

- (b) All unlabelled contractions are due to 0.003 μ g. per ml. of acetylcholine.

Addition of acetylcholine was preceded 30 seconds earlier by:

At A_1 - 20 μ g. per ml. 2-(2'-aminoethyl)-5-hydroxythionaphthen.

At A_2 - 50 μ g. " " "

At A_3 - 100 μ g. " " "

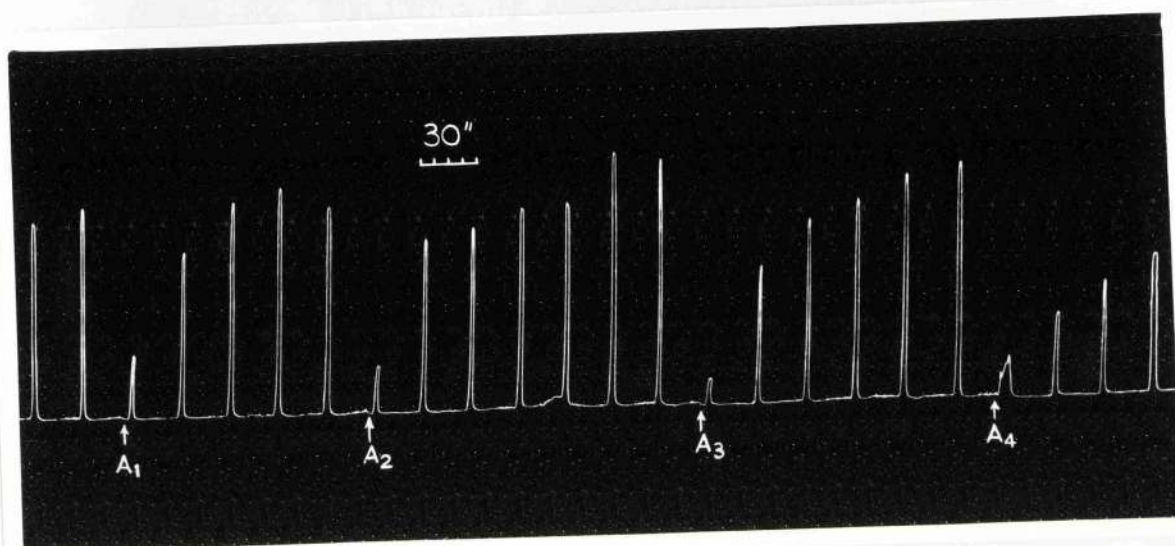


Fig. 66.

The isolated guinea pig ileum.

All unlabelled contractions are due to 0.025 $\mu\text{g.}$ per ml. of histamine.

Addition of histamine was preceded 30 seconds earlier by:-

At A_1 - 25 $\mu\text{g.}$ per ml. 5-amino-2-(piperidinomethyl) thionaphthen (XVII).

At A_2 - 50 $\mu\text{g.}$ " " " "

At A_3 - 100 $\mu\text{g.}$ " " " "

At A_4 - Effect of 500 $\mu\text{g.}$ per ml. " "

All the gramine-like isosteres completely or incompletely inhibited the stimulant response of the isolated guinea pig ileum to acetylcholine (0.01 to 0.5 μ g. per ml.). Compound V was the most potent and at dose levels of from 5 to 10 μ g. per ml. inhibited the stimulant response to acetylcholine. Compound III was very weak and was effective at dose levels of from 50 to 500 μ g. per ml. (Fig. 67a, page 158). Compound I was active at dose levels of from 10 to 20 μ g. per ml. Next in order of potency came compound IV which inhibited the stimulant effect of acetylcholine at a dose level of 100 μ g. per ml. (Fig. 67b, page 158). At dose levels of from 5 to 50 μ g. per ml., compounds II and VII completely or incompletely inhibited the stimulant response to acetylcholine (Fig. 68, page 159). At dose levels of from 10 to 20 μ g. per ml. compound VI did not inhibit at all, or incompletely inhibited (10 to 20 per cent inhibition) the stimulant response to acetylcholine and, at higher concentrations (25 to 50 μ g. per ml.) it caused a direct contraction of the tissue (Fig. 69, page 160).

With the exception of compound VIII, the compounds of Group B incompletely inhibited the stimulant response of the isolated guinea pig ileum to acetylcholine (0.01 to 0.5 μ g. per ml.). A
dose /

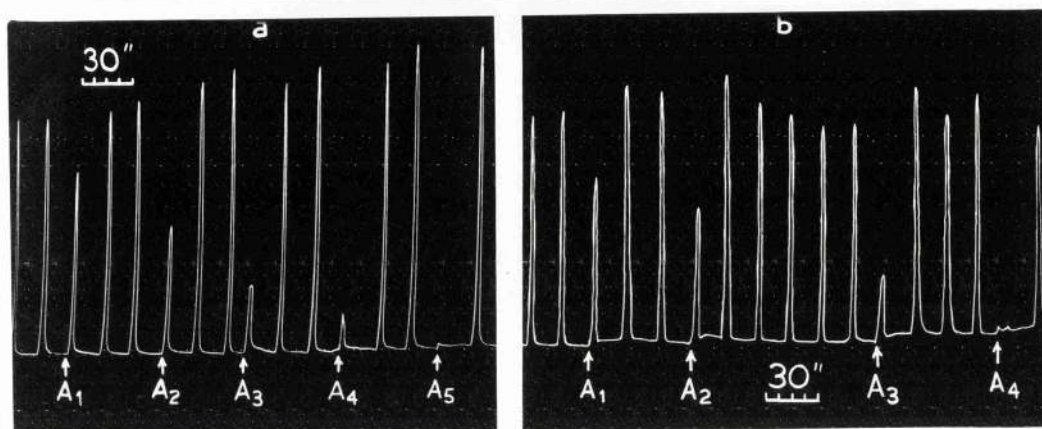


Fig. 67.

The isolated guinea pig ileum.

- (a) All unlabelled contractions are due to 0.02 $\mu\text{g. per ml.}$ of acetylcholine.

Addition of acetylcholine was preceded 30 seconds earlier by:-

At A_1 - 5 $\mu\text{g. per ml.}$	3-(morpholinomethyl) thionaphthen (III).
At A_2 - 10 $\mu\text{g.}$	" "
At A_3 - 20 $\mu\text{g.}$	" "
At A_4 - 50 $\mu\text{g.}$	" "
At A_5 - 100 $\mu\text{g.}$	" "

- (b) All unlabelled contractions are due to 0.05 $\mu\text{g. per ml.}$ of acetylcholine.

Addition of acetylcholine was preceded 30 seconds earlier by:-

At A_1 - 5 $\mu\text{g. per ml.}$	3-(pyrrolidinomethyl) thionaphthen (IV).
At A_2 - 10 $\mu\text{g.}$	" "
At A_3 - 50 $\mu\text{g.}$	" "
At A_4 - 100 $\mu\text{g.}$	" "

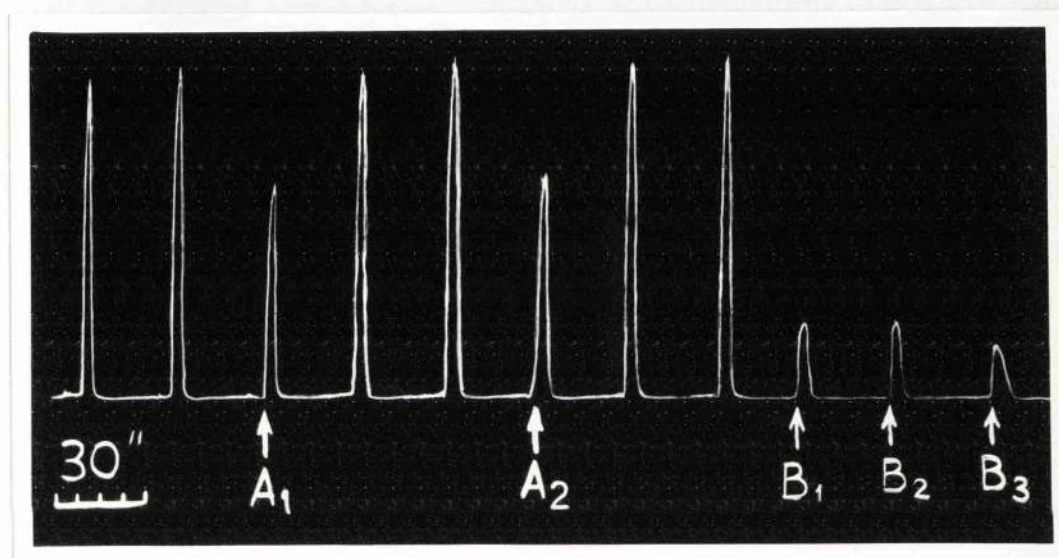


Fig. 68.

The isolated guinea pig ileum.

All unlabelled contractions are due to 0.005 μ g. per ml. of acetylcholine.

Addition of acetylcholine was preceded 30 seconds earlier by:-

At A_1 - 8 μ g. per ml. 3-(dimethylaminomethyl) thionaphthen (II).

At A_2 - 15 μ g. " " "

At B_1 - Effect of 30 μ g. per ml. 3-(dimethylaminomethyl) thionaphthen.

At B_2 - " 50 μ g. " "

At B_3 - " 100 μ g. " "

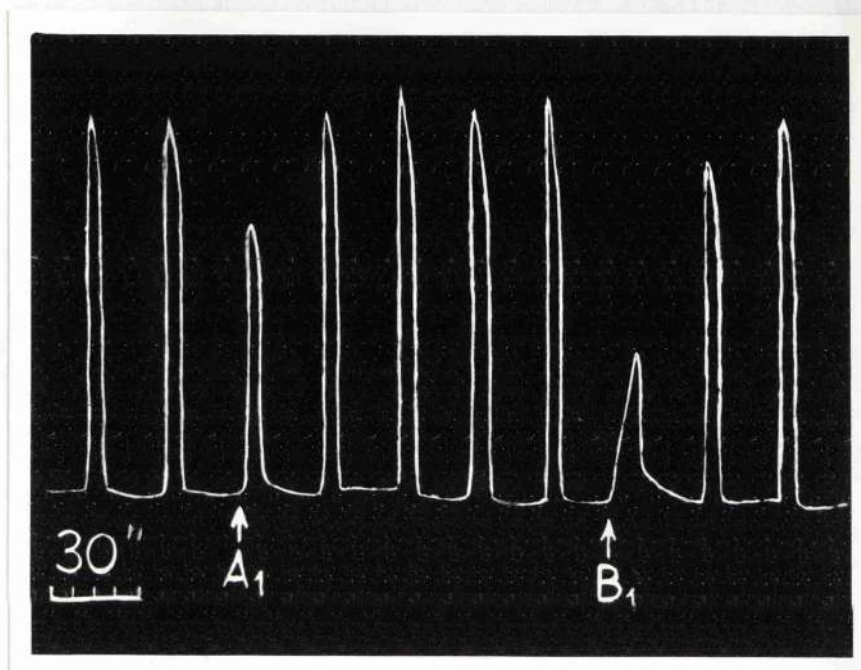


Fig. 69.

The isolated guinea pig ileum.

All unlabelled contractions are due to 0.02 μ g. per ml. of acetylcholine.

Addition of acetylcholine was preceded 30 seconds earlier by:-

At A₁ - 10 μ g. per ml. 3-(2'-methylpiperidinomethyl) thionaphthen
(VI)

At B₁ - Effect of 25 μ g. per ml. 3-(2'-methylpiperidinomethyl)
thionaphthen.

dose levels of from 1 to 2.5 $\mu\text{g. per ml.}$ compound VIII did not show any antagonism to acetylcholine. At higher concentrations than this, it caused a direct contraction of the tissue. At dose levels of from 1 to 2.5 $\mu\text{g. per ml.}$ compound X (Fig. 70, page 162), at dose levels of from 1 to 5 $\mu\text{g. per ml.}$ compound XI, and at dose levels of from 5 to 10 $\mu\text{g. per ml.}$ compound IX incompletely inhibited the stimulant response to acetylcholine. At higher concentrations than these, these compounds caused a direct contraction of the tissue.

At dose levels of from 5 to 25 $\mu\text{g. per ml.}$ compounds XII and XIII partially or completely inhibited the stimulant response of the guinea pig ileum to acetylcholine (0.01 to 0.5 $\mu\text{g. per ml.}$). At a higher concentration (50 $\mu\text{g. per ml.}$) they caused a direct contraction of the tissue and there was no further inhibition of the stimulant response to acetylcholine (Fig. 71, page 163).

At dose levels of from 200 to 400 $\mu\text{g. per ml.}$ compound XV completely inhibited the stimulant response of the guinea pig ileum to acetylcholine (Fig. 72, page 164). At dose levels of from 200 to 500 $\mu\text{g. per ml.}$, compounds XIV, XVI and XVII incompletely inhibited the stimulant response to acetylcholine. At higher concentrations than this, there was no further inhibition, but /

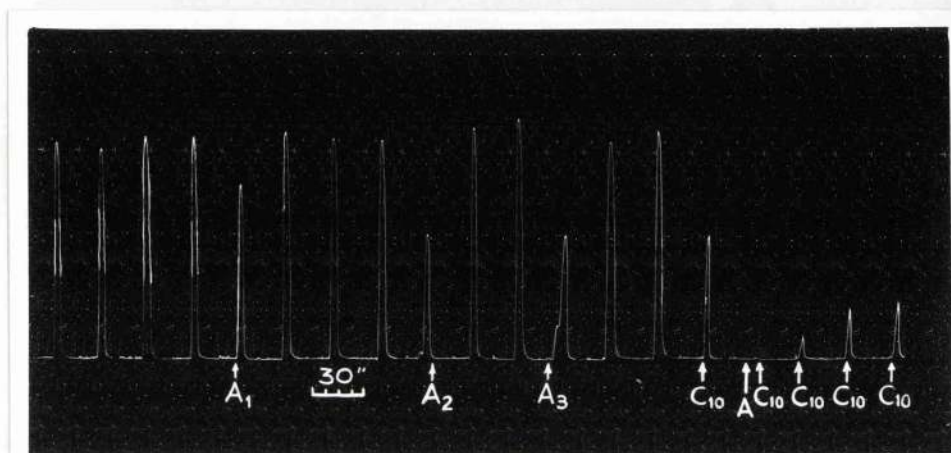


Fig. 70.

The isolated guinea pig ileum.

All unlabelled contractions are due to 0.005 μ g. per ml. of acetylcholine.

Addition of acetylcholine was preceded 30 seconds earlier by:-

At A_1 - 0.5 μ g. per ml. 3-(pyrrolidinomethyl) thionaphthen
methiodide (X).

At A_2 - 1 μ g. " "

At A_3 - 2.5 μ g. " "

At C_{10} - Effect of 5 μ g. per ml. 3-(pyrrolidinomethyl) thionaphthen
methiodide.

At A - Effect of 0.5 μ g. per ml. of atropine followed 30 seconds
later by 5 μ g. per ml. 3-(pyrrolidinomethyl) thionaphthen.
methiodide.

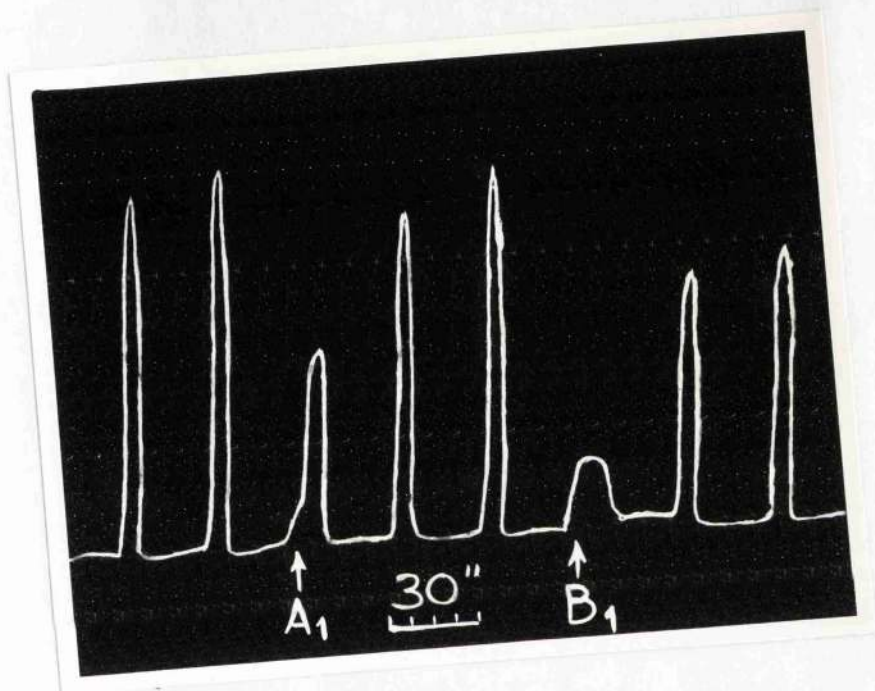


Fig. 71.

The isolated guinea pig ileum.

All unlabelled contractions are due to 0.001 $\mu\text{g.}$ per ml. of acetylcholine.

Addition of acetylcholine was preceded 30 seconds earlier by:-

At A_1 - 4 $\mu\text{g.}$ per ml. 2-(2'-aminopropyl)-5-hydroxythionaphthen
(XIII).

At B_1 - Effect of 10 $\mu\text{g.}$ per ml. 2-(2'-aminopropyl)-5-hydroxythionaphthen.

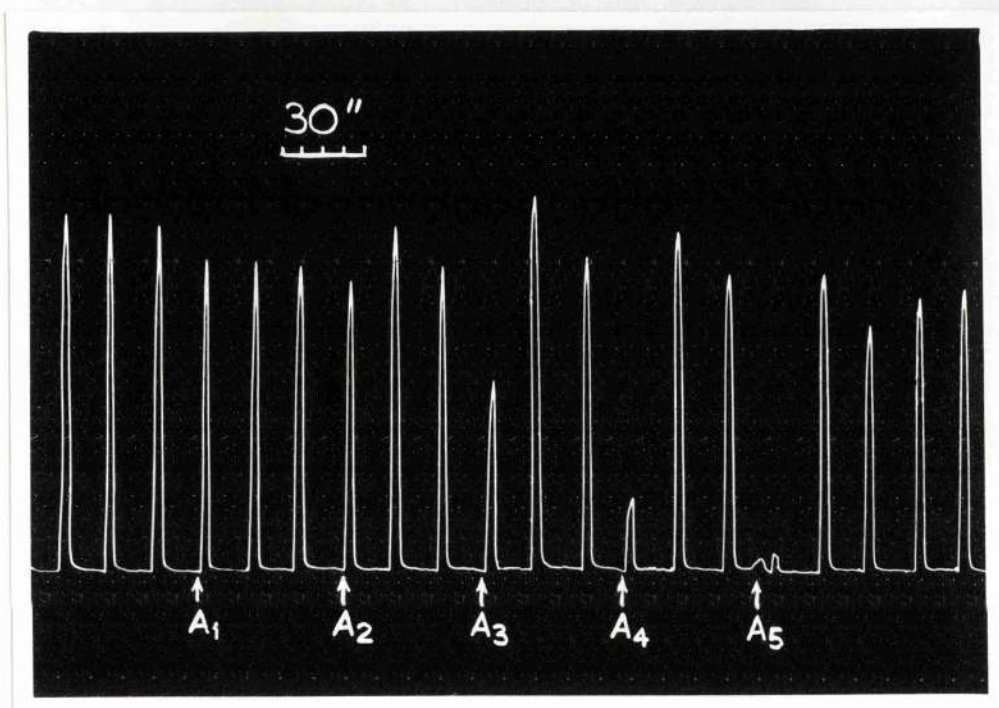


Fig. 72.

The isolated guinea pig ileum.

All contractions are due to 0.005 μ g. per ml. of acetylcholine.

Addition of acetylcholine was preceded 30 seconds earlier by:-

At A_1 - 2.5 μ g. per ml. 5-amino-2-(morpholinomethyl) thionaphthen
(XV).

At A_2 - 25 μ g.	"	"	"	"
At A_3 - 50 μ g.	"	"	"	"
At A_4 - 100 μ g.	"	"	"	"
At A_5 - 200 μ g.	"	"	"	"

but instead these compounds caused a slight contraction of the tissue (Fig. 73, page 166).

(d) Effect of the thionaphthen compounds on the isolated, perfused rat hindquarters

At dose levels of from 0.1 to 0.5 mg. none of the gramine-like isosteres exerted a vasoconstrictor or vasodilator effect in the isolated perfused rat hindquarters. At the same dose levels, they inhibited the vasoconstrictor responses to adrenaline or to noradrenaline (0.1 to 2 μ g.) but there were quantitative differences. Compound III was weakest in this respect. At the same dose levels (0.1 to 0.5 mg.) compounds VI and VII did not inhibit the vasoconstriction due to 5-hydroxytryptamine (0.1 to 2 μ g.). Compounds I, IV and V were, however, effective and compounds II and III had slight antagonistic effects. Tracings obtained from eight different experiments are shown in Figs. 74 to 76, pages 167 to 169.

The quaternary salts of the gramine-like isosteres, at dose levels of from 0.1 to 0.5 mg. had no vasodilator or vasoconstrictor effects. At the same dose levels, antagonism to 5-hydroxytryptamine was not exhibited by compounds VIII and IX. Compounds X and XI incompletely or completely antagonized the vasoconstrictor effects of 5-hydroxytryptamine /

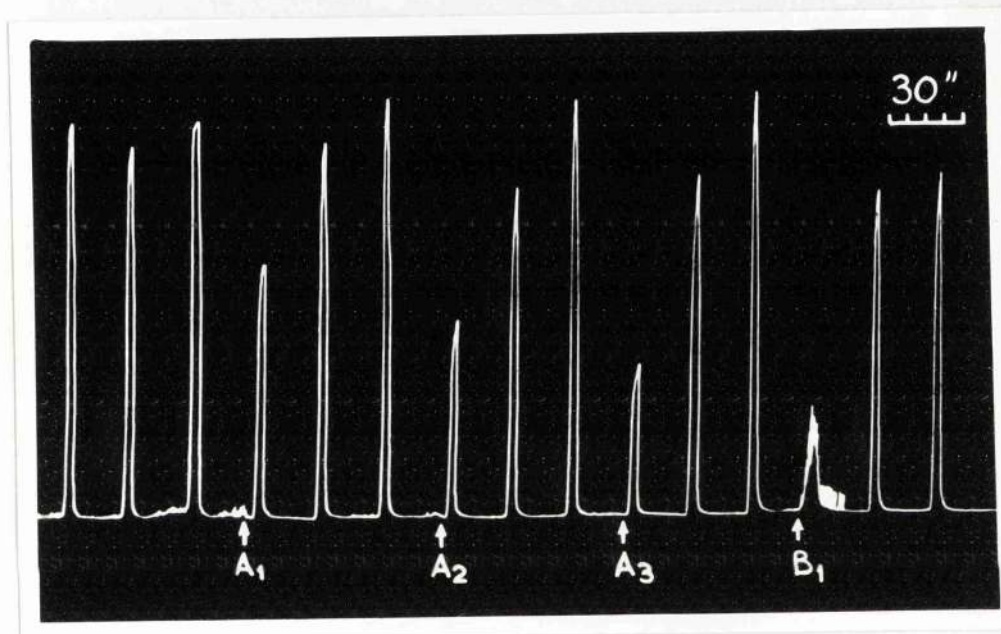


Fig. 73.

The isolated guinea pig ileum.

All unlabelled contractions are due to 0.005 μ g. per ml. of acetylcholine.

Addition of acetylcholine was preceded 30 seconds earlier by:-

At A₁ - 15 μ g. per ml. 5-amino-2-(piperidinomethyl) thionaphthen (XVII).

At A₂ - 30 μ g. " " " "

At A₃ - 50 μ g. " " " "

At B₁ - Effect of 250 μ g. per ml. 5-amino-2-(piperidinomethyl) thionaphthen.

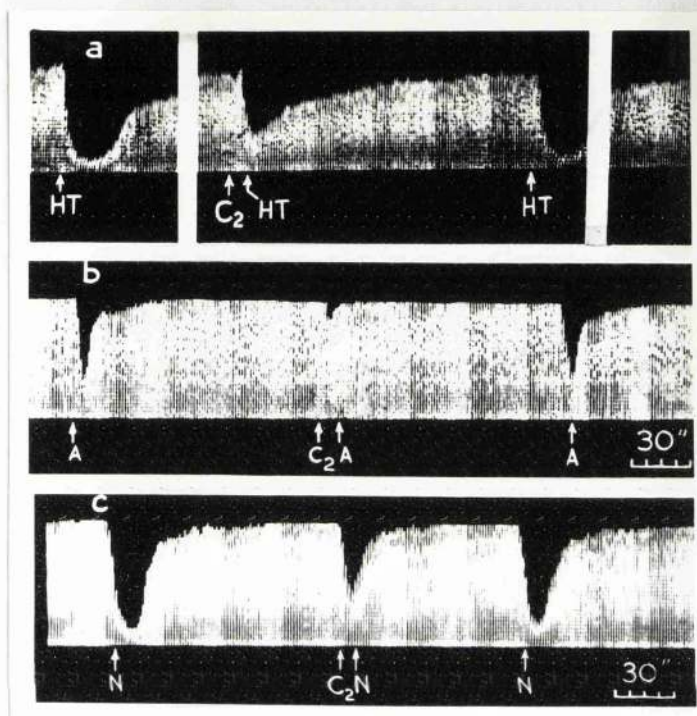


Fig. 74.

Perfusion of the isolated rat hindquarters.

A reduction in the height of the vertical lines indicates vasoconstriction.

(a) At HT - Effect of 2 μ g. 5-hydroxytryptamine.

At C₂ - Effect of 0.1 mg. 3-(dimethylaminomethyl) thionaphthen (II), followed 30 seconds later by 2 μ g. 5-hydroxytryptamine.

(b) At A - Effect of 2 μ g. adrenaline.

At C₂ - Effect of 0.1 mg. 3-(dimethylaminomethyl) thionaphthen followed 30 seconds later by 2 μ g. adrenaline.

(c) At N - Effect of 2 μ g. noradrenaline.

At C₂ - Effect of 0.1 mg. 3-(dimethylaminomethyl) thionaphthen followed 30 seconds later by 2 μ g. noradrenaline.

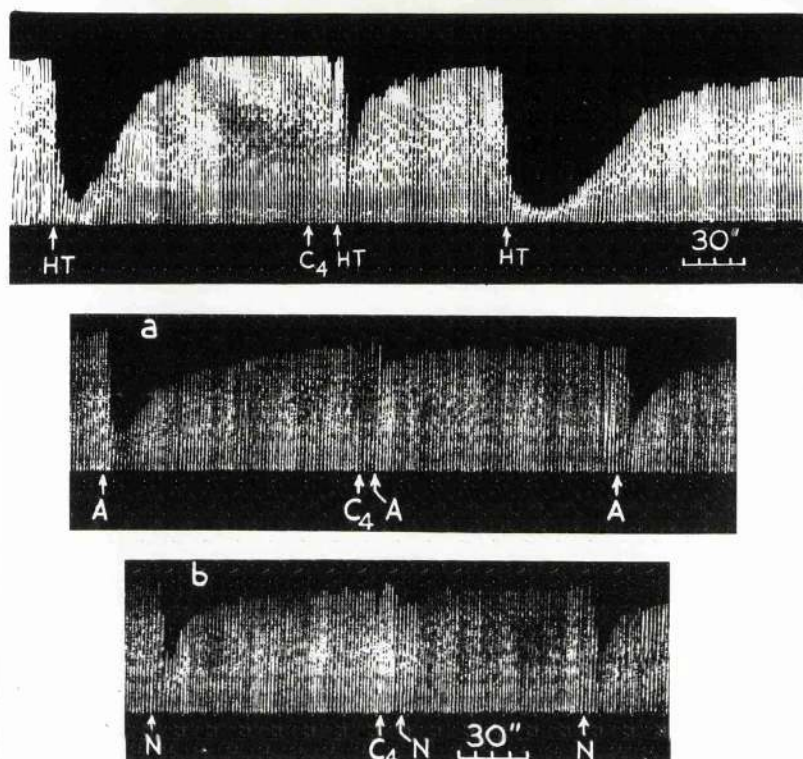


Fig. 75.

Perfusion of the isolated rat hindquarters.

A reduction in the height of the vertical lines indicates vasoconstriction.

Upper tracing.

At HT - Effect of 2 μ g. 5-hydroxytryptamine.

At C₄ - Effect of 0.2 mg. 3-(pyrrolidinomethyl) thionaphthen (IV) followed 30 seconds later by 2 μ g. of 5-hydroxytryptamine.

Lower tracing

(a) At A - Effect of 2 μ g. adrenaline.

At C₄ - Effect of 0.1 mg. 3-(pyrrolidinomethyl) thionaphthen followed 30 seconds later by 2 μ g. adrenaline.

(b) At N - Effect of 2 μ g. noradrenaline.

At C₄ - Effect of 0.1 mg. 3-(pyrrolidinomethyl) thionaphthen followed 30 seconds later by 2 μ g. noradrenaline.

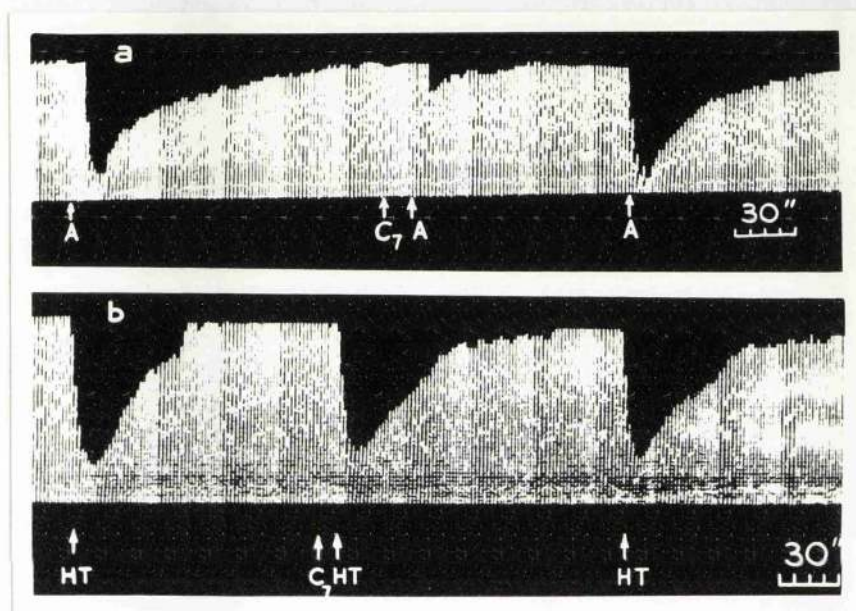


Fig. 76.

Perfusion of the isolated rat hindquarters.

A reduction of the height of the vertical lines indicates vasoconstriction.

(a) At A - Effect of 1.5 µg. adrenaline.

At C₇ - Effect of 0.1 mg. 3-(cyclohexylaminomethyl) thionaphthen (VII) followed 30 seconds later by 1.5 µg. adrenaline.

(b) At HT - Effect of 1.5 µg. 5-hydroxytryptamine.

At C₇ - Effect of 0.1 mg. 3-(cyclohexylaminomethyl) thionaphthen followed 30 seconds later by 1.5 µg. 5-hydroxytryptamine.

5-hydroxytryptamine (0.1 to 2 μ g.). Antagonism to adrenaline and noradrenaline was caused by compounds VIII and X (Fig. 77, page 171). At the same dose levels, compounds IX and XI completely or incompletely antagonized the vasoconstrictor responses to adrenaline and noradrenaline on this preparation (Fig. 78, page 172).

At dose levels of from 0.1 to 0.5 mg. compounds XII and XIII exhibited slight or no antagonism to the vasoconstrictor effects produced by 5-hydroxytryptamine, adrenaline and noradrenaline (0.1 to 2 μ g.) on the isolated, perfused rat hindquarters (Figs. 79, 80, pages 173, 174). They had no direct vasodilator or vasoconstrictor effects upon this tissue.

At dose levels of from 0.1 to 0.5 mg., none of the 5-amino-isogramine-like isosteres inhibited the vasoconstrictor effects of 5-hydroxytryptamine and they had no direct vasoconstrictor or dilator effects. At the same dose levels, they did not inhibit or incompletely inhibited the vasoconstrictor effects caused by adrenaline or noradrenaline (Figs. 81, 82, pages 175, 176).

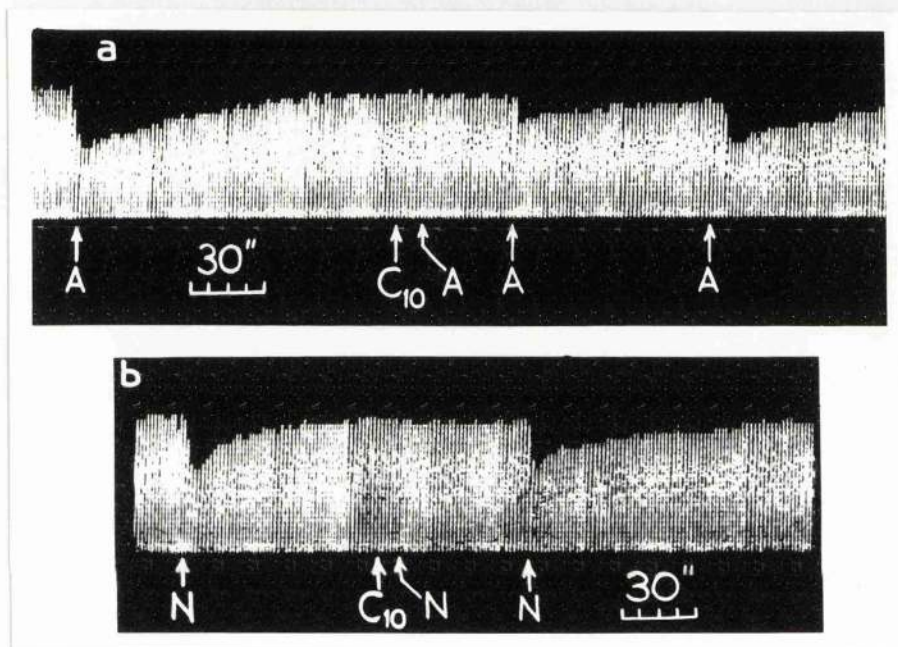


Fig. 77.

Perfusion of the isolated rat hindquarters.

A reduction in the height of the vertical lines indicates vasoconstriction.

(a) At A - Effect of 1.5 μ g. of adrenaline.

At C₁₀ - Effect of 0.5 mg. of 3-(pyrrolidinomethyl) thionaphthen methiodide (X) followed 30 seconds later by 1.5 μ g. adrenaline.

(b) At N - Effect of 2 μ g. noradrenaline.

At C₁₀ - Effect of 0.5 mg. 3-(pyrrolidinomethyl) thionaphthen methiodide followed 30 seconds later by 2 μ g. of noradrenaline.

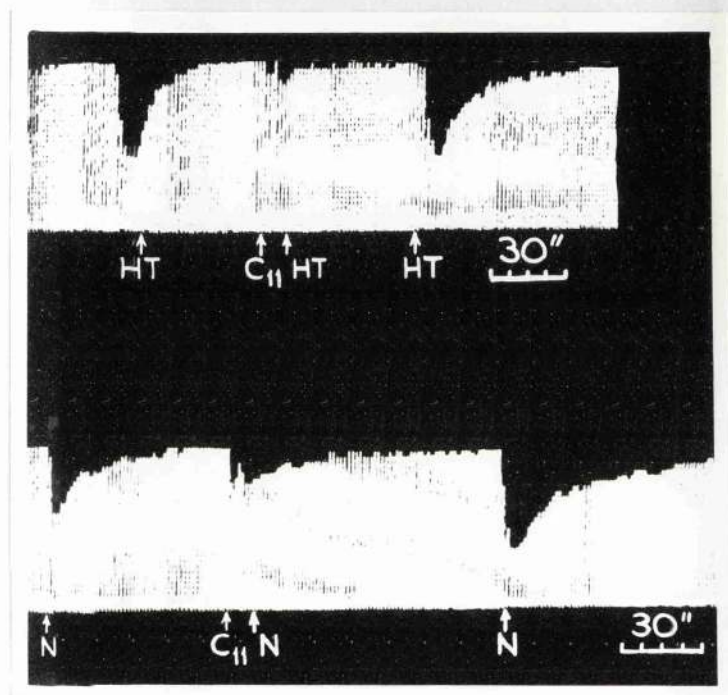


Fig. 78.

Perfusion of the isolated rat hindquarters.

A reduction in the height of the vertical lines indicates vasoconstriction.

At HT - Effect of 1.5 μ g. 5-hydroxytryptamine.

At N - Effect of 2 μ g. noradrenaline.

At C₁₁ - Effect of 0.5 mg. 3-(piperidinomethyl) thionaphthen
methiodide (XI) followed 30 seconds later by 1.5 μ g.
of 5-hydroxytryptamine or by 2 μ g. of noradrenaline.

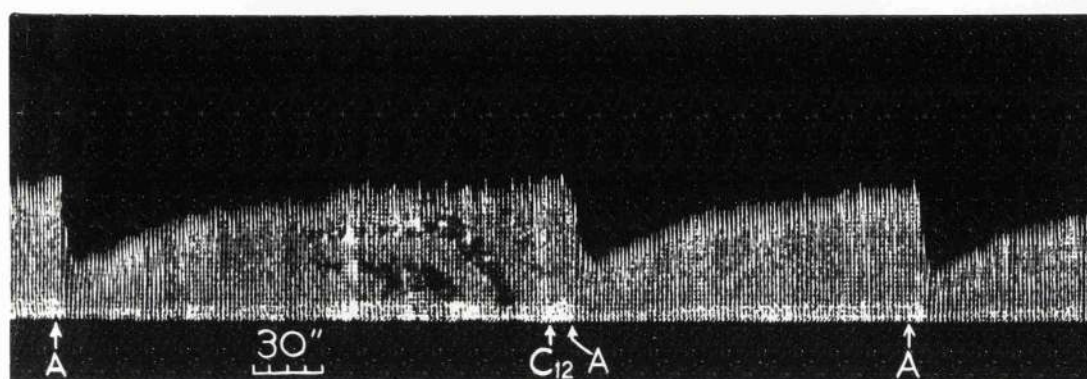


Fig. 79.

Perfusion of the isolated rat hindquarters.

A reduction in the height of the vertical lines indicates vasoconstriction.

At A - Effect of 0.2 μ g. adrenaline.

At C₁₂ - Effect of 0.1 mg. 2-(2'-aminoethyl)-5-hydroxythionaphthen
(XII) followed 30 seconds later by 0.2 μ g. adrenaline.

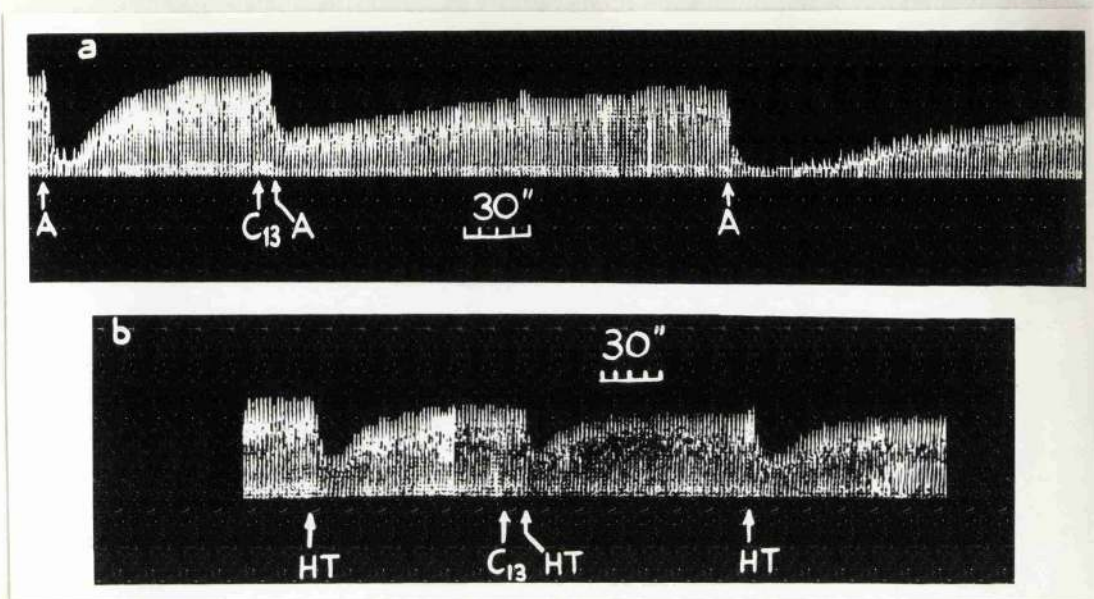


Fig. 80.

Perfusion of the isolated rat hindquarters.

A reduction in the height of the vertical lines indicates vasoconstriction.

(a) At A - effect of 0.5 µg. of adrenaline.

At C₁₃ - effect of 0.2 mg. 2-(2'-aminopropyl)-5-hydroxythionaphthen followed 30 seconds later by 0.5 µg. of adrenaline.

(b) At HT - effect of 0.5 µg. 5-hydroxytryptamine.

At C₁₃ - effect of 0.25 mg. 2-(2'-aminopropyl)-5-hydroxythionaphthen (XIII) followed 30 seconds later by 0.5 µg. of 5-hydroxytryptamine.

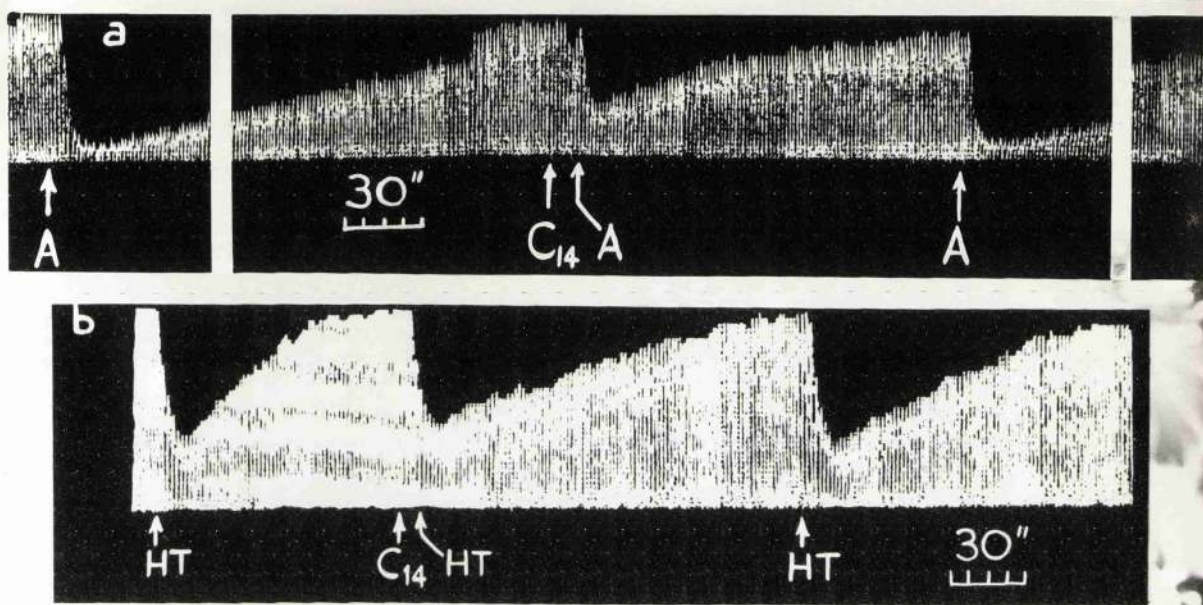


Fig. 81.

Perfusion of the isolated rat hindquarters.

A reduction in height of the vertical lines indicates vasoconstriction.

(a) At A - Effect of 0.5 µg. adrenaline.

At C₁₄ - Effect of 0.5 mg. 5-amino-2-(dimethylaminomethyl) thionaphthen followed 30 seconds later by 0.5 µg. adrenaline.

(b) At HT - Effect of 0.5 µg. 5-hydroxytryptamine.

At C₁₄ - Effect of 0.2 mg. 5-amino-2-(dimethylaminomethyl) thionaphthen (XIV) followed 30 seconds later by 0.5 µg. 5-hydroxytryptamine.

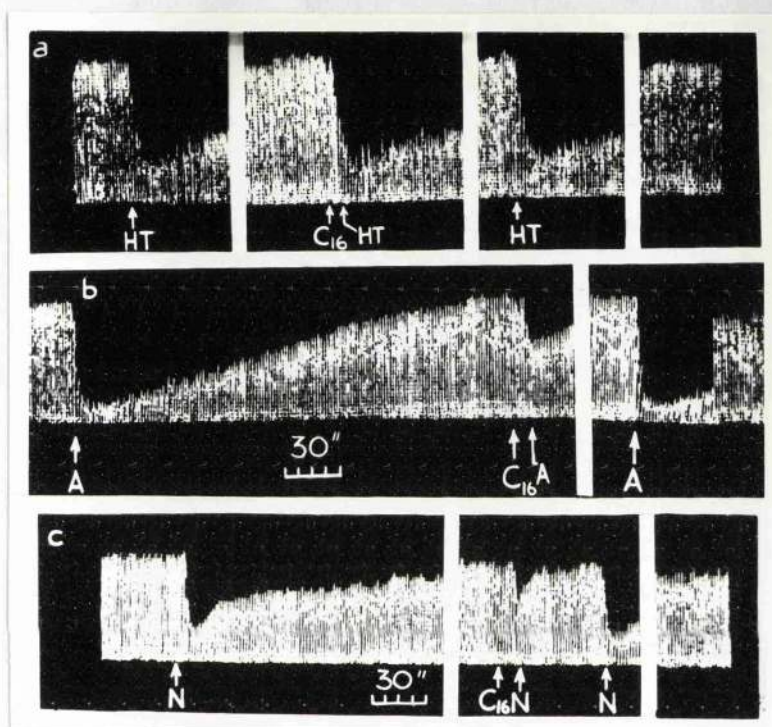


Fig. 82.

Perfusion of the isolated rat hindquarters.

A reduction in height of vertical lines indicates vasoconstriction.

(a) At HT - Effect of 1.5 μ g. 5-hydroxytryptamine.

At C₁₆ - Effect of 0.5 mg. 5-amino-2-(pyrrolidinomethyl) thionaphthen (XVI) followed 30 seconds later by 1.5 μ g. 5-hydroxytryptamine.

(b) At A - Effect of 1 μ g. adrenaline.

At C₁₆ - Effect of 0.5 mg. 5-amino-2-(pyrrolidinomethyl) thionaphthen followed 30 seconds later by 1 μ g. adrenaline.

(c) At N - Effect of 3 μ g. noradrenaline.

At C₁₆ - Effect of 0.2 mg. 5-amino-2-(pyrrolidinomethyl) thionaphthen followed 30 seconds later by 3 μ g. noradrenaline.

II. Effects of the thionaphthen compounds
in intact animals

(a) Effects on the blood pressure of the anaesthetized cat.

At dose levels of from 1 to 5 mg. per kg. all of the gramine-like isosteres caused a fall in the blood pressure of the anaesthetized cat, but did not antagonize the pressor effects of adrenaline (Figs. 83, 84, pages 178, 179).

At dose levels of from 1 to 2 mg. per kg., all the quaternary salts of the gramine-like isosteres caused a slight but definite rise in the level of the cat blood pressure (Fig. 85, page 180).

At dose levels of from 0.1 to 1 mg. per kg., compounds XII and XIII had a direct pressor effect on the cat and this effect was reversed by the previous administration of 1.5 mg. per kg. of 2-N-m-hydroxyphenyl-p-toludinomethylimidazoline (phentolamine) (Fig. 86, page 181).

At dose levels of from 1 to 2 mg. per kg., none of the compounds in Group D had any direct pressor or depressor effects on the blood pressure level of the anaesthetized cat.

(b) Effect on the blood pressure of the anaesthetized rat.

At /

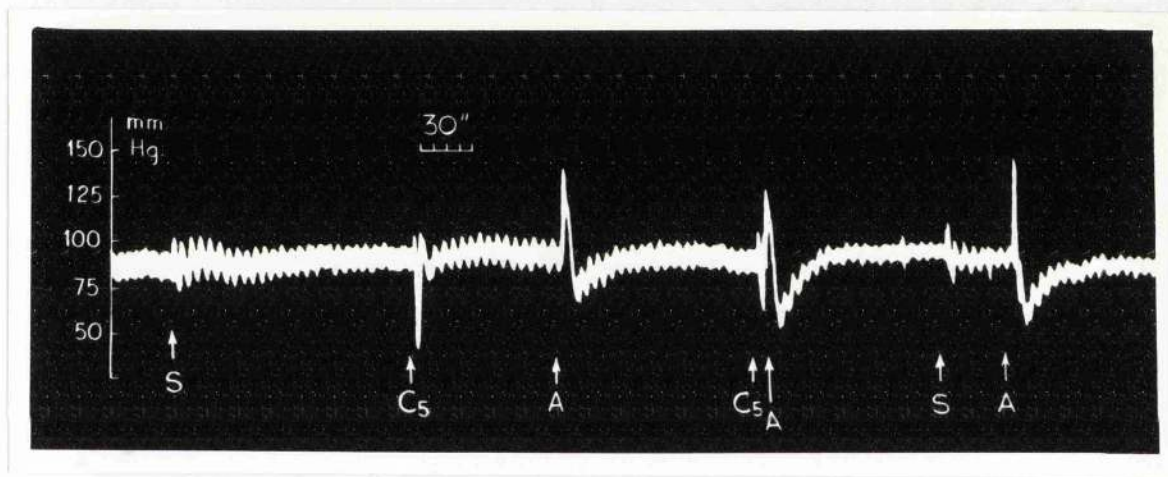


Fig. 83.

Record of the carotid arterial blood pressure of a pentobarbitone-anaesthetized cat.

At S - Effect of 3 ml. normal saline.

At C₅ - Effect of 1 mg. per kg. 3-(piperidinomethyl) thionaphthen (V).

At A - Effect of 2 µg. per kg. adrenaline.

Drugs administered intravenously.

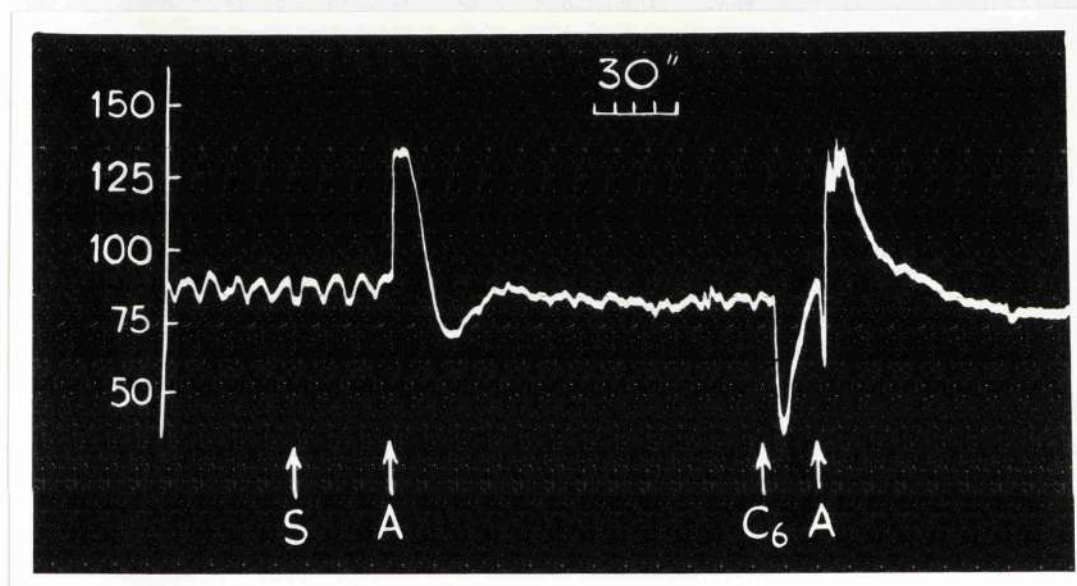


Fig. 84.

Record of the carotid arterial blood pressure of a pentobarbitone-
anaesthetized cat.

At S - Effect of 3 ml. normal saline.

At A - Effect of 2.5 μ g. per kg. adrenaline.

At C₆ - Effect of 1 mg. per kg. 3-(2-methylpiperidinomethyl)
thionaphthen (VI).

Drugs administered intravenously.

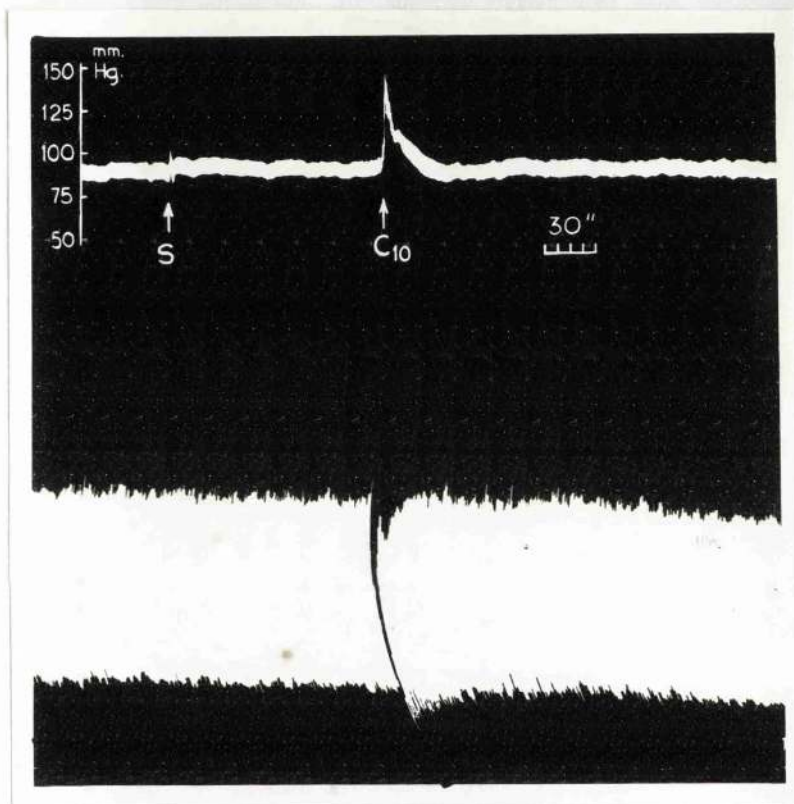


Fig. 85.

Upper tracing - Record of the carotid arterial blood pressure of a pentobarbitone-anaesthetized cat.

Lower tracing - Record of the respiratory movements of the same cat.

At S - Effect of 3 ml. normal saline.

At C₁₀ - Effect of 1 mg. per kg. 3-(pyrrolidinomethyl) thionaphthen methiodide (X).

Drugs administered intravenously.

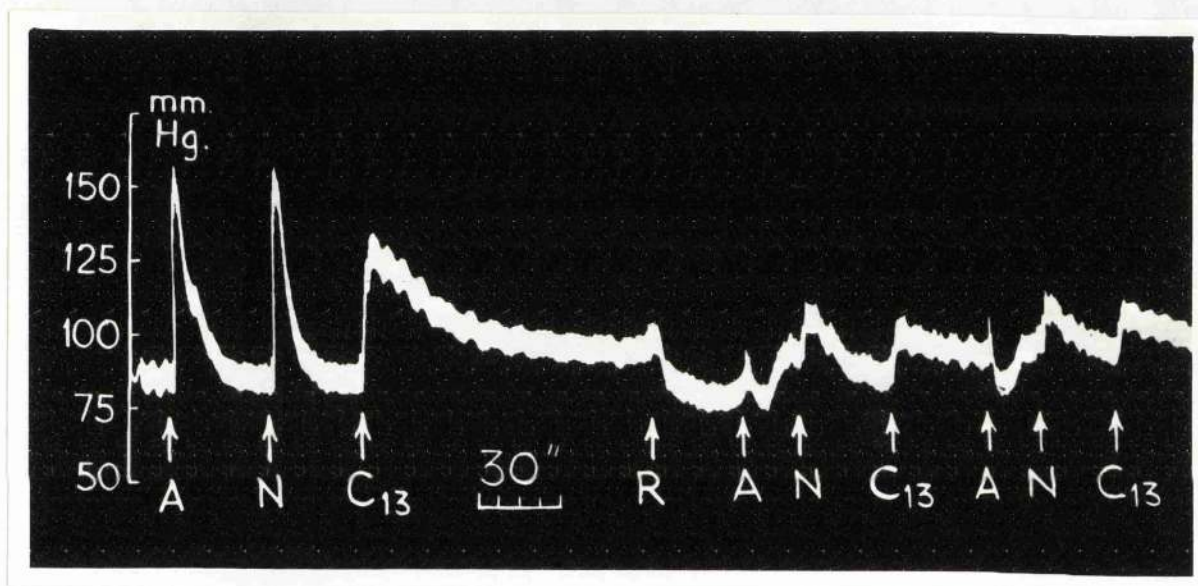


Fig. 86.

Record of the carotid arterial blood pressure of a pentobarbitone-anaesthetized cat.

At A - 1 μ g. per kg. adrenaline.

At N - 1.5 μ g. per kg. noradrenaline.

At C₁₃ - 0.1 mg. per kg. 2-(2'-aminopropyl)-5-hydroxythionaphthen (XIII).

At R - 1.5 mg. per kg. 2-N-m-hydroxyphenyl-p-toluidinomethyl-imidazoline (phentolamine).

Drugs administered intravenously.

At dose levels of from 0.3 to 1.5 mg. per kg. all of the compounds in Group A caused a sharp fall in the blood pressure of the urethane-anaesthetized rat. Compounds I and III were of low potency and caused only a slight fall in the blood pressure level. At the same dose levels, none of the compounds in this series antagonized the pressor effects produced by adrenaline or nor-adrenaline (0.3 to 3.0 μ g. per kg.) (Figs. 87 to 90, pages 183 to 186).

At dose levels of 0.3 to 1.5 mg. per kg. all the quaternary salts of the gramine-like isosteres caused either a slight fall or a slight rise in the blood pressure level and there was very slight or no antagonism to the pressor response to adrenaline (0.3 to 3.0 μ g. per kg.) (Figs. 91, 92, pages 187, 188).

At dose levels of from 0.3 to 1.5 mg. per kg., compounds XII and XIII caused a rise in the level of the blood pressure of the anaesthetized rat and did not antagonize the pressor effect of adrenaline (Fig. 93, page 189).

At dose levels of from 0.3 to 1.5 mg. per kg., all the compounds in Group D caused a slight rise in the blood pressure level of the rat, but did not antagonize the pressor effect of adrenaline /

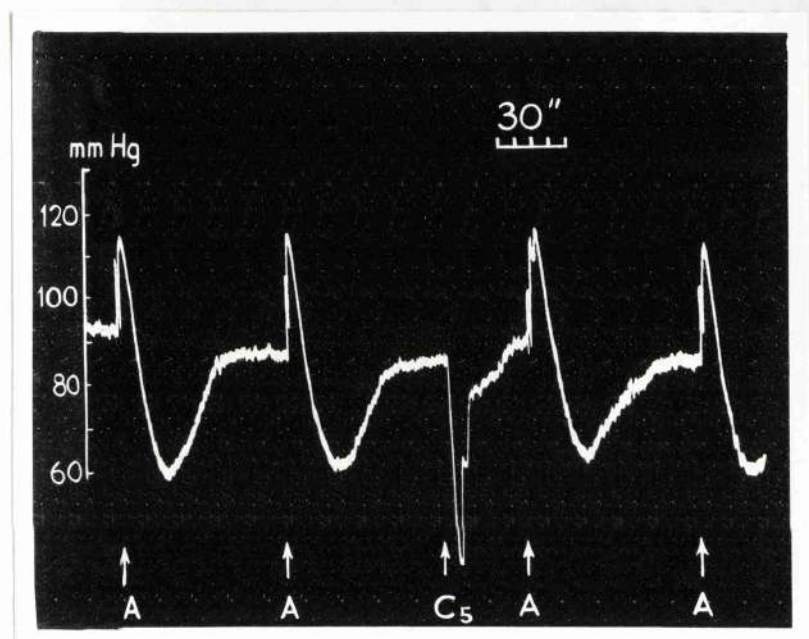


Fig. 87.

Record of the carotid arterial blood pressure of a urethane-anaesthetized rat.

At A - Effect of 4 μ g. per kg. adrenaline.

At C₅ - Effect of 0.4 mg. per kg. 3-(piperidinomethyl)thionaphthen (V).

Drugs administered intravenously.

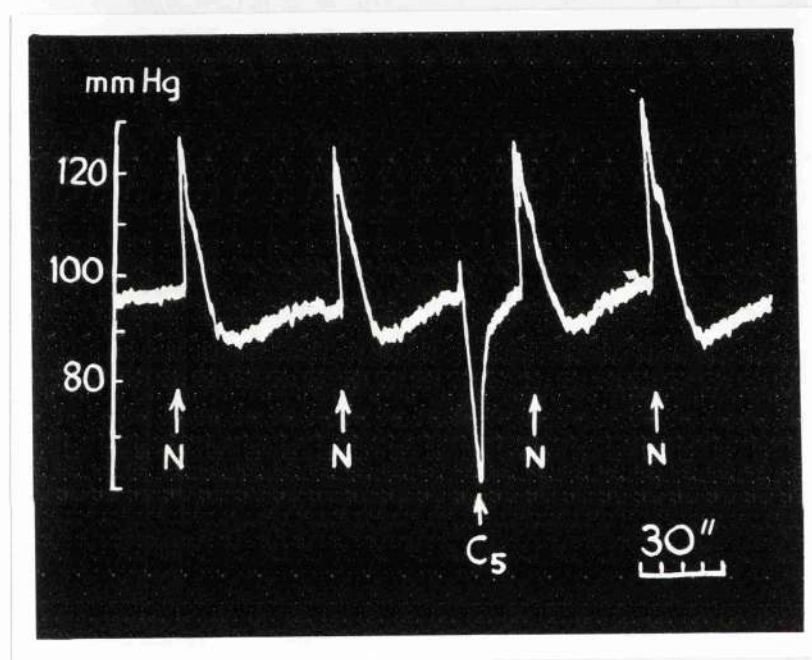


Fig. 88.

Record of the carotid arterial blood pressure of a urethane-anaesthetized rat.

At N - Effect of 7.5 μ g. per kg. noradrenaline.

At C₅ - Effect of 0.3 mg. per kg. 3-(piperidinomethyl)thionaphthen (V).

Drugs administered intravenously.

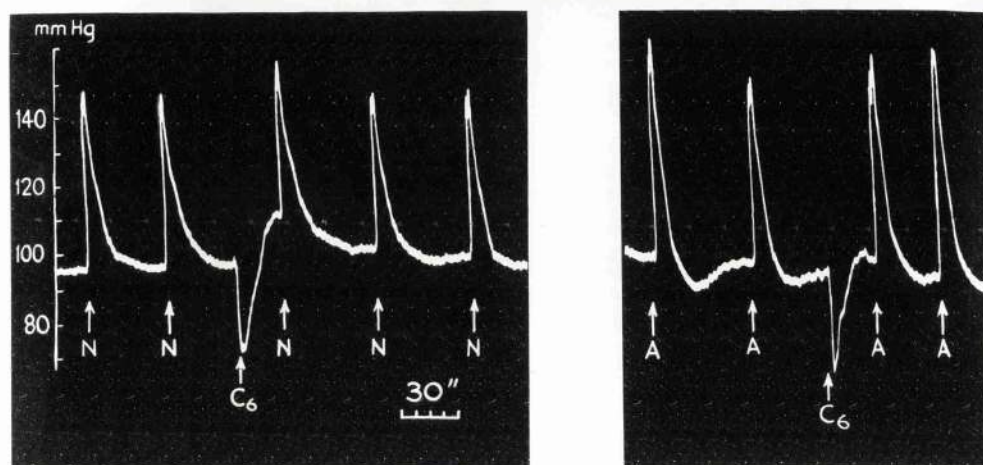


Fig. 89.

Record of the carotid arterial blood pressure of a urethane-anaesthetized rat.

At N - Effect of 4 μ g. per kg. noradrenaline.

At C₆ - Effect of 0.4 mg. per kg. 3-(2'-methylpiperidinomethyl)thionaphthen (VI).

At A - Effect of 4 μ g. per kg. adrenaline.

Drugs administered intravenously.

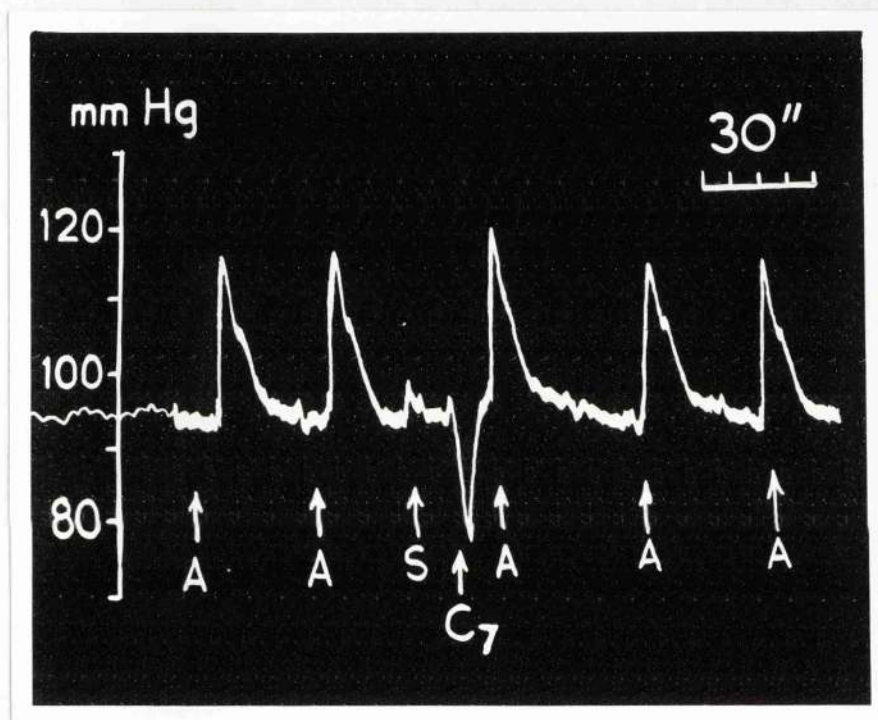


Fig. 90.

Record of the carotid arterial blood pressure of a urethane-anaesthetized rat.

At A - Effect of 2 μ g. per kg. adrenaline.

At S - Effect of 0.2 ml. normal saline.

At C₇ - Effect of 0.8 mg. per kg. 3-(cyclohexylaminomethyl)thionaphthen (VII).

Drugs administered intravenously.

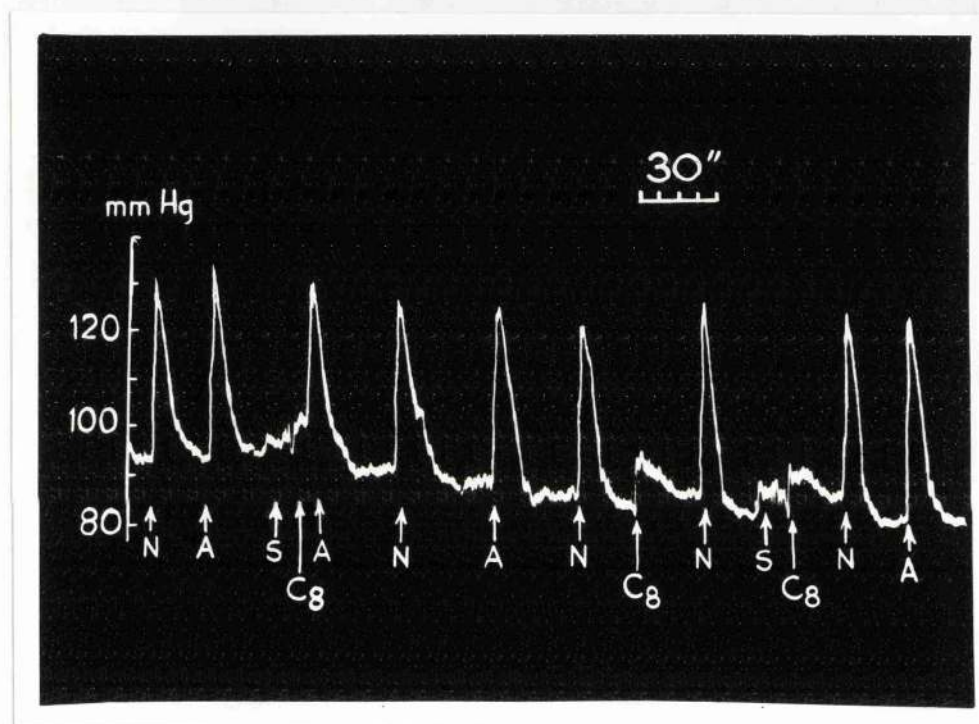


Fig. 91.

Record of the carotid arterial blood pressure of a urethane-anaesthetized rat.

At N - Effect of 3 μ g. per kg. noradrenaline.

At A - " 2 μ g. per kg. adrenaline.

At S - " 0.2 ml. normal saline.

At C₈ - " 0.4 mg. per kg. 3-(dimethylaminomethyl)thionaphthen methiodide (VIII).

Drugs administered intravenously.

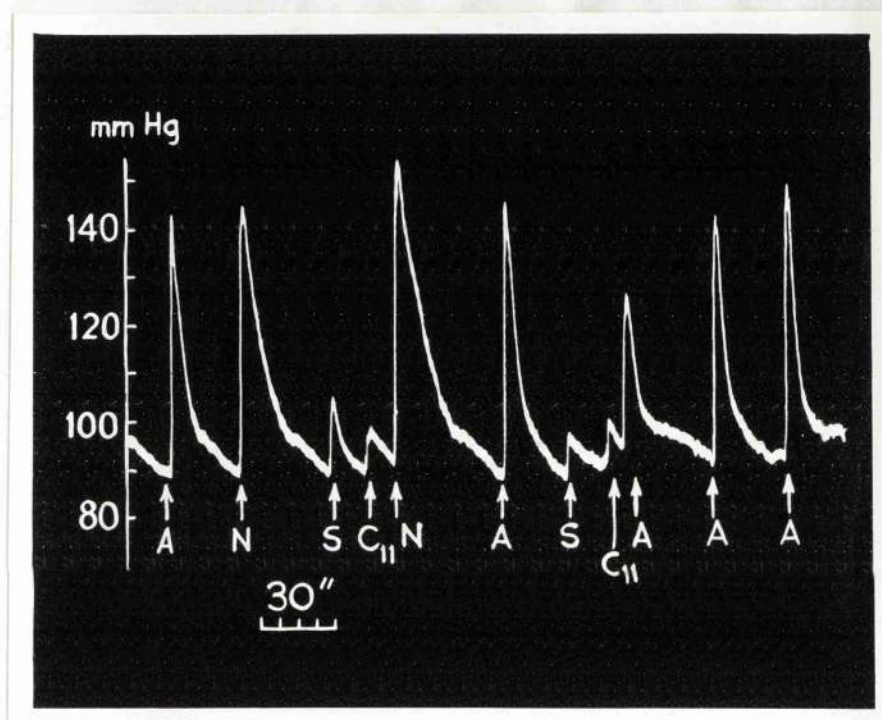


Fig. 92.

Record of the carotid arterial blood pressure of a urethane-anaesthetized rat.

At A - Effect of 2 μ g. per kg. adrenaline.

At N - " 4 μ g. per kg. noradrenaline.

At S - " 0.2 ml. of normal saline.

At C₁₁ - " 0.4 mg. per kg. 3-(piperidinomethyl)
thionaphthen methiodide (XI).

Drugs administered intravenously.

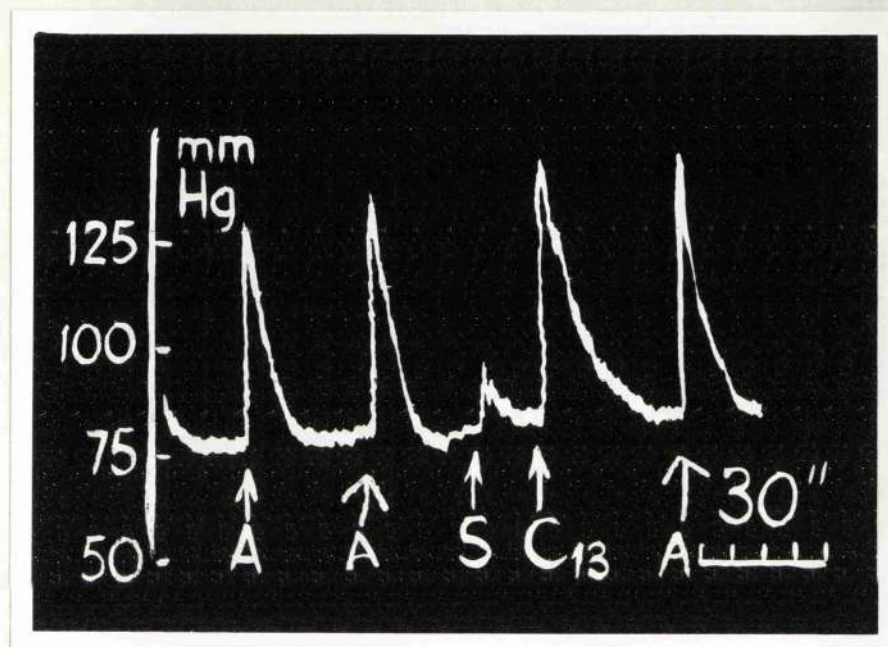


Fig. 93.

Record of the carotid arterial blood pressure of a urethane-anaesthetized rat.

At A - Effect of 0.6 μ g. per kg. adrenaline.

At S - " 0.2 ml. normal saline.

At C₁₃ - " 0.3 mg. per kg. 2-(2'-aminopropyl)-5-hydroxythionaphthen(XIII).

Drugs administered intravenously.

adrenaline (0.3 to 3.0 μ g. per kg.) (Figs. 94 to 96, pages 191 to 193).

(c) Toxic effects on mice

At dose levels of up to 150 mg. per kg. given by intraperitoneal injection, none of the gramine-like isosteres (Group A) had any apparent toxic effects.

Compounds VIII, X and XI in Group B produced toxic effects. Within 30 minutes of injection there was hyperpnoea followed by severe tonic convulsive movements after which the mice died. The LD 50 of compound XI was 57.5 mg. per kg.; of compound X 62.5 mg. per kg., and of compound VIII 67.5 mg. per kg. The results are shown numerically in Table 1, page 194, and graphically in Figs. 97 to 99, pages 195 to 197. At dose levels of up to 150 mg. per kg., compound IX showed no apparent toxic effects in mice.

At dose levels of up to 150 mg. per kg. given by intraperitoneal injection, none of the compounds in Groups C and D showed any apparent toxic effects in mice.

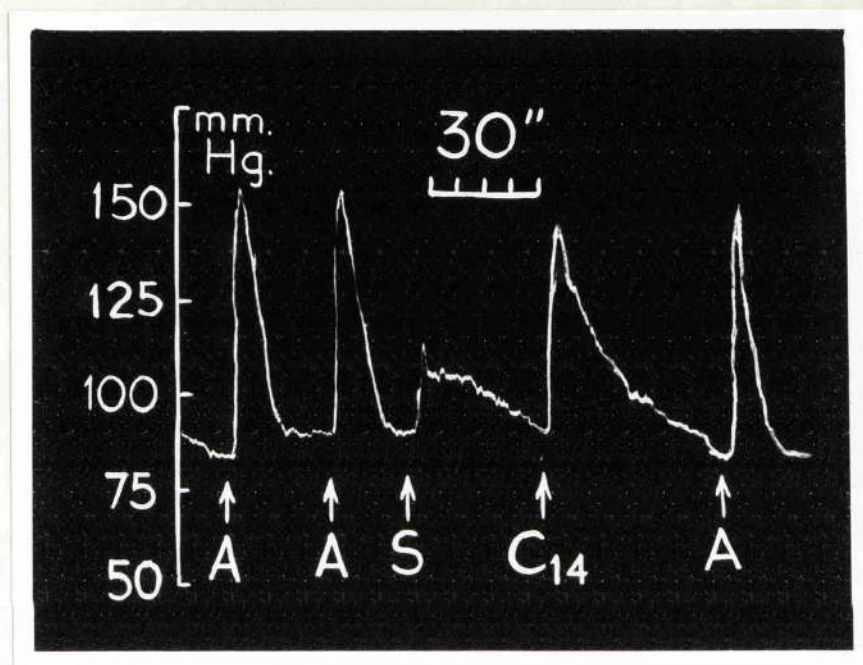


Fig. 94.

Record of the carotid arterial blood pressure of urethane-anaesthetized rat.

At A - Effect of 6 μ g. per kg. adrenaline.

At S - " 0.2 ml. normal saline.

At C₁₄ - " 0.4 mg. per kg. 5-amino-2-(dimethyl-aminomethyl) thionaphthen (XIV).

Drugs administered intravenously.

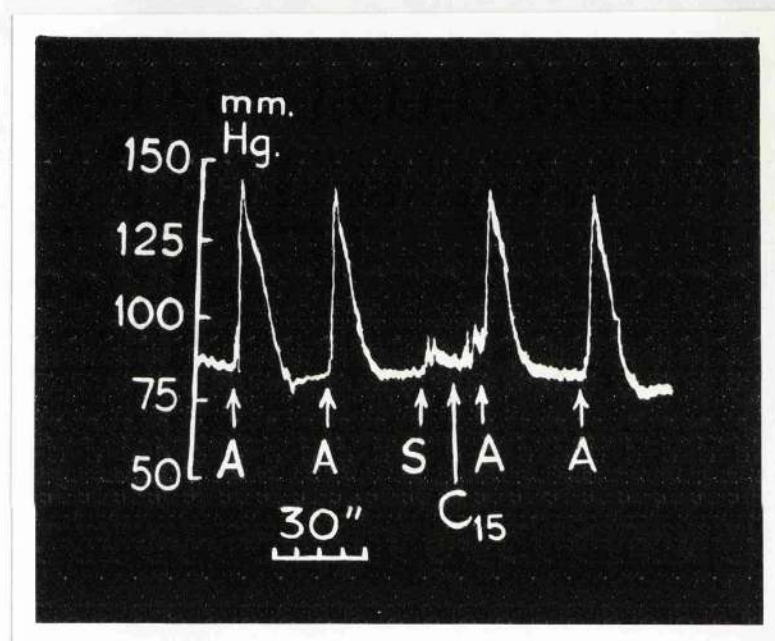


Fig. 95.

Record of the carotid arterial blood pressure of a urethane-anaesthetized rat.

- At A - Effect of 8 μ g. per kg. adrenaline.
 At S - " 0.2 ml. normal saline.
 At C₁₅ - " 0.8 mg. per kg. 5-amino-2-(morpholinomethyl) thionaphthen (XV).

Drugs administered intravenously.

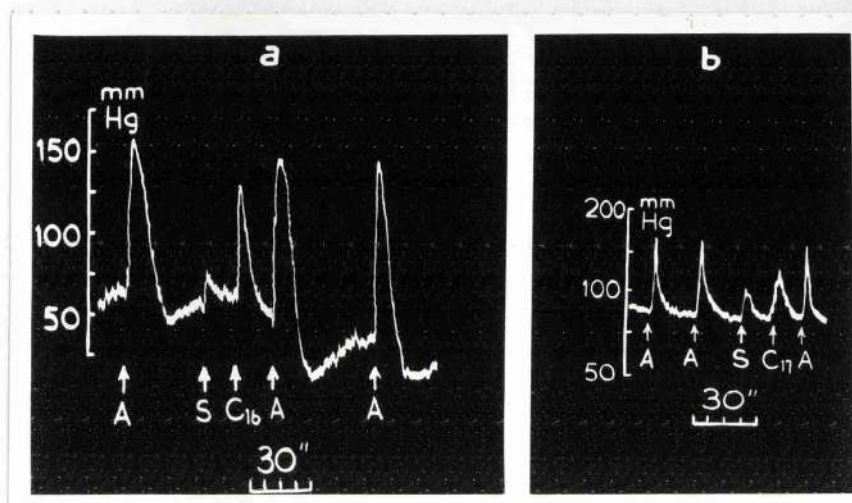


Fig. 96.

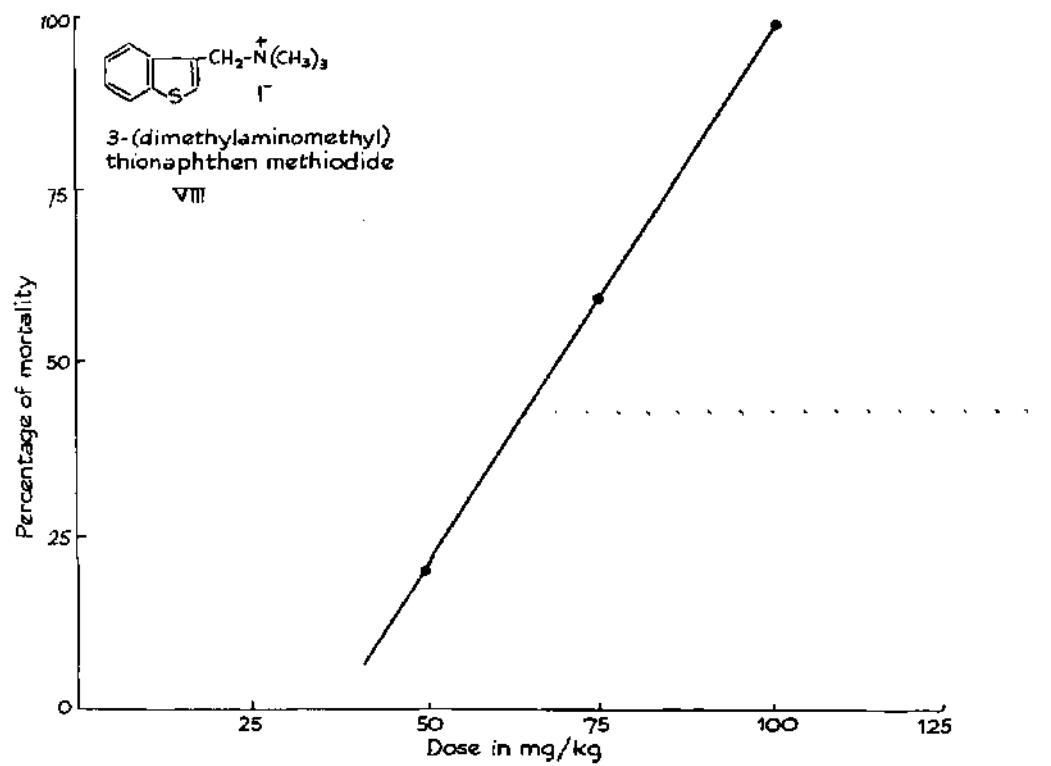
Record of the carotid arterial blood pressure of a urethane-
anaesthetized rat.

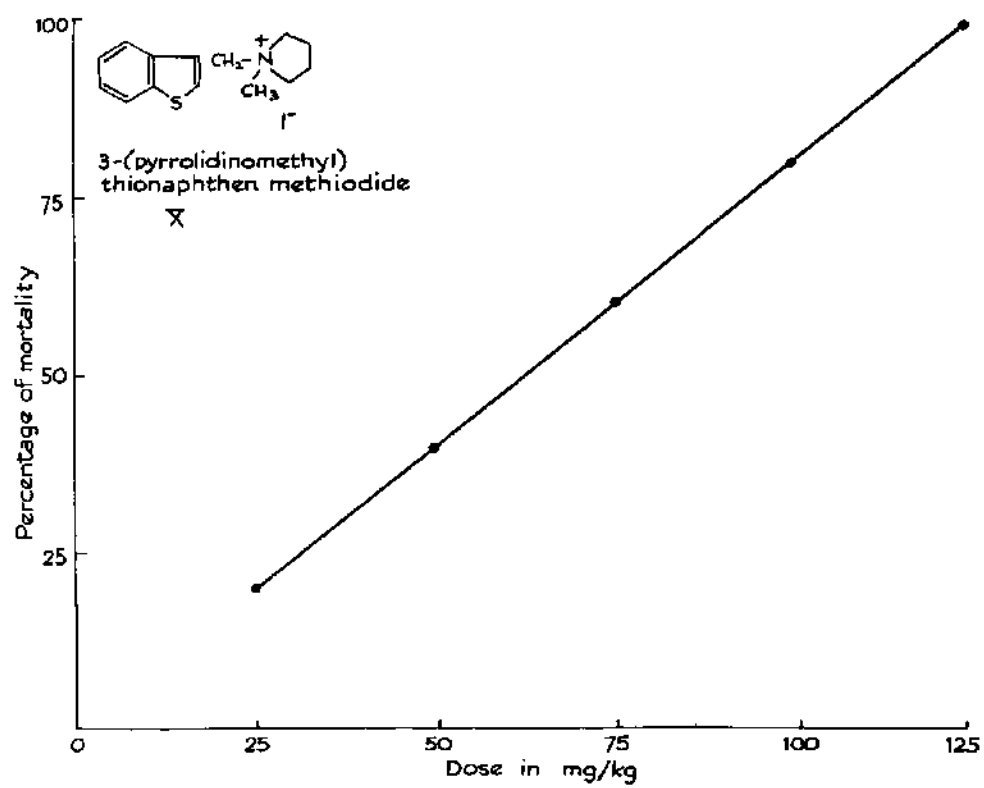
- (a) At A - Effect of 0.3 μ g. per kg. adrenaline.
 At S - Effect of 0.2 ml. normal saline.
 At C₁₆ - Effect of 0.6 mg. per kg. 5-amino-2-
 (pyrrolidinomethyl) thionaphthen (XVI).
- (b) At A - Effect of 0.3 μ g. per kg. adrenaline.
 At S - Effect of 0.2 ml. normal saline.
 At C₁₇ - Effect of 0.6 mg. per kg. 5-amino-2-
 (piperidinomethyl) thionaphthen (XVII).

Drugs administered intravenously.

TABLE 1.
MORTALITY IN MICE

Dose of drug mg. per kg.	percentage mortality		
	Compound VIII	Compound X	Compound XI
25	0	20	0
50	20	40	40
75	60	60	80
100	100	80	100
125	-	100	-

Fig. 97.

Fig. 98.

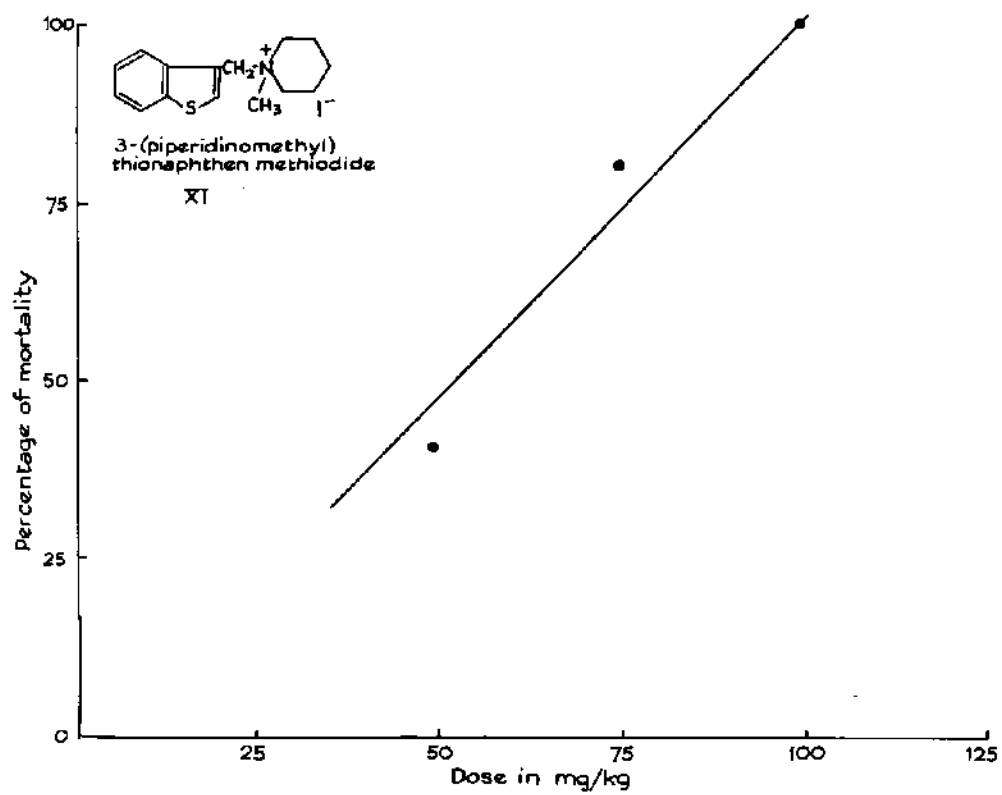


Fig. 99.

III. Results of experiments with the thionaphthen compounds on the isolated rat jejunum using Ariëns' technique.

With the exception of compound X, which was available in quantities sufficient for one only experiment, all were tested on more than two individual preparations. Typical experimental tracings are shown in Figs. 100 to 105, pages 201 to 206, and graphical representations of some of the experimental tracings are given in Figs. 106 to 112, pages 207 to 213.

From the results it is difficult to say if the antagonism exhibited by these compounds is either competitive or non-competitive. If the compound under investigation caused no contraction of the isolated rat jejunum, the cumulative log. dose-response curves for 5-hydroxytryptamine in the presence of various concentrations of the compound were shifted along the abscissa and the height of the maximum response was reduced, it was considered that the drug under test exhibited a purely non-competitive type of antagonism. But, if the compound caused a contraction of the tissue; the cumulative log. concentration curve for 5-hydroxytryptamine in the presence of various concentrations of the drug under test was shifted along the abscissa and the height of the maximum response reduced, it /

it was considered that the compound exhibited an agonistic action and also produced a non-competitive block. It is interesting to note that at low concentrations, some of the compounds themselves caused a contraction of the isolated rat jejunum but, as the concentrations were increased, the height of the contractions was decreased (Figs. 101, 107, pages 202, 208).

Compounds II, III and VII in Group A, VIII and X in Group B, all the compounds in Group C and compound XV in Group D exhibited a purely non-competitive type of antagonism towards 5-hydroxy-tryptamine when tested on the isolated rat jejunum.

Compounds I and VI in Group A, XI in Group B, and XIV, XVI and XVII in Group D exhibited both agonistic properties and a non-competitive type of antagonism. Compounds IV and V in Group A showed, however, variable activity. Compound V exhibited a purely non-competitive antagonism in two different experiments but in another two experiments it showed both agonistic properties and non-competitive antagonism. Compound IX could not be assigned to any particular category. It is clear from the graph (Fig. 108, page 209) that at a concentration of $13.92 \mu \text{ Mol./L.}$, it displaced the cumulative log dose-response curve for 5-hydroxytryptamine along the abscissa and the maximum height /

height was also reduced, indicating a non-competitive block. At higher concentrations ($139.2 \mu \text{ Mol./L.}$ and $1392 \mu \text{ Mol./L.}$), although the cumulative log dose response curves were displaced along the abscissa, the maximum height was increased and not reduced.

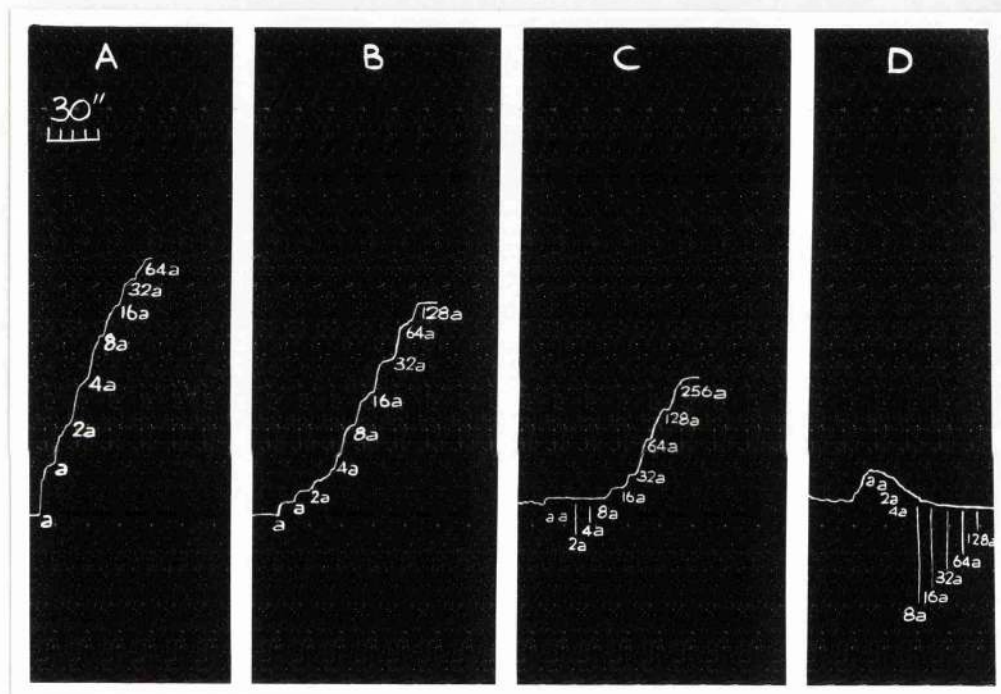


Fig. 100.

The isolated rat jejunum

Experimental, cumulative log dose-response curves, based on the Ariens procedure, for 5-hydroxytryptamine alone (A), and in the presence of 2.5 μ Mol./L. (B), 25 μ Mol./L. (C) and 125.3 μ Mol./L. (D), respectively of 3-(aminomethyl) thionaphthen (I).

Dose a = 0.00568 μ Mol./L. Other doses are multiples of a, as shown on the tracings.

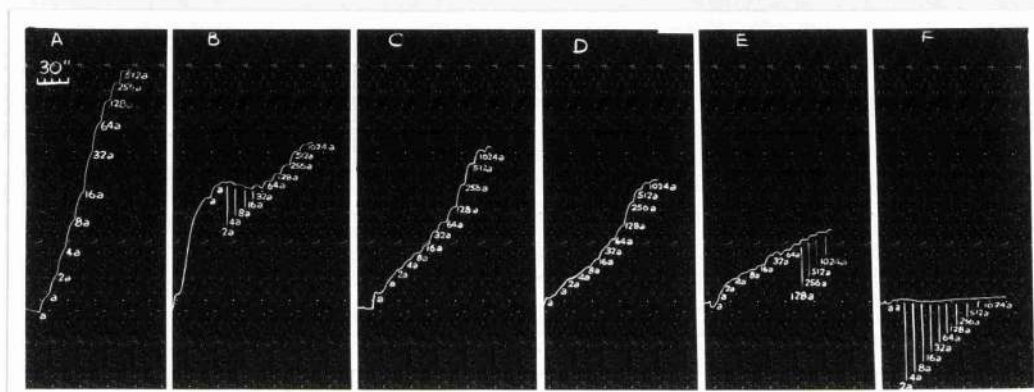


Fig. 101.

The isolated rat jejunum.

Experimental, cumulative log dose-response curves, based on the Ariens procedure, for 5-hydroxytryptamine alone (A), and in the presence of 1.869 μ Mol./L. (B), 3.738 μ Mol./L. (C), 7.476 μ Mol./L. (D), 14.952 μ Mol./L. (E) and 29.904 μ Mol./L. (F) respectively of 3-(piperidinomethyl) thionaphthen (V).

Dose a = 0.00568 μ Mol./L. Other doses are multiples of a, as shown on the tracings.

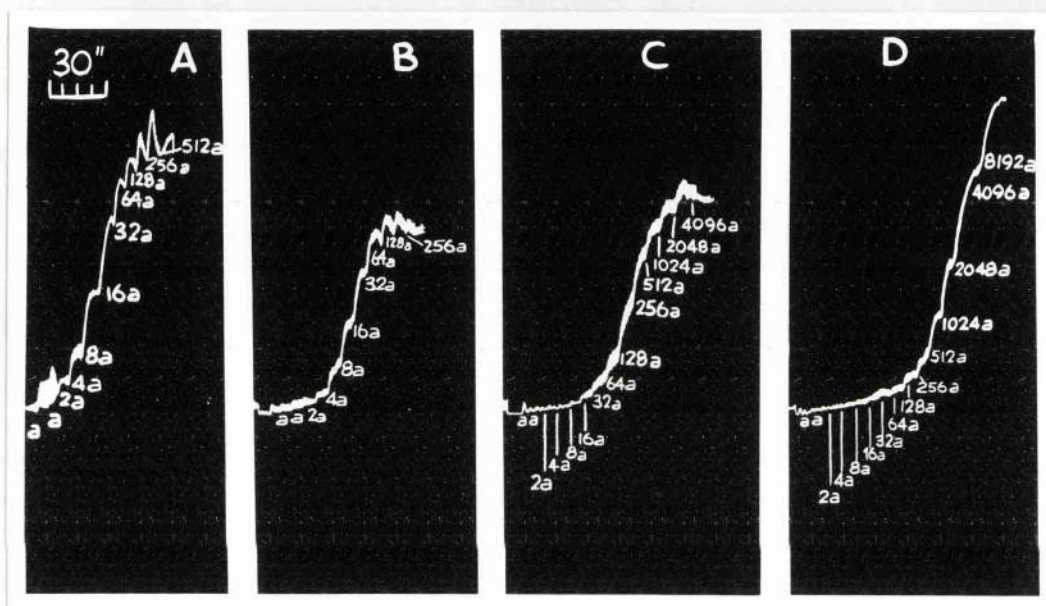


Fig. 102.

The isolated rat jejunum.

Experimental, cumulative log dose-response curves, based on the Ariens procedure, for 5-hydroxytryptamine alone (A), and in the presence of 13.9 μ Mol./L. (B), 139.2 μ Mol./L. (C), and 1392 μ Mol./L. (D) respectively of 3-(morpholinomethyl) thionaphthen methiodide (IX).

Dose a = 0.00568 μ Mol./L. Other doses are multiples of a, as shown on the tracings.

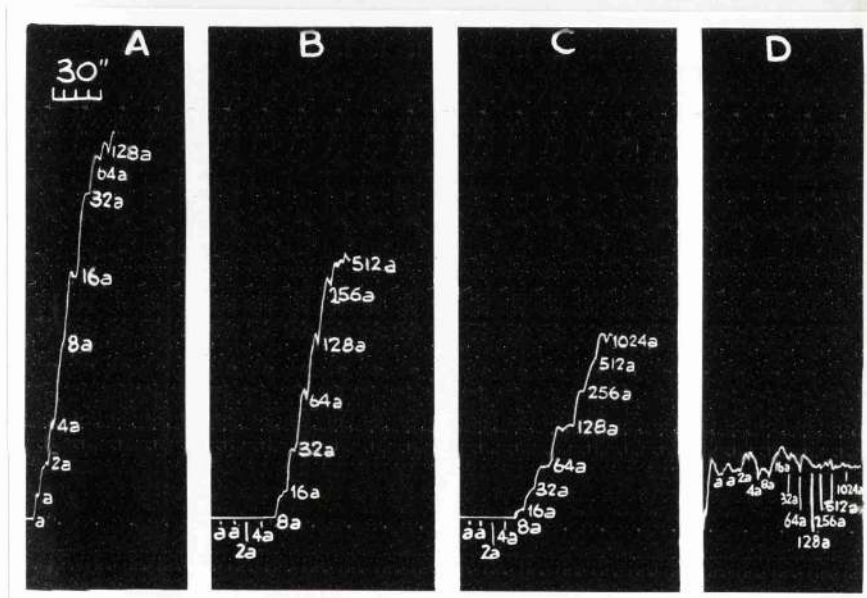


Fig. 103.

The isolated rat jejunum.

Experimental, cumulative log dose-response curves, based on the Ariens procedure, for 5-hydroxytryptamine alone (A), and in the presence of 13.9 μ Mol./L. (B), 278.4 μ Mol./L. (C), and 1392 μ Mol./L. (D) respectively of 3-(pyrrolidinomethyl) thionaphthen methiodide (X).

Dose a = 0.00568 μ Mol./L. Other doses are multiples of a, as shown on the tracings.

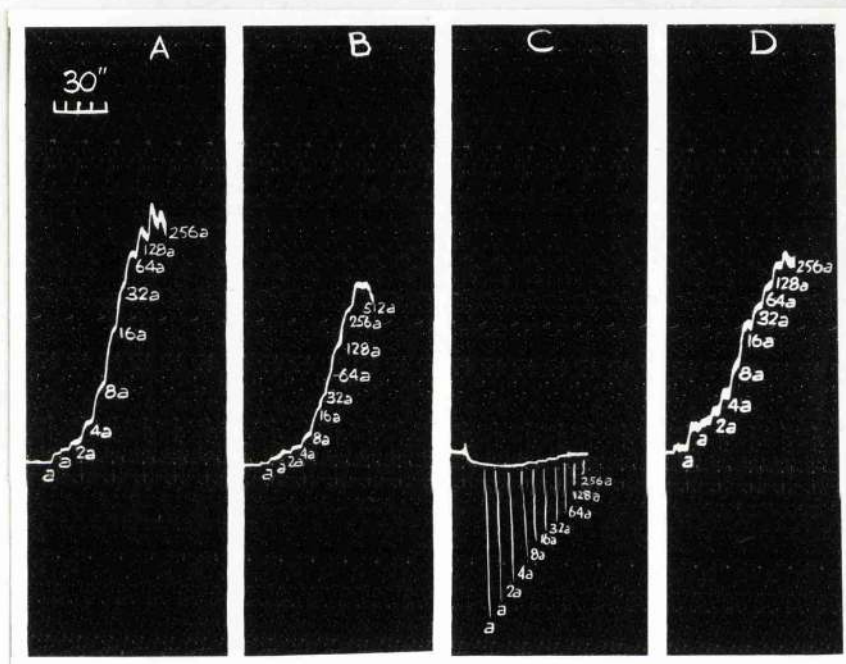


Fig. 104.

The isolated rat jejunum.

Experimental, cumulative log dose-response curves, based on the Ariëns procedure, for 5-hydroxytryptamine alone (A and D), and in the presence of 13.9 μ Mol./L. (B), 278.4 μ Mol./L. (C), and 1392 μ Mol./L. (D) respectively of 2-(2'-aminoethyl)-5-hydroxythionaphthen (XII).

Dose a = 0.00568 μ Mol./L. Other doses are multiples of a, as shown on the tracings.

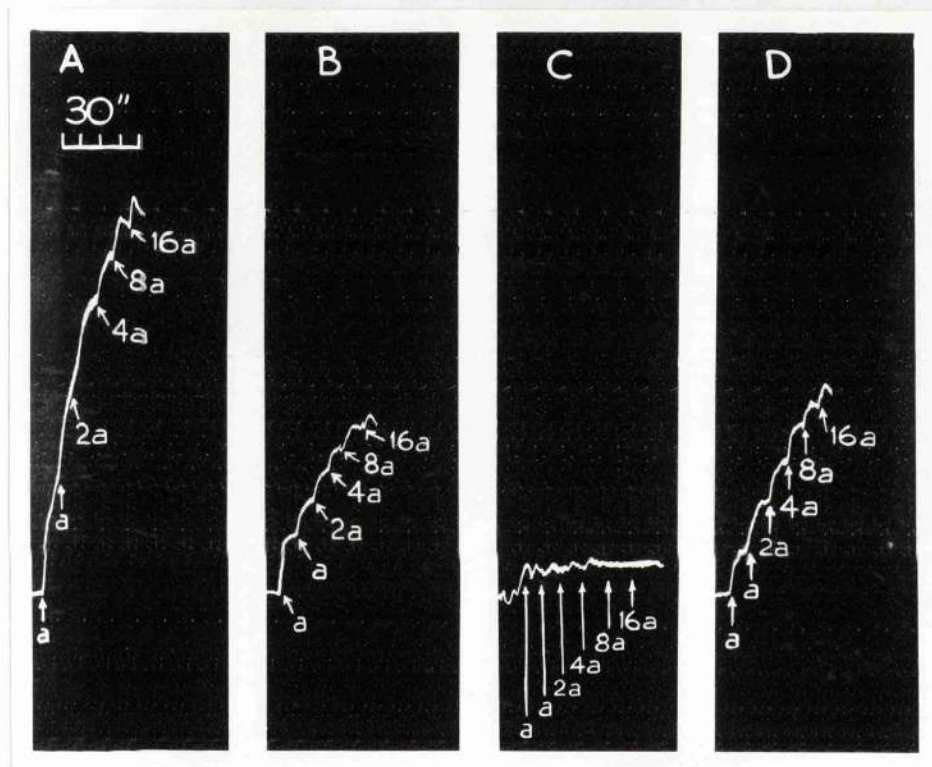


Fig. 105.

The isolated rat jejunum.

Experimental, cumulative log dose-response curves, based on the Ariens procedure, for 5-hydroxytryptamine alone (A and D), and in the presence of 12.69 μ Mol./L. (B) and 126.9 μ Mol./L. (C) respectively of 5-amino-2-(pyrrolidinomethyl) thionaphthen (XVI). Dose a = 0.00568 μ Mol./L. Other doses are multiples of a, as shown on the tracings.

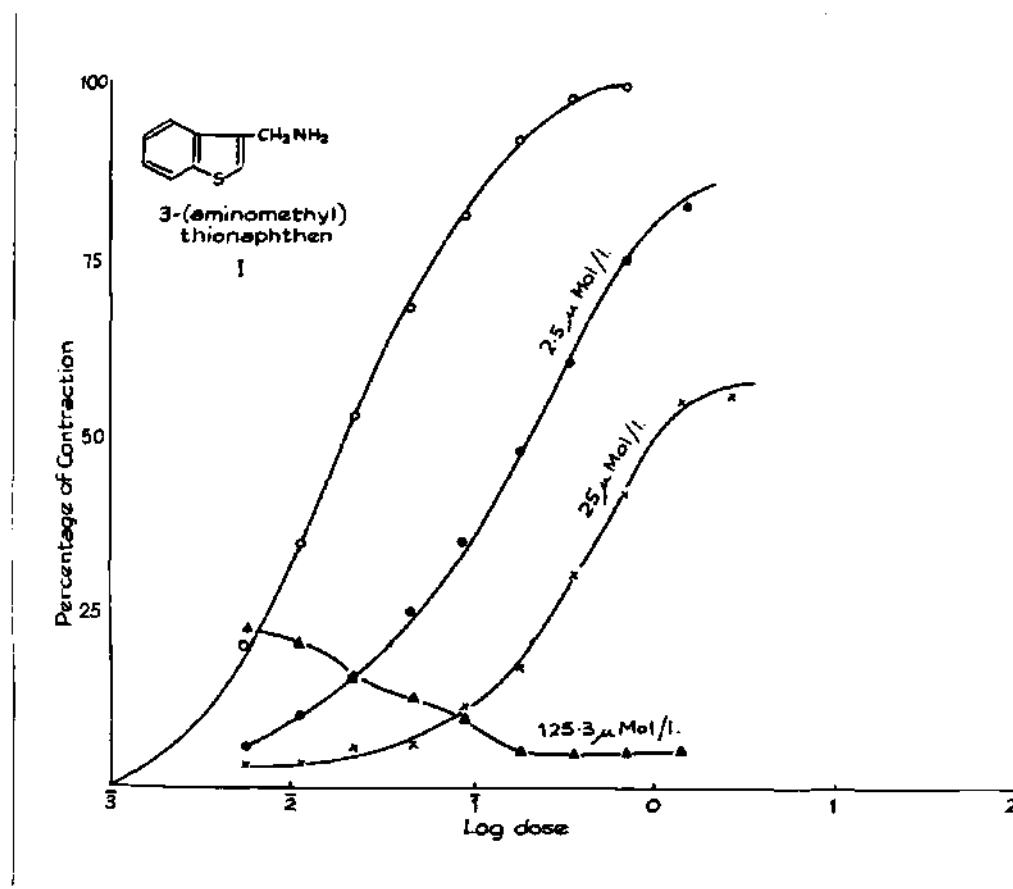


Fig. 106.

The isolated rat jejunum.

Cumulative log dose response curves for 5-hydroxytryptamine alone (o-o-o) and in the presence of 2.5 μ Mol./L. (●-●-●), 25.0 μ Mol./L. (x-x-x) and 125.3 μ Mol./L. (▲-▲-▲) respectively of 3-(aminomethyl)thionaphthen (I).

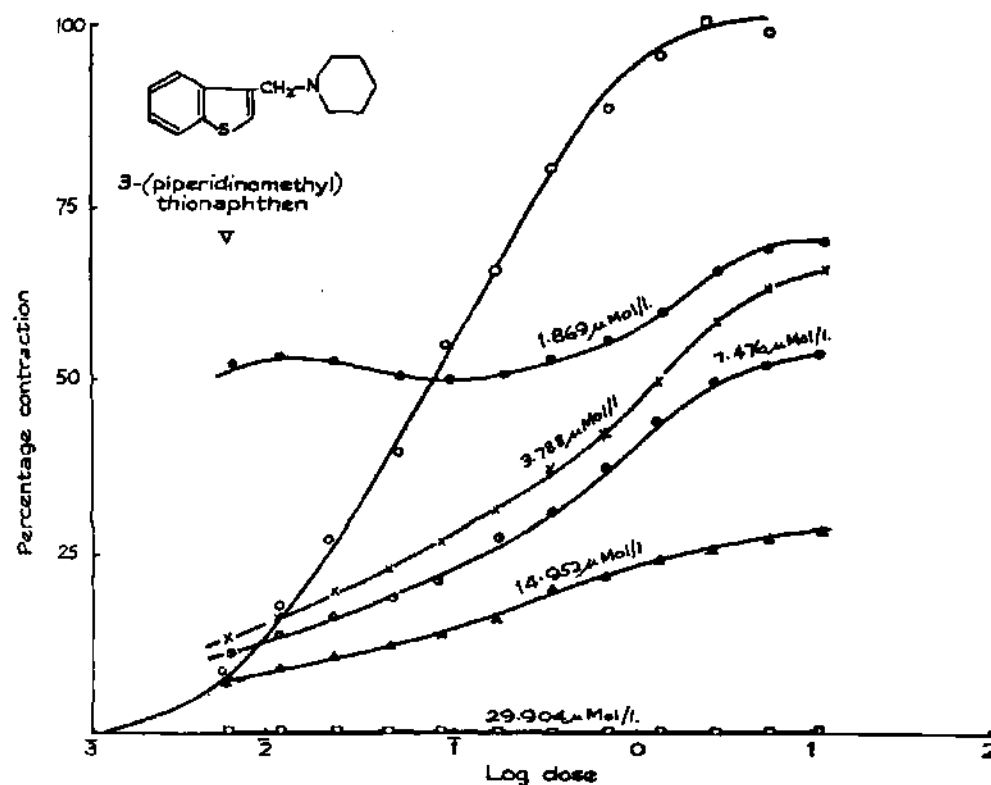


Fig. 107.

The isolated rat jejunum.

Cumulative log dose-response curves for 5-hydroxytryptamine alone (o-o-o) and in the presence of 1.869 μ Mol./L. (●-●-●), 3.738 μ Mol./L. (x-x-x), 7.476 μ Mol./L. (○-○-○), 14.952 μ Mol./L. (▲-▲-▲) and 29.904 μ Mol./L. (□-□-□) respectively of 3-(piperidinomethyl) thionaphthen (V).

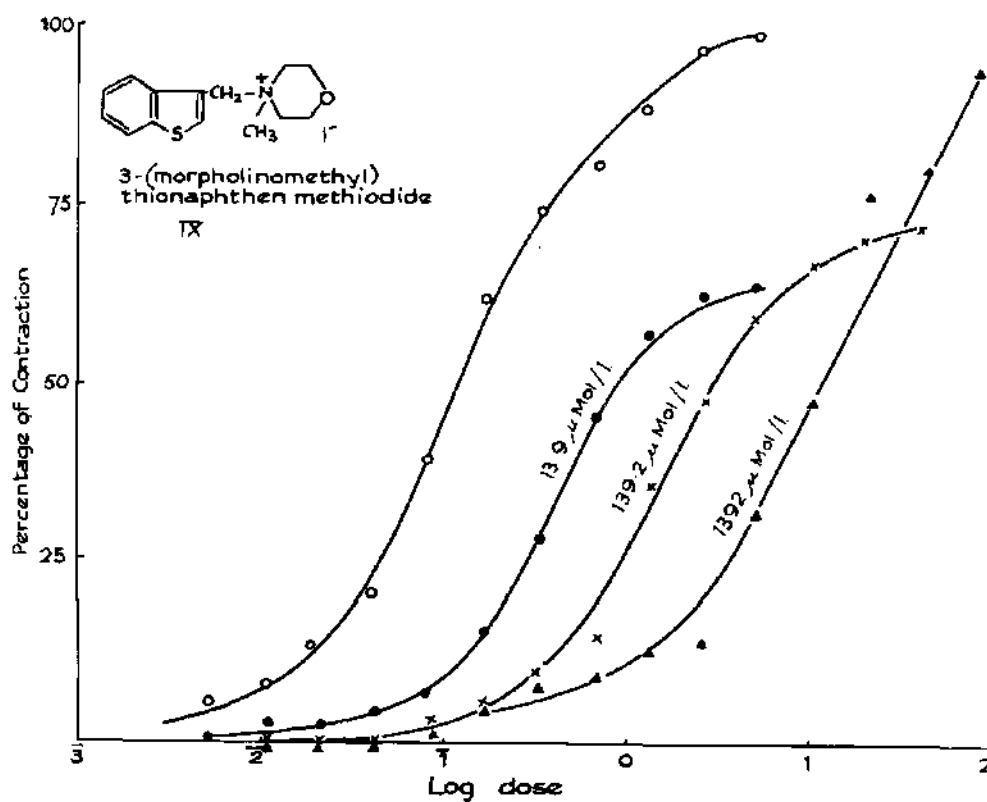


Fig. 108.

The isolated rat jejunum.

Cumulative log dose response curves for 5-hydroxytryptamine alone (o-o-o) and in the presence of 13.9 μ Mol./L. (●-●-●), 139.2 μ Mol./L. (x-x-x) and 1392 μ Mol./L. (▲-▲-▲) respectively of 3-(morpholinomethyl) thionaphthen methiodide (IX).

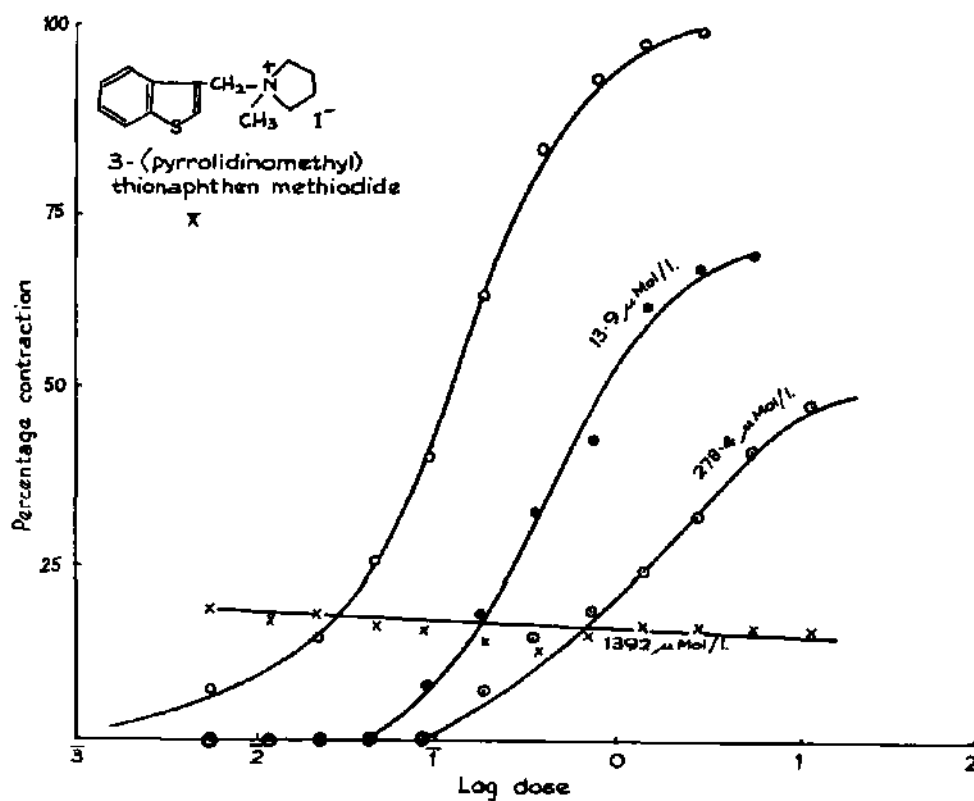


Fig. 109.

The isolated rat jejunum.

Cumulative log dose-response curves for 5-hydroxytryptamine alone (o-o-o) and in the presence of 13.9 μ Mol./L. (•-•-•), 278.4 μ Mol./L. (◐-◐-◐) and 1392 μ Mol./L. (x-x-x) respectively of 3-(pyrrolidinomethyl) thionaphthen methiodide (X).

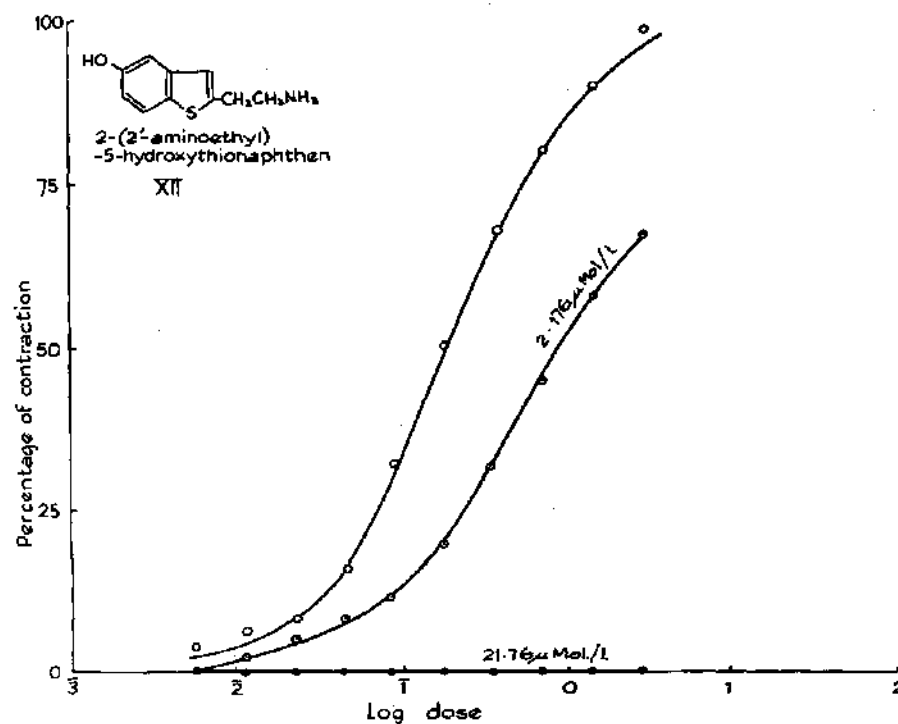


Fig. 110.

The isolated rat jejunum.

Cumulative log dose-response curves for 5-hydroxytryptamine alone (o-o-o) and in the presence of 2.176 μ Mol./L. (o-o-o) and 21.76 μ Mol./L. (●-●-●) respectively of 2-(2'-aminoethyl)-5-hydroxythionaphthen (XII).

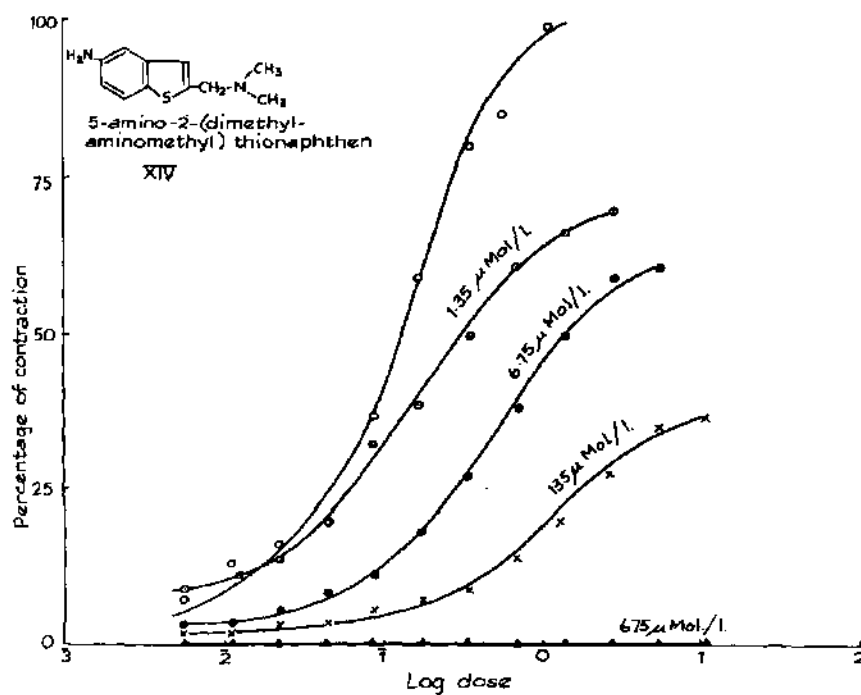


Fig. 111.

The isolated rat jejunum.

Cumulative log dose-response curves for 5-hydroxytryptamine alone (o-o-o) and in the presence of 1.35 μ Mol./L. (o-o-o), 6.75 μ Mol./L. (●-●-●), 135 μ Mol./L. (x-x-x) and 675 μ Mol./L. (▲-▲-▲) respectively of 5-amino-2-(dimethylaminomethyl) thionaphthen (XIV).

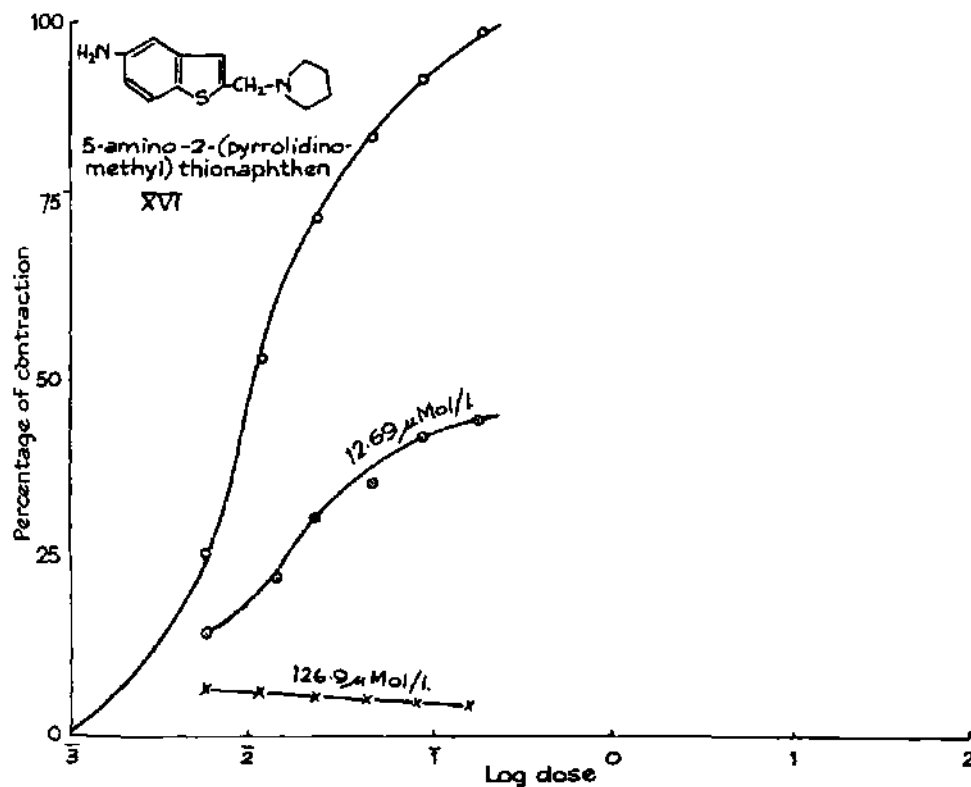


Fig. 112.

The isolated rat jejunum.

Cumulative log dose-response curves for 5-hydroxytryptamine alone (o-o-o) and in the presence of 12.69 μ Mol./L. (e-e-e) and 126.9 μ Mol./L. (x-x-x) respectively of 5-amino-2-(pyrrolidinomethyl) thionaphthen (XVI).

DISCUSSION.

Pages 214 to 242.

D I S C U S S I O N

Substances of diverse chemical composition are capable of preventing or antagonizing the pharmacological actions of 5-hydroxytryptamine both in vitro and in vivo. These include lysergic acid diethylamide, chlorpromazine, dibenamine, reserpine, atropine and morphine. Although the exact physiological role of 5-hydroxytryptamine itself has not been clearly established, 5-hydroxytryptamine antagonists are of pharmacological interest for several reasons. Firstly, they are of value in distinguishing 5-hydroxytryptamine from other active substances present in tissue extracts such as histamine and acetylcholine. Secondly, they may contribute to existing knowledge of the mechanism of the action of 5-hydroxytryptamine as well as of drugs believed to act by the liberation of this compound from its binding sites, e.g., reserpine. Thirdly, they may be of potential value in the prevention or treatment of diseases associated with an excess of 5-hydroxytryptamine in the body (e.g., malignant carcinoid tumours).

Present-day knowledge concerning the mode of action of 5-hydroxytryptamine has been obtained, to a large extent, by means of /

of 5-hydroxytryptamine antagonists and by using isolated tissue preparations such as the rat uterus and the guinea pig ileum. From the results of studies of this kind, it has been postulated that two types of 5-hydroxytryptamine receptors exist in the guinea pig ileum, namely the "M" and "D" receptors (Gaddum and Picarelli, 1957) and these have been differentiated by the use of the 5-hydroxytryptamine antagonists, namely morphine, atropine and methadone on the one hand and dibenzylene, lysergic acid diethylamide and 5-benzylxygramine on the other. In addition to the possible existence of more than one type of specific receptor for 5-hydroxytryptamine in the same tissue, variations in the effects of 5-hydroxytryptamine itself on the same tissue must also be considered. Thus Gaddum (1953a) and Rocha e Silva and his associates (1953) have shown that, in low concentrations, 5-hydroxytryptamine stimulates the isolated guinea pig ileum and at higher concentrations, causes autoinhibition. Furthermore, the nature of the antagonism of certain substances to 5-hydroxytryptamine has further complicated the picture since these compounds not only antagonize the actions of 5-hydroxytryptamine itself, but themselves produce agonistic effects (Woolley and Shaw, 1953a; Shaw and Woolley, 1954; Gaddum and his associates, 1955; /

1955; and Barlow and Khan, 1959a and b).

The picture is further complicated since many indole derivatives, in addition to antagonizing the actions of 5-hydroxytryptamine, are also capable of antagonizing the actions of other pharmacological agents. For example, Gaddum and Hameed (1954) observed that on the isolated rat uterus, guinea pig ileum and on the blood vessels of the perfused rabbit ear, many ergot alkaloids antagonized both 5-hydroxytryptamine and adrenaline. It has been reported that 5-benzyloxy-N',N-dimethyl-tryptamine antagonizes the stimulant actions of 5-hydroxytryptamine, histamine and acetylcholine on the isolated guinea pig ileum, although its antihistaminic and antimuscarinic activities were less than its anti-5-hydroxytryptaminic effects (Barlow and Khan, 1959c). Other indole derivatives such as 3-(2-dibutylaminoethyl) indole exhibit synergism with both 5-hydroxytryptamine and acetylcholine on the isolated guinea pig ileum (Barlow and Khan, 1959a).

A similar pattern of non-selectivity has emerged from the present study of a group of thionaphthen derivatives in which the -NH- group of the indole nucleus has been replaced by a sulphur atom. For example, with the exception of compound VI, all the members of Group A were found to antagonize the stimulant action of /

of 5-hydroxytryptamine on the isolated rat uterus. On the isolated rat fundus strip, however, many of the compounds (including compound VI) were found to potentiate the stimulant response to 5-hydroxytryptamine. In a series of four separate experiments, compound VI, in identical bath concentrations, was found in two cases to exhibit incomplete antagonism to 5-hydroxytryptamine and in the other two experiments to potentiate the 5-hydroxytryptamine-induced contractions of the isolated rat uterus (Figs. 17, 18, pages 94, 95). It is of interest, that a similar variability has been reported using 3-(aminopropyl)-1-methyl indole (Barlow and Khan, 1959b). In addition to their failure to produce a uniform pharmacological response in various tissue preparations, the thionaphthen compounds investigated were found to be more potent in antagonizing the stimulant responses to histamine than those to 5-hydroxytryptamine or to acetylcholine on the isolated guinea pig ileum. At higher concentrations than those required to antagonize the stimulant responses to these spasmogens, the compounds themselves caused contractions of the tissue which were partly or completely inhibited by lysergic acid diethylamide, mepyramine or atropine.

Due to their lack of selectivity, it is difficult to interpret the precise mechanism of action of the thionaphthen compounds investigated. Moreover, the derivation of structure-action relationships /

relationships is itself complicated and beset by many hazards (Ing, 1959 and Reid, 1960). In spite of these difficulties, however, an attempt has been made to investigate the mode of action of the compounds studied using conventional and other pharmacological techniques.

The synthesis of compounds bearing an isosteric relationship to an existing drug is now one of the accepted approaches to the design of new drugs. Although the application of this concept has not been entirely successful in producing drugs with more desirable pharmacological properties than the parent compounds, some successful attempts have been reported among the thiophen and thionaphthen derivatives. Thus Crook and Davies (1937) have shown that thionaphthen-3-acetic acid possesses plant growth-promoting activity similar to that of its naturally occurring isostere, indole-3-acetic acid, although it is less potent. 5-Methyl-4,7-thionaphthenquinone, which is isosteric with 2-methyl-1,4-naphthaquinone (menadione) has weak vitamin K-like activity (Tarbell, Fukushima and Dam, 1945); the pressor activity of β -2-thienylethylamine has been shown to be similar to that of β -phenylethylamine in the spinal cat (Tainter, 1930) and β -2-thienylisopropylamine, an isostere of β -phenylisopropylamine (amphetamine), /

(amphetamine), is as potent as amphetamine in raising the blood pressure of the anaesthetized dog (Alles and Feigen, 1941). On the other hand, this approach has failed in some cases. For example, 2,2-bis-(2'-thienyl)-1,1,1-trichloroethane which is isosteric with 2,2-bis-(p-chlorophenyl)-1,1,1-trichloroethane (DDT) was found to be inactive against the house fly (Prill, Synerholm and Harzell, 1946).

The influence of isosterism as applied to a group of thionaphthen compounds has been assessed in this thesis. The compounds involved were III, V and VI in Group A, which are the isosteres of 3-(morpholinomethyl) indole, 3-(piperidinomethyl) indole and 3-(2'-methylpiperidinomethyl) indole respectively, and compound XII in Group C which is isosteric with 2-(2'-aminoethyl)-5-hydroxyindole. The pharmacology of the indole derivatives has already been reported (De Jong and Van Proosdij-Hartzema, 1952 and Bartaccini and Zamboni, 1961). The structural formulae and the pharmacological properties of the indole derivatives and of the corresponding thionaphthen compounds are shown in Fig. 113, page 220, and summarised in Table 2, page 221. A comparative study on the isolated rat uterus, guinea pig ileum, blood pressure of the anaesthetized cat and rat, blood vessels of the perfused rat hindquarters and the effect of the injection of the compounds into mice revealed that the introduction of a sulphur atom in place of the -NH- group in the indole molecule brought about /

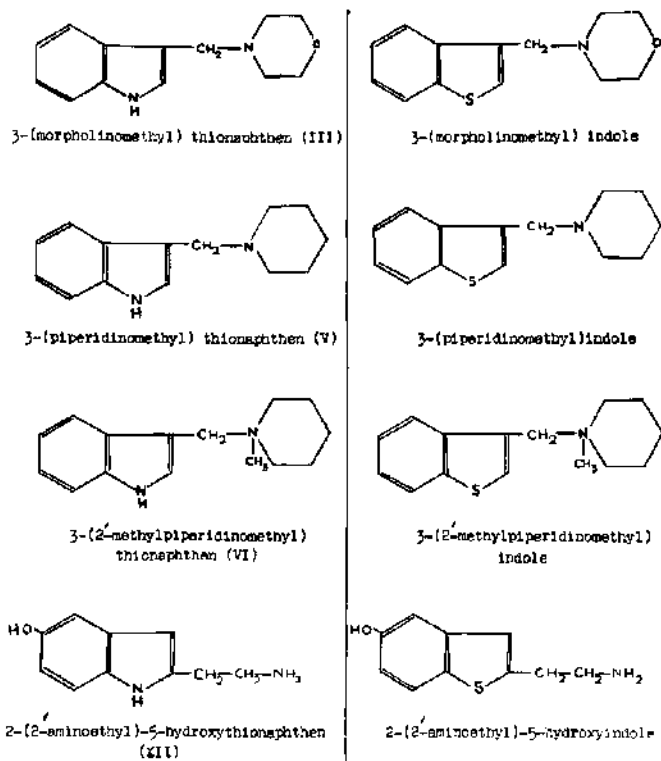
Fig. 113.

TABLE 2.

A comparative pharmacological evaluation of some indole-containing compounds and their corresponding thionaphthen isosteres

Pharmacological test objects	3-(morpholinomethyl)		3-(piperidinomethyl)		3-(2'-methylpiperidinomethyl)	
	Indole	Thionaphthen (III)	Indole	Thionaphthen (V)	Indole	Thionaphthen (VI)
Isolated rat uterus	contraction	no contraction	contraction	no contraction	contraction	*
Isolated guinea pig ileum	contraction	no contraction	contraction	contraction	contraction	contraction
Isolated perfused rat hindquarters	vasoconstriction	no vasoconstriction	vasoconstriction	no vasoconstriction	vasoconstriction	no vasoconstriction
Toxic effects (restlessness & convulsions) in mice	toxic symptoms	no toxic symptoms	toxic symptoms	no toxic symptoms	toxic symptoms	no toxic symptoms
Blood pressure of anaesthetized cat	rise	fall	rise	fall	rise	fall
Blood pressure of anaesthetized rat.	not reported	fall	not reported	fall	not reported	fall
Blood pressure of anaesthetized rabbit	prolonged hypotension	not done	no change	not done	no change	not done

* Caused no contraction of its own but exhibited slight potentiation or incomplete inhibition to 5-hydroxytryptamine-induced contractions and also induced spontaneous activity in the tissue (Figs. 17, 18, pages 94, 95).

Note: The pharmacological properties of 2-(2'-aminoethyl)-5-hydroxy indole and the corresponding thionaphthen isostere has been described in page 236.

about a general reduction in the stimulant properties. Thus, while 3-(morpholinomethyl) indole, 3-(piperidinomethyl) indole and 3-(2'-methylpiperidinomethyl) indole exhibited a powerful stimulant action on the isolated rat uterus, caused constriction of the blood vessels of the perfused isolated rat hindquarters and produced toxic symptoms in mice, the corresponding thionaphthen isosteres exhibited no stimulant properties on the isolated rat uterus, caused no vasoconstriction in the perfused rat hindquarters and did not produce any toxic effects in mice when injected intraperitoneally.

The change in the activity from mainly agonistic (indole-containing compounds) to mainly antagonistic (thionaphthen compounds) properties may be viewed as a reduction in the intensity of the specific fields of forces between the drug molecule and the receptors (i.e., the intrinsic activity) which are believed to initiate the biological response. The increased size of the sulphur atom as compared with the nitrogen atom may bring about an increased distribution of cationic charges and consequently a reduction in the degree of charge intensity available for drug-receptor complex formation and this in turn would be expected to decrease the ability of the sulphur-containing compounds to evoke a positive biological response, i.e., a stimulant action. A quantitative measure of this effect (the intrinsic activity) was not /

not however obtainable because of the variability of the response of the isolated rat jejunum towards the thionaphthen compounds and the failure of experiments using other tissues. The greater availability of p electrons for bonding in the nitrogen atom as compared with the sulphur atom, might also be expected to contribute towards a greater degree of drug-receptor interaction.

It may be concluded that in the case of the thionaphthen isosteres, isosteric replacement has produced compounds possessing slight or no stimulant properties when compared with their parent compounds. In other words, the two groups of compounds are qualitatively and quantitatively dissimilar in their pharmacological properties which indicates that the application of isosterism to the thionaphthen compounds has failed to produce compounds of similar biological activity.

The compounds comprising Group A are also of interest as antagonists to 5-hydroxytryptamine, histamine and acetylcholine on the guinea pig ileum. They represent a series of compounds closely related to gramine and some of its derivatives. Hence their general pharmacological properties might be expected to resemble those of the gramine series. Gramine itself, in keeping with other indole-derivatives, exhibits the typical variability in pharmacological / .

pharmacological activity characteristic of drugs of this class. Thus it has been reported to possess convulsant activity and parasympathomimetic actions (Supniewski and Serafinowna, 1937) and anti-adrenaline actions on the blood pressure of the anaesthetised cat and on certain smooth muscle preparations (Powell and Chen, 1945). Moreover, it has been shown to exhibit both 5-hydroxytryptamine-like and anti-5-hydroxytryptamine properties (Powell, Swanson and Chen, 1955; Gaddum and Hameed, 1954; Gaddum and his associates, 1955; Erspamer, 1953a, 1954a; and Bertaccini and Zamboni, 1961) - further illustrations of its multiplicity of actions.

With the exception of compounds I and III in Group A, the other compounds studied showed very weak stimulant properties on the isolated guinea pig ileum, although in many cases reproducible and measurable responses could not be obtained. Frequently the response to a second and higher dose of the drug was considerably less than that to the first (Figs. 38, 48, pages 120, 133). This type of activity may be due to autoinhibition, i.e., the drug appears to be depressing, inhibiting or antagonizing its own stimulant action. When a large dose of the drug is added to a bath containing a piece of isolated guinea pig ileum it produces a stimulant action, after which the tissue becomes insensitive to further /

further additions. This phenomenon may be due to the exhaustion, directly or indirectly, by the drug of tissue stores of essential metabolites which may bring about the stimulant action. Alternatively, compounds producing autoinhibition may interact simultaneously with two different types of receptors and possess therefore multiple actions - behaving as agonists at lower concentrations and as antagonists at higher concentrations. This kind of dual behaviour has been reported by Ariëns, Van Rossum and Simonis (1957) among some alkyl trimethylammonium salts on the isolated frog rectus abdominis muscle. Autoinhibition has been previously observed by many workers using 5-hydroxytryptamine, acetylcholine and histamine on the isolated guinea pig ileum. For example, Gaddum (1953a) and Rocha e Silva and his associates (1953) observed that large doses of 5-hydroxytryptamine were capable, when left in contact with the isolated guinea pig ileum, of abolishing the stimulant effects of subsequent doses of 5-hydroxytryptamine. Recently, Paton (1961) has introduced the term "desensitization" to describe this type of activity which takes place when isolated guinea pig ileum is exposed to high doses of acetylcholine or histamine. He pointed out that desensitization was not the property of specific receptors because for example after a large dose of acetylcholine, the responses to both histamine and acetylcholine /

acetylcholine were reduced. The same type of response has been observed with compound II (Fig. 38, page 120), which not only exhibited autoinhibition, but also reduced the response of the tissue to added histamine. Paton (1961) suggested that the loss of intracellular potassium was responsible for desensitization.

The nature of the stimulant properties of the thionaphthen compounds has been investigated by attempting to antagonize the stimulant responses to them by using lysergic acid diethylamide, mepyramine and atropine. From the results obtained, however, it is very difficult to say whether these compounds are 5-hydroxytryptamine-like, histamine-like or acetylcholine-like.

The nature of the stimulant action of the thionaphthen compounds becomes more understandable when the mode of action of 5-hydroxytryptamine, histamine and acetylcholine and their antagonism by lysergic acid diethylamide, mepyramine and atropine respectively on the isolated guinea pig ileum are considered. It has been proposed by Rocha e Silva and his associates (1953) that in the guinea pig ileum there exist specific 5-hydroxytryptamine receptors which are distinct from those for histamine and acetylcholine. These workers also suggested that 5-hydroxytryptamine acted on the post-ganglionic cholinergic fibres of the intramural nervous system. /

system. Although mepyramine and atropine are specific in antagonizing the contractions produced by histamine or acetylcholine respectively, the former substances at higher concentrations also inhibit the contractions caused by 5-hydroxytryptamine on the isolated guinea pig ileum. Robertson (1953) considers, however, that atropine reduces markedly the stimulant response to 5-hydroxytryptamine in concentrations which also effect the response of acetylcholine while others report that concentrations which abolish the effect of acetylcholine and carbachol have but little effect upon stimulant responses to 5-hydroxytryptamine (Gaddum, 1953a; and Gaddum and Hameed, 1954). It has been reported that mepyramine abolished the stimulant effect of histamine in doses that have slight or no effect on the stimulant response to 5-hydroxytryptamine (Gaddum and Hameed, 1954).

From Figs. 39a, b, page 122, it is difficult to say whether the compound II has any histamine-like or acetylcholine-like properties. Furthermore, since this compound antagonized the contractions produced by histamine, acetylcholine and 5-hydroxytryptamine, doubt is cast on the assumption that specific receptors for 5-hydroxytryptamine exist in the guinea pig ileum.

The introduction of a piperidinomethyl group in the thionaphthen molecule (compound V) led to the appearance of a different /

different type of activity. Since only mepyramine, but not atropine or lysergic acid diethylamide inhibited the contraction produced by compound V, it may be suggested that it resembled histamine. It is interesting, however, that this compound completely inhibited the stimulant responses to 5-hydroxytryptamine or acetylcholine and exhibited an incomplete inhibition of the stimulant response to histamine on the guinea pig ileum. With such variable activity, it is difficult to obtain any evidence of a structure-activity relationship or of an action upon a specific receptor.

The consistent lack of activity of compound VI towards the effects of 5-hydroxytryptamine on the guinea pig ileum, rat hindquarters, and to some extent, on the rat uterus, may however be due to the presence of the 2'-methylpiperidinomethyl group in the thionaphthen molecule.

In contrast to the anti-5-hydroxytryptamine activity of the thionaphthen compounds on the isolated guinea pig ileum, rat uterus and rat hindquarters, the potentiation of the 5-hydroxytryptamine contraction on the isolated rat fundus strips is interesting. This may be explained in several ways. Firstly, the thionaphthen compounds, when added to the organ bath containing the isolated rat fundus strip, may release quantities of 5-hydroxytryptamine from /

from the tissue stores which may not be sufficiently large to cause any contraction on their own, but when sufficient quantities of 5-hydroxytryptamine are added, the small quantity that is liberated may be enough to cause potentiation of the effects of the added 5-hydroxytryptamine. Secondly, the thionaphthen compounds may release another stimulant substance (e.g. potassium ions or bradykinin) which may enhance the action of 5-hydroxytryptamine. Thirdly, the presence of the compound may increase the sensitivity of receptors for 5-hydroxytryptamine thus producing an increased response. Fourthly, the thionaphthen compounds, in the presence of 5-hydroxytryptamine, on the rat fundus strip may preferentially occupy the sites of loss or acceptor sites as indicated by Cavallito (1959). Since the acceptor sites are now occupied (by the thionaphthen compounds) the 5-hydroxytryptamine added might conceivably gain preferential access to the hypothetical receptor sites thus producing a positive biological response greater than that produced in the absence of the thionaphthen compounds.

It is known that tryptamine is less potent than 5-hydroxytryptamine in producing contractions of the isolated rat fundus strip. Vane (1959) has reported that the rat fundus contains a monoamine oxidase which, in homogenates, was able to inactivate both tryptamine and 5-hydroxytryptamine to about the same degree.

Vane /

Vane (1959) has shown that monoamine oxidase inhibitors potentiated the action of tryptamine but not the action of 5-hydroxytryptamine on the isolated rat fundus strip and suggested that, due to its polar hydroxyl group, 5-hydroxytryptamine does not enter the cell. If it is supposed that 5-hydroxytryptamine does not enter the cell (Vane, 1959), the potentiating effect of the thionaphthen compounds upon 5-hydroxytryptamine cannot be due to the blockade of the action of monoamine oxidase which is found intracellularly in the rat fundus strip and which, when blocked by iproniazid, does not cause any increased response to the standard dose of 5-hydroxytryptamine. Barlow (1961) has, however, suggested that the amine oxidase of the rat fundus appears to be a mixture of at least two types of enzyme, one of which has a higher affinity for 5-hydroxytryptamine than the other. It may be the case that the thionaphthen compounds produce their potentiating action by virtue of their ability to block this enzyme.

Although the compounds of Group A caused a sharp fall in the blood pressure level of the anaesthetized cat or rat which was not antagonized by adrenaline or noradrenaline, these compounds exhibited antagonism to the vasoconstrictor effects of adrenaline or noradrenaline on isolated tissue preparations. In the isolated rat hindquarters, the loss of neurogenic vascular tone may be responsible /

responsible for the lack of a direct vasodilator effect.

The quaternary ammonium bases (Group B) seemed worthy of pharmacological study in view of the biological properties of gramine methosulphate and cinobufotenine. Moreover, a number of quaternary ammonium compounds of diverse chemical structure including certain thionaphthen derivatives, e.g., the methobromide of the 2-diethylaminoethyl ester of cyclopentyl(2-thienyl) glycolic acid, exert cholinolytic activity on the isolated rabbit ileum (Ludueno and Lands, 1954). This quaternary ester on intravenous injection, also produced toxic symptoms in mice (LD 50, 16 mg./kg.). Ludueno and Lands concluded that quaternary ammonium compounds possessed greater cholinolytic activity than the corresponding tertiary amines. On this basis, considering the fact that the gramine-like isosteres possess atropine-like properties, on the isolated guinea pig ileum, one might expect that the corresponding quaternary salts would possess an enhanced degree of cholinolytic activity. In fact the quaternary salts were found to be less potent in this respect than the corresponding parent compounds.

Gaddum and his associates (1955) observed that in low concentrations, gramine methosulphate made the isolated rat uterus more sensitive to 5-hydroxytryptamine but higher concentrations produced initially powerful contractions of the tissue, after which /

which it became insensitive to 5-hydroxytryptamine. On the other hand, cinobufotenine was observed to mimic the action of 5-hydroxytryptamine on both the isolated rat uterus and the guinea pig ileum but it was less potent than 5-hydroxytryptamine itself. The stimulant response of the guinea pig ileum to cinobufotenine was diminished in the presence of excess of 5-hydroxytryptamine or by hexamethonium. Hence these workers suggested that cinobufotenine acted on both 5-hydroxytryptamine and nicotinic receptors. Cinobufotenine is one half as active as 5-hydroxytryptamine giving a qualitatively similar response on the blood pressure of the anaesthetised dog. In the case of the cat, however, it produced a pressor response in contrast to the depressor effect of 5-hydroxytryptamine (Page and McCubbin, 1953, and Powell, Swanson and Chen, 1955). Although cinobufotenine was found to be twenty times more potent than 5-hydroxytryptamine on the isolated rabbit ileum, very low concentrations of the former antagonized the stimulant response to 5-hydroxytryptamine on the same tissue. In contrast to the stimulant response to 5-hydroxytryptamine, on the cat uterus in situ, cinobufotenine had a relaxant action on the same tissue (Powell, Swanson and Chen, 1955). Thus cinobufotenine has multiple pharmacological actions.

The compounds of Group B comprise a series of gramine-like isosteres /

isosteres in which the nitrogen has been quaternized. The introduction of quaternary nitrogen in the molecule has produced changes in pharmacological activity such as an overall reduction in the antagonistic properties and the emergence of agonistic-stimulant activity when compared with the tertiary amines. The greater ionization of quaternary compounds may cause the formation of strong electrostatic bonds with the receptor's anionic groups and this may enhance the stimulant properties of the quaternary compounds when compared to the corresponding tertiary amines.

Although the quaternization of the gramine-like isosteres (Group B) did not cause much alteration in the biological activity upon the isolated rat uterus, rat fundus strip and with respect to antihistaminic properties on the guinea pig ileum, there was a considerable change in their anti-5-hydroxytryptaminic and anti-muscarinic activity and an increase in the stimulant properties of these compounds on the isolated guinea pig ileum. (Figs. 51, 52, pages 137, 138). Thus 3-(morpholinomethyl) thionaphthen methiodide (IX) did not antagonize the stimulant response to 5-hydroxytryptamine but caused a contraction of the tissue whereas the corresponding tertiary amine, 3-(morpholinomethyl) thionaphthen (III) completely antagonized the stimulant response to 5-hydroxytryptamine and did not induce any stimulant effect. Compounds VIII and /

and X did not antagonize the stimulant response to 5-hydroxytryptamine on the guinea pig ileum. On the other hand, introduction of a piperidinomethyl group in the thionaphthen molecule (compound XI) led to the appearance of antagonism towards 5-hydroxytryptamine, histamine and acetylcholine on the same tissue. Although the quaternary compounds caused contraction of the guinea pig ileum, even large doses of these compounds did not produce any autoinhibiting properties like the tertiary amines (Group A) (Figs. 51, 52, 52a, pages 137 to 139). Compound VIII did not exhibit antimuscarinic activity. Since lysergic acid diethylamide did not antagonize the stimulant response to compounds VIII and X on the isolated guinea pig ileum, it can probably be concluded that these compounds had no 5-hydroxytryptamine-like activity. On the other hand, the stimulant effect due to the quaternary gramine-like isosteres was antagonized by atropine or mepyramine, indicating acetylcholine-like or histamine-like properties. These observations indicate that quaternization tends to destroy the 5-hydroxytryptamine-like and anti-5-hydroxytryptamine properties. In contrast to the tertiary amines, the quaternary compounds exhibited a pressor effect on the blood pressure level of the anaesthetised cat and rat and produced toxic effects in mice.

The compounds of Group C are not strictly the isosteres of 5-hydroxytryptamine nor its homologues since they incorporate an isomerisation of the side chain in the 2-position. Such isomerisation by itself can produce antimetabolites. For example, Kornfeld (1951) observed that isotryptophan in a dilution of 1 to 100 caused complete inhibition of the growth of Streptococcus viridans in the presence of a 1 to 40000 dilution of tryptophan. 5-Hydroxyisotryptophan is claimed to have some "neuro-physiological properties" (Geigy, 1958. British Patent, 804, 237). Other indole compounds which possess the side chain in the 2-position, have been reported. These include 5-hydroxyisotryptamine. It is known that lack of an -OH- group as in tryptamine is accompanied by a strong reduction in biological activity when compared with 5-hydroxytryptamine. Recently Bertaccini and Zamboni (1961) have reported the stimulant activity of certain derivatives of tryptamine and 5-hydroxytryptamine on the isolated rat uterus, the guinea pig ileum and rabbit ear preparations. They observed that shifting the side chain from position 3 to position 2 produced compounds with decreased biological activity. It is interesting to note, however, that the reduction of stimulant activity observed in 5-hydroxyisotryptamine was more conspicuous than that in its analogue lacking the hydroxyl group. Thus Bertaccini and Zamboni (1961) found that isotryptamine appeared to be more potent than /

than 5-hydroxytryptamine.

Among the members of Group C, compound XII is the isostere of 5-hydroxyisotryptamine in which a sulphur atom has been introduced in place of the -NH- group. The structural formulae of these compounds are shown in Fig. 113, page 220. Although Bertaccini and Zamboni (1961) have reported that 2-(2'-aminoethyl)-5-hydroxyindole possesses a slight stimulant action (less than one thousandth part of that of 5-hydroxytryptamine) on the isolated rat uterus, its isostere, compound XII did not exhibit any stimulant properties on this tissue. This again supports the view that the introduction of a sulphur atom in place of an -NH- group decreases the stimulant properties of the drug. Among this group, compound XIII is of interest on account of its anti-5-hydroxytryptaminic properties on the isolated guinea pig ileum. Thus low concentrations of this compound did not antagonize 5-hydroxytryptamine. Higher concentrations of this drug given alone, exhibited autoinhibition and this was unspecific because it not only inhibited its own stimulant action but also made the tissue insensitive to added 5-hydroxytryptamine. An explanation for this type of activity has already been given in pages 228 and 229. It is apparent from the results, that the introduction of the aminoethyl group into the 2-position in the 5-hydroxythionaphthen /

5-hydroxythionaphthen molecule (to produce compound XII) gave a compound which inhibited the stimulant response to 5-hydroxytryptamine on the isolated rat fundus strip whereas the introduction of 2'-aminopropyl function gave a compound which caused a powerful contraction of the same tissue and greatly increased the muscle tone. It is very difficult to explain the mode of action of these compounds on the tissue. The pressor activity of these compounds could be seen both in the anaesthetised and spinal cat, and this may be due to a sympathomimetic action since phentolamine reduced the pressor effects of adrenaline, noradrenaline and also of compound XII (Fig. 86, page 181).

The compounds in the last Group (D) also show the isomeric shift of the side chain into the 2-position and in addition possess a 5-amino function which in the indole series promotes an antagonistic action towards 5-hydroxytryptamine (Woolley and Shaw, 1952a; Shaw and Woolley, 1954; and Erspamer, 1955).

The isosteric shift of the side chain into the 2-position and the introduction of a 5-amino group into the thionaphthen molecule produced compounds possessing very weak antagonistic properties towards 5-hydroxytryptamine, histamine and acetylcholine. These compounds did not antagonize the vasoconstriction produced by 5-hydroxytryptamine, although they incompletely inhibited /

inhibited the response to adrenaline or noradrenaline on the isolated perfused rat hindquarters. Decreased biological activity among analogues of tryptamine has been reported when the side chain was shifted from the 3 to the 2 position (Bertaccini and Zamboni, 1961). The only interesting compound in this group is compound XV which inhibited the stimulant response to 5-hydroxytryptamine on the isolated rat fundus strip, whereas the others potentiated the effect of 5-hydroxytryptamine. An explanation for this potentiation has already been suggested (pages 228 and 229).

It is difficult to account for the antagonism shown by compound XV to the stimulant response to 5-hydroxytryptamine on the isolated rat fundus strip. It is tempting to relate this activity to the morpholinomethyl group in the 2-position since compound III of Group A, which possesses a similar group in the 3-position, potentiated the stimulant response to 5-hydroxytryptamine on the same tissue. The presence, however, of an amino group in the 5-position in compound XV cannot be ignored and, since it has been shown (Bertaccini and Zamboni, 1961) that the introduction of a 5-amino group into the 5-hydroxytryptamine molecule, reduced the stimulant properties of this compound, the 5-amino group may also contribute to the antagonism shown.

From the results of experiments using the more conventional techniques, /

techniques, it is rather difficult to say whether the thionaphthen compounds investigated are 5-hydroxytryptamine-like, histamine-like, acetylcholine-like or adrenaline-like or, whether they are 5-hydroxytryptamine antagonists, anti-histaminic, anti-muscarinic or adrenergic blockers. From the results, it is apparent that replacement of the nitrogen atom of the indole ring system by the larger sulphur atom does not increase the selectivity for the hypothetical 5-hydroxytryptamine receptors. Moreover, shifting the side chain from the 3 to the 2 position and the introduction of an amino function into the 5 position decreased the biological activity of the compounds. Furthermore, the dose ratio values for 5-hydroxytryptamine varied greatly from one experiment to another. Due to this lack of selectivity, and also because the compounds did not form a regular series, no correlation between the chemical structure and biological activity could be obtained. In view of these difficulties and due to the paradoxical effects of these compounds, an attempt was made to investigate the type of antagonism produced by the thionaphthen compounds towards 5-hydroxytryptamine. This approach was based on the work of Van Rossum and Ariëns (1959) which has been described in pages 54 to 56 of this thesis. Although this method of approach does not afford a full explanation of the precise mode of action of the thionaphthen /

thionaphthen compounds investigated, it gives some idea of the type of antagonism exhibited by these compounds towards 5-hydroxy-tryptamine-induced contractions on the isolated rat jejunum.

The results obtained using Ariëns' experimental procedure show that certain compounds (I, VI, XI, XIV, XVI and XVII) possess some agonistic properties and show non-competitive antagonism but the majority of them have only an affinity, exhibiting a purely non-competitive antagonism. The tissues showed a variable response to the thionaphthen compounds investigated. For example, compound V in two different experiments exhibited a purely non-competitive antagonism but in the other experiments, it showed an agonistic action and non-competitive antagonism. Moreover, the shape and slope of the cumulative log. dose-response curves for compound IX could not be explained on the basis of Ariëns' approach (Fig. 108, page 209). The non-competitive antagonism, the variable response of the tissue and the evidence from the results using the classic experimental procedures suggest that the thionaphthen compounds are unselective in their pharmacological properties.

Although there is some indirect evidence for the presence of 5-hydroxytryptamine receptors which are distinct from those of /

of adrenaline, histamine, acetylcholine, bradykinin and oxytocin (Rocha e Silva and his associates, 1953; Meier, Tripod and Wirz, 1957; and Gaddum and Picarelli, 1957), the nature of these has not been pictorially represented as has those for muscarine, morphine or acetylcholine. Due to the non-selectivity of the thionaphthen compounds as shown in the possession of both agonistic and antagonistic properties towards the actions of 5-hydroxytryptamine, acetylcholine, histamine, adrenaline and noradrenaline, and also due to the inability to correlate the structure with biological activity, it is difficult to suggest any hypothetical structure for the 5-hydroxytryptamine receptor or indeed to say anything in support of its existence. The possibility, however, that 5-hydroxytryptamine partly, or in some organs predominantly, acts upon receptors sensitive to the better established neurotransmitter substances cannot be excluded. The fact that many chemically dissimilar molecules antagonize the actions of 5-hydroxytryptamine does not support the assumption that 5-hydroxytryptamine has well-characterised, uniform receptor sites. This means that there may be no specific 5-hydroxytryptamine receptors and if this is true, it is difficult to assign a role to 5-hydroxytryptamine in the function of smooth muscle.

The application of the concept of bioisosterism with respect /

respect to the thionaphthen compounds studied has so far failed to produce compounds of similar biological activity and also indicates that the thionaphthen compounds investigated in this thesis were unselective in their pharmacological actions when compared to the related indole compounds.

SUMMARY

Pages 243 to 245.

S U M M A R Y

In the introductory chapter a review is presented of the literature bearing upon the pharmacology of some biologically active indolic compounds and in particular 5-hydroxytryptamine. There is in addition a short historical account of the discovery and identification of 5-hydroxytryptamine. A discussion of the receptor theory of drug action and its bearing upon the action of 5-hydroxytryptamine and its antagonists is included.

In Chapter II, the materials and the experimental techniques used to investigate the pharmacological properties of a number of thionaphthen derivatives bearing an isosteric relationship to gramine and 5-hydroxytryptamine are described. The results of these experiments are also included. A method based upon that described by Van Rossum and Ariëns (1959) to investigate drug-receptor interaction was also employed, use being made of the isolated rat jejunum as a test preparation.

The compounds investigated were found to be unselective in their pharmacological actions and many of them not only antagonized 5-hydroxytryptamine, histamine and acetylcholine on the isolated guinea pig ileum but at higher concentrations, they themselves exhibited /

exhibited a stimulant action on this tissue. This stimulant response was partly or completely antagonized by lysergic acid diethylamide, mepyramine and atropine. On the isolated rat uterus, all of the compounds investigated antagonized the stimulant response to 5-hydroxytryptamine and did not produce any stimulant action. On the other hand, using the rat fundus strip, many of the compounds potentiated the actions of 5-hydroxytryptamine.

The gramine-like isosteres (Group A) reduced the blood pressure level of the anaesthetized cat or rat whereas quaternary salts of gramine-like isosteres, isosteres of 5-hydroxyisotryptamine and 5-amino-isogramine-like isosteres (Groups B, C and D) caused no change or a rise, in the blood pressure level.

None of the compounds investigated influenced the pressor response to adrenaline or noradrenaline. On the other hand, many of the thionaphthen compounds antagonized the vasoconstriction caused by adrenaline, noradrenaline or 5-hydroxytryptamine on the isolated perfused rat hindquarters.

The compounds of Group B were toxic when injected into mice.

The results obtained using Ariëns' experimental procedure indicated /

indicated that many of the compounds exhibited a purely non-competitive antagonism to 5-hydroxytryptamine and that some showed both agonistic properties and non-competitive antagonism.

From the results (Chapter III) it is apparent that the application of the concept of bioisosterism with respect to the thionaphthen derivatives studied has failed to produce compounds of similar biological activity and also indicates that the thionaphthen compounds investigated in this thesis were unselective in their pharmacological actions when compared to the related indole compounds.

K

APPENDIX I Page 246.

A P P E N D I X I

In Table 5 (page 246a) are given the formulae of the saline solutions used in the experimental work described in this thesis. All the chemicals used were of "Analar" quality and only glass distilled water was used. In some cases, aqueous stock solutions of certain salts were prepared to facilitate the rapid preparation of a saline solution. With the exception of sodium bicarbonate solution, these stock solutions could be used for about two weeks after their preparation. Sodium bicarbonate stock solution was freshly prepared every three or four days. Glucose was added as the solid to each batch of saline solution.

TABLE 5Formulae of Physiological Saline Solutions

Salts (g. per litre)	Tyrode's Solution	Locke's Solution	de Jalon's Solution
Sodium chloride	8.0	9.0	9.0
Potassium chloride	0.2	0.42	0.42
Calcium chloride anhydrous	0.2	0.24	0.06
Sodium hydrogen phosphate dihydrate	0.05	-	-
Sodium bicarbonate	1.0	0.5	0.5
Magnesium chloride	0.1	-	-
Glucose	2.0	1.0	1.0

R E F E R E N C E S .

Pages 247 to 295.

R E F E R E N C E S

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APPENDIX II.

The muscle relaxant actions of certain volatile anaesthetics, tetrahydrofuran, ethylene glycol dimethyl ether and nitrous oxide in the spinal cat.

INTRODUCTION

Pages 290 to 304.

A P P E N D I X I I

The muscle relaxant actions of certain volatile anaesthetics, tetrahydrofuran, ethylene glycol dimethyl ether and nitrous oxide in the spinal cat.

INTRODUCTION

Although the use of diethyl ether as an anaesthetic agent dates from 1842, it was not until 1914 that Auer and Meltzer demonstrated its peripheral depressant action upon neuromuscular transmission. When administered to dogs, diethyl ether decreased the height of the contraction of the gastrocnemius muscle produced by indirect electrical stimulation via the sciatic nerve and it was suggested by these workers that diethyl ether acted on the motor end plate in a manner similar to curare. The peripheral depressant action of ether upon neuromuscular transmission has been confirmed by many workers. Thus Githens and Meltzer (1914a) observed the irritability of the phrenic nerve and the diaphragm to be reduced during diethyl ether anaesthesia and were of the opinion that the cessation of respiration during diethyl ether anaesthesia was partly due to a peripheral action. Poulsen and Secher (1949) reported /

reported that the peripheral action of diethyl ether was located at the motor end plate. Secher (1951c), using the isolated rat phrenic nerve-diaphragm muscle preparation, suggested however that diethyl ether influenced the neuromuscular junction in a definite order of effect, namely, the motor end plate, then the motor nerve and finally the muscle cell. He concluded that in anaesthetic concentrations, diethyl ether acted chiefly on the motor end plate. The mode of action of diethyl ether at the neuromuscular junction, on motor nerve fibres, ganglion and muscle cells and its effect on acetylcholine and anticholinesterases has been studied by many workers using isolated tissue preparations and intact animals, but the precise nature of the mechanism by which ether produces relaxation of skeletal muscle is not fully understood.

Effects of diethyl ether upon the
actions of acetylcholine.

It was observed (Simonart and Simonart, 1934) that contractions of the cat gastrocnemius muscle-sciatic nerve preparation induced by intravenous injection of acetylcholine could no longer be elicited during diethyl ether anaesthesia. In contrast to this, Brown, Dale and Feldberg (1936) found that diethyl ether anaesthesia did not decrease contractions of the gastrocnemius muscle elicited by /

by intra-arterial injection of acetylcholine in spinal cats. Gross and Cullen (1943), in experiments using the gastrocnemius muscle-sciatic nerve preparation of the dog found that diethyl ether was capable of inhibiting the contractions due both to intra-arterial injection of acetylcholine and to indirect stimulation of the muscle via the sciatic nerve. In contrast to this, Ettinger, Brown and Megill (1941) observed that diethyl ether potentiated the stimulant action of acetylcholine on the isolated frog rectus abdominis muscle. Although Torda (1943b) observed that the sensitivity of the isolated frog rectus abdominis muscle to acetylcholine was increased by low concentrations of diethyl ether and chloroform, she also demonstrated that higher concentrations of diethyl ether or chloroform inhibited the stimulant action of acetylcholine or that they themselves exerted a stimulant effect on this preparation.

The influence of diethyl ether on
cholinesterase activity.

The influence of diethyl ether on cholinesterase activity has also been investigated, but once again the results are contradictory. It has been reported (Adriani and Rovenstine, 1941) that diethyl ether anaesthesia does not inhibit the activity of human /

human serum cholinesterase. Miquel (1946) observed that at anaesthetic concentrations, neither diethyl ether nor chloroform inhibited cat serum cholinesterase but in concentrations higher than those found in the blood during deep surgical anaesthesia, both drugs inhibited serum cholinesterase in vitro. Bernheim and Bernheim (1936) showed that the activity of dog and rat brain cholinesterase was inhibited by diethyl ether and by chloroform. Ettinger, Brown and Megill (1941) suggested that the diethyl ether-potentiation of the acetylcholine-stimulation of the isolated frog rectus muscle was due to anticholinesterase activity. Torda (1943a) used a chemical method for estimating the cholinesterase activity of ground cat muscle and found that both diethyl ether and chloroform had anticholinesterase activity. Gazzarrini (1954) has, however, reported that diethyl ether anaesthesia increased the hepatic and serum cholinesterase levels in the rat.

The mechanism of the peripheral action of diethyl ether has been studied on intact animals and isolated tissue preparations by the use of anticholinesterases. Simonart and Simonart (1934), for example, observed that in the cat, eserine potentiated the contraction of the gastrocnemius muscle to injected acetylcholine during diethyl ether anaesthesia. Although Brown and his associates /

associates (1936) found that eserine increased the contraction of the gastrocnemius muscle induced by indirect stimulation via the sciatic nerve, they could not demonstrate this effect during diethyl ether anaesthesia. Gross and Cullen (1943), however, using diethyl ether or cyclopropane-anaesthetised dogs to investigate the effects of neostigmine on the contraction of the gastrocnemius muscle induced by indirect stimulation, found that neostigmine could increase the magnitude of the muscular contraction which the anaesthetics had reduced. Similar effects were observed by Poulsen and Secher (1949) using neostigmine in rabbits. Although both Naess (1950c) and Secher (1951b), confirmed the effect of neostigmine using the flexor digitorum longus muscle of the rabbit and isolated rat phrenic nerve-diaphragm preparations, they also observed that higher concentrations of neostigmine potentiated the neuromuscular block produced by diethyl ether and therefore suggested that diethyl ether and tubocurarine acted by different mechanisms.

The effects of diethyl ether and other
anaesthetics upon nerve fibres and ganglia.

The effects of diethyl ether on nerve fibres and ganglia have also been studied. Forbes, McIntosh and Sefton (1916) recorded the action potentials of motor nerves during diethyl ether anaesthesia. They observed that the action potentials remained unchanged /

unchanged at concentrations of diethyl ether which were deep enough to cause cessation of respiration. On the other hand, Wright (1947) demonstrated that dilute diethyl ether vapour depolarised the peroneal and tibial nerves of cats and rabbits and that the action potential was decreased. He also observed that concentrated diethyl ether vapour produced no depolarisation of the nerves and suggested that diethyl ether could block the conduction not only as a result of depolarisation, but also by acting directly on the conducting mechanism. Heinbecker and Bartley (1940) observed that diethyl ether reduced the amplitude and the area of the action potential of frog nerves and noted also that it blocked nerve conduction. Van Harreveld (1947) reported that diethyl ether caused a uniform depolarisation of the entire neuron in the spinal cord of cats. It has been demonstrated that diethyl ether and chloroform at anaesthetic concentrations, depressed synaptic transmission in the cat (Holaday and Larrabee, 1951; and Larrabee and Posternak, 1952). Using the cat stellate ganglion in situ, Normann and Löfström (1955) demonstrated that diethyl ether and tubocurarine had a depressant effect on ganglionic transmission. Although cyclopropane had a weak depressant action, its effects varied from animal to animal and also from one administration to another in the same animal.

Effects of diethyl ether on striated muscle cells.

Naess (1950d) investigated the changes in the action potential of striated muscle under diethyl ether anaesthesia and found that the amplitude was reduced. The reduction of amplitude was more pronounced at higher frequencies of stimulation.

Effect of diethyl ether and other
volatile anaesthetics on potassium.

It has been observed (Torda, 1944) that diethyl ether at anaesthetic concentrations increased, and in higher concentrations, decreased the responses of striated muscle to potassium. She also reported that both low and high concentrations of chloroform increased the muscle responses to potassium. Lorković (1959) observed that the response of the rectus abdominis muscle of the frog to potassium ions was in two phases, namely the twitch fibre and slow fibre reactions. He reported that diethyl ether depressed the twitch fibre reaction, while the slow fibre reaction of the muscle to potassium was increased.

It has been shown that diethyl ether decreases the serum concentration of potassium in dogs (Gerschman and Marenzi, 1933). On the other hand, Kiersz (1948) observed that during the excitatory stage of diethyl ether anaesthesia, there was an increased /

increased serum concentration of potassium in dogs. This was followed by a decrease during the next hour. Davson and Reiner (1942) have shown, however, that there was an increased potassium content in the cat erythrocytes during diethyl ether anaesthesia. Recently Mir (1962), using potassium-42, has observed that diethyl ether caused a marked increase in the release and a decrease in the uptake of potassium ions by isolated strips of rat diaphragm and isolated frog sartorius muscles. It has also been reported (Mir, 1962) that concentrations of diethyl ether which were sufficient to cause inhibition of neuromuscular transmission in the rat phrenic nerve-diaphragm preparation and in the cat gastrocnemius muscle preparation, were capable of causing an increase in the release of potassium.

Effect of diethyl ether, chloroform, halothane, cyclopropane and nitrous oxide on the neuromuscular block produced by tubocurarine.

With the introduction of curare as an adjunct to anaesthesia (Griffith and Johnson, 1942) a synergism was found to exist between diethyl ether and curare. The nature of the peripheral muscle relaxant action of diethyl ether alone, and together with non-depolarising agents such as tubocurarine, has been studied by a number of workers (Cullen, 1944; Schallek, 1946; Pick and Richards, 1947; Watland, Long, Pittinger and Cullen, 1957; Naess, /

Naess, 1950a,b; and Secher, 1951a,b). Thus Cullen (1944) reported that tubocurarine was more effective as a muscular relaxant in combination with diethyl ether than in combination with other inhalation anaesthetics such as cyclopropane and nitrous oxide. Schallek (1946) investigated the action potential of rat skeletal muscle during diethyl ether anaesthesia and found that diethyl ether potentiated the effect of tubocurarine. Pick and Richards (1947) attributed the potentiating effect of diethyl ether upon curare in mice to a central curare action rather than a peripheral one. From experiments upon diethyl ether-induced neuromuscular block in the rabbit digitorum longus muscle-sciatic nerve preparation, Naess (1950b) found that diethyl ether potentiated the effect of curare. Secher (1951a), using the isolated rat phrenic nerve-diaphragm muscle preparation, suggested that diethyl ether and tubocurarine had additive effects and did not show potentiation. It has been observed that in the rabbit, diethyl ether anaesthesia reduced the height of the contraction of the gastrocnemius muscle induced both by indirect single shocks and tetanic stimulation via the sciatic nerve and it has also been shown that diethyl ether potentiated neuromuscular block produced by tubocurarine (Watland and his associates, 1957).

The mode of the peripheral depressant action of chloroform
on /

on neuromuscular transmission and its synergistic action with tubocurarine has been investigated by many workers but the results are contradictory. In contrast to Githens and Meltzer (1914b) who observed no change in the irritability of motor nerves during chloroform anaesthesia, Naess (1950a), using the flexor digitorum longus muscle-sciatic nerve preparation of the rabbit, reported that deep chloroform anaesthesia reduced the magnitude of muscular contractions induced by indirect stimulation via the sciatic nerve. It has been shown (Torda, 1945b) that chloroform causes a contraction of the isolated frog rectus abdominis muscle. Secher (1951d) found that chloroform initially increased the muscular contraction due to indirect stimulation via the phrenic nerve and then reduced it. Watland and his associates (1957) have reported that in the rabbit, deep chloroform anaesthesia increased the height of the gastrocnemius muscle contraction. It has been shown by many workers that chloroform increased neuromuscular block produced by tubocurarine (Naess, 1950a; Secher, 1951d; and Watland and his associates, 1957). On the other hand, Lang, Kimura and Unna (1951) could not demonstrate any synergism between chloroform and tubocurarine.

Few investigations have been made upon the effects of cyclopropane, halothane and nitrous oxide on neuromuscular transmission or on their effects on the neuromuscular block produced by tubocurarine. /

tubocurarine. It is known that cyclopropane increases the magnitude of the muscular contraction in response to indirect single stimulation (Watland and his associates, 1957) and also that it potentiates the action of tubocurarine on neuromuscular transmission. It has been shown that a combination of tubocurarine with cyclopropane increased the paralysis and the death rate in mice over that observed with cyclopropane alone (Lang and his associates, 1951). Although halothane showed no significant effect on neuromuscular transmission, it potentiated the action of tubocurarine (Watland and his associates, 1957; and Burn, Epstein, Feigan and Paton, 1957). Nitrous oxide did not have any demonstrable effect on neuromuscular transmission and did not potentiate the action of tubocurarine (Naess, 1950a).

Diethyl ether and some related
compounds - anaesthetic properties.

Diethyl ether has been in clinical use for over a century and is considered by the majority of anaesthetists to be the safest of the volatile anaesthetic agents. Other volatile aliphatic ethers and certain halogenated hydrocarbons have also been used and their anaesthetic properties have inevitably been compared with those of diethyl ether (Krantz, Carr, Musser and Sauerwald, 1947; Sadove, Wyant and Cletcher, 1955; Krantz, Carr, Lu and Bell, 1953;

Dundee, Linde and Dripps, 1957; Dundee and Dripps, 1957; and Raventós, 1956). Table 3, page 302, shows the structural formulae, the physical constants and compares the anaesthetic properties of certain volatile ethers and some halogenated hydrocarbons.

It has been reported that when tested in various species of animals, n-propyl methyl ether and ethyl vinyl ether were more potent in their anaesthetic properties than the structurally related diethyl ether (Krantz, Evans, Carr and Kibler, 1946; and Krantz, Carr, Musser and Sauerwald, 1947). The margin of safety of ethyl vinyl ether was found to be slightly greater than that of diethyl ether (Krantz and his associates, 1947). Ethyl vinyl ether has been used clinically and the anaesthetic properties of this compound have been compared with diethyl ether and divinyl ether (Sadove, Wyant and Cletcher, 1955) and it was observed that although the induction of anaesthesia and the recovery were more rapid with divinyl ether and ethyl vinyl ether than with diethyl ether, the muscular relaxation was poor.

Halogenated compounds.

In the past few years there has arisen a considerable interest in the use of volatile fluorinated hydrocarbons for anaesthetic purposes. One of the more important of these is trifluoroethyl vinyl /

TABLE 3 Page 302

TABLE 3.

A comparison of the anaesthetic properties of certain volatile aliphatic ethers and halogenated hydrocarbons

	ETHERS					HALOGENATED HYDROCARBONS		
	Diethyl	Methyl-n-propyl	Ethylvinyl	Divinyl	Trifluoroethylvinyl	Trichloroethylene	Halothane	Chloroform
FORMULA	$\text{CH}_3\text{CH}_2\text{OCH}_2\text{CH}_3$	$\text{CH}_3\text{OCH}_2\text{CH}_2\text{CH}_3$	$\text{CH}_3\text{CH}_2\text{OCH}=\text{CH}_2$	$\text{CH}_2=\text{CHOCH}=\text{CH}_2$	$\text{CF}_3\text{CH}_2\text{-O-CH}=\text{CH}_2$	$\text{CHCl}=\text{CCl}_2$	CF_3CHClBr	CHCl_3
Molecular weight	72.12	74.0	72.0	70.09	126.0	131.4	197.39	119.39
Boiling point *	34.6	39.0	35.8	28.4	42.7	87.0	50.2	61.26
Specific gravity *	0.71	0.73	0.76	0.77	1.13	1.47	1.86	1.49
Oil/water solubility *	3.2	10.0	45.0	45.0	91.0	34.4	330.0	100.0
Concentrations for surgical anaesthesia. * Inhaled gas (Vol/100 ml)	3 to 10	-	4	4 to 12	3 to 8	1 to 5	1 to 3	1 to 2
Concentrations for surgical anaesthesia. Blood (mg./100 ml.) *	50 to 150	-	about 25	30 to 40	10 to 40	6 to 12	7 to 12	25 to 70
Approx. muscular relaxant activity in terms of diethyl ether.		poor	poor	moderate or equivalent potent.	poor	poor	equivalent potent	equivalent potent

Cont'd. /

TABLE 3 (Cont'd.).

Approximate anaesthetic potency in terms of diethyl ether		more potent	more potent	more potent	less potent	less potent	more potent	more potent
Approximate speed of induction in terms of diethyl ether		more rapid	more rapid	more rapid	less rapid	less rapid	more rapid	more rapid

* Data after Dundee (1958).

Other data was obtained from various sources including - Krantz, Carr, Musser and Sauerwald (1947)

Sadove, Wyant and Cletcher (1955)

Sadove, Balagot and Linde (1956)

Dundee, Linde and Dripps (1957)

Atkinson (1960)

Bamforth, Siebecker, Steinham and Orth (1960)

Drill (1958) and

Goodman and Gillman (1956).

vinyl ether ("Fluorenar").

Trifluoroethyl vinyl ether is approximately equipotent with diethyl ether in dogs and monkeys (Krantz, Carr and Bell, 1953). It has been used clinically for various types of surgical operations but it was found to possess poor muscle relaxant activity, a poor margin of safety and a respiratory depressant action (Dundee, Linde and Dripps, 1957; Park, Truitt and Krantz, 1957; and Dundee and Dripps, 1957).

The anaesthetic effects of trichloroethylene, another halogenated hydrocarbon, have been reviewed by Atkinson (1960) who concluded that this substance was less potent than diethyl ether, possessed poorer muscular relaxant properties and also possessed a respiratory depressant action. This compound is notable, however, for its potent analgesic action and is used in midwifery for this purpose.

Raventós (1956) studied a series of fluorinated hydrocarbons and observed that in experimental animals, 1,1,1-trifluoro-2-bromo-2-chloroethane (halothane, fluothane) was a more potent anaesthetic than diethyl ether or chloroform. Although chloroform is a more potent anaesthetic than ether, there has been considerable objection to its use because of its alleged cardiotoxic action. Chloroform and halothane have been compared directly by means of

a blind technique. It has been found that the anaesthetic potency, changes in the blood pressure, pulse rate and respiration and the incidence of complications during anaesthesia were similar with respect to both drugs (Bamforth, Siebecker, Steinhaws and Orth, 1960).

The studies described in this section of the thesis were carried out to investigate the effects of a group of volatile aliphatic ethers, certain volatile halogenated hydrocarbons, nitrous oxide and cyclopropane on the contractions of the gastrocnemius muscle induced by indirect stimulation via the sciatic nerve in the spinal cat. An investigation has also been made of the effects of these compounds on the neuromuscular block induced by tubocurarine in the spinal cat.

MATERIALS

Page, 305.

M A T E R I A L S

The following drugs were used in the investigation which is described in this section of the thesis:-

(+)- Tubocurarine which is described as tubocurarine.

Diethyl ether	Allyl ethyl ether
Methyl-n-propyl ether	Ethylene glycol dimethyl ether
Di-n-propyl ether	Tetrahydrofuran
Di-isopropyl ether	Trichloroethylene
N-butyl methyl ether	Halothane
N-butyl ethyl ether	Chloroform
Ethyl vinyl ether	Cyclopropane
Divinyl ether	Nitrous oxide

METHOD

Pages 306 to 315

M E T H O D

Cats of either sex, weighing between 2.0 and 4.0 kg., were anaesthetised by means of intra-peritoneal injection of thiopentone sodium. Fresh solutions of this drug (50 mg. per ml.) were employed for each experiment and a dose of 50 mg./kg. was usually adequate for the production of surgical anaesthesia in 10 to 20 minutes. Simultaneously, 1 mg./kg. of atropine sulphate solution in water for injection was administered to minimise any respiratory distress caused by excess secretion of mucus in the respiratory passages.

The anaesthetised cat was laid on its back upon a warmed operating table and the legs and head secured by means of strings. The fur and the skin covering the region between the upper part of the sternum and the apex of the mandible were removed. The fascia covering the trachea was divided in the mid-line and the trachea freed from the surrounding tissue by blunt dissection. A blunt aneurysm needle carrying a piece of strong thread was passed between the muscles of the neck and around the trachea. By means of a scalpel, a transverse incision was made in the trachea and holding the cut edge by means of forceps, a tracheal cannula was inserted and tied into place. The tracheal cannula could be connected /

connected by rubber tubing to an "Ideal" respiration pump providing artificial respiration, while the amount of air entering and leaving the cannula could be controlled by means of an adjustable sleeve.

The common carotid artery on each side of the trachea was then dissected, freed from accompanying vagosympathetic fibres and other tissues, and tied as near to the head as possible.

The cat was then turned over on to its ventral side, the legs again secured and the neck supported on a wooden block in which there was a narrow groove to keep the tracheal cannula in position. The head of the animal was left free and unsecured. The fur and the skin covering the back of the neck were removed and, by means of a scalpel, a mid-line incision was made extending from the position of the foramen magnum to the level of the third or fourth cervical vertebra. Using retractors and dividing the muscles covering the vertebral column by means of blunt dissection, the first, second and third cervical vertebrae were freed and exposed. A strong linen thread was passed around the vertebral column at a point between the second and third cervical vertebrae and tied very firmly. This compressed the vertebral arteries and reduced subsequent haemorrhage. The vertebral column was then transected by means of bone forceps at the position of the second cervical /

cervical vertebra. Due to the previous compression of the vertebral arteries, bleeding at this stage of the experiment was not excessive and was further reduced by the application at the point of transection of the vertebral column, of cotton wool swabs soaked in hot normal saline. A probe 3 to 4 mm. in diameter was inserted through the foramen magnum into the skull at the point of transection of the vertebral column and the brain destroyed. The probe was withdrawn and plasticine used to plug the foramen magnum to prevent further loss of blood. The skin over the back of the neck was sewn by means of strong thread and the animal again turned on its back.

The skin of the anterolateral part of the neck was removed and the external jugular vein exposed on one side and freed from fascia and surrounding tissue. A cotton thread was tied around the cephalic end and a bulldog clip was put on to the cardiac aspect. A small transverse incision was made in the dilated vessel by means of sharp-pointed iris scissors. A vein cannula was filled with a solution of heparin and the pointed end inserted into the vein towards the heart and secured by means of cotton thread tied around the vein. The cannula was connected by rubber tubing to a 50 ml. burette containing normal saline solution, taking care to /

to remove all air bubbles in the system. The bulldog clip was removed and a small volume of normal saline from the burette allowed to flow through the vein into the circulation to ensure free flow. A bulldog clip was now placed on the cardiac aspect of one of the ligated common carotid arteries and a thread passed around the artery. A small transverse incision was made in the artery by means of sharp-pointed iris scissors and the pointed end of a polythene cannula filled with a solution of heparin was inserted towards the heart and secured. The cannula was connected by rubber tubing to a mercury manometer and the system, freed from air, filled with heparinized normal saline. The pressure in the manometer was raised to approximately 80 mm. of mercury, the bulldog clip removed and the system allowed to equilibrate. A writing flag on one arm of the manometer recorded the blood pressure on a moving smoked surface.

One leg was then prepared for indirect stimulation of the gastrocnemius muscle via the sciatic nerve. The leg was held with its long axis perpendicular to the operating table and fixed rigidly by means of two clamps (Fig. 114, page 309a) one at the knee joint and the other at the ankle. The skin over the Achilles tendon was cut away and the tendon freed from surrounding tissue. A strong linen thread was tied around the tendon near to its insertion. /

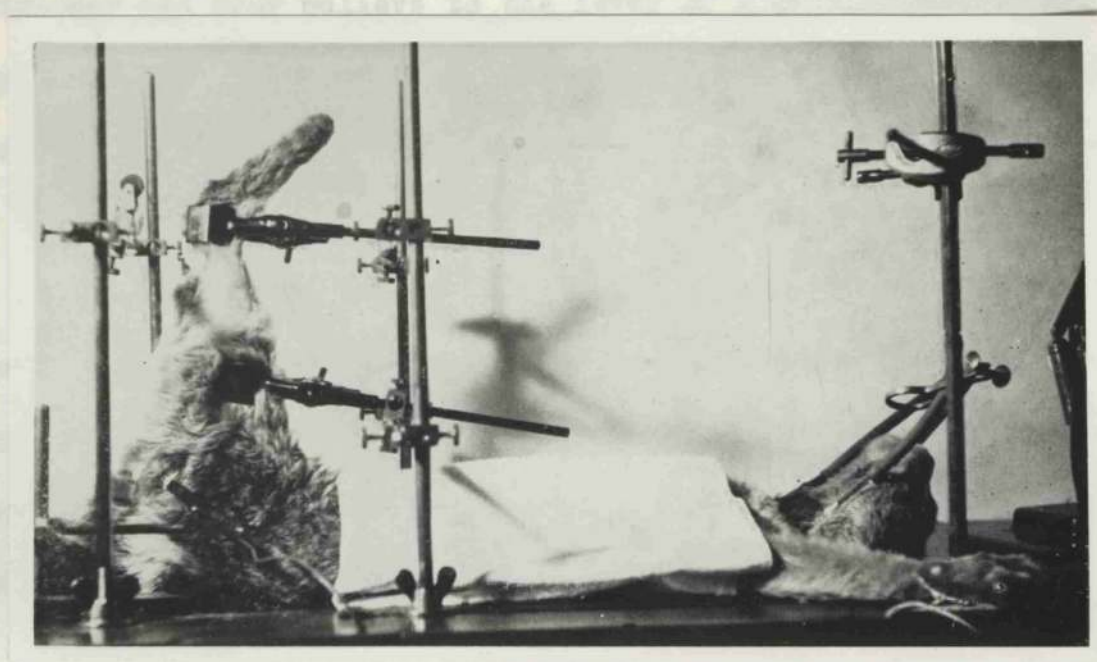


Fig. 114.

Experimental "set up" of a spinal cat for the recording of the contractions of the gastrocnemius muscle induced by indirect stimulation via the sciatic nerve.

insertion. Another longer thread was sewn through the tendon proximal to the first and tied firmly. The tendon was then severed between the threads. The long thread attached to the Achilles tendon was led over pulleys to the lever of a Brown-Schuster myograph, the writing point of which was adjusted to record the contractions on a moving smoked surface. The fur and the skin covering the dorsal aspect of the lower part of the thigh were cut away and the hamstring muscles separated by blunt dissection exposing the sciatic nerve. A pair of shielded electrodes was fixed in place around the nerve and stimulation by square impulses carried out, using a Dobbie McInnes stimulator at a frequency of 6 to 8 per minute, at 10 to 25 volts, the pulse width being 2.0 to 3.0 msec. The tension placed upon the muscle varied between 0.2 and 0.3 kg. but in any one experiment the tension, voltage, frequency and pulse width remained constant throughout.

Solutions of drugs in normal saline were injected by means of a 1 ml. tuberculin syringe into a rubber tube connecting the burette and the jugular vein cannula. 3 ml. of saline were run in from the burette after each injection of drug, to ensure that all the drug, properly mixed in normal saline, had entered the circulation.

The apparatus used to deliver certain standard vapour mixtures of volatile liquids in oxygen was essentially that of Kochman (1912) as modified by Raventós (1956). A diagrammatic sketch, not drawn to scale, is shown in Fig. 115, page 311a.

The apparatus consisted essentially of a vaporisation chamber (1) composed of a large boiling tube $1\frac{1}{2}$ " diameter by 9" long maintained at the boiling point of the volatile liquid under test by means of a water bath. The open end of the boiling tube was firmly sealed by a rubber stopper through which passed two glass tubes $\frac{3}{8}$ " diameter and one capillary as shown in Fig. 115, page 311a. One of the glass tubes was surrounded by a glass jacket enclosing a vacuum and reached almost to the bottom of the chamber. This tube allowed oxygen at a predetermined, constant, metered rate to be introduced into the chamber. The volatile liquid under test was forced into the chamber at a steady controlled rate through the capillary tube (2), it now fell on the glass jacket where it was vaporised and the resulting vapour, together with the oxygen passed out of the chamber via the second glass tube. The volatile liquid itself was contained in a cylindrical glass vessel (3) connected by means of a capillary tube which protruded approximately $\frac{1}{2}$ " into the upper part of the vessel as shown in Fig. 115, page 311a. Mercury was forced /

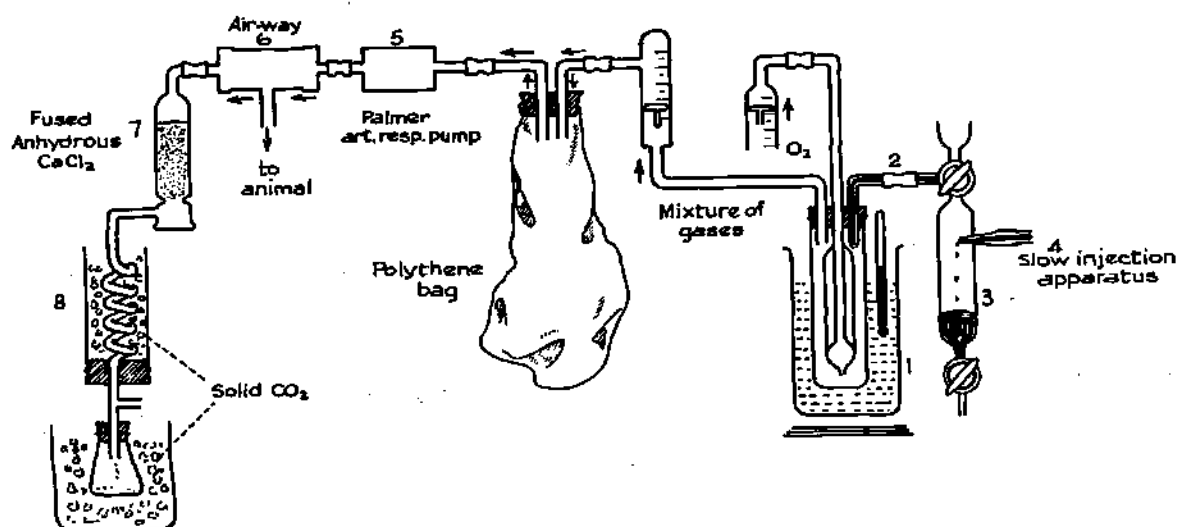


Fig. 115

Diagram (not to scale) of apparatus used to administer known concentrations of anaesthetic vapours, in oxygen, to the spinal cat.

See description in the text, pages 311 to 314 for key to the numbered pieces of apparatus.

forced into the vessel through the capillary tube at a constant, predetermined rate by means of a Palmer's slow injection apparatus (4) and displaced an equal volume of volatile liquid into the vaporization chamber. The vessel could be emptied by means of a stopcock at the bottom.

The outlet of the vaporization chamber was connected through a rotometer, which controlled the rate of flow of mixture, to a polythene bag of approximately 15 litres capacity. The mouth of the bag was secured around a large rubber stopper by means of a jubilee clip through which passed an inlet and an outlet tube. The vapour mixture was stored in this bag. Both the outlet and inlet could be closed to the outside, sealing off the bag and preventing the escape of vapour to the atmosphere. The outlet tube from the polythene bag was connected by means of rubber tubing to the inlet of an "Ideal" respiration pump (5). By this means, the animal under artificial respiration was made to breathe the gaseous mixture of known composition and when the pump was disconnected from the system, the animal breathed atmospheric air.

When the vapour mixture under test was being administered to the animal, the vapour contents of the bag were pumped through the /

the inlet valve of a double respiration valve chamber (Airway) (6) to the tracheal cannula, the sleeve of which was completely closed and the exhaled vapour passed through the outlet valve of the airway to a drying column (7) of anhydrous calcium chloride as shown in the Fig. 115, page 311a. From this, the dry vapours passed through a glass coil (8) surrounded by solid carbon dioxide where the vapour condensed and the liquid was recovered.

PROCEDURE

A standard amount of tubocurarine in water for injection was injected into the animal via the external jugular vein, the sciatic nerve was stimulated electrically and contractions of the gastrocnemius muscle elicited, as previously described. When the response of the muscle had returned to normal, the process was repeated until two consecutive doses of tubocurarine produced approximately the same quantitative effect. The dose chosen was that which would produce 20 to 50% muscular relaxation. This took several hours to accomplish. When the response to tubocurarine had thus been stabilized, the gaseous mixture was administered in the following manner. The bag was filled with the gaseous mixture under test and the outlet tube from it connected to the "Ideal" respiration pump. The rubber tubing connecting the pump to the tracheal /

tracheal cannula was disconnected from the latter and reconnected to the inlet valve of a double respiration valve chamber (Airway) (6). The sleeve of the cannula was then closed. The tracheal cannula was meanwhile connected to the middle arm of the airway (6) as shown in Fig. 115, page 311a while the outlet valve of the airway was connected to the drying tower. This procedure was carried out as quickly as possible to prevent respiratory collapse due to the interruption of the flow of gas to the animal.

The gas mixture was administered for about ten minutes and then the same dose of tubocurarine again given. The administration of the mixture was continued for a further five minutes. The pump was then disconnected from the polythene bag, the airway connection removed from the tracheal cannula and the rubber tubing joining the pump to the airway disconnected from the latter and re-connected to the tracheal cannula. The sleeve of the tracheal cannula was then adjusted to permit a sufficient respiratory excursion to take place.

The cycle of events could then be repeated using different concentrations of vapour. In each case, however, it was essential to stabilise the response of the animal to tubocurarine before administering the mixture.

The /

The concentration of the vapour mixture was calculated from the gas laws. The volume of vapour produced from a certain volume of volatile liquid was calculated using the following formula:-

$$\frac{V \times \text{S.G.}}{\text{M.W.}} \times 22.4 = \text{Litre vapour/min. at S.T.P.}$$

where V is the volume of a liquid vaporized/minute, S.G. the specific gravity and M. W. the molecular weight of the compound.

Let the slow injection apparatus deliver 0.192 ml. of diethyl ether per minute, and let the percentage of diethyl ether vapour required be 5 in oxygen.

Specimen Calculation:-

Diethyl ether

S. G. = 0.713, M. W. = 74.12, V. = 0.192 ml./min.
Room temperature = 27°C.

Substituting in the above formula:-

$$\frac{0.192 \times 0.713}{74.12} \times \frac{22.4}{1} \times \frac{273}{300} \times \frac{747.5}{760} \times \frac{1000}{1}$$

= 37.03 ml. of diethyl vapour produced in one minute.

Now, let x be the volume of oxygen required per minute,

$$\therefore \frac{95\%}{5\%} = \frac{x}{37.03}$$

or, x = 703.57 ml. of oxygen required per minute.

RESULTS

Pages 316 to 327

R E S U L T S

The results of a series of experiments with a group of volatile ethers, some volatile halogenated hydrocarbons, cyclopropane and nitrous oxide upon the height of contraction of the gastrocnemius muscle of the spinal cat in response to indirect stimulation via the sciatic nerve and the effects of these compounds on the neuromuscular block induced by tubocurarine on the same preparation are shown in Table 4, page 317. Some typical experimental records are shown in Figs. 116 to 122, pages 318 to 324.

On the basis of their chemical structure, the compounds investigated have been divided into five groups. The results are presented under two main headings; namely, the effect of the compounds studied on the contractions of the gastrocnemius muscle in response to indirect stimulation via the sciatic nerve and their actions on the neuromuscular block produced by tubocurarine.

With the exception of the halogenated hydrocarbons, cyclopropane, nitrous oxide, tetrahydrofuran and ethylene glycol dimethyl ether, all the compounds investigated caused muscle relaxation. This varied in intensity from animal to animal.

I. /

TABLE 4 Page 317

The effects of a group of volatile aliphatic ethers, certain volatile halogenated hydrocarbons, nitrous oxide, and cyclopropane on neuromuscular transmission in the spinal cat with studies on the neuromuscular block produced by tubocurarine in spinal cats.

Drugs and Formulae	Mol. Wt.	Specific gravity	Boiling point	Experiment	Percentage in oxygen	Effect on the magnitude of contraction of the gastrocnemius muscle induced by indirect stimulation <u>via</u> sciatic nerve	Effect on neuro-muscular block produced by tubocurarine
1. Diethyl ether $\text{CH}_3\text{CH}_2\text{OCH}_2\text{CH}_3$	74.12	0.713	34.6	A B C D E	1.5 3.0 6.0 15.0 20.0	no change " " reduction "	potentiation " " " "
2. Methyl-n-propyl ether (neothyl) $\text{CH}_3\text{OCH}_2\text{CH}_2\text{CH}_3$	74.0	0.7	39.0	A B	1.25 2.5 5.0 20.0	slight reduction " no change slight reduction	potentiation " " "
3. Di-n-propyl ether $(\text{CH}_3\text{CH}_2\text{CH}_2)_2\text{O}$	102.0	0.736	90.5	A B	1.0 1.5 3.0 6.0	no change reduction " "	potentiation " " "
4. Di-isopropyl ether $(\text{CH}_3)_2\text{CHOCH}(\text{CH}_3)_2$	102.17	0.735	68.0	A B	1.0 5.0 5.0	reduction complete abolition " "	potentiation " "
5. N-butyl methyl ether $\text{CH}_3\text{O}(\text{CH}_2)_3\text{CH}_3$	88.15	0.764	70.3	A B	5.0 10.0 5.0 20.0	no change " reduction "	potentiation " " "
6. N-butyl ethyl ether	102.0	0.752	92.0	A B	1.0 5.0 5.0	reduction " no change	potentiation " " (cont'd)

TABLE 4 (Cont'd.).

15.	cyclopropane $\begin{array}{c} \text{CH}_2 \\ \\ \text{CH}_2 - \text{CH}_2 \end{array}$	42.08	0.720	- 34	A B	5 10	no change "	potentiation "
16.	Nitrous oxide	44	1.226 at -89°C.	-88.49	A B	80 100 100	no change " "	slight potent'ion " "

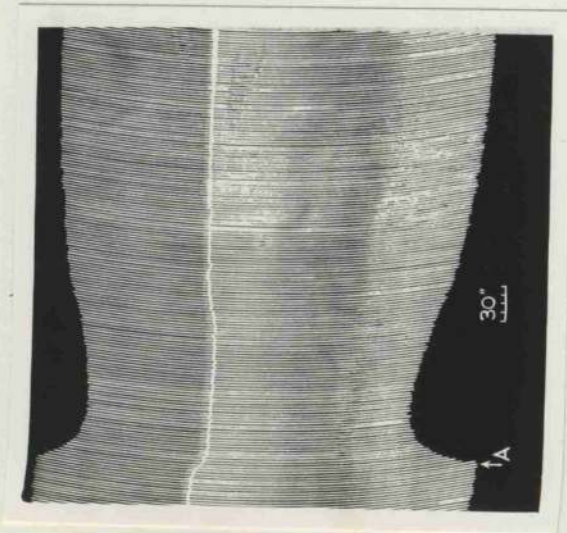


Fig. 116 (a)

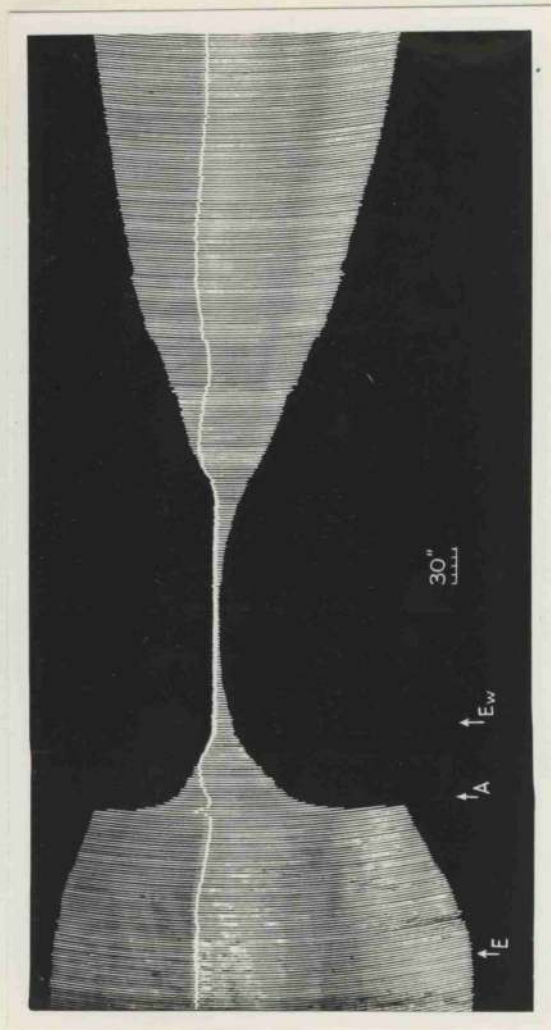


Fig. 116 (b)

Contractions of the gastrocnemius muscle of the spinal cat induced by indirect stimulation via the sciatic nerve. Contractions downwards.

- (a) At A, 75 μ g. per kg. tubocurarine.
- (b) At E, 15% v/v of diethyl ether vapour in oxygen.
- At A, 75 μ g. per kg. tubocurarine.
- At Ew, diethyl ether vapour in oxygen withdrawn.

Tubocurarine administered intravenously.

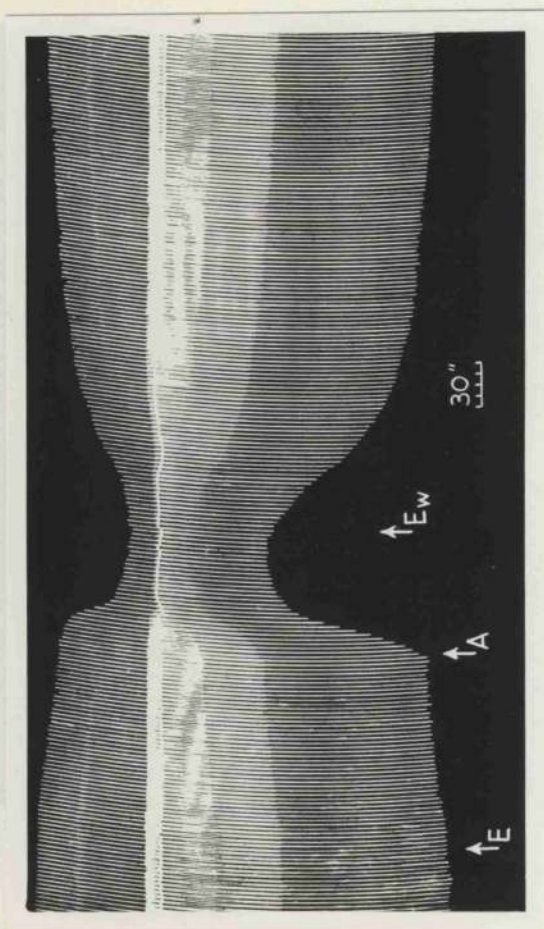


Fig. 117 (a)

Fig. 117 (b)

Contractions of the gastrocnemius muscle of the spinal cat induced by indirect stimulation via the sciatic nerve. Contractions downwards.

(a) At A, 50 μ g. per kg. tubocurarine.

(b) At E, 2.5% v/v of methyl-n-propyl ether vapour in oxygen.

At A, 50 μ g. per kg. tubocurarine.

At Ew, methyl-n-propyl ether vapour in oxygen withdrawn.

Tubocurarine administered intravenously.

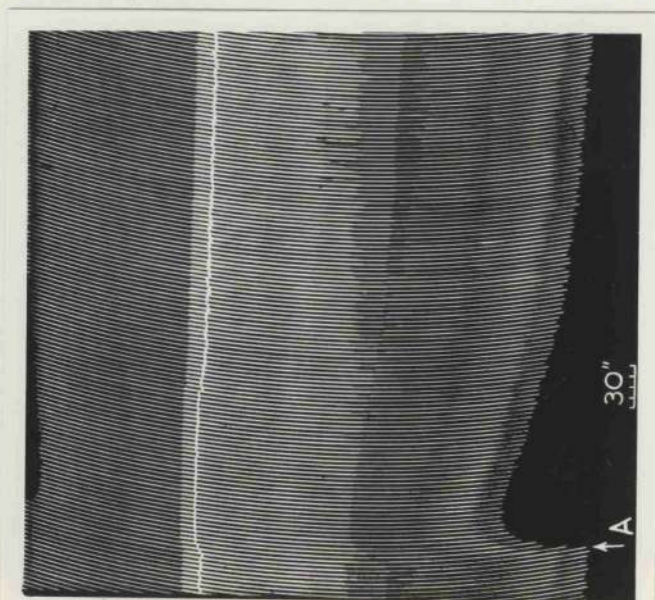


Fig. 118 (a)

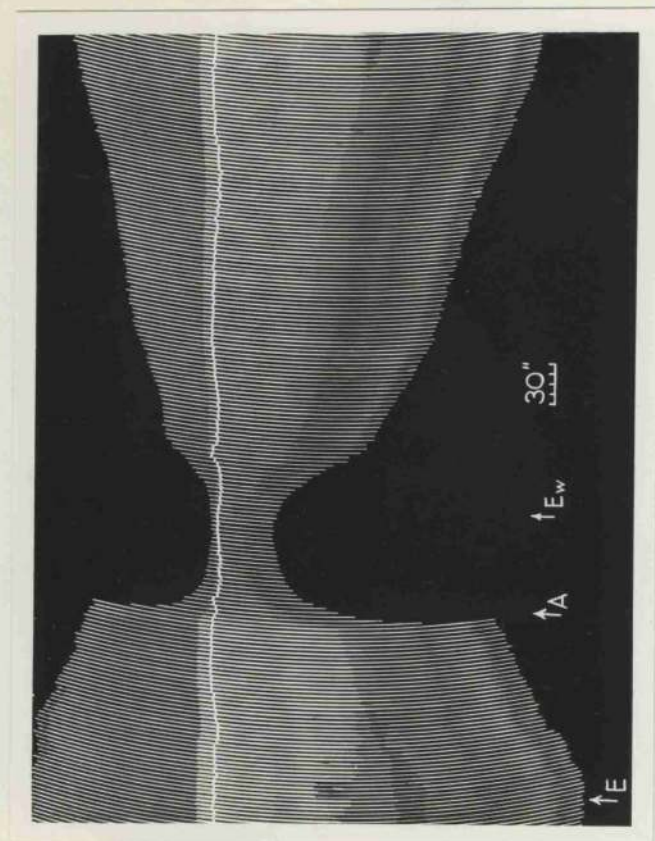


Fig. 118 (b)

Contractions of the gastrocnemius muscle of the spinal cat induced by indirect stimulation via the sciatic nerve. Contractions downwards.

- (a) At A, 100 μ g. per kg. tubocurarine.
- (b) At E, 3% v/v of di-n-propyl ether vapour in oxygen.
At A, 100 μ g. per kg. tubocurarine.
At Ew, di-n-propyl ether vapour in oxygen withdrawn.

Tubocurarine administered intravenously.

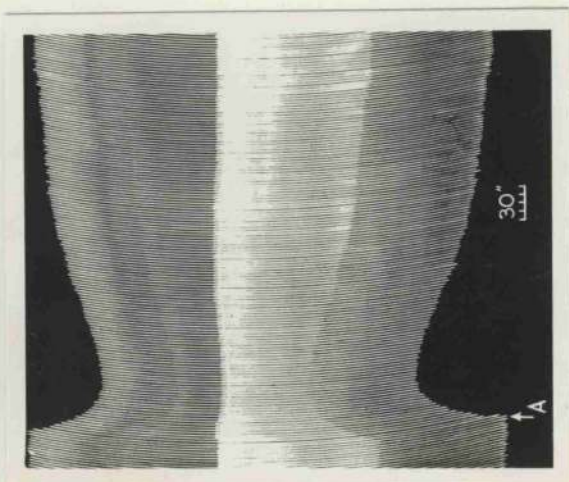


Fig. 119 (a)

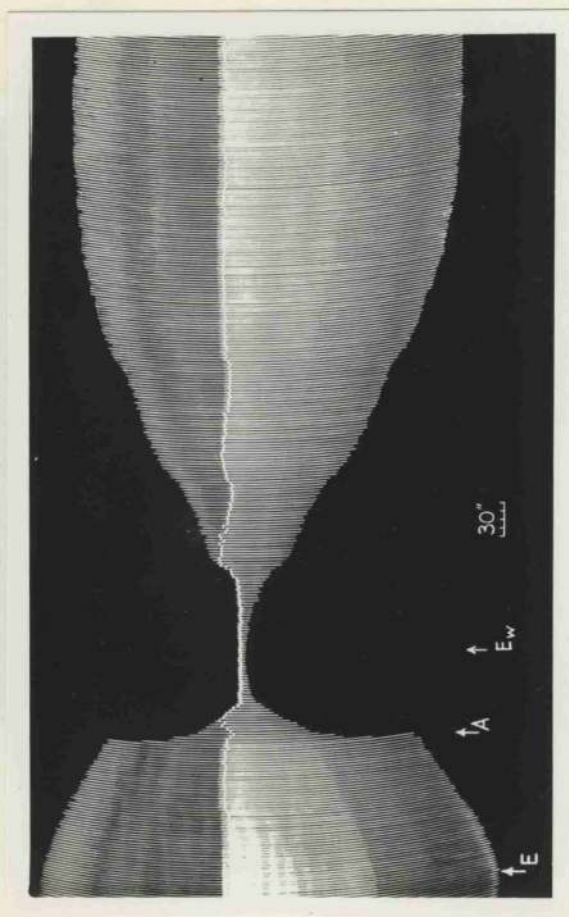


Fig. 119 (b)

Contractions of the gastrocnemius muscle of the spinal cat induced by indirect stimulation via the sciatic nerve. Contractions downwards.

- (a) At A, 40 μ g. per kg. tubocurarine.
- (b) At E, 1.5% v/v of di-isopropyl ether vapour in oxygen.
At A, 40 μ g. per kg. tubocurarine.
At Ew, di-isopropyl ether vapour in oxygen withdrawn.

Tubocurarine administered intravenously.

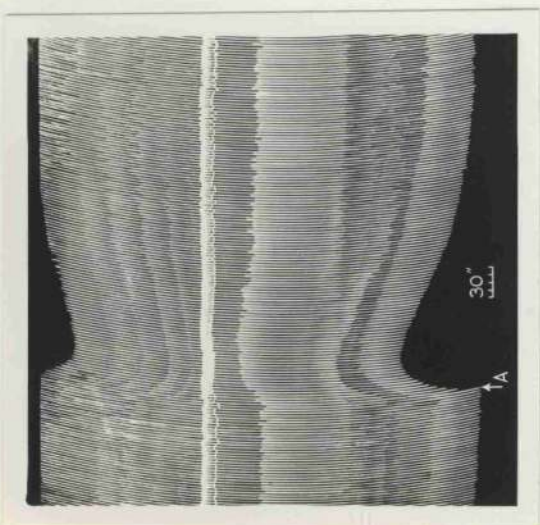


Fig. 120(a)

Contractions of the gastrocnemius muscle of the spinal cat induced by indirect stimulation via the sciatic nerve.

(a) At A, 100 μ g. per kg. tubocurarine.

(b) At E, 2.5% v/v of ethyl vinyl ether vapour in oxygen.

At A, 100 μ g. per kg. tubocurarine.

At Ew, ethyl vinyl ether vapour in oxygen withdrawn.

Tubocurarine administered intravenously.

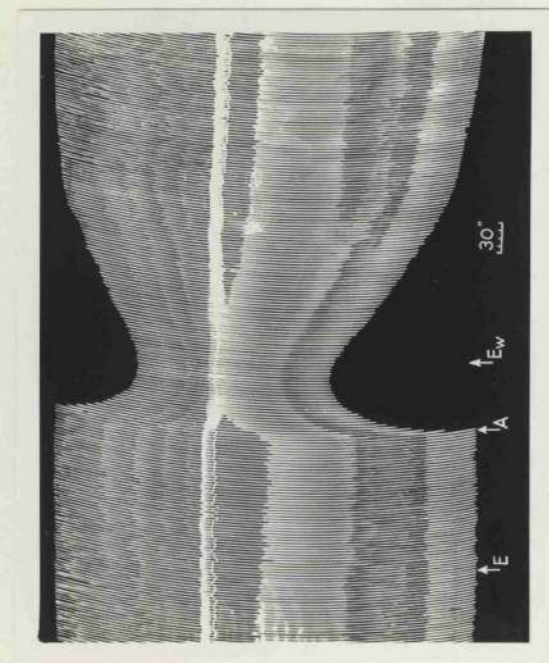


Fig. 120(b)

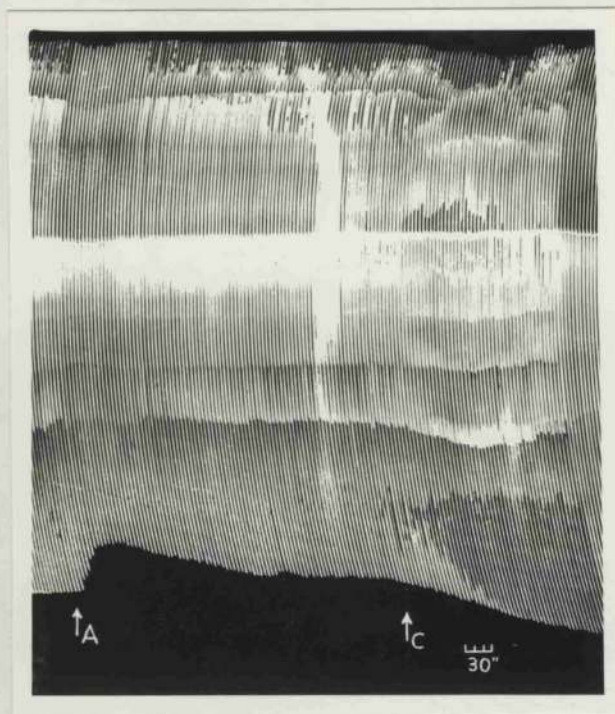


Fig. 121.

Contraction of the gastrocnemius muscle of the spinal cat induced by indirect stimulation via the sciatic nerve.

Contractions downwards.

At A, 50 μ g. per kg. tubocurarine.

At C, 4% v/v chloroform vapour in oxygen.

Tubocurarine administered intravenously.

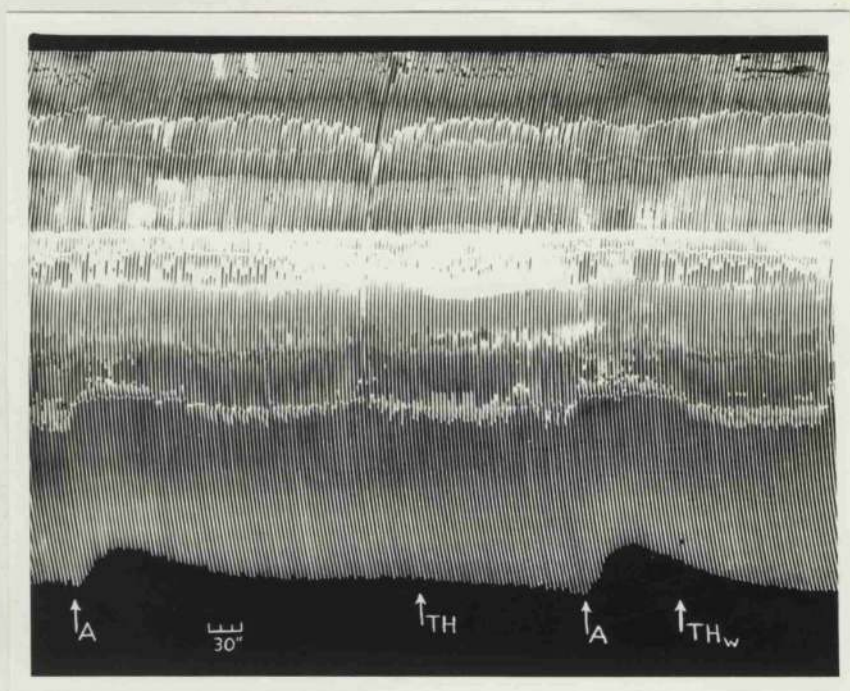


Fig. 122.

Contraction of the gastrocnemius muscle of the spinal cat induced by indirect stimulation via the sciatic nerve.

Contractions downwards.

At A, 75 μ g. per kg. tubocurarine.

At TH, 10% v/v tetrahydrofuran vapour in oxygen.

At THw, tetrahydrofuran vapour in oxygen withdrawn.

Tubocurarine administered intravenously.

I. Effect on the magnitude of the contraction of the gastrocnemius muscle.

(a) Volatile, aliphatic, saturated mono ethers.

Among the volatile, aliphatic, saturated mono ethers studied, di-isopropyl ether was the most potent and at concentrations of 5% v/v in oxygen it caused a complete abolition of the contraction of the gastrocnemius muscle induced by indirect stimulation via the sciatic nerve. N-butyl ethyl ether at concentrations ranging from 5 to 40% v/v in oxygen in one experiment caused no reduction in the height of muscular contraction, while in another two experiments, a concentration of 5% v/v in oxygen caused a reduction. A similar variable effect was observed with n-butyl methyl ether and methyl-n-propyl ether. Diethyl ether up to 6% v/v in oxygen did not affect the height of the muscular contraction but at concentrations between 15 and 20% v/v in oxygen, it caused a reduction in twitch height.

(b) Volatile, aliphatic, unsaturated mono ethers.

All the volatile, aliphatic, unsaturated mono ethers studied, at concentrations ranging from 2.5 to 20% v/v in oxygen caused little or no reduction in the height of contraction of the gastrocnemius muscle in response to indirect stimulation via the sciatic nerve /

nerve (Fig. 120, page 322).

(c) Halogenated hydrocarbons.

In all cases, the halogenated, volatile hydrocarbons at concentrations ranging from 1.5 to 10% v/v in oxygen slightly increased the magnitude of contraction of the gastrocnemius muscle induced by indirect stimulation via the sciatic nerve. Fig. 121, page 323, represents an experimental record of this type using chloroform at a concentration of 4% v/v in oxygen.

(d) Tetrahydrofuran and ethylene glycol dimethyl ether.

At concentrations ranging from 5 to 20% v/v in oxygen, tetrahydrofuran and ethylene glycol dimethyl ether did not alter the height of contraction of the gastrocnemius muscle induced by indirect stimulation via the sciatic nerve. An experimental record of a typical experiment using tetrahydrofuran is shown in Fig. 122, page 324.

(e) Cyclopropane and nitrous oxide

Cyclopropane (at 10% v/v in oxygen) and nitrous oxide (at 80% v/v in oxygen) caused no change in the height of contraction of the gastrocnemius muscle in response to indirect stimulation via the sciatic nerve.

II. Effect on the neuromuscular block produced by tubocurarine.

With the exception of tetrahydrofuran and ethylene glycol dimethyl ether (Fig. 122, page 324) all the saturated and unsaturated ethers, the halogenated hydrocarbons, cyclopropane and nitrous oxide potentiated the neuromuscular block produced by tubocurarine. This appeared to be independent of their ability to cause muscular relaxation when used alone. Thus, for example, ethyl vinyl ether, cyclopropane and nitrous oxide did not cause muscular relaxation but all the three substances potentiated the action of tubocurarine (Table 4, page 317). At a concentration which did not possess any muscle relaxant activity, some of the compounds investigated potentiated the neuromuscular block produced by tubocurarine. For example, although diethyl ether and n-butyl methyl ether at concentrations of 1.5% and 5% v/v in oxygen respectively caused no change in the height of muscular contraction of the gastrocnemius muscle in response to indirect stimulation via the sciatic nerve, these compounds potentiated the action of tubocurarine (Table 4, page 317). It is interesting to note that although the halogenated hydrocarbons slightly increased the magnitude of the muscular contractions of the gastrocnemius muscle induced by indirect stimulation via the sciatic nerve, they potentiated the neuromuscular block produced by tubocurarine.

CONCLUSION

Pages 328 to 332

CONCLUSION

With the exception of tetrahydrofuran and ethylene glycol dimethyl ether, all the compounds reported in the present investigation potentiated the neuromuscular block produced by tubocurarine. Thus, while tubocurarine at a dose of 75 µg. per kg. consistently produced an approximately 33 per cent reduction in the height of the muscular contraction, the parallel administration and presence in the blood of diethyl ether (Fig. 116, page 318) completely abolished the response of the muscle. Furthermore, many of the compounds investigated themselves produced muscle relaxant actions when used alone. It would be incorrect to ascribe the muscle relaxant actions to the presence of the ether-oxygen function alone, since tetrahydrofuran and ethylene glycol dimethyl ether, which possess the ether-oxygen function do not appear to possess this property. The role of this ether-oxygen link in muscle relaxant compounds can therefore be only an auxiliary one, modifying the degree of pharmacological activity of the molecule as a whole. By means of Ariëns' approach to drug receptor interaction, it has been observed (Van Rossum and Ariëns, 1959, and Muir, 1962) that the ether-oxygen link in aliphatic bis- and polyonium polymethylene compounds modified the affinity (i.e., the ability of the drug to form /

form the drug receptor complex of the compounds rather than the intrinsic activity. Bovet, Bovet-Nitti, Guarino and Fusco (1948) have also reported that the presence of oxygen in certain phenolic ethers played no significant role in their muscle relaxant activity. There are moreover qualitative pharmacological differences between tetrahydrofuran and ethylene glycol dimethyl ether on the one hand, and the other volatile substances studied on the other, which cannot be attributed to the absence or presence of an ether-oxygen link. It is indeed most likely that an accumulation of circumstances may influence the type, intensity and duration of muscle relaxant activity of a compound (Foldes, 1959 and Cavallito, 1959). Among these, an optimum oil/water solubility ratio must be taken into account (Cavallito, 1959). In general, compounds with a high water solubility possess low muscular activity. The reverse is, however, not always true. It seems that the muscle relaxant activity of volatile anaesthetics does not parallel the oil/water solubility. For example, the oil/water solubilities of ethyl vinyl ether and divinyl ether are 15 times greater than that of diethyl ether, but they possess poor muscle relaxant properties. Although the oil/water solubilities of chloroform and halothane are 30 times and 100 times greater than that of diethyl ether respectively, these compounds are equipotent with diethyl ether with/

with respect to muscle relaxant properties. Another example is seen with trifluoroethyl vinyl ether which is a poor muscle relaxant although its oil/water solubility ratio is approximately the same as that of chloroform.

The potentiating actions of the compounds studied on the muscle relaxant action of tubocurarine appeared to be independent of their "direct" muscle relaxant properties. Thus ethyl vinyl ether, cyclopropane and nitrous oxide, which had no muscle relaxant actions when used alone, potentiated the action of tubocurarine. The ability of certain compounds, themselves without any muscle relaxant properties, to intensify the action of tubocurarine and other neuromuscular blocking agents has been attributed to their preferential adsorption at sites of loss, thereby enabling the active compounds to gain easier access to the active receptor sites. In this way, the degree of loss at inactive receptor sites by the pharmacologically active compounds is minimised and thus the drug effects are intensified. This view has found experimental support using hexafluorenum in combination with lipophilic substances such as dibenzylamine, diethyl ether and pentobarbitone (Cavallito, Arrowood and O'Dell, 1956 and Cavallito, 1959). The chlorhydrate of β -diethylaminoethyldiphenylpropylacetate (SKF 525-A) also potentiates the action of decamethonium, tubocurarine and gallamine by the same mechanism (Bovet, Bovet-Nitti, Bettschart and/

and Scognamiglio, 1956). However, it has been observed that anaesthetic agents such as diethyl ether, chloroform and halothane increase the potassium release from skeletal muscle preparations (Mir, 1962). The potentiating action of several of the compounds investigated in this thesis on tubocurarine, may be due to a high local concentration of potassium causing an intense and persistent depolarization sufficient to overcome the effects of the tubocurarine. It may then exert a depolarizing block of its own. It may also be the case that the loss of large quantities of potassium from the cell may cause paralysis (or inactivation) of the cellular contractile mechanism. A further possibility is that the potassium loss induced by these volatile substances may render more easy the access of tubocurarine to the active receptor sites.

The results of the present investigation are incomplete and experiments are being continued in an attempt to contribute more information on the mode of action at the neuromuscular junction of the compounds studied. It is therefore difficult, with the limited information available, to say much more.

Investigations are being continued in order to estimate the concentration of the volatile substances in the blood at various/

various time intervals during the administration of the vapours in oxygen. By correlating the blood concentration of these substances with the neuromuscular blocking activity it is hoped to define more clearly their actions.

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BIOLOGICALLY ACTIVE THIONAPHTHEN DERIVATIVES

AND

AN INVESTIGATION OF THE EFFECTS OF SOME
VOLATILE ANAESTHETICS ON THE MUSCLE
RELAXANT ACTIONS OF TUBOCURARINE

By

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This thesis describes an investigation of the pharmacological properties of a series of thionaphthen compounds and the effects of certain volatile substances on the muscle relaxant actions of tubocurarine.

The thionaphthen compounds are the isosteres of gramine and its analogues differing in the replacement of -NH- by a sulphur atom.

These compounds fall logically into four groups:-

Group A. Gramine-like isosteres.

Group B. Quaternary gramine-like isosteres.

Group C. Isosteres of 5-hydroxyisotryptamine.

Group D. 5-amino-isogramine-like isosteres.

The work was undertaken to investigate whether or not the thionaphthen compounds were more or less specific in their actions than the corresponding indole compounds as this information might be used to obtain further information on the existence, or otherwise, of specific receptors for the indole derivatives.

Experiments/

Experiments were carried out using isolated smooth muscle preparations and intact animals. An additional study was also made using Ariens' technique.

Experimental evidence suggests that the thionaphthen compounds investigated were unselective in their pharmacological actions and many of them not only antagonized 5-hydroxytryptamine, histamine and acetylcholine on the isolated guinea pig ileum but at higher concentrations, they themselves exhibited a stimulant action on this tissue. This stimulant response was partly or completely antagonized by lysergic-acid diethylamide, atropine and mepyramine. In contrast to the anti-5-hydroxytryptamine properties on the isolated guinea pig ileum, rat uterus and isolated perfused rat headquarters, many of the thionaphthen compounds potentiated the actions of 5-hydroxytryptamine on the isolated rat fundus strip. The gramine-like isosteres (Group A) reduced the blood pressure level of the anaesthetized cat or rat whereas the quaternary salts of the gramine-like isosteres, the isosteres of 5-hydroxyisotryptamine and the 5-amino-isogramine-like isosteres (Groups B, C and D) caused no change, or a rise in the blood pressure level. None of the compounds investigated influenced the pressor response to adrenaline or noradrenaline. On the other hand, many of the compounds antagonized the vasoconstriction caused by adrenaline, noradrenaline or 5-hydroxytryptamine on the isolated perfused rat hindquarters. The results obtained using Ariens' experimental procedure indicated that many/

many of the compounds exhibited a purely non-competitive antagonism to 5-hydroxytryptamine.

From the data presented, it was concluded that the application of the concept of bioisosterism with respect to the thionaphthen compounds studied has failed to produce derivatives of similar biological activity and also indicates that the thionaphthen compounds investigated in this thesis were unselective in their pharmacological actions when compared to the related indolic compounds.

Studies of the effects of a group of volatile liquid anaesthetics and of nitrous oxide upon the muscle relaxant actions of tubocurarine were made using the spinal cat. A known concentration of the vapour in oxygen was administered to the animal and the effect on the height of contraction of the gastrocnemius muscle both in the absence and in the presence of tubocurarine was observed.

It was concluded that, with the exception of tetrahydrofuran and ethylene glycol dimethyl ether, all the compounds potentiated the neuromuscular block produced by tubocurarine. Some exhibited a muscle relaxant action themselves. Di-isopropyl ether was the most potent in this respect. The potentiating action on tubocurarine was independent of the muscle relaxant action. Thus, although the halogenated hydrocarbons slightly increased the magnitude of the muscular contraction, they potentiated the neuromuscular block produced by tubocurarine. The potentiating action was not considered to be due to the presence of the ether oxygen link or related to the oil/water solubility ratio. The effect/

effect can most logically be attributed to their preferential adsorption at sites of loss thus enabling tubocurarine to gain greater access to the active receptor sites. It was also suggested that the loss of intracellular potassium might be a contributing factor.