A STUDY OF THE PHARMACOLOGY OF THE ALIMENTARY TRACT OF THE DOMESTIC FOWL.

By

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TO MY WIFE - MARIA

DECLARATION

In compliance with the Edinburgh University Regulation 2.4.15, I, the undersigned, hereby declare that this thesis has been composed by myself and that the material in it is my own.

In compliance with Regulations 2.4.11 and 5.7, reprints of two papers and a communication to the British Pharmacological Society describing some of the material in this thesis are enclosed as an appendix. Other parts of the experimental work dealing with the chick isolated nerve-oesophagus preparation have been accepted for publication by the Editors of the British Journal of Pharmacology and will most probably appear in the June issue of this Journal under my name. In addition the experimental comparisons of MJ-1999 and propranolol were communicated to the British Pharmacological Society this Spring together with Dr. A.L.Bartlet whose contribution is acknowledged in the thesis.

TIGANI HASSAN

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Appendix: Publications:-

Hassan, T.(1967). Effects of stimulation of the cervical vagus and descending oesophageal nerves on the alimentary tract of the domestic fowl. Zentbl. Vet Med.A., 14, 854-861.

Hassan, T.(1968). A hyoscine-resistant contraction of the chicken isolated oesophagus to stimulation of the vagus and descending oesophageal nerves. Br. J. Pharmac., 34, 205-206P.

Bartlet, A.L. & Hassan, T.(1968). The action of physostigmine and the distribution of cholinesterases in the chicken oesophagus. Br. J. Pharmac., 33, 531-536.

SUMMARY

In vivo Study of the Upper Alimentary Tract of the Fowl

1. Vagal stimulation produced contractions of the pre-crop oesophagus, crop and stomachs (proventriculus and gizzard) and a fall in arterial blood pressure. Stimulation of the descending oesophageal nerve produced contractions of the pre-crop oesophagus and crop but had no effect on the stomachs or blood pressure.

2. The residual response of the crop to high cervical vagal stimulation after cutting the recurrent nerve was very small, suggesting that the recurrent nerve supplied the main efferent vagal fibres to the crop. The response of the blood pressure to high cervical vagal stimulation was not affected by cutting the recurrent branch of the vagus and stimulation of the cut recurrent nerve had no effect on the blood pressure, suggesting an absence of cardiac vagal fibres in the recurrent nerve.

3. The effects of stimulation of the vagus or descending oesophageal nerve were potentiated by physostigmine and abolished by hyoscine, suggesting that these nerves are cholinergic; but hexamethonium did not abolish responses to nerve stimulation.

4. In decerebrate chickens not anaesthetized with pentobarbitone, intravenous hyoscine abolished the contractions of the oesophagus to vagal or descending oesophageal nerve stimulation.

The Chicken Isolated Oesophagus

1. Contractions of the chicken isolated oesophagus produced by stimulation of the vagus and descending oesophageal nerves were potentiated by physostigmine and abolished by hyoscine (1 to 100 μg/ml) if the duration of stimulation was less than 5 sec, but prolonged stimulation produced contractions not antagonized by hyoscine. The contractions were not abolished by hexamethonium or tubocurarine even when the nerves were stimulated for less than 5 sec.

2. The contractions to nerve stimulation were abolished by cocaine and nerve section, showing that the plain muscle was not stimulated directly. Bretylium, mepyramine and methysergide did not antagonize the contraction to nerve stimulation, however, suggesting a lack of involvement of noradrenaline, histamine and 5-hydroxytryptamine, respectively, in the transmission of impulses producing the hyoscine-resistant response.

3. Hyoscine-resistant contractions of the oesophagus to nerve stimulation were obtained from thin external muscle preparations, and previous intravenous injection of hyoscine into chicks did not prevent subsequently isolated oesophageal preparations from contracting to nerve stimulation, nor did exposure to hyaluronidase modify the effect of hyoscine on the contractions to nerve stimulation, suggesting that resistance to complete block by hyoscine is probably not due to an inability of the drug to diffuse into the preparations.

4. Prolonged nerve stimulation of an isolated oesophagus preparation did not produce a contraction of a preparation of isolated guinea-pig ileum

or post-crop chick oesophagus suspended in the same organ bath.

5. It seems possible that small amounts of a slow contracting substance were released from the stimulated nerves together with acetylcholine, although such a substance could not be pharmacologically detected in the bathing solution.

Selection of Drugs for Blocking Adrenergic Neurones and α- and β-Receptors

1. Stimulation of the Remak nerve produced a contraction of the rectum but when the tone of the preparation was raised with acetyl-β-methylcholine, nerve stimulation produced a transient contraction followed by a relaxation. Pharmacological characterization of the relaxing fibres of the Remak nerve necessitated an in vitro evaluation of drugs which block adrenergic neurones and α- and β-receptors.

2. Propranolol was 26 times as potent as MJ-1999 in blocking the hypogastric nerve of the guinea-pig, but it was 550 times as potent in antagonizing the action of isoprenaline on the β-receptors of the chick rectum, showing that propranolol was the drug which was most likely to block β-receptors without producing a nerve block. Moreover, propranolol (0.05 μg/ml) did not antagonize contractions of the chick rectum to acetylcholine or histamine whereas MJ-1999, at a concentration which was equipotent with propranolol (0.05 μg/ml) in blocking isoprenaline, antagonized both agonists. Thus propranolol (0.05 μg/ml) was selected for experiments with the chick Remak nerve-rectum preparation.

3. Tolazoline, but not phentolamine, potentiated contractions of the guinea-pig vas deferens to hypogastric nerve stimulation, making it an unsuitable drug for antagonizing the action of a neurotransmitter at α-receptors. Furthermore, tolazoline (10 μg/ml) antagonized histamine acting on the chick rectum, whereas phentolamine (0.1 μg/ml), which was equipotent with tolazoline on the rat seminal vesicle, did not. Phentolamine (0.1 μg/ml) was therefore selected for blocking the α-receptors of the chick rectum.

4. Bretylium (10 μg/ml) blocked contractions of the guinea-pig vas deferens to hypogastric nerve stimulation, but it did not antagonize contractions of the guinea-pig oesophagus to vagal stimulation or contractions of the chick rectum to acetylcholine or histamine. Thus bretylium (10 μg/ml) produced a specific block of adrenergic neurones and was used for this purpose in experiments with the chick isolated Remak nerve-rectum preparation.

The Chick Isolated Remak Nerve-rectum Preparation

1. Contractions of the rectum to Remak nerve stimulation resisted complete block by hyoscine as did the contractions of the chick isolated oesophagus to vagal and descending oesophageal nerve stimulation, but the rectal contractions, unlike the oesophageal contractions, were usually abolished by hexamethonium and tubocurarine.

2. Phenylephrine, which typically stimulates α-receptors, produced

relaxations of the rectum which were greatly inhibited by propranolol but not phentolamine. A small residual relaxation to phenylephrine persisted in the presence of propranolol, and this was abolished by phentolamine. This suggests that nearly all of the sympathomimetic receptors of the chick rectum were stimulated by phenylephrine and blocked by propranolol, although these two drugs do not normally combine with the same receptor.

3. Propranolol only antagonized noradrenaline when isoprenaline and phenylephrine were tested on the same preparation. Furthermore, antagonism of the relaxation of the rectum to Remak nerve stimulation by propranolol was greatest in experiments where isoprenaline and phenylephrine were tested. This suggests that isoprenaline and/or phenylephrine had an influence on the combination of noradrenaline and/or propranolol with the sympathomimetic receptors.

4. Relaxations of the rectum to Remak nerve stimulation were abolished by a combination of propranolol and phentolamine, and were inhibited by bretylium or by pretreatment with reserpine, suggesting the release of a noradrenaline-like substance from sympathetic fibres contributed by the coeliac plexus.

Records obtained from the upper alimentary tract were of the circular muscles *in vivo* but of the longitudinal muscles *in vitro*.

INTRODUCTION

REVIEW OF LITERATURE

The literature dealing with the general pharmacology of the alimentary tract of the domestic fowl (Gallus domesticus) was reviewed in a previous thesis (Hassan, 1966).

ATROPINE-RESISTANT RESPONSES OF PLAIN MUSCLE TO STIMULATION OF PARASYMPATHETIC NERVES

In the experiments to be described in the present thesis contractions of the chicken oesophagus, crop, proventriculus and gizzard to stimulation of the vagus and descending oesophageal nerves were abolished by atropine or hyoscine in vivo, whereas the contractions of the isolated oesophagus to stimulation of these nerves resisted complete block by either drug. Furthermore, contractions of the chick isolated rectum produced by stimulation of the Remak nerve exhibited a similar hyoscine-fastness. Failure of atropine to abolish responses of the alimentary tract and urinary bladder to parasympathetic nerve stimulation in other species has been described before, both in vitro and in vivo, and a review of some of these observations follows.

I. ALIMENTARY TRACT

A. IN VIVO

Jacobj(1891) observed that the excitation of the dog's alimentary tract produced by vagal stimulation in vivo exhibited resistance to block by atropine. Similarly Bayliss and Starling(1899) showed that the contractions of the anaesthetized dog's duodenum and ileum produced by vagal stimulation were only partly antagonized by an injection of 4 mg of atropine, the residual contractions remaining unaffected by a higher dose of 30 mg of atropine. Cushny(1910) observed that the excitation of the dog's intestine elicited by vagal stimulation in vivo was not affected by an injection of 2 mg of atropine, a dose which abolished the excitation of this organ produced by pilocarpine. In later experiments Henderson (1923) investigated the effect of atropine on the excitation of the intestine produced by cervical or thoracic vagal stimulation in anaesthetized dogs. Atropine, 0.5 to 1 mg, always decreased the tonus of the intestine but even 30 mg of the drug did not abolish the response to nerve stimulation.

Although the motor response of the alimentary tract to vagal stimulation seems to be atropine-resistant in dogs in vivo, it was blocked by atropine in the spinal cat(Harrison & McSwiney,1936). Published information about the effect of atropine on responses of the alimentary tract of other species to parasympathetic nerve stimulation

in vivo is very scanty, however, apart from the work of Straub & Stefansson(1937) who found that the excitation of the guinea-pig small intestine evoked by vagal stimulation was blocked by atropine in doses as small as 10 μg/kg injected intravenously. Hobbiger & Lessin(1955) found that in rabbits devoid of serum atropinesterase, previously determined manometrically, an intravenous injection of atropine sulphate, 0.25 mg/kg, produced a lasting inhibition of the contraction of the stomach to supramaximal stimulation of the vagus nerve. However, in rabbits in which serum atropinesterase was present, atropine, 0.25 to 1 mg/kg, produced only a transient and weak block of the response of the stomach to vagal stimulation.

B. IN VITRO

a. Responses to Extrinsic Nerve Stimulation

Resistance to block by atropine of contractions of the isolated alimentary tract to parasympathetic extrinsic nerve stimulation has not been described in the available literature.

b. Responses to Intrinsic Nerve Stimulation

i) Transmural Stimulation of Intrinsic Nerves

The response of isolated preparations to transmural stimulation is generally believed to be due to excitation of postganglionic neurones (Paton,1955). Munro(1953) found that transmural electrical stimulation

of isolated segments of proximal and terminal guinea-pig small intestine produced a quick transient contraction, the voltage being 30 to 80V and stimulus applied for 2 sec every 20 sec. Exposure of the preparations to atropine sulphate, 0.13 μg/ml, for 2 to 3 min, reduced or abolished the rapid contractions, but in the continuous presence of atropine a residual response appeared consisting of a small, quick contraction followed by a second slower contraction. The secondary contraction gradually increased in size, but it was still preceded by the slight twitch. At this stage the preparation no longer responded to added acetylcholine. Although the atropine-resistant response could have been due to stimulation of adrenergic terminal fibres since adrenaline also contracted the terminal ileum(Munro, 1951,1952), the author thought this unlikely as the atropine-resistant response was not antagonized by ergotoxin. However, Paton(1955) using guinea-pig isolated ileum preparations contracting to transmural electrical stimulation found that the rapid twitches of the preparation elicited by single shocks of 0.5 msec duration and 5 to 15V potential were abolished by small concentrations of atropine (0.01 μg/ml).

Recently Ambache & Freeman(1969) described atropine-resistant contractions of the separated, plexus-containing, longitudinal muscle of the guinea-pig isolated ileum to transmural stimulation (27V, pulse width 0.1 to 0.2 msec). Single electrical shocks produced a twitch response which was abolished by atropine, 4 to 10 ng/ml, as in the experiments of Paton (1955). However, in the presence of atropine, delayed spasms were

revealed by tetanic stimulation at a frequency of 50 c/s for 1 sec; these spasms were not antagonized by concentrations of atropine up to 1 μg/ml and only slightly depressed by 10 μg/ml of the drug. The atropine-resistant response was similar to that described by Munro(1953) and was not affected by drugs which antagonise histamine, 5-hydroxytryptamine or prostaglandins, although it was abolished or reduced by the nerve blocking drug tetrodotoxin, 0.1 to 0.2 μg/ml. Ambache & Freeman(1969) concluded that the tetanic spasms were mediated by the release of a substance ('tetanic spasmogen') from nerves in Auerbach's plexus which was not any of the above amines or a prostaglandin.

ii) Stimulation of Intrinsic Nerves by Drugs

A number of workers have found that the contractions of the longitudinal muscle of the rabbit isolated intestine produced by nicotine resisted complete block by atropine(Euler, 1945; Ambache & Edwards, 1951; Ellis & Rasmussen, 1951). Although contractions of the rabbit intestine to nicotine resisted block by concentrations of atropine as high as 100 μg/ml, the contractions of the preparation produced by acetylcholine or muscarine were invariably blocked by atropine (Ambache & Edwards,1951). However, Ambache & Robertson(1953) later obtained results which were at variance with these observations in that the contractions to nicotine were greatly reduced or abolished by atropine, 0.05 to 0.2 μg/ml, in two out of five preparations. Ambache(1955) in a later review concluded that the failure of atropine to block contractions of rabbit intestines to nicotine could

not be accounted for by the destruction of atropine by atropinesterase in the preparations as fresh concentrations of atropine were added 1 min before each exposure to nicotine, and the action of muscarine was abolished under the same conditions. Thus he considered it likely that nicotine stimulated cholinergic motor neurones in the wall of the rabbit's intestine and that the usual inability of atropine to inhibit these responses was due to the "close proximity" of the nerve endings to the receptors, as previously suggested by Dale & Gaddum(1930). In support of the view that both atropine-sensitive and atropine-resistant contractions to nicotine were due to an action on cholinergic neurones was the finding that botulinum toxin always abolished the response of the rabbit intestine to this drug (Ambache & Lessin, 1955).

The effect of atropine on the response of the rabbit intestine to some other neuronal stimulants was similar to its effect on the response to nicotine. Thus the contractions of the longitudinal muscle of the isolated intestine produced by piperidine, which has nicotine-like pharmacological actions(Euler,1945), and by the neuronal stimulant bromophenyl-ethers of choline(Ambache & Robertson,1953), exhibited resistance to block by atropine. Feldberg(1951) found that contractions of the rabbit intestine to barium were more resistant to block by atropine than contractions to acetylcholine. The relative resistance to atropine of the responses to barium was thought to be due to acetylcholine being released from the stimulated nerve endings in such close contact with the muscle fibres that it was less susceptible to the effect of atropine than

added acetylcholine; alternatively, the transmitter substance might have been similar to the atropine-resistant smooth muscle stimulating substance (Darmstoff) released from the frog's stomach as a result of vagal stimulation(Vogt, 1949).

In other species such as the guinea-pig or cat, contractions of isolated pieces of alimentary tract produced by nicotine were abolished by small concentrations of atropine however (Emmelin & Feldberg, 1947; Tripod, 1949; Ellis, 1951; Ambache & Edwards, 1951; Ambache & Robertson, 1953).

II. URINARY BLADDER

A. IN VIVO

Langley(1911) found that the contractions of the urinary bladder of the anaesthetized cat produced by electrical stimulation of the pelvic nerves resisted complete block by atropine. Similarly Henderson & Roepke(1934, 1935) found that the contractions of this organ to pelvic nerve stimulation were not affected by doses of atropine sufficient to abolish the contractions produced by acetylcholine. Their experiments were carried out on dogs and cats anaesthetized with morphine and chloralose or with urethane maintained with ether. After a delay of 1 to 2 sec electrical stimulation of the pelvic nerve produced a sustained contraction of the bladder which relaxed slowly following cessation of the stimulation. Intraarterial injection of acetylcholine produced a contraction which was similar to that produced by nerve stimulation except

that its onset was slower. Injection of atropine, 100 μg/kg, partially antagonised the response to nerve stimulation, only a transient residual contraction being obtained after atropine. The response to acetylcholine was abolished by this dose of atropine, however, but increasing the dose of acetylcholine 10 to 100 times produced a transient contraction similar to that produced by nerve stimulation after atropine. The authors suggested that the response to a large dose of acetylcholine was due to the drug stimulating ganglia in the bladder; and concluded that the atropine-sensitive "tonal" response to pelvic nerve stimulation was mediated by cholinergic nerve fibres whereas the initial contractile response was produced by a neurotransmitter other than acetylcholine.

The effect of atropine on the contractions of the urinary bladder to pelvic nerve stimulation was also studied by Edge(1955) using cats anaesthetised with chloralose. The bladder was made to contract to stimulation of the pelvic and hypogastric nerves. Contractions to pelvic nerve stimulation were potentiated by atropine, 100 μg/kg, within 1 min of its injection and this effect lasted for at least 20 min. Hexamethonium, 0.25 mg/kg, antagonized the response to pelvic nerve stimulation, the onset of the action of the drug and its duration being similar to those of atropine. On the other hand, the contractions to hypogastric nerve stimulation were not affected by either atropine or hexamethonium. Dihydroergotamine abolished the contractions to hypogastric nerve stimulation but did not antagonize those to pelvic nerve stimulation; thus the failure of atropine to inhibit contractions of the bladder to pelvic

nerve stimulation was not due to the stimulation of excitatory adrenergic fibres in the pelvic nerve. An injection of acetylcholine produced a contraction of the bladder which was abolished by atropine although a large dose of acetylcholine still produced a transient contraction. The atropine-resistant contraction to acetylcholine was nearly abolished by a large dose of hexamethonium suggesting a stimulant action of acetylcholine on the ganglia of the bladder after injection of atropine, and supporting the view previously expressed by Henderson & Roepke(1934) regarding the effect of acetylcholine on the dog's bladder.

Ursillo (1961) recorded isotonic and isometric contractions of the urinary bladder to electrical stimulation of the pelvic nerve in bitches anaesthetized with dial-urethane. The height of the contractions produced by supramaximal stimulation at a frequency of 15 c/s was only partially antagonized by atropine, 1 to 10 mg, injected into the abdominal aorta. The contraction produced during prolonged stimulation of the pelvic nerve was still well sustained after the injection of atropine. The discrepancy between this observation and that reported by Henderson & Roepke(1934, 1935) was thought to be due to fatigue of the preparation caused by a high frequency of stimulation in Henderson & Roepke's experiments (which was not defined); hence the apparent inhibition of the "tonal" response by atropine.

Chen, Portman & Wickel(1953) studied the effect of atropine on contractions of the anaesthetized dog's urinary bladder to the ganglion stimulant DMPP (1, 1-dimethyl-4-phenylpiperazinium iodide). Atropine, 2.5 μg to 10 mg/kg, injected intravenously, only slightly suppressed the

response of the bladder to 40 μg/kg of DMPP, although it abolished the contractions of the preparation produced by carbachol. However, atropine at doses above 10 mg/kg produced a marked suppression of the contractions to DMPP, an effect which the authors attributed to a partial ganglionic blocking action and a partial antispasmodic action of atropine as these doses of atropine also suppressed the hypertension produced by DMPP acting on sympathetic ganglia or by adrenaline. The results obtained by using weaker doses of atropine were thus taken to be comparable with those of Henderson & Roepke(1934, 1935).

Resistance to block by atropine of contractions of the urinary bladder to intraarterial DMPP and pelvic nerve stimulation was also reported by Gyermek(1961) using anaesthetized dogs and cats. An intravenous injection of atropine, 1 to 2 mg/kg, partially antagonized the contractions produced by DMPP or pelvic nerve stimulation whereas the contractions to muscarine or acetyl-β-methylcholine were abolished. Hexamethonium, 1 mg/kg, abolished the residual atropine-resistant contractions to DMPP and nerve stimulation. These results led the author to postulate the existence of specific receptors in the plain muscle of the urinary bladder which behaved functionally like autonomic ganglia. Thus the motor innervation of the bladder supplied by the pelvic nerves was regarded to be wholly cholinergic in opposition to the view held by Henderson & Roepke(1934, 1935).

Garret(1963) showed that contractions of the spinal dog's urinary bladder to intravenous DMPP were not affected by a dose of atropine which

antagonised the fall in blood pressure produced by acetylcholine or vagal stimulation. In these experiments the hypogastric nerves were cut on both sides of the bladder so that DMPP only produced contraction of the bladder through its action on the parasympathetic ganglia of the pelvic nerves.

B. IN VITRO

Ursillo & Clark(1956) were the first to describe an isolated preparation of urinary bladder with parasympathetic nerve attached. A strip of the detrusor muscle from the rabbit urinary bladder about 2 mm thick, was suspended in an organ bath filled with Krebs solution bubbled with 5% CO_2 in O_2 and kept at 37°C. The preparation supported a tension of 0.5 to 1g and the contractions to nerve stimulation were magnified 20 times. The nerve fibres supplying this strip were dissected together with the adjoining blood vessels and passed through platinum wire loop electrodes which were immersed in the bathing solution. These nerves were found to be postganglionic as histological examination of sections of the bladder wall revealed only a few scattered ganglionic cells and the response to nerve stimulation was not antagonised by the ganglion blocking drugs tetraethylammonium and hexamethonium; moreover, the ganglion-stimulant DMPP did not usually produce a contraction of the preparation. The contractions of the preparation to stimulation of the nerve (12V, 1 msec and 30 c/s applied for 5 sec every 2 min) were partially antagonised by atropine, 2 ng/ml, but the residual atropine-resistant contraction was always obtained even after exposure to atropine, 20 μg/ml. The

contractions of the bladder produced by acetylcholine were abolished by atropine, however. It was observed that the time for the onset or offset of a steady state of antagonism by atropine, was 8 times longer for block of nerve stimulation than for acetylcholine. Physostigmine potentiated the contraction to submaximal nerve stimulation and reversed the partial antagonism produced by atropine. The effects of atropine and physostigmine on the responses of the bladder to nerve stimulation led the authors to conclude that at least a portion of the response of the nerve-bladder strip was cholinergic.

Similar atropine-resistant contractions of the isolated urinary bladder to pelvic nerve stimulation have been reported in other species such as the ringtail possum (Burnstock & Campbell, 1963), the toad (Burnstock, O'shea & Wood, 1963) and the rat (Hukovic , Rand & Vanov, 1965). Furthermore, transmural stimulation of the isolated bladder produced contractions which resisted complete block by atropine in rats (Carpenter, 1963) and in guinea-pigs(Chesher & Thorp 1965).

METHODS

I. CHEMICALS

The drugs used were: acetylcholine chloride (B.D.H.), acetyl-β-methylcholine chloride (Koch-Light), atropine sulphate (B.D.H.), Bretylium tosylate (Burroughs Wellcome & Co.), carbachol chloride (carbamylcholine chloride, B.D.H.), cocaine hydrochloride (T. & H.Smith), Halothane (I.C.I.), heparin (Boots), hexamethonium bromide (May & Baker), histamine acid phosphate (B.D.H.), hyaluronidase (Fisons Pharmaceuticals), hyoscine hydrobromide (B.D.H.), isoprenaline hydrochloride (Ward Blenkinsop & Co.), meclofenamic acid (Parke Davis & Co.), MJ-1999 (4'- (2-isopropylamino-1-hydroxyethyl) methanesulphonanilide hydrochloride, Mead Johnson & Co.), mepyramine maleate (May & Baker), methysergide bimaleate (Sandoz), *l*-noradrenaline bitartrate (Koch-Light), pentobarbitone sodium (Abbott Laboratories), phentolamine mesylate (Ciba), *l*-phenylephrine hydrochloride (Koch-Light), physostigmine salicylate (B.D.H.), pilocarpine hydrochloride (B.D.H.), propranolol hydrochloride (1-isopropylamino-3- (1-naphthyloxy)-2-propanol hydrochloride, I.C.I.), reserpine (Ciba), tetramethylammonium chloride (B.D.H.), tolazoline hydrochloride (2-benzyl-4,5-imidazoline hydrochloride, Koch-Light) and tubocurarine chloride (Burroughs Wellcome & Co.). The doses of drugs in the text, tables and figures refer to the above compounds.

The Krebs' solution was made by dissolving salts of the A.R.grade in de-ionized water. Its composition was as described by Krebs & Henseleit (1932), except that the concentration of calcium was halved in the

experiments with the chick isolated oesophagus. The solution contained (g/l): NaCl 6.9, KCl 0.35, $CaCl_2$ 0.28, KH_2PO_4 0.14, $MgSO_4$ 0.11, $NaHCO_3$ 2.1 and dextrose 2.

Chemicals used in the cholinesterase staining were: acetylthiocholine iodide (Koch-Light), n-butyrylthiocholine iodide (Koch-Light), copper sulphate ($CuSO_4$, $5H_2O$, A.R., B.D.H.), DFP (diisopropyl phosphorofluoridate, Koch-Light), glycine (A.R., B.D.H.), sodium acetate (hydrated salt, A.R., B.D.H.), sodium sulphate (Na_2SO_4, $10H_2O$, A.R., B.D.H.) and sodium sulphide (M.A.R.,B.D.H.).

II. IN VIVO PREPARATIONS

A. THE ANAESTHETIZED FOWL

Cockerels (Brown or White Leghorn) weighing 0.8 to 2 kg were fasted overnight and anaesthetized with pentobarbitone sodium (30 to 35 mg/kg, intravenously). Sometimes cockerels were lightly anaesthetized with halothane and the skull quickly trephined over the cerebrum which was then sucked out with a pump and replaced by cotton gauze to stop bleeding, 60 to 90 min being allowed for halothane to be removed before start of an experiment. The anaesthetized or decerebrate birds were secured on their backs and kept warm.

The trachea, jugular vein and carotid or brachiocephalic artery were cannulated. The arterial blood pressure was recorded with a mercury

manometer, or with a *Greer micromanometer(Greer, 1958) operating a pen recorder. Drugs dissolved in 0.9% W/V NaCl were injected into the venous cannula and washed into the circulation with 2 ml of saline run into the cannula from a burette.

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* A Greer micromanometer is a pressure transducer consisting essentially of a reflective diaphragm mounted in a sealed capsule so that pressure may be applied to either side. Light from a single bulb is reflected by mirrors to illuminate both faces of the diaphragm and is then reflected on two photocells. When the pressure on both sides of the diaphragm is equal, the width of the reflected beam is the same on each side and thus the intensity seen by the cells is equal; the bridge circuit, in which the cells are wired, thus remains balanced. If, however, the pressures are unequal the light tends to be focused on one cell and "defocused" on the other, and this results in an out-of-balance current flowing in the bridge proportional to the pressure differential across the diaphragm.

Thin rubber balloons attached to polythene tubing (10 mm external diameter) were introduced through a fistula in the post-crop oesophagus into the stomachs (proventriculus and gizzard), and through the mouth into the crop and pre-crop oesophagus, the exact location of the balloons being verified at autopsy. The balloons were connected by rubber tubing to water manometers and filled with 4 to 8 ml of air; when inflated their external diameter was 20 to 30 mm. On completing the dissection, heparin (100 to 200 u./kg) was given intravenously. Pressure changes in the water manometers attached to the balloons were recorded on a smoked drum with a frontal writing lever attached to a tambour, or, by means of a Greer micromanometer operating a pen recorder.

The vagi and glossopharyngeal nerves were cut proximally on either side of the neck and the branches of the vagi to the glossopharngeal nerves and of the glossopharyngeal nerves to the pharynx were severed (Fig. 1). A pair of silver electrodes was hooked around the peripheral end of a cut vagus or glossopharyngeal (descending oesophageal) nerve, the nerves being kept moist with cotton wool soaked in warm liquid paraffin. The nerves were stimulated for 5 sec in every 2 to 5 min with a train of square wave pulses: width 8 to 10 msec, frequency 20 to 50 c/s and the voltage adjusted to give submaximal responses.

In some experiments the bird was artificially ventilated and the thorax opened with bone forceps to expose the thoracic vagus and recurrent nerves. The recurrent branch of the left vagus was cut near its origin, and the vagal trunk was cut just cranial to the origin of the recurrent nerve and

at a level about the middle of the pre-crop oesophagus (Fig. 1). Stimulating electrodes were placed around the peripheral ends of the severed nerves and the stimuli applied as above.

B. ANAESTHESIA OF THE RABBIT CORNEA

In each rabbit a drug solution was instilled into one eye and 0.9% W/V NaCl into the other. Both corneae were touched 5 times with the blunt end of a glass rod at intervals of 2, 4, 6, 10, 15, 20, 25 and 30 min after instillation of the solutions, the number of blinks from each cornea being recorded in each instance. Each drug was tested in five rabbits, and the mean per cent inhibition of the corneal reflex for each solution at each time interval was calculated and plotted on the ordinate of a graph, the time from instillation of the drugs being plotted on the abscissa.

III. ISOLATED PREPARATIONS

A. CHICK OESOPHAGUS

Brown Leghorn Chicks aged 1 to 14 days were killed by an air embolus and the whole pre-crop oesophagus together with the right jugular vein and the accompanying nerves rapidly removed and placed in Krebs' solution. A nerve-muscle preparation was made by ligating the vagus, descending

oesophageal nerve and jugular vein at the level of the pharynx and separating them from the pre-crop oesophagus 1 cm of their length. A preparation of separated external muscle with nerve attached was made by everting an isolated nerve-oesophagus preparation on a glass rod, peeling away the mucosa and reverting the tubular muscularis externa. The oesophagus was set up in a 40ml organ bath filled with Krebs' solution, so that its oral end was tied with a thread to a frontal writing lever and the lower end tied to a supporting glass rod. The Krebs' solution in the bath was bubbled with a mixture of 5% CO_2 in oxygen. The organ bath and glass warming coils were immersed in a water tank kept at 35°C by means of a toluene-mercury thermostat and heating element. The nerves were drawn through an electrode, similar to that described by Burn & Rand (1960), immersed in the bathing solution. Contractions of the longitudinal muscle of the preparation were recorded isotonically on a kymograph with a frontal writing lever which exerted a force of 1-2g cm and magnified the contractions four times. Occasionally the post-crop oesophagus or a piece of guinea-pig ileum was suspended in the same organ bath as the nerve-oesophagus preparation such that their longitudinal contractions could be recorded with isotonic levers.

The vagus and descending oesophageal nerves were usually encircled by the same electrodes and stimulated together; and the term "<u>nerve stimulation</u>" in the text and figures always refers to the synchronous stimulation of the two nerves. In some experiments the vagus

or descending oesophageal nerve was freed from other tissues and stimulated separately; in referring to these experiments the terms "vagal stimulation" and "descending oesophageal nerve stimulation" were used.

Unless otherwise specified, the nerves were stimulated with a train of square wave pulses: width 10 msec, frequency 20 c/s and intensity 5V, which usually produced maximal responses. Trains of stimuli were applied for periods varying from 3 to 90 sec in every 2 to 15 min.

B. CHICK RECTUM

Chicks (Brown Leghorn) aged 1 to 3 weeks were decapitated and bled so as to facilitate the dissection. The abdomen was opened with a pair of rough scissors and the whole rectum together with the Remak nerve and adjoining blood vessels (Fig.2) removed and placed in a petri dish containing Krebs' solution. The contents of the rectum were washed out with Krebs' solution. To make a nerve-rectum preparation the Remak nerve and caudal mesenteric vein were tied with cotton and freed along 0.5 to 1 cm of their length from the caecal end of the rectum. The posterior end of the rectum was cut at the level of the apex of the Bursa of Fabricius to exclude cloacal striated muscle. The preparation with open ends was suspended in a 40 ml organ bath containing Krebs' solution bubbled with 5% CO2 in O_2 and maintained at 35°C. The method of recording contractions of the longitudinal muscle of the preparation, and of nerve stimulation was similar to that described for the nerve-oesophagus preparations.

The Remak nerve was stimulated with a train of square wave pulses: width 1 to 10 msec, frequency 20 c/s and intensity 5 to 10V. Trains of stimuli were applied for periods varying from 15 to 60 sec in every 5 to 15 min.

C. GUINEA-PIG VAS DEFERENS

Male guinea-pigs were stunned by a blow on the head and bled. The abdomen was opened in the midline and the vasa deferentia with hypogastric nerves attached dissected by the method of Hukovic(1961). The preparations were mounted in organ baths filled with Krebs' solution kept at 35°C and bubbled with a mixture of 5% carbon dioxide in oxygen, contractions being recorded with an isotonic lever carrying a frontal writing point and exerting a force of 1-2 gm on the preparation. The hypogastric nerve was drawn through the channel of a pair of electrodes similar to that described by Burn & Rand(1960); and in some experiments a Perspex gutter carrying two parallel platinum wires for transmural stimulation (Birmingham & Wilson, 1963) was placed round the vas deferens. The hypogastric nerve was stimulated with 0.1 msec pulses at 30 c/s and the preparation was stimulated transmurally with 0.5 msec pulses at a frequency of 50 c/s, the voltage being submaximal for both methods of stimulation. The stimulation was applied for 5 sec in every 5 min, either through the hypogastric nerve alone, or alternating the transmural stimulation with hypogastric nerve stimulation.

In a few experiments a preparation of vas deferens without the hypo-

gastric nerves was made for the purpose of studying the action of noradrenaline. These preparations produced steady contractions when exposed to noradrenaline(10 μg/ml) for 30 sec in every 5 min.

D. GUINEA-PIG OESOPHAGUS

A preparation of isolated oesophagus with vagus nerve attached was made from guine-pigs as described by Bartlet(1968a). The oesophagus with open ends was suspended in an organ bath filled with Krebs' solution kept at 35°C and bubbled with 5% carbon dioxide in oxygen, contractions of the longitudinal muscles being recorded with an isotonic lever carrying a frontal writing point and exerting a force of 2.5 g cm on the preparation. The vagus nerve was drawn through bipolar platinum electrodes and stimulated with bursts of 0.1 msec pulses at 30 c/s for 5 sec in every 5 min, the voltage being adjusted to give submaximal responses.

E. RAT SEMINAL VESICLE

Male rats were stunned by a blow on the head and bled. The abdomen was opened in the midline and the seminal vesicles exposed. The glandular tissue adherent to the curved blind end of the seminal vesicle was dissected away and the organ removed and placed in a petridish containing Krebs' solution where it soon emptied itself of the seminal contents. The preparation was suspended in an organ bath filled with Krebs' solution and its contractions to noradrenaline recorded as for

the guinea-pig isolated vas deferens preparation.

III. METHOD OF TESTING DRUGS ON ISOLATED PREPARATIONS

Stock solutions of drugs were made with de-ionized water and kept in the refrigerator at 5°C for not more than a week. Meclofenamic acid (10 mg/ml) was freshly prepared by dissolving the drug in 10 ml of de-ionized water with the addition of 1 drop of 5N NaOH and adjusting the solution to a pH of 8.7 with 0.1N HCl. Reserpine was dissolved in 20% W/V ascorbic acid and used on the same day. The drugs were diluted to the required concentrations with 0.9% W/V NaCl just before use. Drugs were added to the organ bath with an all-glass syringe in a volume not exceeding 1% of that of the organ bath.

Agonists were left in contact with isolated preparations for specified periods of time and the solution in the bath changed twice after each test, the tests usually being repeated every 5 to 10 min.

When the responses to agonists became steady, an antagonist was added to the bathing solution and its concentration maintained throughout the period of test. Whenever possible antagonism was measured in terms of the dose ratio(Gaddum, Hameed, Hathway & Stephens, 1955), which is the ratio of equi-active concentrations of agonist in the presence and absence of the antagonist, the measurements being made when the antagonism became steady.

IV. HISTOCHEMISTRY

The method used for the localisation of cholinesterase in tissue sections was that introduced by Koelle and Friedenwald(1949) and modified by Lewis(1961) and by Krnjevic and Silver(1965). Details of some unpublished modifications were kindly supplied by Dr. A.Silver.

The stock solutions required in this technique were: isotonic sodium sulphate (38 g/L Na_2SO_4, 10 H_2O, A.R.), fixative (10% V/V Formalin, A.R., in isotonic sodium sulphate), M/10 copper sulphate (25g/L Cu SO_4, 5 H_2O, A.R.), N/1 sodium acetate (136 g/L of hydrated salt, A.R.) and N/1 acetic acid (57 ml/L glacial acetic acid). Salts were dissolved in distilled water.

The incubation medium was prepared by dissolving 100 mg of acetylthiocholine iodide, or 110 mg of butyrylthiocholine iodide, in 4 ml distilled water and precipitating the solute with 7ml of M/10 copper sulphate. The initial portion of copper sulphate was added a drop at a time with shaking after each addition. After completion of the addition of copper sulphate, the solution was allowed to stand for 10 min before being centrifuged for 15 to 20 min at 2,000 rev/min. 10 ml of the supernatant fluid was then pipetted off and 62 mg glycine added to it. When the glycine had dissolved, sufficient N/1 sodium acetate (about 3.5 ml) was added to adjust the pH to 5.4, using a glass electrode.

The sulphide solution was made by rapidly dissolving 2 to 3 g of sodium sulphide, M.A.R., in N/5 acetic acid, 45ml of acid being used for each gram

of sodium sulphide. The pH of the final solution was checked with a glass electrode and was within the range 5 to 6. The solution was then stored in a screw-capped bottle.

Both the incubation medium and the sulphide solution were prepared the day before use and were stored in stoppered bottles at 4°C. The incubation medium was made first to avoid it reacting with sulphide vapour in the room.

To fix the tissues, the isolated oesophagus was placed in 10% Formalin for 4 hr at 4°C. It was then transferred to another bottle containing 20% V/V ethanol and kept at 4°C for 12 hr to 10 days. Small pieces of fixed tissue were frozen with carbon dioxide on a freezing microtome and sections cut at a thickness of 40 microns. The sections were transferred to a petri dish containing distilled water. 4 to 5 sections were then transferred by means of a camel hair brush into each of a number of perspex haemagglutination tray 'cups' half-filled with incubation medium. The tray was covered with a sheet of glass to prevent evaporation and to exclude dust. Sections were incubated at room temperature for 5 hr.

Some sections were placed in 0.25M solution of sodium sulphate containing DFP, 10 μg/ml, for 30 min before being incubated in a medium which contained, in addition to the substrate, the inhibitor at the concentration mentioned. Incubation of sections with butyrylthiocholine produced staining at sites of pseudocholinesterase activity only, whereas when acetylthiocholine was used as substrate, staining was produced at sites of true and pseudocholinesterase activities. DFP, 10 μg/ml, inhibited the activity of

pseudocholinesterase only.

After the incubation, the medium was carefully removed from the cups with a pipette and the sections washed twice with distilled water. Each group of sections was then transferred to another cup and washed once more with distilled water for a total time of 5 to 7 min. The water was pipetted off and replaced with sulphide solution which was left in contact with the sections for 2 to 3 min. The sulphide was then pipetted off and discarded and the cups refilled with distilled water.

To mount the sections, a very small quantity of glycerine albumen (George T.Gurr) was smeared onto a clean glass slide. Sections were transferred individually to the appropriate slide with a large camel hair brush and laid flat with the aid of a smaller brush. Excess water was removed from the slide with blotting paper and the rest allowed to evaporate in air at room temperature. When the slide was dry enough, it was placed in a beaker of absolute alcohol for 2 to 3 min and then transferred to xylene for 2 to 3 min. The sections were mounted from xylene into Neutral Mounting Medium (George T.Gurr) and were covered with clean glass cover slips.

V. STATISTICAL ANALYSIS OF RESULTS

When more than two observations were made, the values given in the text and tables refer to means with standard errors and the number of observations in parenthesis. The significance of differences between means was estimated by the "t" test(Emmens,1948) and probability (P) values are given.

A value for (P) of more than 0.05 is considered to be not significant. Regression lines were calculated by the method of least squares and the probability (P) of significant regression was computed by analysis of variance(Emmens, 1948).

Chapter 1.

PHARMACOLOGY OF THE UPPER ALIMENTARY TRACT OF THE ANAESTHETIZED FOWL

RESULTS

I. SPONTANEOUS MOVEMENTS OF THE OESOPHAGUS, CROP AND STOMACHS

The pre-crop oesophagus exhibited small rhythmic spontaneous movements. Sometimes large contractions which were about 15 times as high as the regular rhythmic contractions appeared every 8 to 10 min. The larger contractions were followed by relaxations before the smaller rhythmic movements were resumed (Fig. 3). On the other hand the crop was usually quiescent.

The stomachs (proventriculus and gizzard) contracted spontaneously but at a variable rate. The contractions were more frequent at the beginning of the experiment, and the effects of drugs and nerve stimulation were therefore studied 30 to 60 min after the dissection. The spontaneous contractions of the stomachs usually occurred every 3 to 5 min but occasionally contractions occurred every 90 sec, the proventriculus always contracting just before the gizzard. The contraction was often brief and monophasic but biphasic or triphasic contractions were also observed (Fig. 3).

II. EFFECT OF NERVE STIMULATION ON THE UPPER ALIMENTARY TRACT

Stimulation of the vagus nerve produced contractions of the pre-crop oesophagus, crop, proventriculus and gizzard, and a fall in arterial blood pressure (Fig. 4). The effects of stimulating the right or left vagi were similar. Stimulation of the right or left descending oesophageal nerve produced a contraction of the pre-crop oesophagus and crop but had no effect on the stomachs or blood pressure (Fig. 4). The contractions of the oesophagus and crop were prompt and sustained throughout the stimulation, but the onset of the response of the stomachs was delayed for about 3 sec and the initial transient contraction was usually followed by other contractions for 5 to 10 min after cessation of stimulation.

III. EFFECTS OF NERVE TRANSECTION AND CHRONIC DENERVATION

To trace pathways of efferent vagal fibres innervating the upper alimentary tract, the vagal trunk was cut at different levels and the responses to high cervical vagal stimulation recorded. Only the left vagus was cut at the thoracic level as it was the more accessible.

Transection of the recurrent branch of the vagus reduced the response of the crop, but not that of the pre-crop oesophagus, stomachs or blood pressure to high cervical vagal stimulation. Stimulation of the severed recurrent nerve produced a contraction of the crop equal in height to that produced by stimulation of the vagus before cutting its recurrent branch

but it did not have an effect on the pre-crop oesophagus or blood pressure (Fig. 5). However, when the stimuli were initially applied to the vagal trunk just cranial to the recurrent nerve the contractions of the crop were abolished after cutting the recurrent nerve(two experiments).

Transection of the vagal trunk near and cranial to the origin of the recurrent nerve abolished the response of the stomachs and blood pressure to high cervical vagal stimulation. Severing the vagus at a level about the middle of the pre-crop oesophagus abolished the response of the crop to high cervical vagal stimulation.

Contractions of the pre-crop oesophagus and crop produced by stimulation of the descending oesophageal nerve were not affected by transection of the vagus nerve at the levels described above.

Further experiments were made to find out whether the responses of the pre-crop oesophagus and crop to stimulation of the descending oesophageal nerve were partly due to stimulation of fibres contributed by the corresponding vagus nerve. In two cockerels anaesthetised with pentobarbitone sodium the left vagal trunk at the base of the skull and its branch to the glossophayngeal nerve were cut and allowed to degenerate. Three weeks after the operation the birds were anaesthetized and the effects of stimulation of the degenerate vagus nerve and the corresponding descending oesophageal nerve on the pre-crop oesophagus, crop and blood pressure were studied. The right vagus and descending oesophageal nerves were also stimulated. High cervical stimulation of the degenerate vagus produced no effect, but stimulation of the normal vagus produced contractions

of the pre-crop oesophagus and crop and a fall in arterial blood pressure. Stimulation of the descending oesophageal nerve corresponding to the degenerate vagus produced a contraction of the pre-crop oesophagus in both experiments and a contraction of the crop in one.

Additional evidence of degeneration of the cut vagus was obtained histologically. Both the intact and severed cervical vagi were removed at the end of the experiment and fixed in formol-saline. Degenerate myelin was demonstrated in sections made from the cut vagus by the Chiffelle and Putt's fat-soluble dye method and the Swank-Davenport osmium tetroxide method(Culling, 1957).

IV. EFFECTS OF DRUGS ON RESPONSES TO NERVE STIMULATION

Since the vagus and descending oesophageal nerves belong anatomically to the parasympathetic nervous system(Hsieh, 1951; Watanabe, 1960, 1964) the following experiments were made to find out whether the two nerves would behave pharmacologically as cholinergic nerves. For this purpose, pharmacological agents which are known to modify the actions of acetylcholine were used.

A. ATROPINE AND HYOSCINE

In five experiments hyoscine, 0.1 to 1 mg/kg, abolished the responses of the pre-crop oesophagus, crop, stomachs and arterial blood pressure to vagal and descending oesophageal nerve stimulation (Fig. 6). In four other experiments intravenous injection of atropine, 0.1 to 1 mg/kg, also blocked

the responses to stimulation of these nerves. The full effects of atropine and hyoscine usually developed within 5 min of injecting the drugs.

B. PHYSOSTIGMINE

The cholinesterase inhibitor, physostigmine, 0.2 mg/kg, potentiated the response of the upper alimentary tract and blood pressure to vagal or descending oesophageal nerve stimulation (four experiments). The effect of physostigmine on the duration of the responses was more pronounced than on their magnitude. Higher doses of physostigmine produced spasm of the organs which was abolished by atropine or hyoscine, 0.5 mg/kg (two experiments).

C. HEXAMETHONIUM

Hexamethonium, a ganglion blocking drug(Paton & Zaimis,1949) did not abolish the contractions of the upper alimentary tract to vagal or descending oesophageal nerve stimulation. In three experiments hexamethonium, 5 to 10 mg/kg, only partly reduced the heights of the contractions of the pre-crop oesophagus and crop; and in two other experiments, hexamethonium, 10 and 50 mg/kg, respectively, had no effect on the contractions. In two further experiments, however, hexamethonium, 10 mg/kg, potentiated the effects of vagal and descending oesophageal nerve stimulation on the pre-crop oesophagus and crop, but lowered the blood pressure and partly reduced the vasodepression produced by vagal stimulation. In both these experiments a further dose of hexamethonium, 50 mg/kg, abolished the vaso-

depression produced by vagal stimulation but had no further effect on the responses of the oesophagus and crop to vagal or descending oesophageal nerve stimulation (Fig. 7).

Contractions of the pre-crop oesophagus and crop to the ganglion stimulant tetramethylammonium, 0.25 mg/kg, were abolished by hexamethonium, 5 mg/kg(two experiments).

V. EFFECTS OF ACETYLCHOLINE, CARBACHOL AND PILOCARPINE ON THE UPPER ALIMENTARY TRACT.

The antagonism of the responses of the upper alimentary tract to vagal and descending oesophageal nerve stimulation by atropine or hyoscine and their potentiation by physostigmine showed the cholinergic nature of these nerves. The following experiments were carried out with acetylcholine and other parasympathomimetic drugs in the expectation that these drugs would mimic the actions of the neurotransmitter on the alimentary tract and blood pressure.

Intravenous injection of acetylcholine, 50 to 100 μg, or carbachol, 5 to 10 μg, produced a fall in the arterial blood pressure and contractions of the oesophagus, crop, and stomachs (five experiments). The effect of carbachol lasted longer than that of acetylcholine and was accompanied by profuse diarrhoea, but acetylcholine made the bird struggle and was sometimes fatal; thus acetylcholine was tested last in most of the experiments. Pilocarpine, 250 to 500 μg, produced effects on the alimentary canal and blood pressure similar to those produced by acetylcholine and carbachol. The fall in blood pressure produced by the drugs was followed by a rise

in blood pressure, whereas vagal stimulation produced a vasodepressor response only. Atropine, 0.5 mg/kg, abolished the effects of these drugs on the upper alimentary tract and blood pressure (three experiments).

DISCUSSION

The excitatory response to vagal stimulation of the oesophagus, crop, proventriculus and gizzard supports the anatomical evidence of the innervation of these structures(Watanabe, 1960). Stimulation of the peripheral end of the severed vagus below the vestigial diaphragm produced an increased motility of the intestines(Nolf, 1934a), suggesting that most of the alimentary tract of the domestic fowl is innervated by excitatory vagal fibres.

Stimulation of the descending oesophageal nerve produced contractions of the pre-crop oesophagus and crop but had no effect on the stomachs or blood pressure. This supports the anatomical evidence that the descending oesophageal nerve does not extend beyond the cervical part of the alimentary tract (Hsieh, 1951; Watanabe, 1964).

Since transection of the recurrent nerve did not affect the vaso-depression produced by vagal stimulation and since the blood pressure was not affected by stimulation of the recurrent nerve, the recurrent fibres to the heart described by Watanabe(1960) must have been absent or insufficiently inhibitory to affect the blood pressure. Although the response of the crop to high cervical vagal stimulation was much reduced after cutting the recurrent nerve, stimulation of the recurrent nerve produced contraction of the crop, which was similar in height to that

produced by stimulation of the vagus nerve with its recurrent branch intact. This suggests that the recurrent nerve supplied the main efferent vagal fibres to the crop.

Stimulation of the recurrent nerve did not produce an effect on the pre-crop oesophagus. This was in agreement with the finding that the recurrent nerve does not extend cranially beyond the crop(Hsieh, 1951; Watanabe, 1964) and supports the use of the term "recurrent nerve" (Fedde, Burger & Kitchell, 1963) instead of "recurrent laryngeal nerve".

The persistence of the responses of the pre-crop oesophagus and crop to stimulation of the descending oesophageal nerve following chronic degeneration of the corresponding vagus nerve indicates that the descending oesophageal nerve could function independently.

The actions of the vagus and descending oesophageal nerves on the alimentary tract and arterial blood pressure were potentiated by physostigmine and abolished by atropine or hyoscine. This suggests that these nerves supplied cholinergic fibres.

Large doses of hexamethonium did not abolish the response of the oesophagus or crop to stimulation of the vagus or descending oesophageal nerves. It is possible that some postganglionic fibres were stimulated, or, that transmission at the synapses was mediated by direct electrical coupling(Martin & Pilar, 1963 a, b) as the response to stimulation of the nerves was very rapid; alternatively synaptic transmission might be mediated by a chemical transmitter not having nicotine-like properties (Dale, 1914).

Chapter 2.

THE CHICKEN ISOLATED OESOPHAGUS

R E S U L T S

I. THE RESPONSE TO NERVE STIMULATION AND ITS MODIFICATION BY PHYSOSTIGMINE AND ANTI-ACETYLCHOLINE DRUGS

Stimulation of the vagus and descending oesophageal nerves, separately or synchronously, produced a contraction of the oesophagus within 1 to 2 sec of application of the stimulus. On prolonged stimulation, the preparation contracted for 10 to 20 sec and then partially relaxed to about half the initial response. Sometimes the response remained at this level with increased rhythmicity (Fig. 8); in other preparations a second smaller contraction developed. After cessation of stimulation, relaxation was complete within 15 sec. The responses were reproducible for over 2 hr when the nerve was stimulated for 90 sec at intervals of not less than 5 min.

A. PHYSOSTIGMINE

Exposure of the oesophagus to physostigmine, 0.1 μg/ml, for 15 min, potentiated the height of the contractions produced by 5 sec of nerve stimulation by a mean of 21.3±4.3% ($n=2$, $P<0.05$).

B. HEXAMETHONIUM AND TUBOCURARINE

Hexamethonium, 5 μg/ml, did not appreciably reduce the height of the contractions of the oesophagus to nerve stimulation after 60 min exposure to the drug (four experiments), but in six experiments exposure to hexamethonium, 50 μg/ml, for 60 min, reduced the height of the contractions by a mean of 8.5±3.0% ($P<0.05$). This mean includes the results of two experiments in which hexamethonium was without effect.

Tubocurarine, 5 or 50 μg/ml, added to the organ bath for 30 min, did not antagonize the response of the oesophagus to nerve stimulation (two experiments in each instance).

C. HYOSCINE AND ATROPINE

Exposure of the oesophagus to hyoscine or atropine, 1 μg/ml or more, for 30 min, abolished the contraction to nerve stimulation when the period of stimulation was less than 5 sec but not when the nerves were stimulated for longer periods. Hyoscine was no more effective in blocking the response to prolonged nerve stimulation when its concentration was raised to 100 μg/ml. The resistance of the contraction to hyoscine or atropine blockade was also observed in five experiments when the nerves were stimulated with 1 msec pulses at 10 c/s instead of with the usual stimulus parameters (see Methods). When the effect of hyoscine became steady the preparation contracted to nerve stimulation after a delay of 4 to 7 sec, the contraction rapidly reaching a peak (Fig. 9) which was usually lower than that of the

contraction in the absence of hyoscine. On application of a prolonged train of stimuli, the hyoscine-resistant contraction was only maintained for about 10 sec, the preparation relaxing to the baseline without exhibiting a sustained high tone or second contraction as observed in the absence of hyoscine (Fig.8).

II. FACTORS POSSIBLY INFLUENCING THE ACTION OF HYOSCINE

A. PENTOBARBITONE SODIUM AND AGE OF BIRDS

It was shown in the previous Chapter that in vivo, the contraction of the oesophagus to vagal or descending oesophageal nerve stimulation was abolished by intravenous hyoscine. Although the muscles recorded from were different *in vivo* and *in vitro*, two factors which might account for the anomaly between the effects of hyoscine in the present experiments and in the earlier in vivo experiments were that pentobarbitone sodium blocked the hyoscine-resistant response in the in vivo experiments, or that the birds used in the in vivo and in vitro experiments were different in age. To find out whether the anaesthetic and/or the maturity of the birds precluded a hyoscine-resistant oesophageal response to nerve stimulation, the in vivo experiments were repeated with unanaesthetised decerebrate birds, and hyoscine was tested on isolated preparations made from mature chickens.

In decerebrate chickens, contractions of the oesophagus to vagal and descending oesophageal nerve stimulation were abolished by intravenous injection of hyoscine, 100 μg/kg (four experiments) even when the duration of stimulation was as long as 30 or 45 sec (Fig. 10). In one of these

experiments the administration of pentobarbitone sodium, 30 mg/kg, did not affect the response of the oesophagus to stimulation of the descending oesophageal nerve although the contraction was subsequently abolished by hyoscine, 100 μg/kg.

On three isolated oesophageal preparations made from 10-month old cockerels hyoscine, 100 μg/ml, abolished the contraction produced by nerve stimulation of short duration (3 to 5 sec), but a hyoscine-resistant contraction was produced on prolonged stimulation.

Thus it seemed that the appearance of a hyoscine-resistant response of the oesophagus to nerve stimulation was associated with the in vitro preparations only. Three possibilities were thought likely to account for this phenomenon. First, the oesophageal wall may be stimulated directly by passage of current through the bathing solution or through the plain muscle of the jugular vein which was encircled by the stimulating electrodes; second, hyoscine added to the organ bath may not diffuse to all the receptors responding to acetylcholine released from the nerves; and third, it is possible that a hyoscine-resistant neurotransmitter was released together with acetylcholine. To examine these possibilities the following experiments were carried out in the continuous presence of hyoscine, 100 μg/ml.

B. DIRECT STIMULATION OF THE PLAIN MUSCLE

To ascertain that the oesophageal wall was not stimulated directly by passage of current through the bathing solution or jugular vein, the

preparation was exposed to cocaine or the nerves were cut near the oesophagus and the stimulation repeated. Furthermore, in some experiments the vagus and descending oesophageal nerve were dissected free from the vein and all other tissues before stimulation.

Exposure of the preparation to cocaine, 50 μg/ml, for 30 min, abolished the hyoscine-resistant response to nerve stimulation (three experiments). Similarly after cutting the nerves the preparation no longer contracted to nerve stimulation in the presence of hyoscine whereas it still contracted to potassium chloride (three experiments).

Prolonged stimulation of the vagus (four experiments) or descending oesophageal nerve (three experiments) produced a hyoscine-resistant contraction of the oesophagus. However, the response to vagal stimulation in the presence of hyoscine was not as steadily reproducible as that produced by stimulation of the descending oesophageal nerve or the two nerves together.

C. DIFUSSION BARRIER

The possibility that hyoscine failed to reach receptors responding to acetylcholine released from the nerves was investigated by exposing the preparation to a combination of hyoscine and hyaluronidase, an enzyme which promotes the spread of drugs into tissues; and by testing hyoscine on a thinner preparation of the separated muscularis externa with nerve attached. Moreover, hyoscine was injected intravenously into chicks from which isolated nerve-oesophagus preparations were subsequently made.

In four experiments hyoscine-resistant contractions of the oesophagus

to nerve stimulation were still obtained after exposure of the preparation to hyaluronidase, 0.3 to 30 i.u./ml, for 15 to 60 min.

Similarly, contractions of the separated external muscle to nerve stimulation resisted complete block by hyoscine (four experiments).

Furthermore, when hyoscine, 100 μg/g, was injected intravenously into six chicks 5 to 10 min before making isolated nerve-oesophagus preparations, the preparations contracted to nerve stimulation when they were made although acetylcholine, 10 μg/ml, did not produce contractions until 1 hr or more had elapsed. Three of these chicks had been anaesthetized with halothane and a fourth was killed with halothane before making the nerve-oesophagus preparations.

D. RELEASE OF A NON-CHOLINERGIC SUBSTANCE

This possibility was investigated by testing antagonists of some naturally occurring substances on the hyoscine-resistant response, and by attempting to detect a pharmacologically active substance in the bathing solution.

Exposure of the oesophagus to mepyramine (0.1 to 10 μg/ml, for 30 min, three experiments) or to methysergide (0.1 to 10 μg/ml, for 30 min, two experiments) which are antagonists of histamine and 5-hydroxytryptamine, respectively, had no effect on the hyoscine-resistant response to nerve stimulation. Similarly bretylium, which blocks responses of isolated tissues to sympathetic nerve stimulation(Boura & Green, 1959), did not antagonize the hyoscine-resistant response of the oesophagus to nerve

stimulation after 90 min exposure to 10 μg/ml of the drug (three experiments). Furthermore, 30 min exposure to 10 μg/ml of meclofenamic acid, which antagonizes the actions of kinins and related substances on guinea-pig lungs(Collier & James, 1967), did not affect the hyoscine-resistant contractions of the oesophagus to nerve stimulation (four experiments).

In five experiments, isolated oesophageal preparations were made to contract to nerve stimulation in the presence of hyoscine, 100 μg/ml, the train of stimuli being applied continuously for 30 min. In three of these experiments a piece of isolated guinea-pig ileum was suspended in the same organ bath as the nerve-oesophagus preparation, pieces of post-crop chick oesophagus being suspended in the organ bath with the other two nerve-muscle preparations. Neither the guinea-pig ileum nor the post-crop chick oesophagus contracted during this prolonged nerve stimulation.

III. CHOLINESTERASE STAINING

The inability of hexamethonium or tubocurarine to abolish the contraction of the oesophagus to nerve stimulation raised the question of the presence or absence of ganglia in the innervation of this organ. However, histochemical staining of cholinesterases has shown that Auerbach's plexus is present in the external muscle layers of the chick oesophagus, although no ganglia could be demonstrated in the mucosa in this way(Hassan, 1966). This was probably due to a masking of the staining attributable to mucosal

true cholinesterase by the dense staining produced by the activity of pseudocholinesterase which was present in the muscularis mucosae and mucosal glands.

In the present experiments sections were incubated with acetylthiocholine after treatment with DFP, 10 μg/ml, which inhibits pseudocholinesterase(Krenjevic & Silver, 1965), so that staining due to mucosal true cholinesterase, and thus ganglia, would become apparent. Control sections were incubated with acetylthiocholine alone.

In sections incubated with acetylthiocholine and DFP, a few ganglia were demonstrated in the muscularis mucosae (Fig. 11a), but in sections incubated with acetylthiocholine alone ganglia could not be demonstrated in this layer as the staining due to true cholinesterase activity was masked by the heavier staining attributable to pseudocholinesterase activity (Fig. 11b). Sections incubated with butyrylthiocholine alone confirmed the presence of pseudocholinesterase in the muscularis mucosae and glands (Fig. 12).

DISCUSSION

The contractions of the isolated oesophagus to nerve stimulation for a short period (5 sec or less) were potentiated by physostigmine and abolished by hyoscine or atropine, suggesting the release of an acetylcholine-like substance from the nerves.

The contractions of the isolated oesophagus to prolonged nerve stimulation in the presence of hyoscine could not have been due to passage of

current through the plain muscle of the jugular vein which was encircled by the stimulating electrodes since a hyoscine-resistant contraction was observed on stimulation of either nerve dissected free from contiguous tissues. Nor could the effect have been due to passage of current through the bathing solution, since a response to nerve stimulation was not obtained after cutting the nerves or exposure to cocaine. The response did not seem to be due to stimulation of adrenergic fibres for it was not affected by bretylium. Similarly, it is unlikely that the hyoscine-resistant contraction was due to the release of histamine, 5-hydroxytryptamine or bradykinin since it was not affected by antagonists of these substances.

In decerebrate chickens, contractions of the oesophagus to nerve stimulation were abolished by intravenous hyoscine. These preparations were left for 1 hr after brief anaesthesia with halothane for the anaesthetic to be removed before recording contractions of the oesophagus. Thus it seems improbable that pentobarbitone sodium masked a hyoscine-resistant response to nerve stimulation in the previous in vivo experiments (Chapter 1.), although it has been reported that some barbituric acid derivatives block some effects of vagal stimulation on the cat's heart and alimentary tract in vivo(Garry, 1930; Brown & Garry, 1932).

Hyoscine-resistant contractions were obtained from isolated oesophageal preparations made from the adult fowl, showing that a difference in age of the birds did not account for the difference in effectiveness of hyoscine in the in vitro and in vivo experiments.

It is improbable that the slow hyoscine-resistant contraction of the oesophagus to nerve stimulation was produced by direct electrical coupling, since ephaptic transmission is very rapid (Martin & Pilar, 1963a). A more plausible possibility is that hyoscine did not reach all the receptors stimulated by acetylcholine released from nerves in the isolated chicken oesophagus, for, it has been shown that some drugs only combine with receptors in the muscularis mucosae of isolated guinea-pig oesophagus after separation of the external muscle from the mucosa(Bartlet, 1968b,c). However, hyoscine abolished the contractions of the chick oesophagus produced by physostigmine, which apparently stimulated neural structures since its action was blocked by cocaine(Bartlet & Hassan, 1968). Thus in these experiments hyoscine seemed effective in antagonising acetylcholine released from neural structures. Nevertheless, hyoscine did not abolish the contractions to nerve stimulation of a thin separated external muscle preparation made from the chick oesophagus. Moreover, intravenous injection of large doses of hyoscine into chicks before isolation of the nerve-oesophagus preparation was ineffective in abolishing the contraction to nerve stimulation, and exposure of the oesophagus to hyaluronidase, an enzyme which promotes the spread of drugs into tissues, did not alter the effect of hyoscine on responses to nerve stimulation. Thus it is hard to reconcile these results with the assumption that hyoscine failed to reach all the receptors responding to acetylcholine.

It seemed possible that the hyoscine-resistant contraction was due to the release of a slow contracting substance from the nerves together with

acetylcholine. A similar view has recently been put forward by Ambache & Freeman(1969) from their experiments with the separated longitudinal muscle of guinea-pig ileum stimulated transmurally. It might be that in vivo a slow contracting substance could be removed from the vicinity of the nerve terminals by the circulation before it contracted the oesophagus. In vitro, a slow contracting substance released from nerves would only be removed by diffusion and/or metabolism, and if these effects occurred slowly, the substance might accumulate in sufficient amount to produce a contraction. However, pieces of guinea-pig ileum or chick oesophagus did not contract when suspended in the same organ bath as the nerve stimulated chick oesophagus, so that no slow contracting substance could be detected in this way.

It is customary to classify cholinesterases into "true" and "pseudo" cholinesterases(Mendel & Rudney, 1943; Mendel, Mundell & Rudney, 1943). In mammals both enzymes hydrolyse acetylcholine; in addition, true cholinesterase hydrolyzes acetyl-β-methylcholine but not benzoylcholine or butyrylcholine, whereas pseudocholinesterase hydrolyzes benzoylcholine and butyrylcholine but not acetyl-β-methylcholine. Although chicken pseudocholinesterase has been reported to show some differences to the corresponding mammalian enzyme regarding substrate and inhibitor specificity(Earl & Thompson, 1952; Myers, 1953; Blaber & Cuthbert, 1962) the present experiments show that DFP inhibited pseudocholinesterase and not true cholinesterase of this species. Thus in the present experiments DFP could be used to restrict the histochemical staining of cholinesterases

by the thiocholine method to sites of true cholinesterase activity.

Staining of sections of oesophagus for true cholinesterase demonstrated the presence of ganglia in both the externa and the mucosa. Thus the failure of hexamethonium and tubocurarine to abolish the contractions of the oesophagus to nerve stimulation both in vivo and in vitro can not be attributed to an absence of ganglia in the preparation. It is possible that the stimulated nerve fibres do not synapse at the intramural ganglia of the preparation, for, the vagal fibres innervating the external muscle of guinea-pig oesophagus do not synapse with the intramural ganglia of Auerbach's plexus(Bartlet, 1968a). Alternatively, transmission at the synapses may be effected by a substance not having a nicotinic action, or hexamethonium and tubocurarine may not reach ganglionic receptors in the preparations.

According to Bowman & Everett (1964) atropine (0.02 μg/ml and above) abolished the contractions of the chick isolated oesophagus to parasympathetic nerve stimulation. In their experiments the nerves were stimulated with rectangular pulses of 0.5 or 1 msec duration, whereas in the present experiments 10 msec pulses were usually used. However this difference in pulse width does not account for the difference in block by atropine, for, in some of the present experiments in which the pulse width was reduced to 1 msec duration, an atropine-resistant contraction was still obtained. In the experiments of Bowman and Everett(1964) hexamethonium (2 to 4 μg/ml) and other drugs which block ganglia produced a 50 to 80% depression of the height of contraction of the chick isolated oesophagus

produced by vagal stimulation. Moreover, Everett (1966) assumed that the addition of hexamethonium (4 μg/ml) to the Krebs' solution bathing the oesophagus restricted the effective transmural stimulation to the post-ganglionic fibres. This is at variance with the present results where hexamethonium in a concentration of less than 50 μg/ml failed to produce a significant antagonism of the response to vagal stimulation. No plausible explanation can be suggested for these differences in the actions of hexamethonium and atropine as observed by Bowman and Everett(1964) and in the present experiments.

Chapter 3.

SELECTION OF DRUGS FOR BLOCKING ADRENERGIC NEURONES AND α- AND β-RECEPTORS

Some anti-adrenaline drugs were tested on isolated tissues to find concentrations which blocked α- or β-receptors without producing a nerve block and with little or no antagonism of receptors for other agonists. The adrenergic neurone blocking drug, bretylium(Boura & Green, 1959), was similarly tested to find a concentration which blocked sympathetic nerves only. These experiments were carried out with a view to using the blocking drugs in experiments with the chick isolated nerve-rectum preparation.

RESULTS

I. PROPRANOLOL AND MJ-1999

A. THE β-RECEPTOR BLOCKING ACTIONS OF PROPRANOLOL AND MJ-1999

To find out which of the two drugs was the more potent β-receptor antagonist in vitro, their relative potencies as antagonists of isoprenaline relaxations of the rectum were determined. The dose ratios for isoprenaline in the presence of propranolol or MJ-1999 (Table 1) have been plotted on the ordinate of a graph (Fig.13), concentrations of the antagonists being plotted on the abscissa. According to the calculated

regression lines propranolol was 550 times as potent as MJ-1999 in blocking β-receptors since the dose ratio for isoprenaline was 100 in the presence of propranolol, 4 ng/ml, or MJ-1999, 2.2 μg/ml.

B. NERVE BLOCKING ACTIONS OF PROPRANOLOL AND MJ-1999

MJ-1999, unlike propranolol, is reported to lack local anaesthetic action(Lish, Weikel & Dungan, 1965). To find out whether MJ-1999 could be used for anatagonising the activity of a neurotransmitter at β-receptors without producing a nerve block, the drug was compared with propranolol and procaine as a blocking agent against contractions of the guinea-pig vas deferens to hypogastric nerve stimulation.

Both MJ-1999 and propranolol antagonized the contractions of the vas deferens to hypogastric nerve stimulation. The nerve blocking actions of procaine, propranolol and MJ-1999 are depicted in Fig.4, which shows that a 60% reduction in the height of the contraction was produced by 20 min exposure to procaine, 57 μg/ml, propranolol, 5.4 μg/ml, or MJ-1999, 142 μg/ml. Thus propranolol was about 10 times more potent than procaine and 26 times more potent than MJ-1999 in blocking the hypogastric nerve.

If MJ-1999 blocked the hypogastric nerve through a local anaesthetic action, it might anaesthetize the rabbit cornea; thus MJ-1999 was compared as a surface anaesthatic with propranolol and cocaine on the rabbit eye. The effects of the three drugs on the corneal reflex of the rabbit eye are shown in Fig.5, which shows that propranolol (0.2% W/V) and cocaine (2% W/V)

anaesthatized the cornea to a similar degree within 5 min of instillation whereas MJ-1999 tested at 50 times the concentration selected for propranolol had no effect on the cornea.

The results with the rabbit eye threw some doubt as to whether the hypogastric nerve blocking action of MJ-1999 was due to a local anaesthetic action. Thus the experiments with the guinea-pig vas deferens were extended to investigate the site of action of propranolol and MJ-1999 in blocking responses to stimulation of the hypogastric nerve. Concentrations of the two drugs which produced 65-85% reduction in the height of contraction in the previous experiments were used. Preganglionic stimulation of the hypogastric nerve was alternated with postganglionic transmural stimulation(Birmingham & Wilson, 1963) before exposing the vas deferens to MJ-1999 or propranolol.

Exposure of the vas deferens to propranolol, 10 μg/ml, for 20 min, depressed contractions to both hypogastric and transmural stimulation (three experiments) (Fig.16a). However, in four experiments 20 min exposure to propranolol, 5 μg/ml, potentiated or did not affect contractions of the vas deferens to noradrenaline. In two experiments additional exposure to propranolol, 20 μg/ml, did not reduce the height of the contractions to noradrenaline below that of the control responses to the drug (Fig.16b). MJ-1999, 200 μg/ml, produced a marked inhibition of the contractions of the vas deferens to hypogastric nerve stimulation but the contractions to transmural stimulation were hardly affected by the drug (three experiments) (Fig.17).

The dissimilarity between the effects of propranolol and MJ-1999 on the response of the vas deferens to transmural stimulation suggests that the drugs blocked the response of the preparation to hypogastric nerve stimulation through acting at different sites on the nervous pathway. To confirm this suggestion, propranolol and MJ-1999 were tested on the guinea-pig isolated oesophagus contracting to vagal stimulation.

The guinea-pig oesophagus responds to stimulation of the vagus nerve with a twitch followed by a sustained contracture(Bartlet, 1968a). Exposure of the oesophagus to propranolol, 10 μg/ml, for 20 min almost abolished both components of the response of the preparation to vagal stimulation, the per cent reduction of the response being 72±14% (three experiments). However, 20 min exposure of the preparation to MJ-1999, 200 μg/ml, antagonized the contracture but not the twitch, the height of the contracture being reduced by 18±3.5% (n=2, P<0.02). Fig.18(a) and (b) illustrates these effects of propranolol and MJ-1999, respectively, on the response of the guinea-pig oesophagus to vagal stimulation.

C. EFFECTS OF PROPRANOLOL AND MJ-1999 ON CONTRACTIONS OF THE CHICK RECTUM TO ACETYLCHOLINE AND HISTAMINE

The unspecific blocking effects of propranolol and MJ-1999 on receptors for acetylcholine and histamine were studied as a final step in evaluating the comparative usefulness of these drugs.

The data in Table 1 shows that propranolol, 0.05 μg/ml, and MJ-1999,

50 μg/ml, antagonized isoprenaline acting on the chick rectum to a similar degree, so these concentrations of the drugs were compared for antagonism of acetylcholine and histamine. Contractions of the rectum to acetylcholine or histamine were not antagonized by propranolol, 0.05 μg/ml (Table 2). However, when the concentration of propranolol was raised 10 times to 0.5 μg/ml it produced a slight but not statistically significant antagonism of both acetylcholine and histamine, the dose ratio for acetylcholine being 1.5±0.3 (n=2, P>0.2) and that for histamine 1.9±0.3 (n=2, P>0.05). On the other hand MJ-1999, 50 μg/ml, significantly antagonized acetylcholine and histamine, the dose ratio for acetylcholine being 5.2± 1.3 (n=3, P<0.05) and that for histamine 2.1±0.1 (n=2, P<0.01). When the concentration of MJ-1999 was lowered to 5 μg/ml the drug still produced a slight but statistically not significant antagonism of acetylcholine and histamine, the dose ratio for acetylcholine being 1.3± 0.2 (n=3, P>0.2) and that for histamine 1.3±0.1 (n=2, P>0.05).

II. PHENTOLAMINE AND TOLAZOLINE

A. THE α-RECEPTOR BLOCKING ACTIONS OF PHENTOLAMINE AND TOLAZOLINE

The potency of these drugs in antagonizing noradrenaline acting on the rat seminal vesicle was determined. Phentolamine, 1 μg/ml, and tolazoline, 100 μg/ml, were equipotent in antagonizing the contraction of the rat seminal vesicle produced by noradrenaline (10 μg/ml) (Table 1).

B. EFFECTS OF PHENTOLAMINE AND TOLAZOLINE ON RESPONSES TO NERVE STIMULATION

Exposure to phentolamine, 10 μg/ml, for 20 min, had no effect on the contractions of the guinea-pig isolated vas deferens to hypogastric nerve stimulation or oesophagus to vagal stimulation. However, although tolazoline, 100 μg/ml, had no effect on the oesophageal contractions to vagal stimulation, it potentiated markedly the contractions of the vas deferens to hypogastric nerve stimulation (Table 3).

C. EFFECTS OF PHENTOLAMINE AND TOLAZOLINE ON CONTRACTIONS OF THE CHICK RECTUM TO ACETYLCHOLINE AND HISTAMINE

Contractions of the chick rectum produced by acetylcholine or histamine were antagonized by phentolamine, 1 μg/ml (Table 2), the antagonism of histamine being significant ($P<0.02$). Phentolamine, 0.1 μg/ml, appeared to antagonise histamine, but the antagonism was not significant ($P>0.3$). Tolazoline, 100 μg/ml, which was equipotent with phentolamine, 1 μg/ml, in antagonising noradrenaline acting on the rat seminal vesicle, did not antagonise acetylcholine acting on the chick rectum (Table 2), but it antagonized histamine significantly ($P<0.05$). Exposure of the rectum to tolazoline, 10 μg/ml, also antagonized histamine significantly ($P<0.02$).

III. BRETYLIUM

A. THE ADRENERGIC NEURONE BLOCKING ACTION OF BRETYLIUM

The sympathetic nerve blocking action of bretylium was studied on contractions of the guinea-pig isolated vas deferens to hypogastric nerve stimulation. Bretylium was also tested on contractions of the guinea-pig isolated oesophagus to vagal stimulation to ascertain that a concentration of the drug which was effective in blocking the adrenergic neurone was free from cholinergic neurone blocking activity. The height of the contraction of the vas deferens was reduced significantly after 20 min exposure to bretylium, 2 or 10 μg/ml, but these concentrations of the drug had no effect on the responses of the oesophagus to vagal stimulation (Table 3). Bretylium, 20 μg/ml, slightly inhibited the responses of the oesophagus to vagal stimulation, however.

B. EFFECT OF BRETYLIUM ON CONTRACTIONS OF THE CHICK RECTUM TO ACETYLCHOLINE AND HISTAMINE

Exposure of the chick rectum to bretylium, 10 μg/ml, for 20 min, did not antagonize the contractions to acetylcholine (five experiments) or histamine (three experiments).

DISCUSSION

I. PROPRANOLOL AND MJ-1999

The local anaesthatic action of propranolol presents a problem when the drug is used to investigate the possible action of a neurotransmitter at β-receptors. MJ-1999 which is free from local anaesthetic action(Lish et al, 1965) might antagonize the action of a neurotransmitter at β-receptors without blocking its release from the nerves. However, although propranolol was 26 times as potent as MJ-1999 in blocking the hypogastric nerve of the guinea-pig, it was 550 times as potent in antagonizing isoprenaline acting on the chick rectum. Thus propranolol seemed to be the drug which was most likely to block β-receptors without producing a nerve block.

MJ-1999 seemed to block ganglia, for, it blocked responses of the guinea-pig vas deferens to stimulation of the preganglionic hypogastric nerve without affecting responses to postganglionic stimulation. Moreover, the action of MJ-1999 resembled that of hexamethonium(Bartlet, 1968a) in that both drugs antagonized the contracture but not the twitch of the guinea-pig oesophagus produced by vagal stimulation.

MJ-1999 (50 μg/ml) antagonized acetylcholine and histamine acting on the chick rectum. On the other hand propranolol (0.05 μg/ml) did not antagonize acetylcholine or histamine on the rectum although it was equipotent with MJ-1999 (50 μg/ml) in blocking isoprenaline relaxations of the preparation. Thus the β-receptor blocking action of propranolol (0.05 μg/ml) was preferable to that of MJ-1999 (50 μg/ml) since the latter drug

was the more likely to antagonize acetylcholine, histamine and nervous transmission.

II. PHENTOLAMINE AND TOLAZOLINE

Tolazoline (10 μg/ml) and phentolamine (0.1 μg/ml) antagonized the action of noradrenaline on the rat seminal vesicle to about the same degree, but tolazoline (10 μg/ml) antagonized the action of histamine on the chick rectum ($P<0.02$) whereas phentolamine (0.1 μg/ml) did not ($P>0.3$). Moreover, tolazoline (100 μg/ml) markedly potentiated the response of the guinea-pig vas deferens to hypogastric nerve stimulation, making it an unsuitable drug for antagonizing the action of a neurotransmitter at α-receptors. Phentolamine (10 μg/ml) did not have such an effect on the response of the vas deferens to hypogastric nerve stimulation, however, and was therefore selected for blocking the α-receptors of the chick rectum.

II. BRETYLIUM

Bretylium (2 or 10 μg/ml) blocked responses of the guinea-pig vas deferens to hypogastric nerve stimulation without having an effect on responses of the oesophagus to vagal stimulation or antagonising the responses of the chick rectum to acetylcholine and histamine. A higher concentration of bretylium (20 μg/ml) slightly blocked the vagus nerve, however. Thus bretylium (10 μg/ml) seemed to produce a specific blockade of the responses of isolated preparations to adrenergic nerve stimulation.

Chapter 4.

THE CHICK ISOLATED RECTUM WITH REMAK NERVE ATTACHED

The response of the chicken isolated oesophagus to parasympathetic nerve stimulation resists complete block by anti-muscarine or ganglion blocking drugs. The question arose as to whether responses to nerve stimulation of other parts of the chicken alimentary tract are also resistant to blockade by antagonists of acetylcholine and noradrenaline. The chick rectum was chosen for comparison with the oesophagus as it represents the other end of the alimentary tract and has a parasympathetic extrinsic innervation contained in the Remak nerve. Moreover, since the Remak nerve contains sympathetic fibres(Nolf, 1934b), studies with the nerve-rectum preparation have shown whether responses to sympathetic nerve stimulation are atypical in this species.

R E S U L T S

I. RESPONSES OF THE RECTUM TO NERVE STIMULATION, ACETYLCHOLINE AND NORADRENALINE

On stimulating the Remak nerve the rectum contracted after a delay of 1 to 3 sec. The height of the contraction reached a peak after 10 to 15 sec of stimulation and then declined to the baseline. However, when the tone of the preparation was raised by acetyl-β-methylcholine, 2 μg/ml, or

by hyoscine, 100 μg/ml, stimulation of the Remak nerve produced a transient contraction followed by a relaxation which lasted until the cessation of the stimulation. The rectum contracted to acetylcholine, 0.05 to 0.1 μg/ml, and relaxed to noradrenaline, 0.1 to 1 μg/ml.

II. PHARMACOLOGY OF THE CONTRACTION OF THE RECTUM TO REMAK NERVE STIMULATION

The Remak nerve contains fibres originating from the sacral and coeliac plexuses(Nolf, 1934b); thus it seemed likely that the contraction of the rectum was due to stimulation of cholinergic fibres contributed by the sacral plexus since the preparation also contracted to acetylcholine. The following experiments investigated the possible cholinergic nature of the neurotransmitter producing contraction of the rectum to Remak nerve stimulation.

A. EFFECT OF HYOSCINE

Exposure of the preparation to hyoscine, 1 μg/ml(two experiments) or 10 μg/ml(four experiments), for 45 min, did not antagonise the contraction to nerve stimulation. Hyoscine, 100 μg/ml, antagonized the contraction, however, the antagonism usually being fully developed after 30 min exposure to the drug when the contraction became briefer and slower in onset(six experiments). The effect of hyoscine, 100 μg/ml, on the height of the contraction was variable; in three of the experiments the height of

the contraction became so small as not to be readily distinguishable from the spontaneous movements of the preparation (Fig.19a), but in the other three experiments it was only slightly depressed (Fig.19b). Moreover, hyoscine, 100 μg/ml, raised the tone of the preparation in five of the six experiments, and when this occurred, nerve stimulation produced a transient contraction followed by a relaxation (Fig.19a).

B. EFFECTS OF HEXAMETHONIUM AND TUBOCURARINE

Hexamethonium, 5 μg/ml, reduced the height of the contraction of the rectum to Remak nerve stimulation (two experiments). In four experiments hexamethonium, 10 μg/ml, produced a slight rise in the tone of the preparation and abolished the contraction; but in a fifth experiment the contraction to nerve stimulation was not affected after 45 min exposure to the drug at this concentration. In those experiments where hexamethonium abolished the contraction, 30 sec stimulation of the nerve (10 msec, 20 c/s) produced a relaxation (two experiments) or had no effect (two experiments).

Exposure of the rectum to tubocurarine, 10 μg/ml, for 10 to 20 min, abolished the contraction to nerve stimulation (five experiments). Tubocurarine raised the tone of these preparations and when the nerve was stimulated for 30 sec (10 msec, 20 c/s) the preparations relaxed.

III. PHARMACOLOGY OF THE RELAXATION OF THE RECTUM TO REMAK NERVE STIMULATION

In experiments where relaxations of the rectum to Remak nerve stimulation were studied, the tone of the preparations was raised by continuous exposure to acetyl-β-methylcholine, 2 μg/ml.

As the Remak nerve contains fibres originating at the coeliac plexus (Nolf, 1934b) and since noradrenaline produced a relaxation of the rectum, it seemed likely that the relaxation of the preparation to nerve stimulation was due to a release of catecholamines from sympathetic fibres. This possibility was investigated by exposing preparations to phentolamine (which blocks α-receptors), propranolol (a β-receptor antagonist) and to bretylium, an adrenergic neurone blocking drug(Boura & Green, 1959). In addition, a study was made of the responses of preparations made from chicks pretreated with reserpine, which depletes tissues of their catecholamine content(Muscholl & Vogt, 1958).

A. ACTION OF AGONISTS AND ANTAGONISTS ON THE SYMPATHOMIMETIC RECEPTORS OF THE CHICK RECTUM

Before attempting to block the action of the neurotransmitter at the sympathetic receptor sites, it was necessary to investigate the presence of α- and/or β-receptors in the chick rectum. Phentolamine, 0.1 μg/ml, and

propranolol, 0.05 μg/ml, were used to block α- and β-receptors, respectively, in accordance with the results obtained in Chapter 3. The two antagonists were tested on rectal relaxations to phenylephrine, which is reported to stimulate the α-receptor only, and to isoprenaline, the β-receptor stimulant(Ahlquist & Levy, 1959) as well as to noradrenaline.

Phenylephrine (0.5 to 1 μg/ml), isoprenaline (0.5 to 5 ng/ml) or noradrenaline (0.1 to 1 μg/ml) produced matching relaxations of the rectum, the three amines always being tested on each preparation. The onset of the relaxations were prompt and the preparation regained its tone within 1 to 2 min of washing out noradrenaline or phenylephrine, recovery from isoprenaline being slightly more delayed.

In five experiments 20 to 60 min exposure to phentolamine, 0.1 μg/ml, did not antagonize the relaxations of the rectum produced by phenylephrine, noradrenaline or isoprenaline, but propranolol, 0.05 μg/ml, antagonized the relaxations produced by the three sympathomimetic amines. Fig. 20 shows the lack of effect of phentolamine, 0.1 μg/ml, on the response of the rectum to phenylephrine and its antagonism by subsequent exposure to propranolol, 0.05 μg/ml. In six experiments 20 min exposure to propranolol, 0.05 μg/ml, abolished the relaxations produced by isoprenaline and reduced the relaxations produced by noradrenaline and phenylephrine by 71±10% and 82±7% respectively, the residual responses to the amines being briefer than before exposure to propranolol. These mean values include the results of two experiments in which the effects of noradrenaline and phenylephrine were abolished by

propranolol, 0.05 μg/ml (Fig.21). In the four experiments where propranolol-resistant relaxations to noradrenaline and phenylephrine were obtained, increasing the concentrations of the amines about 100 fold did not increase the size of the propranolol-resistant relaxations. In the six experiments, the dose ratios for isoprenaline, noradrenaline and phenylephrine in the presence of propranolol, 0.05 μg/ml, were 1667±366, 238±40 and 1382±647, respectively. Comparison of these dose ratios by Students "t" test has shown that the antagonism of isoprenaline by propranolol, 0.05 μg/ml, was significantly greater than that of noradrenaline ($t_{calc.}$ = 3.865, n = 10, P<0.01), but was not significantly different from that of phenylephrine ($t_{calc.}$ = 0.382, n = 10, P>0.7).

The propranolol-resistant relaxations of the rectum produced by noradrenaline and phenylephrine were abolished by additional exposure to phentolamine, 0.1 μg/ml (four experiments).

These results suggested that the sympathomimetic receptors in the chick rectum are mainly of the β-type, and that the α-type forms such a small proportion of the total number of these receptors that the response to their stimulation becomes apparent only when the β-receptors have been blocked. Thus it was decided that propranolol was the adrenaline antagonist which was most likely to block the relaxation produced by stimulation of the Remak nerve, and was therefore the drug first tested.

B. EFFECTS OF PROPRANOLOL AND PHENTOLAMINE ON RELAXATIONS OF THE RECTUM TO REMAK NERVE STIMULATION

The rectum was made to relax by stimulation of the Remak nerve or with noradrenaline, the tone of the preparation being raised with acetyl-β-methylcholine, 2 μg/ml. Exposure of the preparation to propranolol, 0.05 μg/ml, for 20 min, reduced the relaxations to nerve stimulation by a mean of 22±4% (n = 3, P<0.02) but had no effect on the relaxations produced by noradrenaline. In four further experiments a combination of propranolol, 0.05 μg/ml, and phentolamine, 0.1 μg/ml, abolished the relaxation to nerve stimulation and almost abolished that to noradrenaline, the height of the initial contraction to nerve stimulation being unaffected although its duration was prolonged (Fig.22).

As propranolol markedly antagonized noradrenaline in the earlier experiments where noradrenaline was tested on preparations which were also exposed to phenylephrine and isoprenaline, the failure of propranolol to antagonize noradrenaline when the amine was alternated with stimulation of the nerve seemed to require some further investigation.

Acetyl-β-methylcholine was present in the earlier series of experiments but absent in the later experiments where the nerve was stimulated, so it seemed possible that acetyl-β-methylcholine affected the antagonism of noradrenaline. Thus the experiments with noradrenaline, phenylephrine and isoprenaline were repeated on preparations suspended in Krebs' solution

containing acetyl-β-methylcholine, 2 μg/ml, to investigate this possibility. In three experiments exposure to propranolol, 0.05 μg/ml, abolished the relaxation of the rectum to isoprenaline, and reduced the size of the relaxations to noradrenaline and phenylephrine, the per cent inhibition of the relaxations being 75±7% for noradrenaline and 78±8% for phenylephrine. Propranolol antagonized noradrenaline to the same degree in the presence or absence of acetyl-β-methylcholine, showing that the choline ester did not affect the antagonism of noradrenaline by propranolol.

Some further experiments were made to find out whether phenylephrine and isoprenaline made the relaxation of the rectum to noradrenaline and Remak nerve stimulation more sensitive to block by propranolol. Rectal preparations were made to relax to Remak nerve stimulation and to phenylephrine, isoprenaline and noradrenaline in the continuous presence of acety-β-methylcholine, 2 μg/ml. In five experiments exposure of the preparations to propranolol, 0.05 μg/ml, always abolished the response to isoprenaline, and reduced the responses to phenylephrine, noradrenaline and nerve stimulation by a mean of 65±11%, 56±12% and 76±10%, respectively, the response to nerve stimulation being abolished in two of the experiments. In the present experiments where the rectum was relaxing to noradrenaline, isoprenaline, phenylephrine and nerve stimulation the per cent reduction in the size of the noradrenaline relaxation produced by propranolol, 0.05 μg/ml, was not different from that in the earlier series of experiments in which the preparation relaxed to the three sympathomimetic amines only ($t_{calc.}$ = 0.094, n=9, P>0.9). Thus isoprenaline and phenylephrine did make the

relaxation of the rectum to noradrenaline more sensitive to block by propranolol, regardless of whether or not the Remak nerve was stimulated. Furthermore, the redcution in the size of the relaxation to nerve stimulation produced by propranolol, 0.05 μg/ml, was significantly greater in the experiments where phenylephrine, isoprenaline and noradrenaline were tested than in those where only noradrenaline was alternated with nerve stimulation ($t_{calc.}$=4.505, n=7, P<0.01).

C. EFFECT OF BRETYLIUM

Exposure of the rectum to bretylium, 10 μg/ml, gradually inhibited the relaxations to nerve stimulation. In four experiments the size of the relaxation was reduced by 65.5±2.9% (P<0.001) after 2 hr exposure to bretylium, 10 μg/ml, but the height of the initial transient contractions was hardly affected by the drug at this concentration (Fig.23).

D. EFFECT OF RESERPINE

Reserpine, 5 μg/g, was injected intramuscularly in five chicks on two successive days, the second dose of the drug being injected 24 hr before isolation of a nerve-rectum preparation. Three of the reserpinized preparations responded to Remak nerve stimulation with a contraction only, and did not relax when the duration of stimulation was as long as 1 min (Fig.24). The other two reserpinized preparations responded to 1 min of nerve stimulation with a contraction, which was sustained for 45 sec, followed by a relaxation. In five control preparations nerve

stimulation produced a brief contraction, which was never sustained for more than 15 sec, followed by a relaxation.

DISCUSSION

Since the Remak nerve contains parasympathetic fibres originating at the sacral plexus(Nolf, 1934b) and acetylcholine contracts the rectum, the contraction of the preparation to stimulation of the nerve is likely to be due to stimulation of cholinergic fibres. However, the contraction to nerve stimulation of the isolated rectum, like that of the chick isolated oesophagus, was not abolished by hyoscine. Thus the responses of both isolated extremities of the alimentary tract of the domestic fowl to stimulation of extrinsic parasympathetic nerves are resistant to complete block by hyoscine; this phenomenon has been discussed before in Chapter 2.

Hexamethonium and tubocurarine usually abolished the contractions of the rectum to Remak nerve stimulation although they always failed to abolish contractions of the oesophagus to vagal and descending oesophageal nerve stimulation. Thus although responses of both the rectum and oesophagus to nerve stimulation were atypically resistant to hyoscine, only responses of the oesophagus were atypically resistant to ganglion blocking drugs. The cloacal region, which contains striped muscle, was excluded from the rectum preparation, thus the action of tubocurarine on

the preparation could not have been a neuromuscular blocking action.

Noradrenaline and phenylephrine (which stimulate α-receptors) produced relaxations of the rectum which were antagonized by propranolol but not phentolamine. However, there was a small residual relaxation of the rectum to noradrenaline and phenylephrine in the presence of propranolol, and this was abolished by phentolamine. Thus it seems that the sympathomimetic receptors in the chick rectum are mainly β-receptors, so that relatively higher concentrations of phenylephrine and noradrenaline than of isoprenaline were required to produce relaxations of the preparation. The predominance of β-receptors in this organ may account for its usefulness in detecting adrenaline in sympathin(Mann & West,1950; Vane, 1969).

Propranolol only antagonized noradrenaline when isoprenaline and phenylephrine were tested on the same preparation, suggesting that isoprenaline and/or phenylephrine had an influence on the combination of noradrenaline and/or propranolol with the sympathomimetic receptors. Similarly isoprenaline and phenylephrine seem to influence the action of the adrenergic neurotransmitter, for the antagonism of the relaxation to Remak nerve stimulation by propranolol was greater in experiments where isoprenaline and phenylephrine were tested.

Phentolamine and propranolol together, at concentrations which blocked only α- and β-receptors, respectively, abolished the relaxations of the rectum to nerve stimulation, isoprenaline and phenylephrine not being tested in these experiments. Furthermore, bretylium inhibited the relaxations of the rectum to Remak nerve stimulation, and rectal preparations

made from reserpinized chicks responded to nerve stimulation mainly by contraction. These results suggest that the relaxations to Remak nerve stimulation were produced by an adrenergic neurotransmitter released from sympathetic fibres contributed by the coeliac plexus(Nolf, 1934b), and show that the action of the adrenergic neurotransmitter can be antagonized by α- and β-receptor blocking drugs in this species.

TABLE 1.

ANTAGONISM OF THE α-ACTION OF NORADRENALINE ON THE RAT ISOLATED SEMINAL VESICLE AND THE β-ACTION OF ISOPRENALINE ON THE CHICK ISOLATED RECTUM.

Dose ratios were measured when the antagonism became steady. Where more than two observations have been made, results are quoted as means and standard errors with the number of observations in parenthesis.

ANTAGONIST	CONCENTRATION (μg/ml)	DOSE RATIOS	
		NORADRENALINE CONTRACTIONS	ISOPRENALINE RELAXATIONS
TOLAZOLINE	1.0	2.3, 2.7	
	10	7.5±0.2(4)	
	100	83.8±4.8(4)	1.0(5)
PHENTOLAMINE	0.1	6.0±1.0(4)	
	1.0	41.3±7.0(4)	1.0(3)
PROPRANOLOL	0.0005		15.1±5.6(4)
	0.005		167.5±66.6(4)
	0.05		1390±626(4)
	1.0	1.0(4)	
MJ-1999	0.05		3.7±1.3(4)
	0.5		16.0±2.5(4)
	5.0	1.0(3)	168±16.8(4)
	50.0	1.0(4)	1655±592(4)
	200.0	1.0(4)	

TABLE 2

ANTAGONISM OF ACETYLCHOLINE AND HISTAMINE

CONTRACTIONS OF THE CHICK ISOLATED RECTUM

Dose ratios were measured when the antagonism became steady.

Where more than two observations have been made, results are quoted as means and standard errors with the number of observations in parenthesis.

ANTAGONIST	CONCENTRATION (μg/ml)	DOSE RATIOS	
		ACETYLCHOLINE	HISTAMINE
HYOSCINE	1.0	3400, 3350	1.2±0.1(4)
MEPYRAMINE	0.1		193±71(3)
TOLAZOLINE	10.0		2.4±0.2(3)
	100.0	1.0(4)	8.4±2.0(4)
PHENTOLAMINE	0.01		1.0(3)
	0.1	1.4±0.2(4)	1.2±0.2(4)
	1.0	1.4±0.3(6)	2.4±0.3(4)
	10.0	8.0±3.0(4)	
PROPRANOLOL	0.05	1.0(4)	1.0(3)
	0.5	1.5±0.3(3)	1.9±0.3(3)
MJ-1999	5.0	1.3±0.2(4)	1.3±0.1(3)
	50.0	5.2±1.3(4)	2.1±0.1(3)

TABLE 3

EFFECT OF DRUGS ON CONTRACTIONS TO NERVE STIMULATION OF GUINEA-PIG ISOLATED VAGUS-OESOPHAGUS AND HYPOGASTRIC-VAS DEFERENS PREPARATIONS

Potentiation (+) or inhibition (-) of contractions measured after 20 min exposure to drug.

Where more than two observations have been made, results are quoted as means and standard errors with the number of observations in parenthesis.

DRUG	CONCENTRATION (μg/ml)	VAGUS-OESOPHAGUS PREPARATION	HYPOGASTRIC VAS DEFERENS PREPARATION	P
TOLAZOLINE	100	No effect (3)	+96±24%(3)	<0.1
PHENTOLAMINE	10	No effect (2)	No effect(3)	
BRETYLIUM	2	No effect (3)	-39±2%(3)	<0.01
	10	No effect (3)	-77±5%(3)	<0.01
	20	-8±2%(3)		>0.05

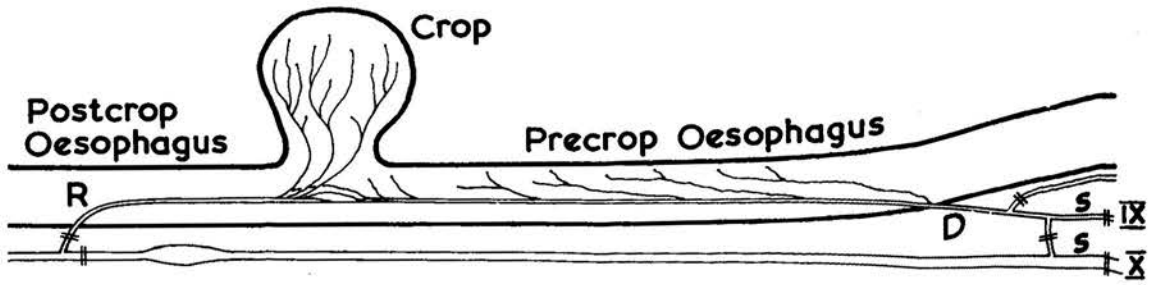


Fig. 1. Diagram of left side of chicken neck. $\overline{X}$, vagus; $\overline{IX}$, glossopharyngeal nerve; D, descending oesophageal nerve; R, recurrent nerve; s, points of stimulation; nerves were cut at cross lines.

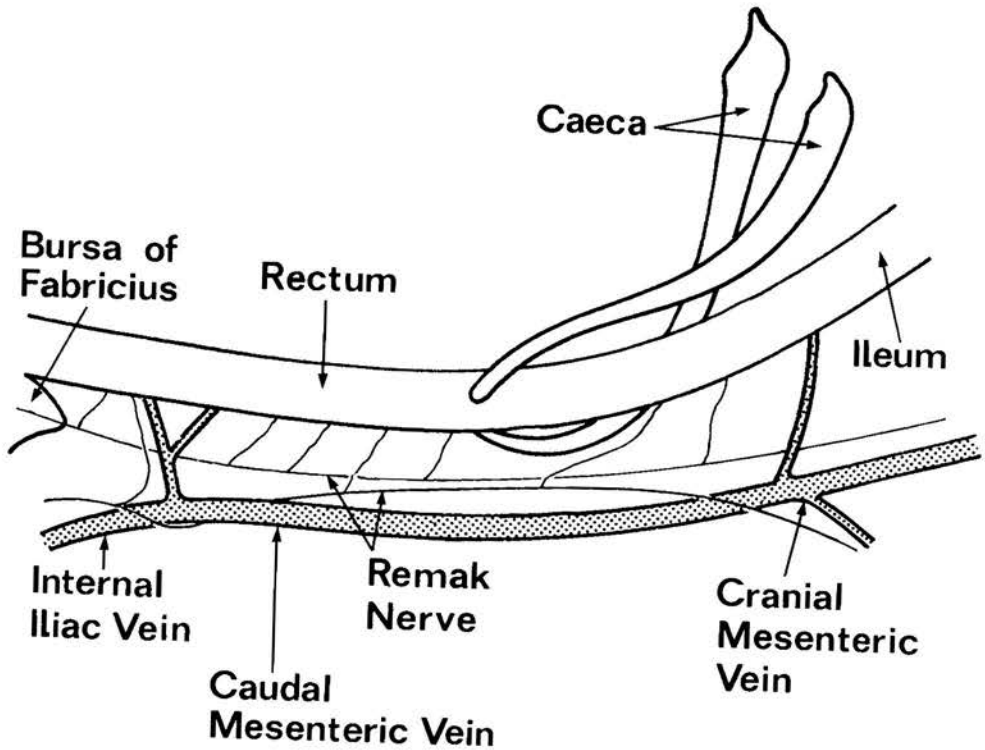


Fig. 2. Diagram of right ventrolateral aspect of the lower abdomen of the chick.

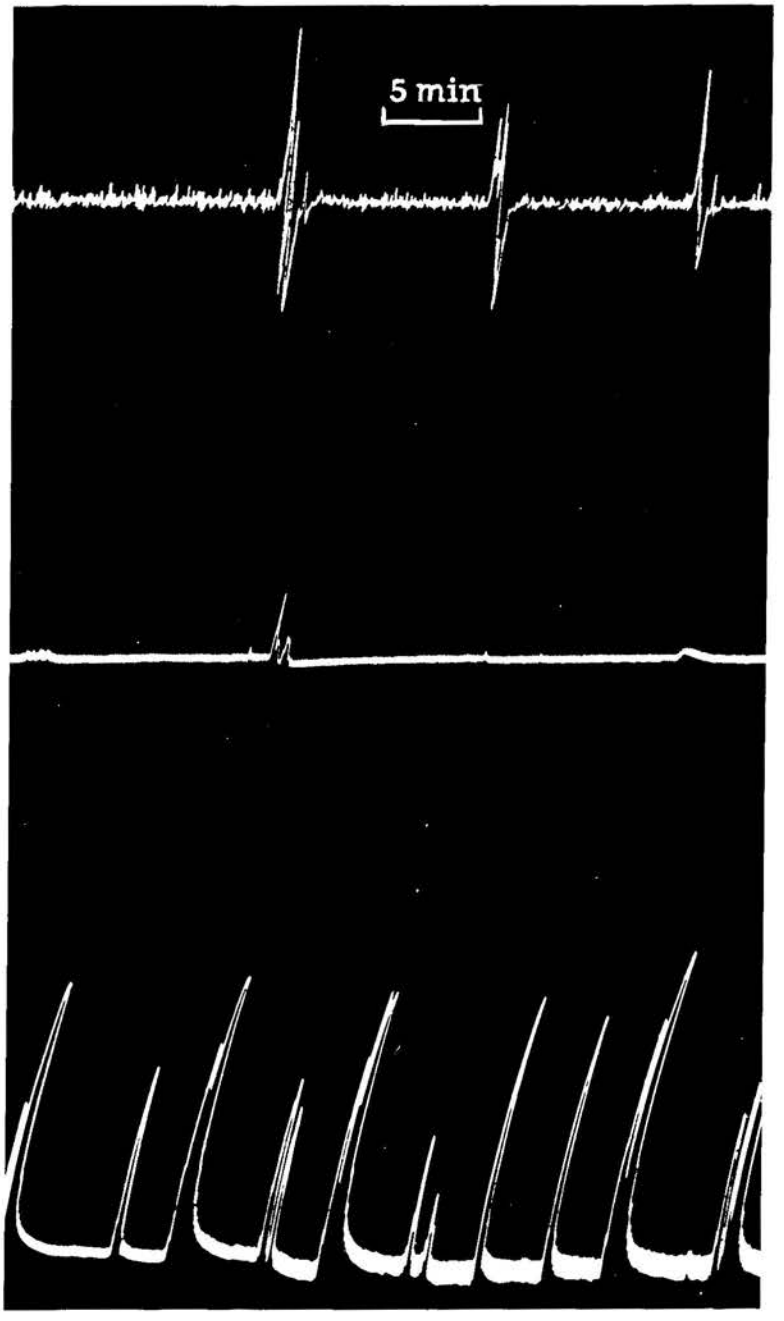


Fig. 5. Cockerel, pentobarbitone. From above down, spontaneous movements of pre-crop oesophagus, crop and gizzard. Note the size and form of the rhythmic contractions of the oesophagus and gizzard, and the quiescence of the crop.

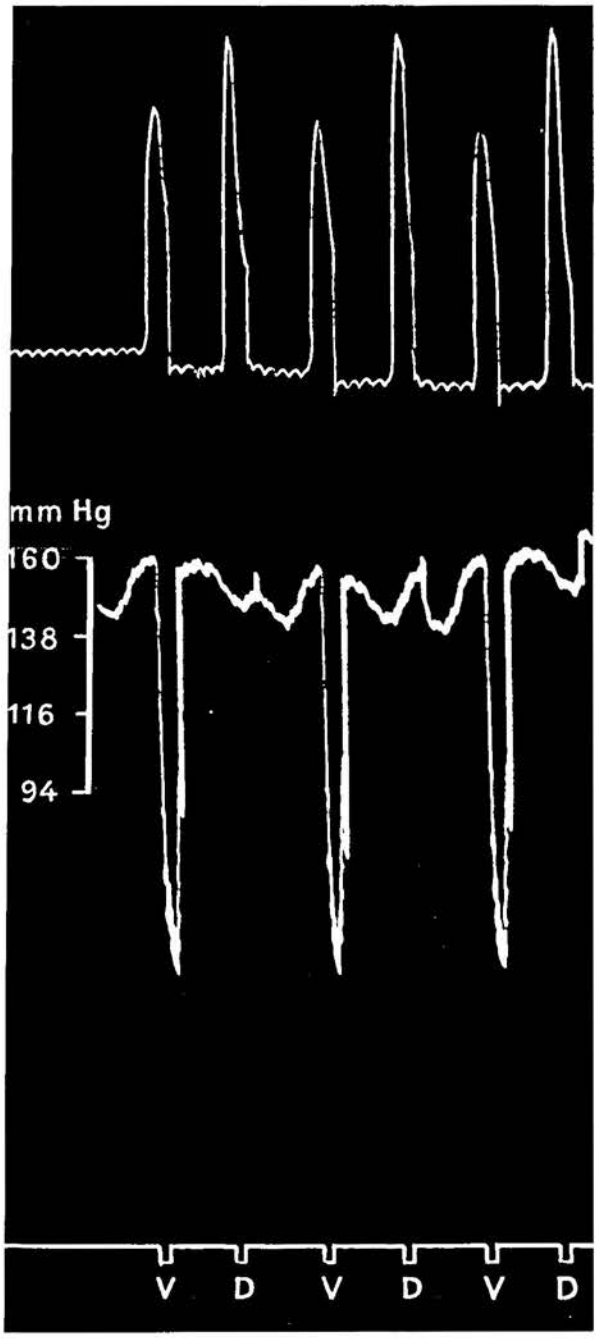


Fig. 4. [illegible], rabbit. From above down, pressure oesophagus, arterial blood pressure and signal recording stimulation of the vagus (V) and descending oesophageal nerve (D) for 5 sec [illegible] min (1 msec, 5 c/s). Stimulation of the vagus nerve affected both the oesophagus and blood pressure, but stimulation of the descending oesophageal nerve affected the oesophagus only.

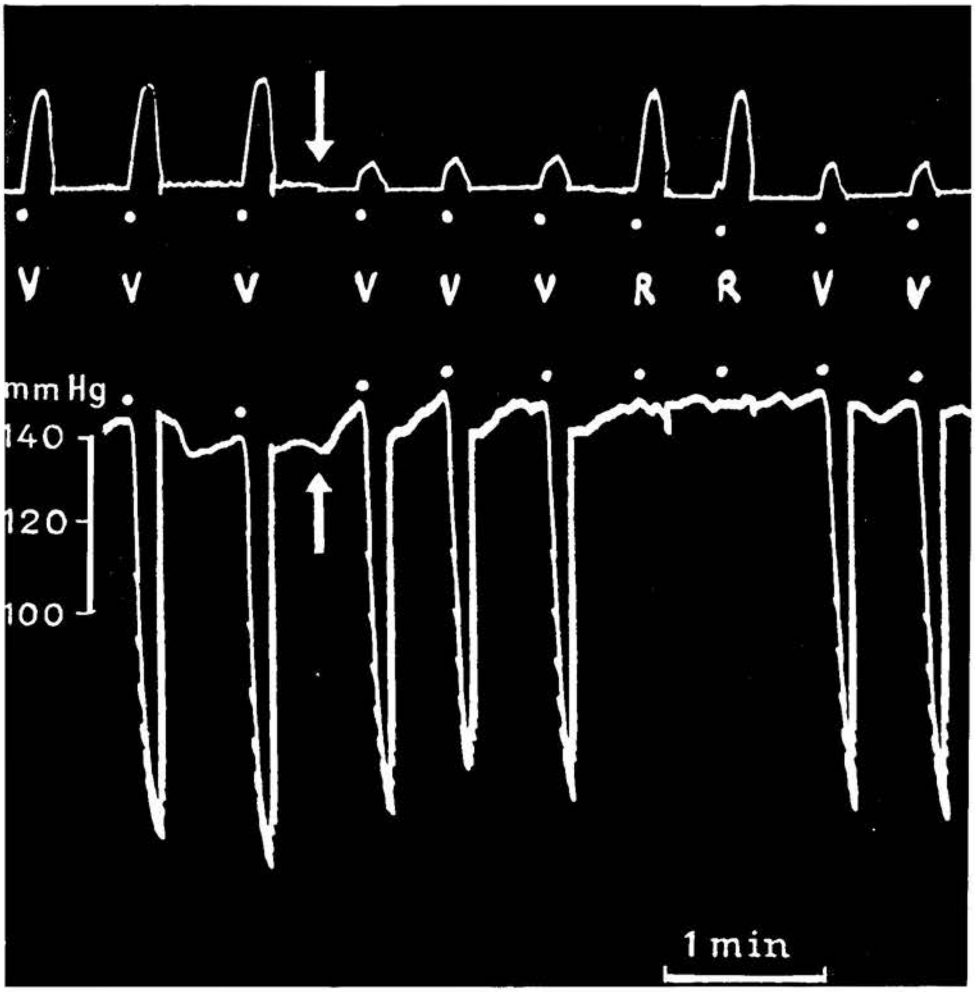


Fig. 5. Cockerel, pentobarbitone. Crop (above) and arterial blood pressure. The left vagus (V) and recurrent nerve (R) were stimulated at [illegible] every [illegible], [illegible]. The recurrent nerve [illegible] severing the recurrent nerve [illegible] the crop contraction to vagus stimulation. Stimulation of the severed recurrent nerve produced a contraction of the crop which was similar to that produced by stimulation of the vagus nerve before severing its recurrent branch, but the stimulation had no effect on the blood pressure.

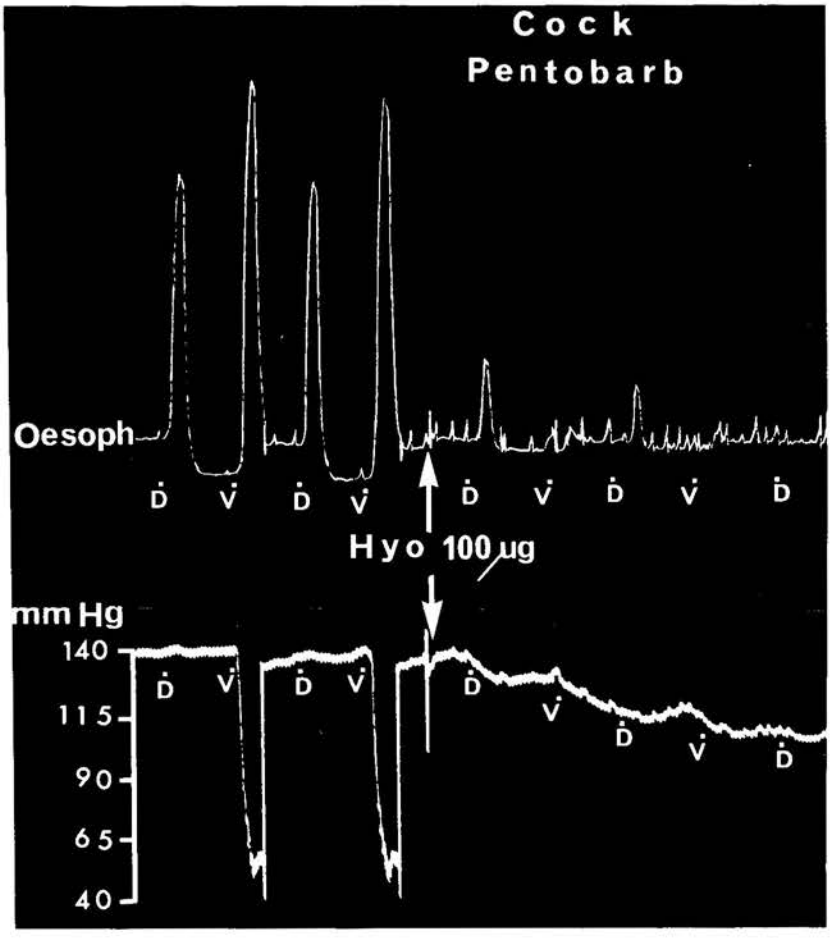
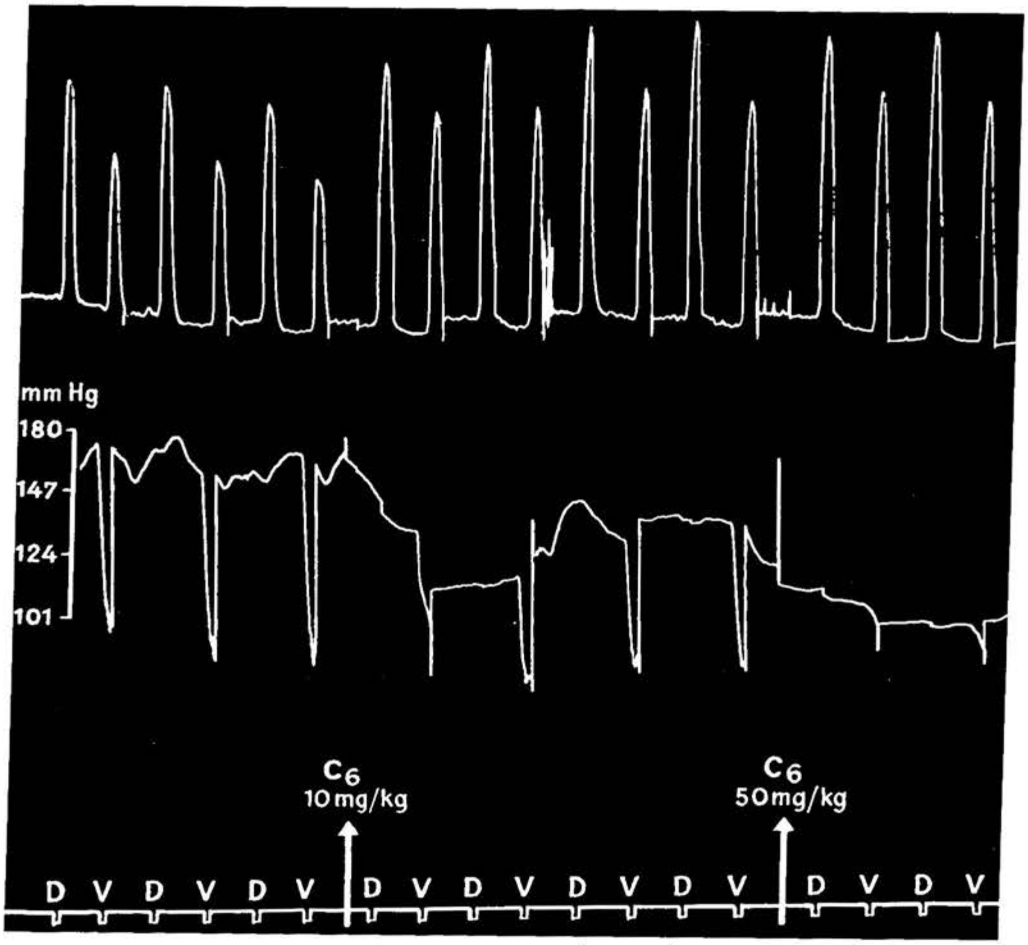
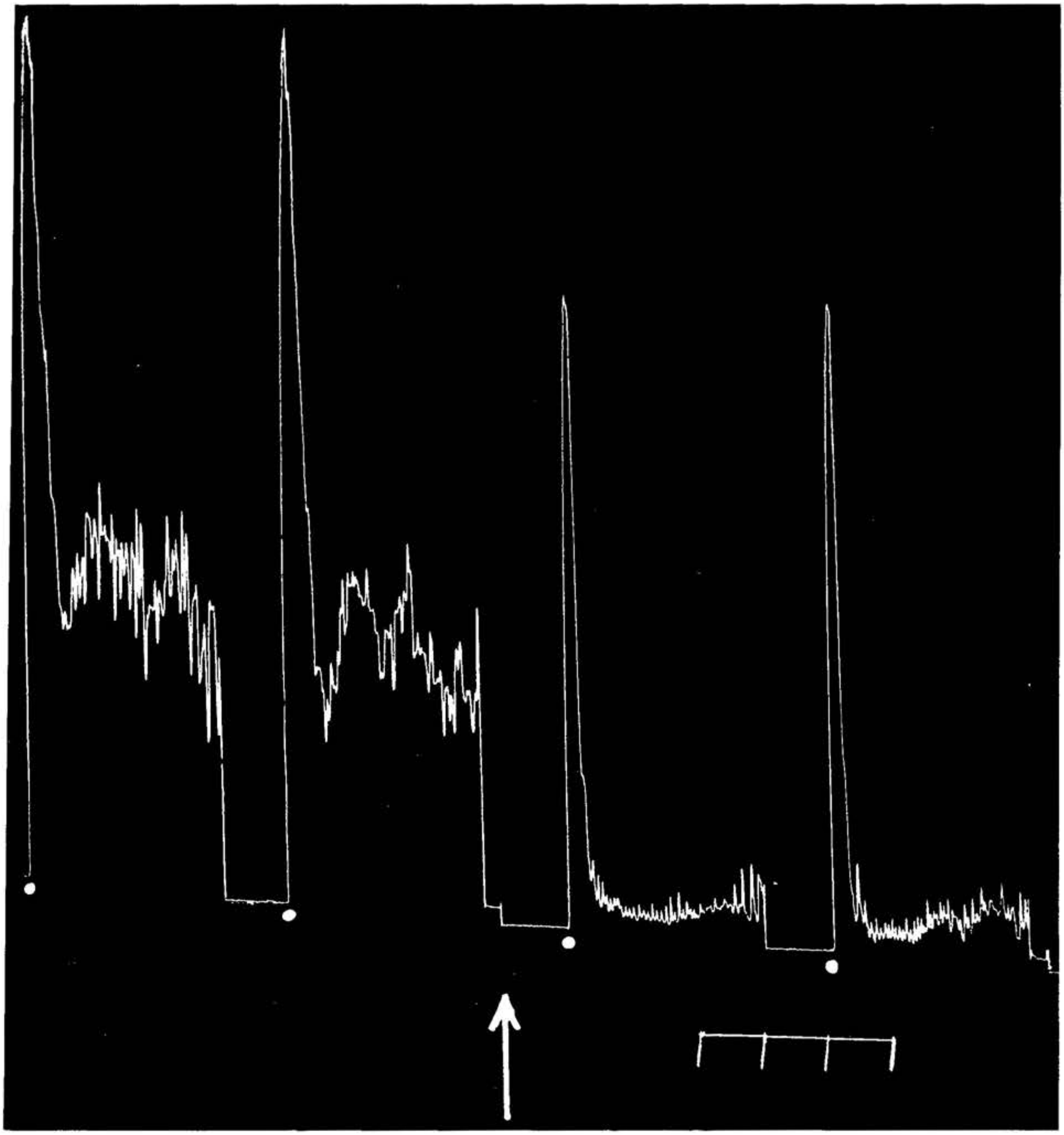


Fig. 4. Cockerel, pentobarbitone. Pre-ganglionic oesophageal (Oesoph) and arterial blood pressure. The vagus (V) and ansa subclavia - vagal nerve (D) were stimulated at the squares for 5 sec in every 5 min (10 msec, 20 c/s). The arrows mark the intravenous injection of hyoscine, 100 µg/kg. Hyoscine abolished the responses to the oesophagus and blood pressure to vagal and descending oesophageal nerve stimulation.



[illegible]. 7. [illegible]cheren, [illegible]nt[illegible]barbitone. [illegible] above down, [illegible]oesophagus, arterial blood pressure [illegible] signal [illegible] [illegible] [illegible] [illegible] [illegible] (V, [illegible] [illegible] oesophageal nerve (D) for [illegible] sec in every [illegible] min [illegible] msec, [illegible]. [illegible] the intravenous injection of hexamethonium (C_6), 10 [illegible], respectively. Hexamethonium, 10 mg/kg, [illegible] the [illegible] contractions [illegible] oesophagus to vagal and [illegible], oesophageal nerve stimulation, lowered the blood pressure and partly [illegible] the vaso-depression produced by vagal stimulation. [illegible] additional dose [illegible] hexamethonium, 50 mg/kg, [illegible] effect [illegible] vagal stimulation on the blood pressure but had no further effect [illegible] the oesophageal contractions.



[illegible] synchronous [illegible] nerves [illegible] in [illegible] [illegible], [illegible], [illegible]. [illegible] to [illegible] end of [illegible] tracing [illegible], [illegible] ml, [illegible]. [illegible] the [illegible] contraction to nerve stimulation, [illegible] the abolition [illegible] the [illegible] rise in [illegible]. [illegible].

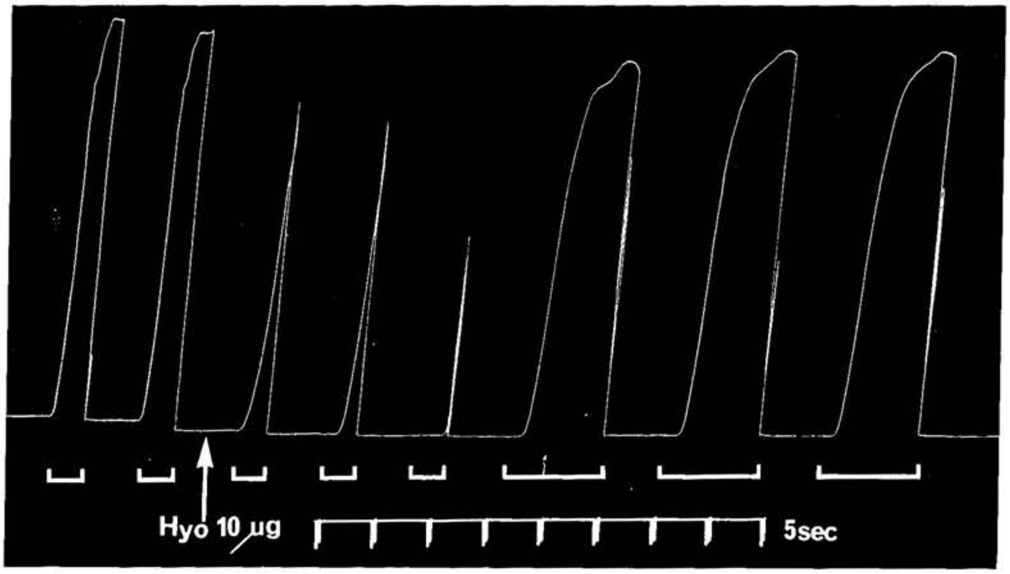


Fig. [illegible]. [illegible]ongitudinal contract[illegible] of chic[illegible] to [illegible] evoked by synchronous stimulati[illegible] vagus and [illegible] nerves ([illegible], [illegible], 57) for [illegible] sec (small [illegible]) [illegible] sec [illegible] every [illegible] min. From th[illegible] the end of the tracing [illegible], [illegible], was present. [illegible] abolished the contracti[illegible] sec [illegible] stimulation [illegible] hyoscine-resistant contracti[illegible] produced by the [illegible] sec stimulation. [illegible], [illegible] sec.

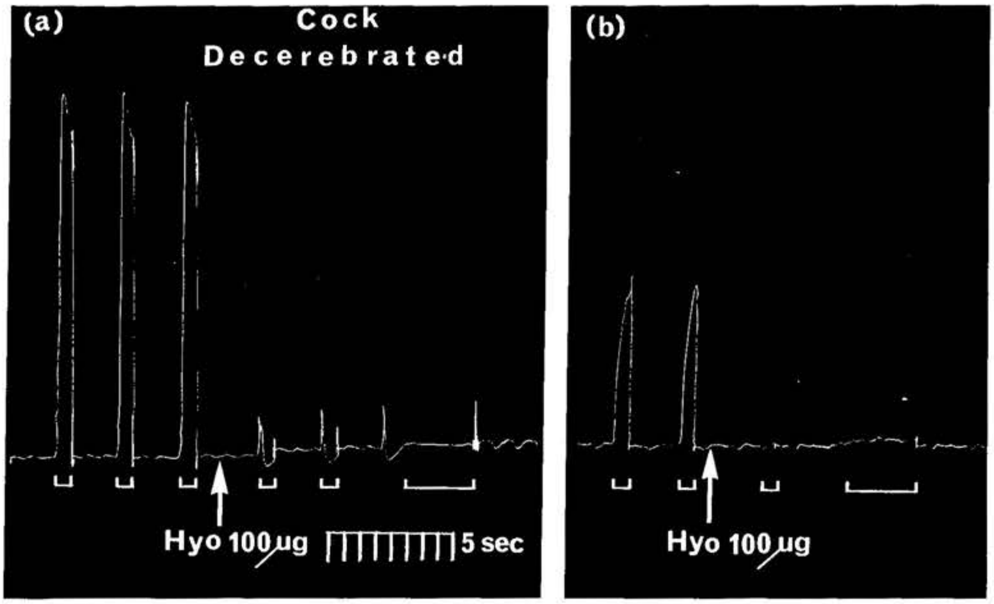


Fig. 18. [illegible]-tambour rec[illegible] of oeso[illegible] contractions of de-
cerebrate cockerel. [illegible] brackets [illegible] stimulation [illegible] the
descending oesophageal nerve at [illegible] intervals (10 msec, [illegible],
5V). [illegible], 100 [illegible], [illegible] injected intra-
venously [illegible] the response of the oesophagus to stimulation of the [illegible]
oesophageal nerve. Time, 5 sec.

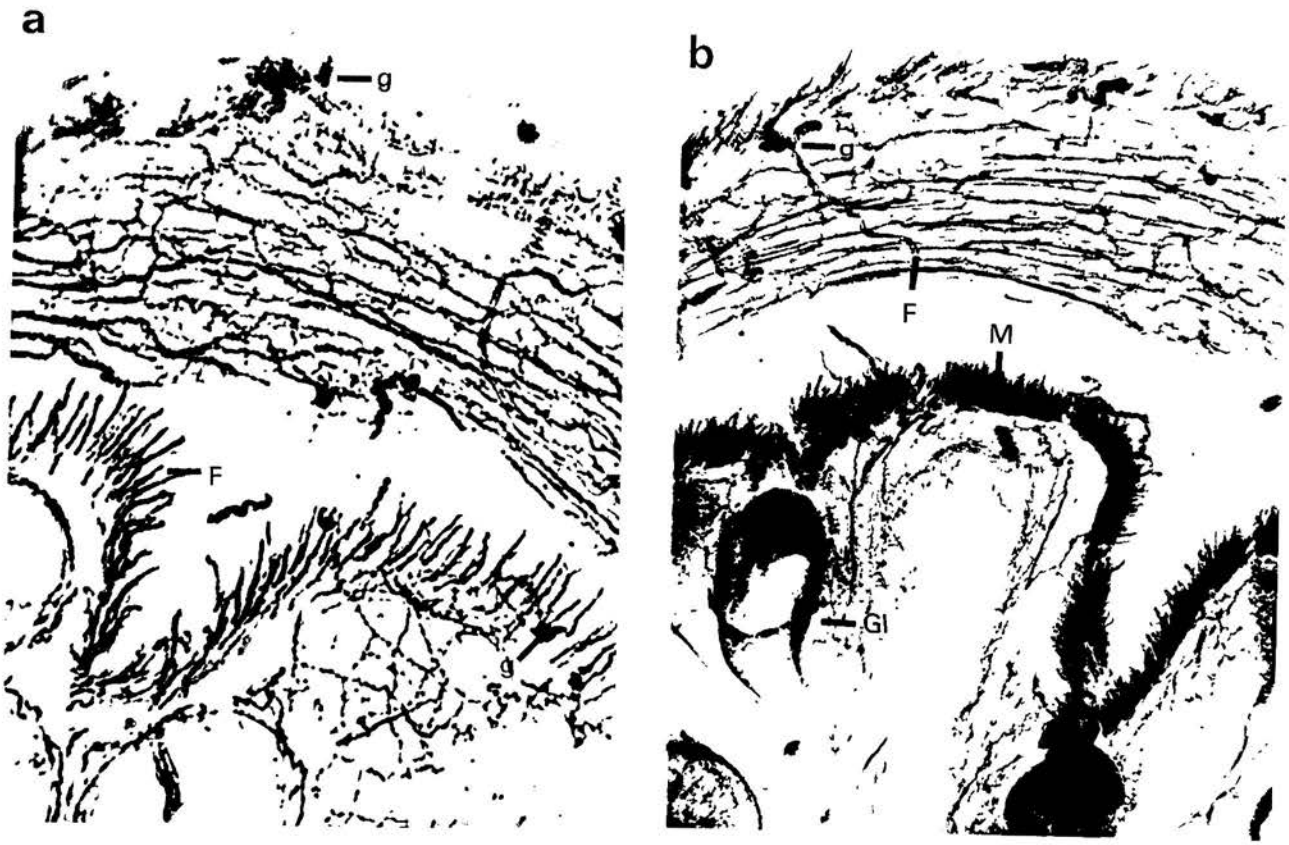


Fig. 11. Transverse section of oesophagus incubated with (a) acetylthiocholine and DFP, 10 μg/ml, and (b) acetylthiocholine alone (X100). Staining depicts cholinesterase activity in ganglia (g), nerve fibres (F), muscularis mucosae (M) and mucosal glands (Gl). Ganglia in the muscularis mucosae are shown most clearly in (a), where the stain is due to the activity of true cholinesterase only.

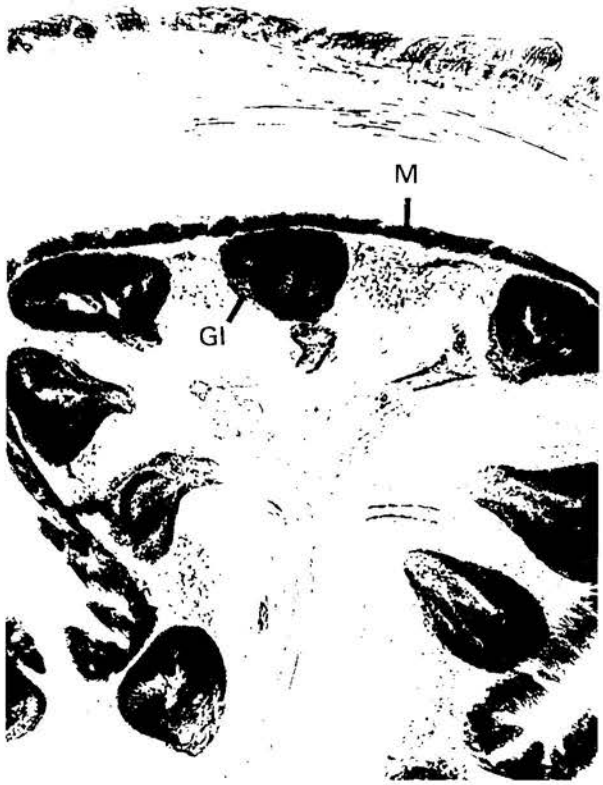


Fig. 12. Transverse section of esophagus incubated with butyrylthiocholine (175). Black staining depicts pseudocholinesterase activity, which is present in the muscularis mucosae (M) and glands (Gl).

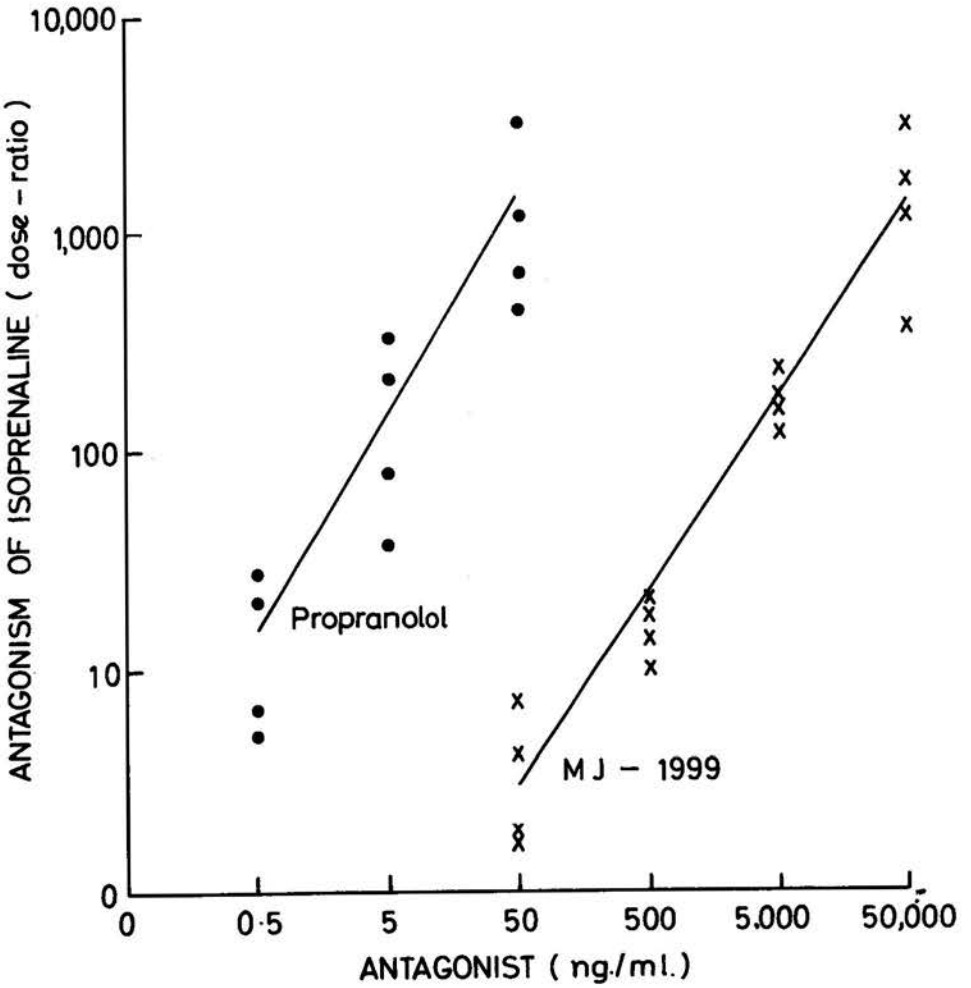


Fig. 13. Antagonism of isoprenaline on the chick isolated rectum. Ordinate, dose ratio for isoprenaline; abscissa, concentrations of antagonists on a log scale (ng/ml). Propranolol was 550 times as potent as MJ-1999 in blocking the isoprenaline relaxations of the rectum.

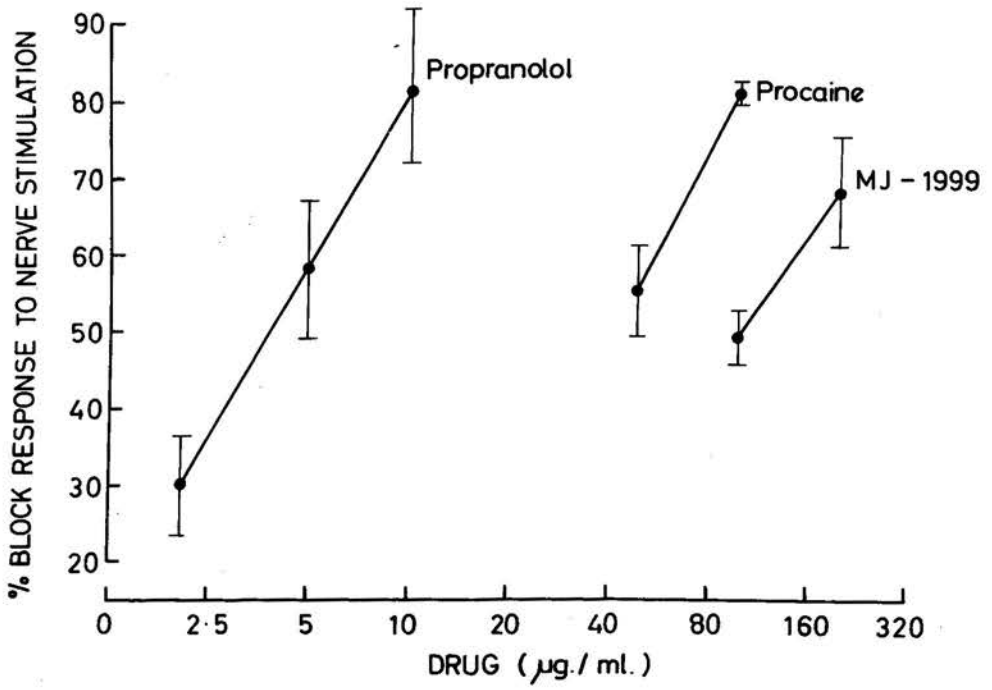


Fig. 14. Guinea-pig isolated hypogastric nerve-vas deferens preparations. Ordinate, blockade of contractions to nerve stimulation (% reduction in height of contraction after 20 min exposure to a drug); abscissa, concentration of drugs on a log scale (µg/ml). Each point is a mean value from four preparations, the vertical bar depicting the standard error of the mean. Propranolol was about 26 times as potent as MJ-1999 and 10 times as potent as procaine in producing a nerve block.

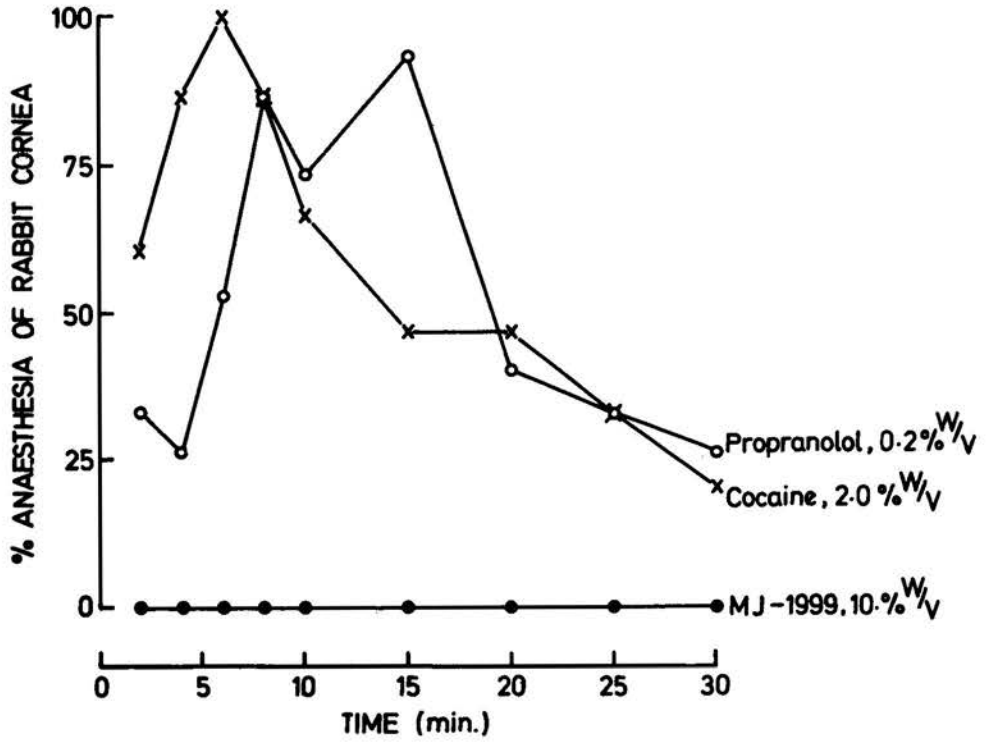
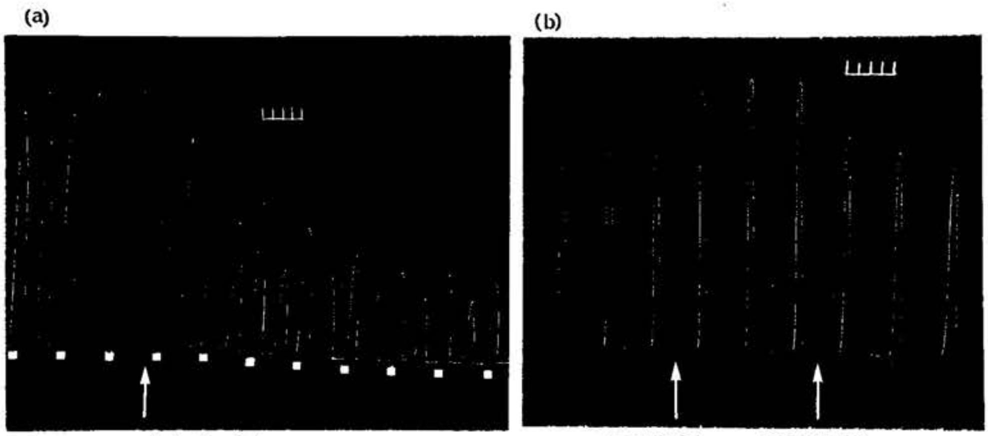


Fig. 15. Surface anaesthesia of rabbit cornea. Ordinate, inhibition of the corneal reflex to touch; abscissa, time from instillation of drugs (min). Each point is the mean of five tests with propranolol (open circles), cocaine (crosses) and MJ-1999 (closed circles). Propranolol (0.2% W/V) and cocaine (2.0% W/V) anaesthetized the cornea but MJ-1999 (10% W/V) was without effect.



[illegible]. (a) Guinea-pig [illegible] vas deferens, [illegible] preparation [illegible], contractions to hypogastric nerve stimulation (0.1 msec, [illegible] c/s, submaximal) alternated with those to [illegible] stimulation (0.5 msec, [illegible] c/s, 30V; at the [illegible] for 5 sec [illegible] min. From the arrow to the end of the tracing propranolol (10 μg/ml) was present. Time, 5 sec. (b), contractions [illegible] by exposure to noradrenaline (10 μg/ml) [illegible] sec in every [illegible] min. At the first arrow propranolol [illegible] was added [illegible] the second its concentration [illegible] raised to [illegible] ml. Time, [illegible] min. Propranolol (10 μg/ml) [illegible] contractions of the vas deferens to transmural and hypogastric nerve stimulation but not to noradrenaline.

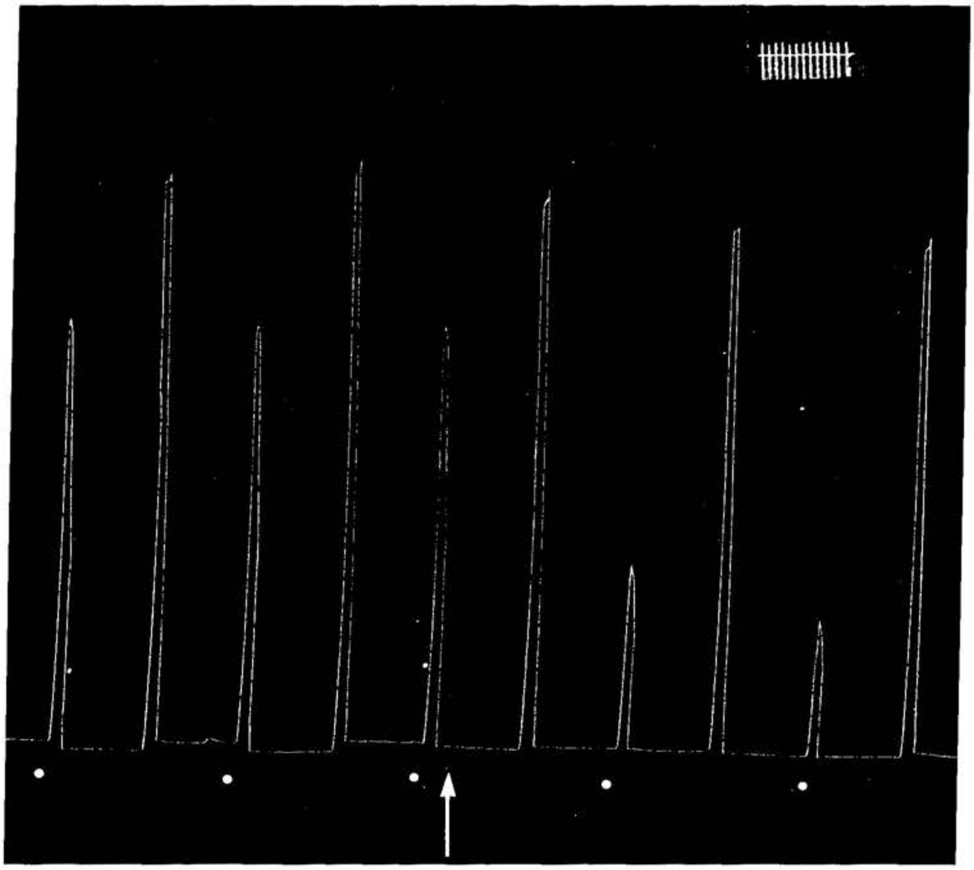


Fig. 17. [illegible] hypogastric nerve-[illegible] preparation. [illegible] hypogastric nerve [illegible] trans- [illegible] msec, [illegible] submaximal; [illegible] marked) for [illegible] sec in every [illegible] min. [illegible] to the [illegible] selectively antagonized [illegible] contractions produced by stimulation [illegible] hypogastric nerve. Time, [illegible] sec.

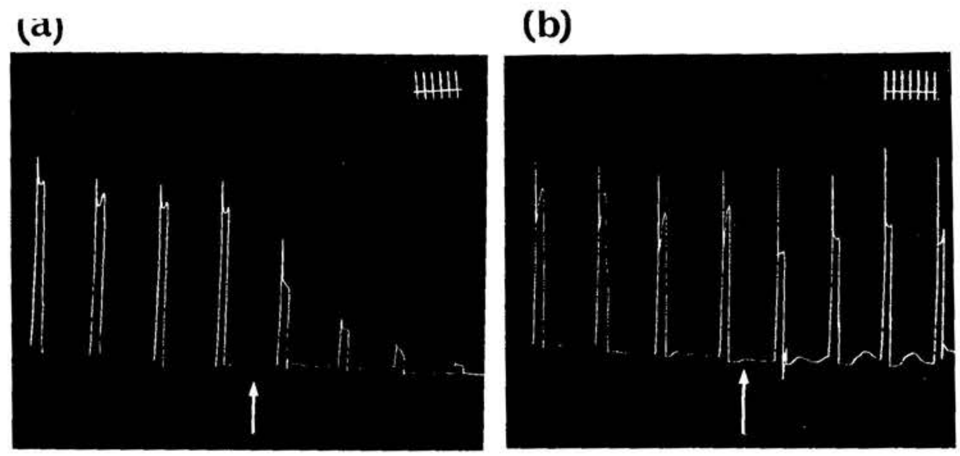


Fig. [illegible]. [illegible] isolated vagus nerve-[illegible], the [illegible]. The nerve was stimulated [illegible] .1 msec, [illegible], supramaximal, for 5 sec in every [illegible]. [illegible], the [illegible] of [illegible] [illegible]-[illegible] ([illegible]) . [illegible] response to vagal stimulation, whereas [illegible]-[illegible] antagonized the contracture but not the twitch. Time, 5 sec.

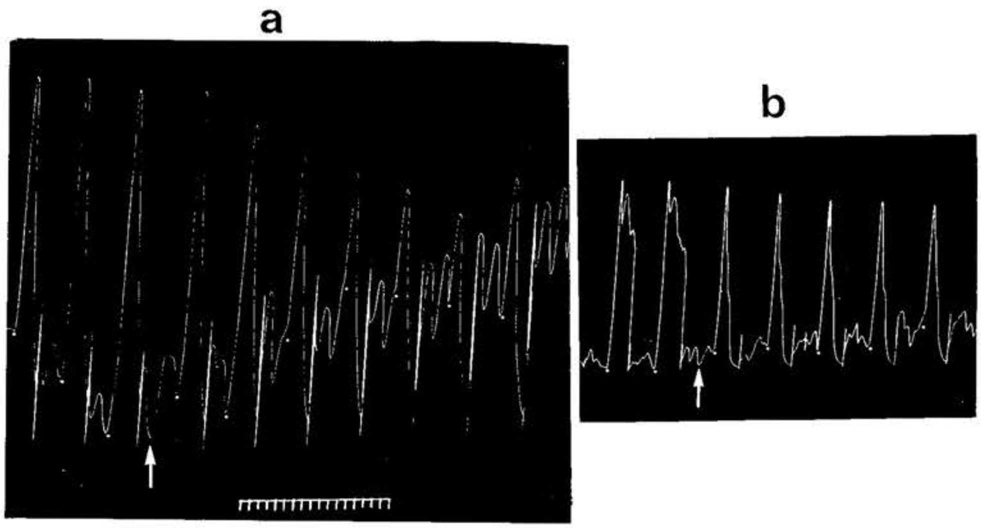


Fig. [illegible]. [illegible]olated [illegible]emak nerve-rectum, t[illegible] [illegible] [illegible]rations. [illegible] dots [illegible] nerve stim[illegible]tion (1[illegible] [illegible], 20 c/s, [illegible] [illegible] sec in every 5 min. [illegible] (1[illegible] [illegible]) was present in [illegible] solution fr[illegible] the arrow t[illegible] the [illegible] of t[illegible] tracing in [illegible] and (b). [illegible] partially [illegible]tagonized the contractions t[illegible] nerve sti[illegible]tion, [illegible] in (a) it r[illegible] the tone [illegible] the [illegible]ration. Time, 10 sec.

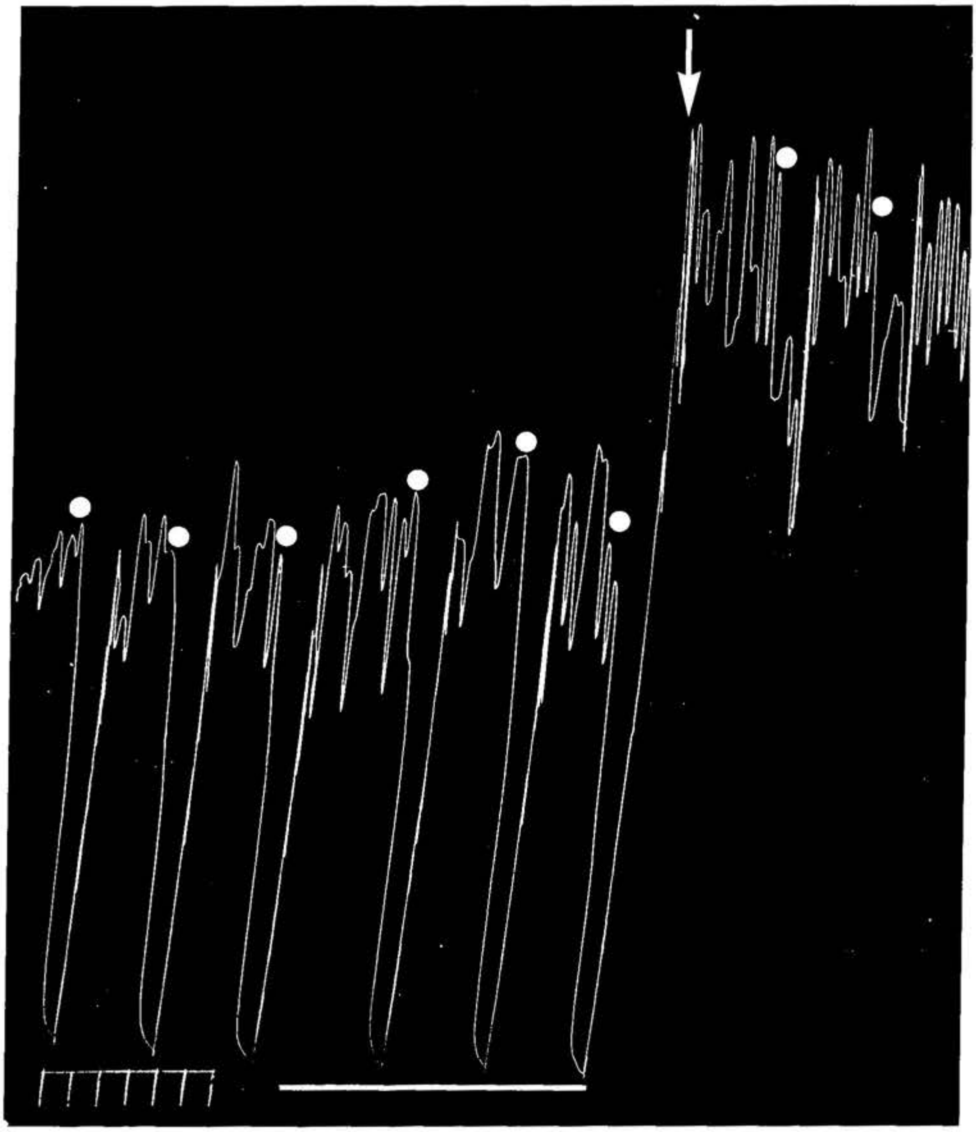


Fig. [illegible]. [illegible] isolated rectum. [illegible] mark the addition of phenylephrine ([illegible] µg/ml) to the organ bath for [illegible] sec in every [illegible] min. [illegible] white bar marks the presence of phentolamine (0.1 [illegible]), and from [illegible] to the end of the tracing propranolol (0.05 [illegible]) was present. Phentolamine [illegible] the relaxations of the rectum to phenylephrine but propranolol [illegible]. Time, 30 sec.

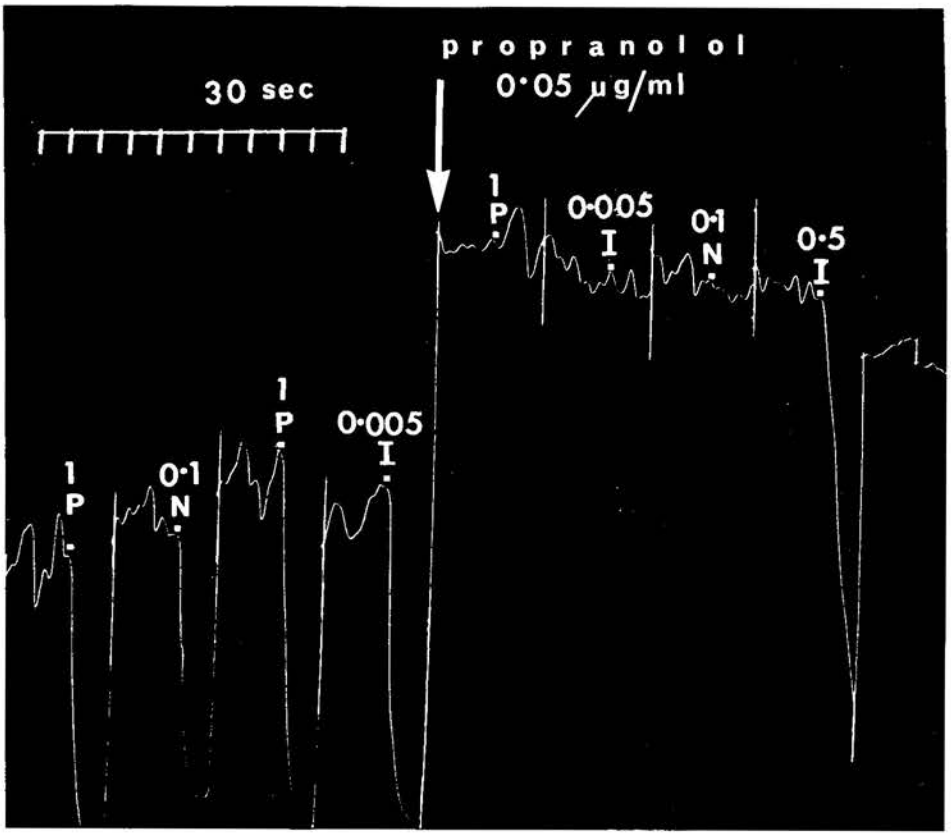


Fig. [illegible]. [illegible] isolated rectum. Phenylephrine (P), noradrenaline (N) and isoprenaline (I) were present [illegible] bath [illegible] every [illegible], the numbers referring to the concentrations [illegible] the [illegible]. From the arrow to the end of the tracing propranolol [illegible] was present. Propranolol [illegible] the relaxation [illegible] isoprenaline, phenylephrine and noradrenaline. Time, [illegible].

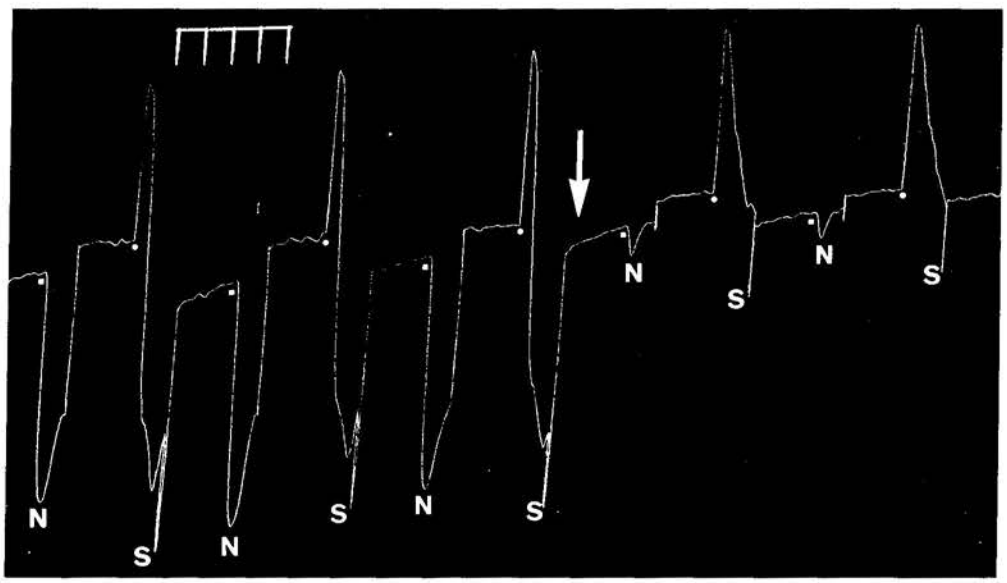


Fig. 1. Isolated Lenek nerve-rectum preparation, tone raised with acetyl-β-methylcholine (2 μg/ml). The nerve was stimulated (2 msec, 20 c/s, 4 V; at "N") for 5 sec and the preparation was exposed to noradrenaline (0.5 μg/ml; at "S") for 30 sec, in every 5 min. From the arrow to the end of the tracing propranolol (0.01 μg/ml) and phentolamine (0.1 μg/ml) were present. These two drugs abolished the relaxations of the rectum to nerve stimulation. Time, 30 sec.

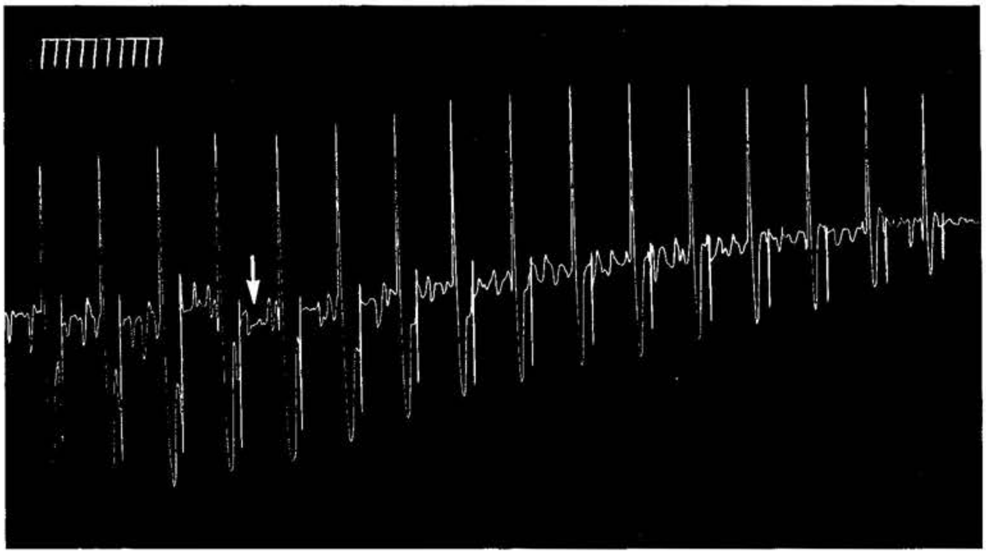


Fig. [illegible]. Isolated Remak nerve-rectum preparation, tone raised with acetyl-β-methylcholine ([illegible] μg/ml). The nerve was stimulated (1 msec, [illegible] c/s, [illegible]) for [illegible] sec in every [illegible] min. From the arrow to the end of the record, bretylium ([illegible] μg/ml) was present. Bretylium selectively inhibited the relaxation to nerve stimulation. Time, [illegible] sec.

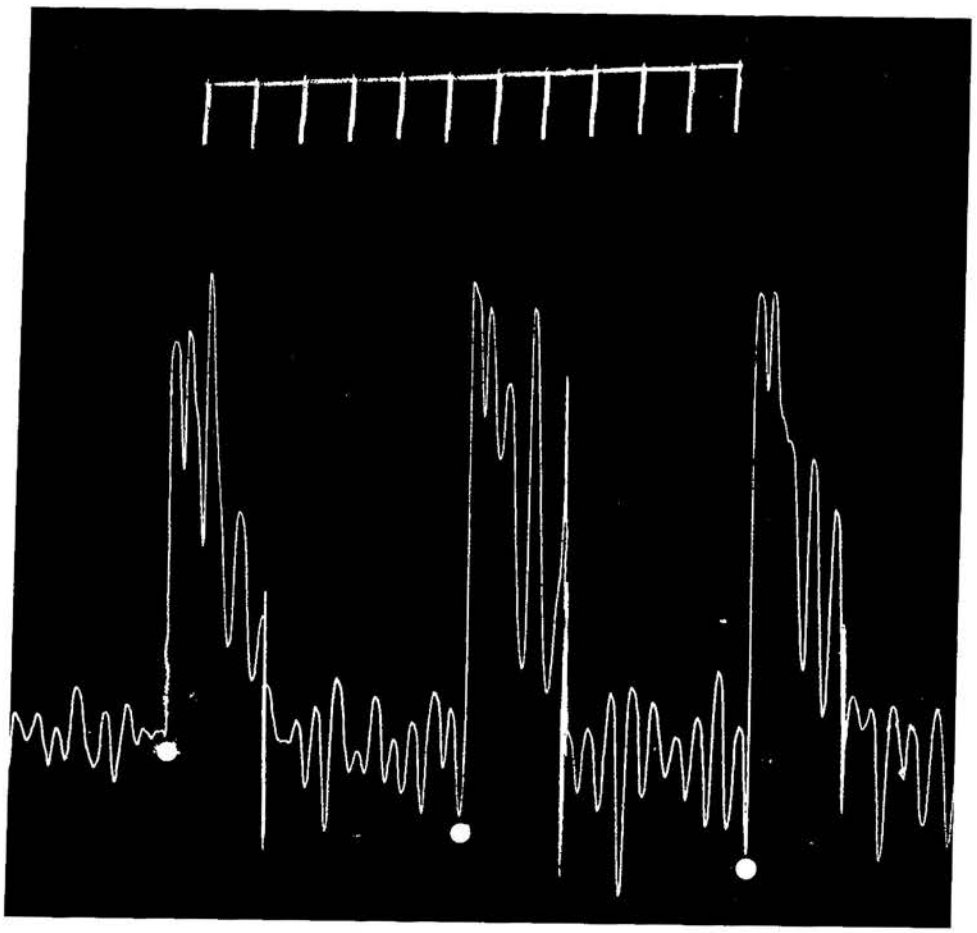


Fig. 4. Isolated [illegible] nerve-muscle preparation from reserpinized chick, tone raised with neostigmine (2 µg/ml). Single twitch mark 1 min of nerve stimulation (1 msec, 2.[illegible] v, 5/) in every 5 min. Stimulation of the nerve only produced a contraction in the reserpinized preparation. Time, 10 sec.

ACKNOWLEDGMENTS

I wish to thank Professor F.Alexander for his helpful suggestions and advice. I am particularly grateful to Dr. A.L.Bartlet for his continuous guidance and his help in the experiments dealing with the evaluation of sympathomimetic antagonists and the pharmacological analysis of the relaxations of the chick rectum to agonists and nerve stimulation.

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Effects of Stimulation of the Cervical Vagus and Descending Oesophageal Nerves on the Alimentary Tract of the Domestic Fowl

By

T. HASSAN

With 6 figures

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Apart from the work of NOLF (1934 a, b, c; 1938) the role of the extrinsic nerves in the activity of the alimentary tract of the domestic fowl has received little attention. In view of the anatomical peculiarities of the fowl's digestive tract (BRADLEY and GRAHAME, 1960) and the innervation of its upper part by two cranial nerves, the vagus and glossopharyngeal (HSIEH, 1951; WATANABE, 1960, 1964) it was thought worthwhile to study the role of these nerves in the activity of various parts of the tract.

Methods

Nerve Effect on Fowl Alimentary Tract

Male birds weighing 0.8—2 kg. were fasted overnight and anaesthetised with Sodium Pentobarbitone (30—35 mg./kg. i. v.). The bird was secured on its back and kept warm. The trachea, jugular vein and carotid or brachiocephalic artery were cannulated. In some experiments, the thorax was opened to expose the thoracic vagal trunk and recurrent nerve and the bird artificially ventilated. Thin rubber balloons attached to polythene tubing (10 mm. ext. diam.) were introduced into the stomachs (proventriculus and gizzard) through a fistula in the post-crop oesophagus, and into the crop and pre-crop oesophagus through the mouth. The balloons were filled with 4—8 ml. of air and when inflated their external diameter was 20—30 mm. Pressure changes in the balloons were recorded kymographically by means of a water manometer-tambour system. The exact location of the balloons was verified at autopsy. Arterial blood pressure was recorded with a mercury manometer. Heparin (100—200 Units/kg. i. v.) was given on completing the dissection.

The vagi and glossopharyngeal nerves were cut proximally on either side of the neck and the branch of the vagus to the glossopharyngeal nerve was severed (Fig. 1). The nerve on the electrodes was kept moist with cotton-wool soaked in warm liquid paraffin. The nerve was stimulated for 5 sec at 25—50 stimuli per second using a square wave stimulator and the voltage was adjusted to give submaximal responses. The pulse duration was fixed at 8 msec.

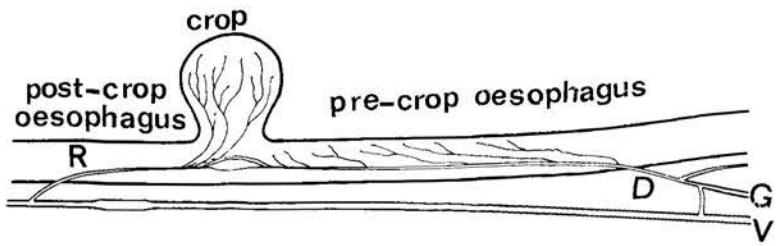

Fig. 1. Diagram of left side of chicken's neck; V, vagus; G, glossopharyngeal nerve; D, descending oesophageal nerve; R, recurrent nerve

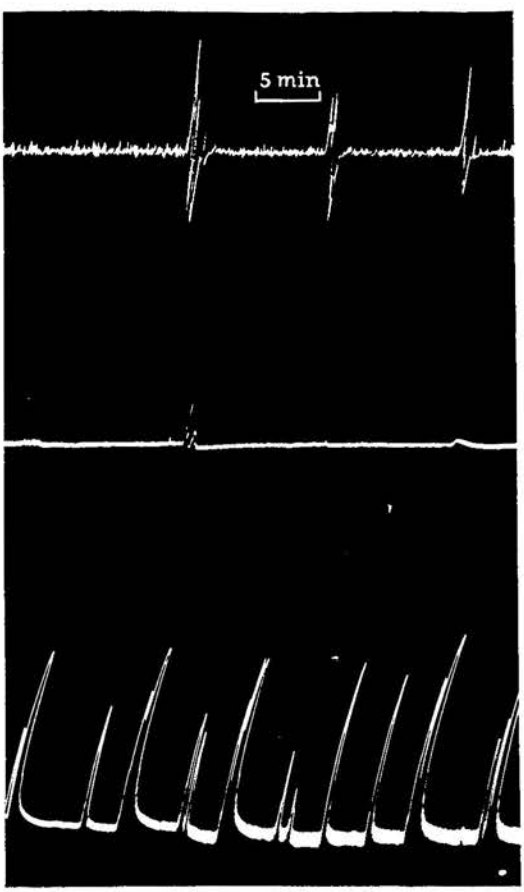

Fig. 2 (left). Cockerel, sodium pentobarbitone. From above down, contractions of pre-crop oesophagus, crop and gizzard

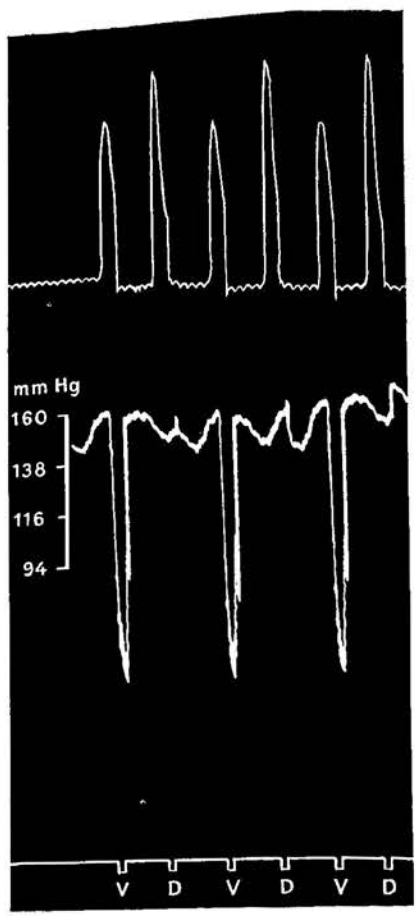

Fig. 3 (right). From above down, pre-crop oesophagus, arterial blood pressure and signal marking stimulation of vagus (V) and descending oesophageal nerve (D) for 5 sec

The drugs used were atropine sulphate, eserine salicylate, hexamethonium bromide, hyoscine hydrobromide and tetramethylammonium chloride. The drugs were dissolved in aqueous NaCl (0.9 g./100 ml.) and injected intravenously in a volume not exceeding 2.8 ml. The doses in the text and figures refer to weights of the above salts.

Results

The oesophagus exhibited rhythmic spontaneous movements but the crop was usually quiescent (Fig. 2). The proventriculus and gizzard (Fig. 2) contracted spontaneously and the rate and amplitude of the contractions varied with individual birds and at different stages during the experiment.

Effect of nerve stimulation

Stimulation of the vagus produced contractions of the precrop oesophagus (Fig. 3) crop, proventriculus and gizzard and a fall in arterial blood pressure. The effects of stimulation of the right and left vagi were similar. Stimulation of the right or left descending oesophageal nerve produced a contraction of the pre-crop oesophagus and crop but had no effect on the stomachs or blood pressure (Fig. 3). The contractions of the oesophagus and crop were prompt and sustained throughout the stimulation, but the onset of the response of the stomachs was usually delayed for about 3 sec. and the initial transient contraction was usually followed by other contractions for 5—10 min. after cessation of stimulation.

Nerve transection

The responses of the pre-crop oesophagus, crop, stomachs and arterial blood pressure to stimulation of the vagus were recorded following transection of the vagal trunk at various levels. At the thoracic level only the left vagus was cut as it was the more accessible.

Transection of the recurrent branch of the vagus reduced the response of the crop, but not that of the pre-crop oesophagus, stomachs or blood pressure (Fig. 4), to high cervical vagal stimulation, and abolished the contraction of the crop produced by stimulation of the vagus just cranial to the recurrent nerve. produced a contraction of the crop similar to that produced by stimulation of the vagus with its recurrent nerve intact but it did not

Stimulation of the severed recurrent nerve

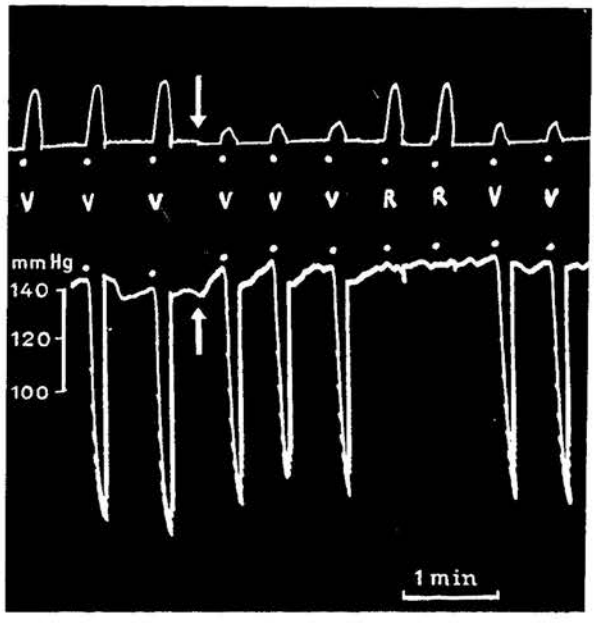

Fig. 4. Crop (top) and arterial blood pressure. The left vagus (V) and recurrent nerve (R) were stimulated at (.) for 5 sec. The recurrent nerve was cut at the arrows

have an effect on the pre-crop oesophagus or blood pressure (Fig. 4). Transection of the vagal trunk near and cranial to the origin of the recurrent nerve abolished the response of the stomachs and blood pressure, but not of the crop, to high cervical vagal stimulation. Severing the vagus at a level about the middle of the pre-crop oesophagus abolished the response of the crop to high cervical vagal stimulation. These transection procedures did not alter the response of the pre-crop oesophagus and crop to stimulation of the descending oesophageal nerve.

Chronic denervation

To test for the presence of vagal fibres in the descending oesophageal nerve the vagus was cut, allowed to degenerate and the responses of the pre-crop oesophagus and crop to stimulation of the corresponding descending oesophageal nerve were recorded. The left vagal trunk at the base of the skull and the branch to the glossopharyngeal nerve were cut in 2 anaesthetised cockerels. Three weeks after the operation, the birds were anaesthetised and the effects of stimulation of the degenerate vagus nerve and the corresponding descending oesophageal nerve on the alimentary tract and blood pressure were studied. The right vagus and descending nerve were also stimulated.

Stimulation of the degenerate vagus had no effect on the pre-crop oesophagus, crop or blood pressure, whereas stimulation of the normal vagus had. Stimulation of the descending oesophageal nerve corresponding to the degenerate vagus produced a contraction of the pre-crop oesophagus in the 2 expts. and a contraction of the crop in one.

Additional evidence of degeneration of the cut vagus was obtained histologically. Both normal and cut cervical vagi were removed at the end of the experiment and fixed in formol-saline. Degenerate myelin was demonstrated in sections made from the cut vagus by the Chiffelle and Putt's fat-soluble dye method and the Swank-Davenport osmium tetroxide method (CULLING, 1957).

Effects of drugs on nerve stimulation

Eserine, 0.2 mg./kg., potentiated the response of the alimentary tract and blood pressure to vagal or descending oesophageal nerve stimulation (4 expts). The duration of the responses was potentiated more than their magnitude. High doses of eserine produced spasm of the organs which was abolished by hyoscine or atropine.

Atropine or hyoscine, 0.1—1 mg./kg., antagonised the effects of vagal and descending oesophageal nerve stimulation on the alimentary tract or arterial blood pressure within 5 min. after injection.

Hexamethonium did not abolish the responses of the alimentary tract to vagal or descending oesophageal nerve stimulations; in 2 expts., 10 and 50 mg./kg. respectively had no effect on the responses to nerve stimulation and in 3 other expts. 5—10 mg./kg. only partly reduced the responses (Fig. 5). In 2 further expts. hexamethonium, 10 mg./kg., potentiated the effects of vagal and descending oesophageal nerve stimulation on the pre-crop oesophagus and crop, but lowered the blood pressure and partly reduced the vasodepression produced by vagal stimulation (Fig. 6). In both these experiments a further dose of hexamethonium, 50 mg./kg., abolished the vasodepression produced by vagal stimulation but had no effect on the responses of the oesophagus and crop to nerve stimulation. Hexamethonium, 5 mg./kg., abolished the contractions of the pre-crop oesophagus and crop produced by the ganglion-stimulant tetramethylammonium (2 expts.).

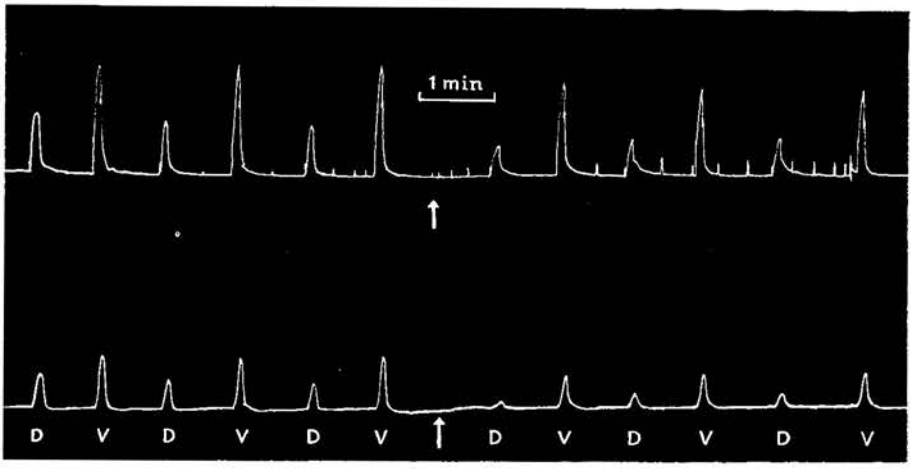

Fig. 5. Contractions of the pre-crop oesophagus (top) and crop produced by stimulation of the vagus (V) and descending oesophageal nerve (D) for 5 sec. Hexamethonium, 10 mg./kg., was injected at the arrow

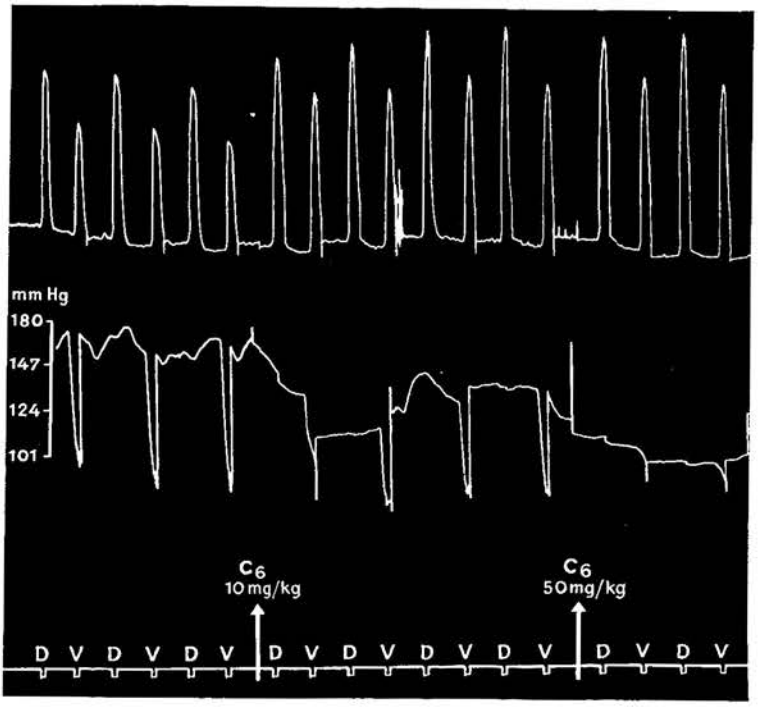

Fig. 6. From above down; — pre-crop oesophagus, arterial blood pressure and signal marking stimulation of vagus (V) and descending oesophageal nerve (D) for 5 sec. The 2 arrows mark the intravenous injection of hexamethonium (C_6), 10 and 50 mg./kg., respectively

Discussion

The excitatory effects of vagus stimulation on the oesophagus, crop, proventriculus and gizzard supports the anatomical evidence of the innervation of these structures (WATANABE, 1960). Since stimulation of the peripheral end of the cut vagus nerve below the diaphragm-like structure produces an increased motility of the intestines (NOLF, 1934 a), it seems that the whole alimentary tract of the domestic fowl is innervated by excitatory vagal fibres.

Stimulation of the descending oesophageal nerve produced contractions of the pre-crop oesophagus and crop but had no effect on the proventriculus, gizzard or blood pressure. This supports the anatomical evidence that the descending nerve does not extend beyond the cervical part of the alimentary tract (HSIEH, 1951; WATANABE, 1964).

Since transection of the recurrent nerve did not affect the vasodepression produced by vagal stimulation and scince the blood pressure was not affected by stimulation of the recurrent nerve, the recurrent fibres to the heart described by WATANABE (1960) must have been absent or insufficiently inhibitory to affect the blood pressure. Although the response of the crop to vagal stimulation was much reduced after cutting the recurrent nerve, stimulation of the recurrent nerve produced a crop contraction similar to that produced by stimulation of the vagus nerve with its recurrent branch intact. This suggests that the recurrent nerve supplied the main efferent vagal fibres to the crop.

Stimulation of the recurrent nerve did not produce an effect on the pre-crop oesophagus. This was in agreement with the finding that the recurrent nerve does not extend cranially beyond the crop (HSIEH, 1951; WATANABE, 1964) and supports the use of the term "recurrent nerve" (FEDDE, BURGER and KITCHELL, 1963) instead of "recurrent laryngeal nerve".

The persistence of the responses of the pre-crop oesophagus and crop to descending oesophageal nerve stimulation following chronic degeneration of the corresponding vagus nerve indicates that the descending oesophageal nerve could function independently.

The actions of the vagus and descending oesophageal nerves on the alimentary tract and arterial blood pressure were potentiated by eserine and antagonised by atropine or hyoscine. This suggests that these nerves supplied cholinergic fibres.

Large doses of hexamethonium did not abolish the effects of stimulation of the vagus or descending oesophageal nerve on the oesophagus or crop. It is possible that some post-ganglionic fibres were stimulated or that transmission at the synapses was mediated by direct electrical coupling (MARTIN and PILAR, 1963 a, b) or by a chemical transmitter other than a nicotine-like substance (DALE, 1914).

Summary

1. Electrical stimulation of the vagus nerve in the fowl produced contractions of the pre-crop oesophagus, crop, proventriculus and gizzard and a fall in arterial blood pressure. Stimulation of the descending oesophageal nerve caused contraction of the pre-crop oesophagus and crop but had no effect on the stomachs or blood pressure.

2. Transection of the recurrent nerve did not abolish the response of the stomachs or arterial blood pressure to high cervical vagal stimulation, and stimulation of the recurrent nerve had no effect on the blood pressure. Transection of the vagal trunk cranial to the recurrent nerve abolished the responses of the stomachs and blood pressure, but not of the pre-crop oesophagus or crop, to high cervical vagal stimulation.

3. Cutting the vagus and allowing it to degenerate did not abolish the actions of the descending oesophageal nerve.

4. Effects of stimulation of the vagus or descending oesophageal nerve were potentiated by eserine, blocked by atropine or hyoscine and variably affected by hexamethonium.

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Zusammenfassung

Reizeffekte des Halsvagus und der absteigenden Ösophagusnerven auf den Verdauungsapparat des Haushuhnes

1. Elektrische Reizung des Nervus vagus beim Haushuhn bewirkte Kontraktionen am präingluvialen Teil des Ösophagus, am Kropf, am Muskel- und Drüsenmagen sowie einen arteriellen Blutdruckabfall. Reizung der absteigenden Ösophagusnerven verursachte Kontraktionen am präingluvialen Teil des Ösophagus und am Kropf, hatte jedoch keinen Effekt auf den Drüsen- und Muskelmagen sowie auf den Blutdruck.

2. Durchschneidung des N. recurrens führte nicht zu einem Ausfall der Reaktionen der beiden Magenabteilungen sowie der Blutdrucksenkung im Gefolge der Reizung des proximalen Halsvagus. Auch hatte die Stimulation des N. recurrens keinen Einfluß auf den Blutdruck. Durchschneidung des Vagus kranial vom Abgang des N. recurrens führte zu einem Ausfall der vagalen Reizeffekte an den beiden Magenabteilungen und am Blutdruck, nicht aber der Effekte am präingluvialen Ösophagus.

3. Durchschneidung des Vagus mit konsekutiver Degeneration desselben hob die Aktivität der absteigenden Ösophagusnerven nicht auf.

4. Die Effekte der Reizung des N. vagus oder der absteigenden Ösophagusnerven wurden durch Eserin verstärkt, durch Atropin oder Hyoszin hingegen blockiert und durch Hexamethonium uneinheitlich beeinflußt.

Résumé

Effets de la stimulation du nerf vague cervicial et des nerfs oesophagiens descendants sur le système digestif de la poule

1. Une stimulation électrique du nerf vague de la poule provoque des contractions de l'oesophage antérieur au jabot, du jabot, du proventricule et du gésier, de même qu'une chute de la pression artérielle. Une stimulation du nerf oesophagien descendant cause une contraction de l'oesophage antérieur au jabot et du jabot, mais n'exerce aucun effet sur les estomacs ou la pression sanguine.

2. La section du nerf récurrent ne supprime pas la réponse des estomacs et de la pression artérielle à une forte stimulation du vague cervical et une stimulation du nerf récurrent n'a aucune répercussion sur la pression sanguine. La section du tronc cranial du vague vers le nerf récurrent abolit les réponses des estomacs et de la pression sanguine à une forte stimulation du vague cervical, mais non les réponses de la portion oesophagienne antérieure au jabot ou du jabot.

3. Si l'on sectionne le vague et qu'on le laisse dégénérer, on ne supprime pas les actions du nerf oesophagien descendant.

4. Les effets de stimulation du vague et du nerf oesophagien descendant sont renforçes par l'ésérine, bloqués par l'atropine ou l'hyoscine et affectés de façon variable par l'hexamethonium.

Resumen

Efectos de la estimulación de los nervios vago cervical y esofágico descendiente sobre el tracto alimenticio de las aves domésticas

1. El estímulo eléctrico del nervio vago en la gallina produce contracciones en el esófago pre-ingluvieico, buche, proventrículos y molleja, así como hipotensión en la sangre arterial. La estimulación del nervio esofágico descendiente ocasiona la contracción del esófago pre-ingluvieico y del buche, pero no surte efecto sobre los estómagos o la presión sanguínea.

2. La sección transversa del nervio recurrente no abule la respuesta de los estómagos o de la presión en la sangre arterial al elevado estímulo vagal cervical, y la estimulación del nervio recurrente no surte efecto sobre la presión sanguínea. La sección transversa del tronco vagal craneal cerca del nervio recurrente abule las respuestas de los estómagos y de la tensión sanguínea, pero no las del esófago pre-ingluvieico o del buche frente a un estímulo vagal cervical considerable.

3. La tajadura del vago y su degeneración no abule las acciones del nervio esofágico descendiente.

4. Los efectos de la estimulación del vago o del nervio esofágico descendiente son reforzados por la eserina, bloqueados por la atropina e hioscina, comportándose el hexametonio de forma variable.

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A hyoscine-resistant contraction of the chicken isolated oesophagus to stimulation of the vagus and descending oesophageal nerves

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Isolated oesophageal preparations with vagus and descending oesophageal nerves attached were made from young and adult chickens (Bartlet & Hassan, 1968a), and the contractions of the longitudinal muscle recorded isotonically. The preparations were suspended in Krebs solution containing half the normal concentration of Ca^{++}, equilibrated with 5% carbon dioxide in oxygen and maintained at 35° C. *In vivo* experiments were carried out on cockerels anaesthetized with halothane and decerebrated; after an interval of 60–90 min contractions of the oesophagus to stimulation of the vagus and descending oesophageal nerves were recorded by a balloon-tambour system (Hassan, 1967). In both *in vitro* and *in vivo* experiments the nerves were stimulated with square wave pulses (width 10 msec, frequency 20 c/s and intensity 5V) the duration of stimulation being 5–15 sec applied every 2–15 min.

Exposure of the isolated oesophagus to hyoscine (1–100 μg/ml.) for 30 min abolished the contraction produced by stimulation of either nerve if the duration of stimulation was less than 5 sec; however, prolonged stimulation produced a delayed contraction which was not antagonized by hyoscine (100 μg/ml.) although cocaine (50 μg/ml.) abolished it (Bartlet & Hassan, 1968b). The hyoscine-resistant contraction was abolished by cutting the nerves, but mepyramine, methysergide or bretylium had no effect on it. Physostigmine (5 μg/ml.) did not have a significant effect on the hyoscine-resistant contraction and physostigmine (50 μg/ml.) antagonized it ($P<0.05$). In six experiments tubocurarine (50 μg/ml.) reduced the height of the hyoscine-resistant contraction by a mean (±S.E. of mean) of 21% (±8, $P<0.05$) and hexamethonium (100 μg/ml.) reduced it by a mean of 59% (±14, $n=3$, $P<0.05$).

In previous experiments with pentobarbitone-anaesthetized chickens, the contraction of the oesophagus *in vivo* produced by stimulation of the vagus and descending oesophageal nerves was abolished by intravenous hyoscine (Hassan, 1967). Decerebrate preparations have now been used to find out whether the pentobarbitone had blocked a hyoscine-resistant response of the oesophagus to nerve stimulation. Hyoscine (100 μg/kg intravenously) abolished the contractions of the oesophagus produced by prolonged stimulation of the vagus and descending oesophageal nerves.

The *in vivo* experiments suggest that the vagus and descending oesophageal nerves are cholinergic, but the *in vitro* experiments suggest that these nerves release acetylcholine which acts on receptors inaccessible to hyoscine added to the organ bath.

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THE ACTION OF PHYSOSTIGMINE AND THE DISTRIBUTION OF CHOLINESTERASES IN THE CHICKEN OESOPHAGUS

BY

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Physostigmine produces a contraction of the muscularis mucosae but not of the external muscle of the isolated guinea-pig oesophagus. The oesophagus of guinea-pigs contains a striped external muscle innervated by vagal fibres which are not blocked by hexamethonium, and a muscularis mucosae consisting of plain muscle innervated by post-ganglionic nerves. This led to the suggestion that contractions produced by physostigmine in the isolated guinea-pig oesophagus involved only post-ganglionic nerves or the muscularis mucosae (Bartlet, 1968a, b). The chicken oesophagus has a plain external muscle and muscularis mucosae (Calhoun, 1933, 1954), and in the present experiments physostigmine increased the tone of both the external muscle and the muscularis mucosae. The possibility that physostigmine acted through non-neuronal structures has been investigated by testing its effect in the presence of cocaine. In addition the histochemical localization of cholinesterases was investigated to depict the site of action of physostigmine.

METHODS

Isolated organs

Preparations of external muscle, muscularis mucosae, oesophagus and oesophagus with nerve attached were made as described by Bartlet & Hassan (1968). The terms "external muscle" and "muscularis mucosae" in the text, table and figures refer to the separated oesophageal layers, and the term "oesophagus" refers to the whole organ. The isolated tissues had open ends and were set up for the recording of contractions in a 40 ml. organ bath filled with the saline-bicarbonate solution of Krebs & Henseleit (1932) with the Ca^{++} halved, gassed with 5% carbon dioxide in oxygen and maintained at 35° C. The preparations were attached to an isotonic frontal writing lever which exerted a force of 2 *g* cm, and the nerves were drawn through an electrode similar to that described by Burn & Rand (1960). The nerve was stimulated for 5–30 sec every 5 min or for 1.5 min every 10 min with square pulses of 10 msec duration, a frequency of 20 stimuli/sec and a voltage adjusted to produce a submaximal or a maximal contraction.

The drugs used were acetylcholine chloride, cocaine hydrochloride, physostigmine salicylate and hyoscine hydrobromide. Quantities of drugs in the text, table and figures refer to the salts.

Histochemistry

Pieces of oesophagus were fixed at 4° C for 4 hr in an isotonic solution of sodium sulphate containing 3.6% v/v formaldehyde. After rinsing the tissue with distilled water, it was transferred to 20% v/v ethanol and kept at 4° C for at least 15 hr and sometimes as long as a week. Sections were cut at a thickness of

40 μ on a freezing microtome. The freshly cut sections were stained for acetylcholinesterase or butyrylcholinesterase by the thiocholine method (Krnjević & Silver, 1965), with incubation in the substrate containing solution being carried out at a pH of 5.4 for 5 hr. Some sections were placed in a solution of physostigmine salicylate (4 μg/ml.) or diisopropyl phosphorofluoridate (DFP) (10 μg/ml.) for 30 min before being incubated in a medium which contained in addition to the substrate the inhibitor at the concentration mentioned. Incubation of sections with butyrylthiocholine produced staining at sites of butyrylcholinesterase activity only, whereas when acetylthiocholine was used as substrate staining was produced at sites of acetylcholinesterase and butyrylcholinesterase activities. DFP (10 μg/ml.) inhibited the activity of butyrylcholinesterase only, and physostigmine salicylate (4 μg/ml.) inhibited both acetylcholinesterase and butyrylcholinesterase activities.

RESULTS

Effect of cocaine on the contractions produced by vagal stimulation, acetylcholine and physostigmine

Exposure of the oesophagus to cocaine (50 μg/ml.) for 20–30 min abolished the contractions produced by submaximal or supramaximal vagal stimulation (seven experiments), and potentiated the contraction of the oesophagus to acetylcholine added to the organ bath.

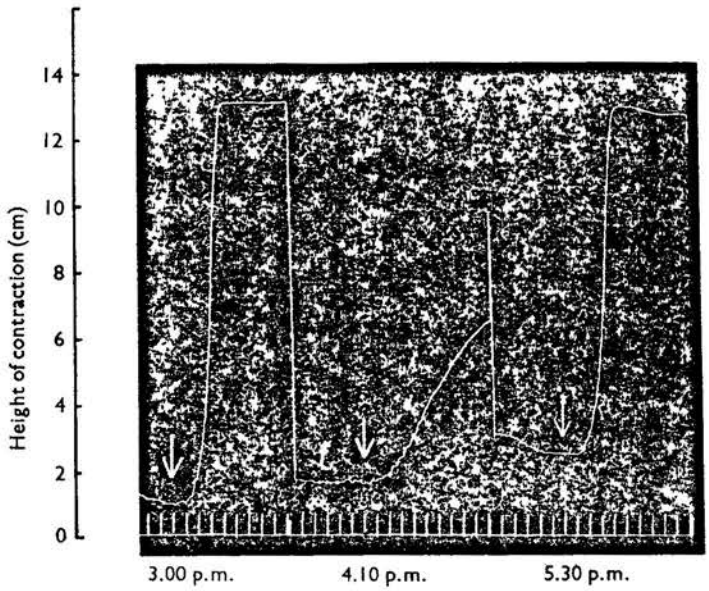


Fig. 1. Contractions of the isolated muscularis mucosae. The arrows mark the addition of physostigmine salicylate (5 μg/ml.) to the organ bath at the times shown. After exposure of the preparation to physostigmine for 5 min the drum was stopped and the drug washed from the organ bath. Cocaine hydrochloride (50 μg/ml.) was present in the organ bath from 3.15 p.m. to 4.15 p.m. Time, 30 sec.

Physostigmine (5 μg/ml.) always produced contractions of the whole oesophagus and external muscle and muscularis mucosae preparations. The preparations contracted after addition of physostigmine to the organ bath after a delay of 0.5–3.5 min, and relaxed only slowly after washing the drug out of the organ bath. Preparations were exposed to

physostigmine long enough to produce a suitable height of contraction—1–3 min in the case of the oesophagus and 5 min in the case of the external muscle or muscularis mucosae preparations. The interval between tests with physostigmine was about one hour and in these conditions a constant response to a given concentration of physostigmine was obtained. Hyoscine (100 ng/ml.) abolished the responses to physostigmine, and exposure to cocaine (50 μg/ml.) for 30–60 min also antagonized it (Fig. 1 and Table 1). The antagonism of physostigmine by cocaine was greater on the oesophagus than on the external muscle or muscularis mucosae preparations ($P<0.01$).

TABLE 1

EFFECT OF COCAINE ON CONTRACTIONS PRODUCED BY PHYSOSTIGMINE

	Residual contractions to physostigmine salicylate (5 μg/ml.) in the presence of cocaine hydrochloride (50 μg/ml.). The results are expressed as % controls. Mean values±S.E. (No. expts.)	*P* (antagonism by cocaine)
Oesophagus	8·8±2·8 (4)	<0·001
External muscle	38·3±2·8 (7)	<0·001
Muscularis mucosae	44·0±5·7 (5)	<0·001

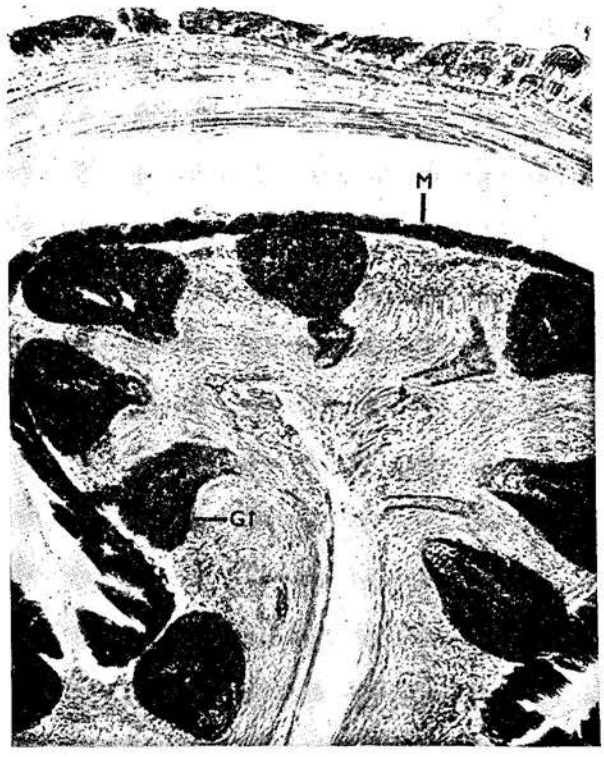
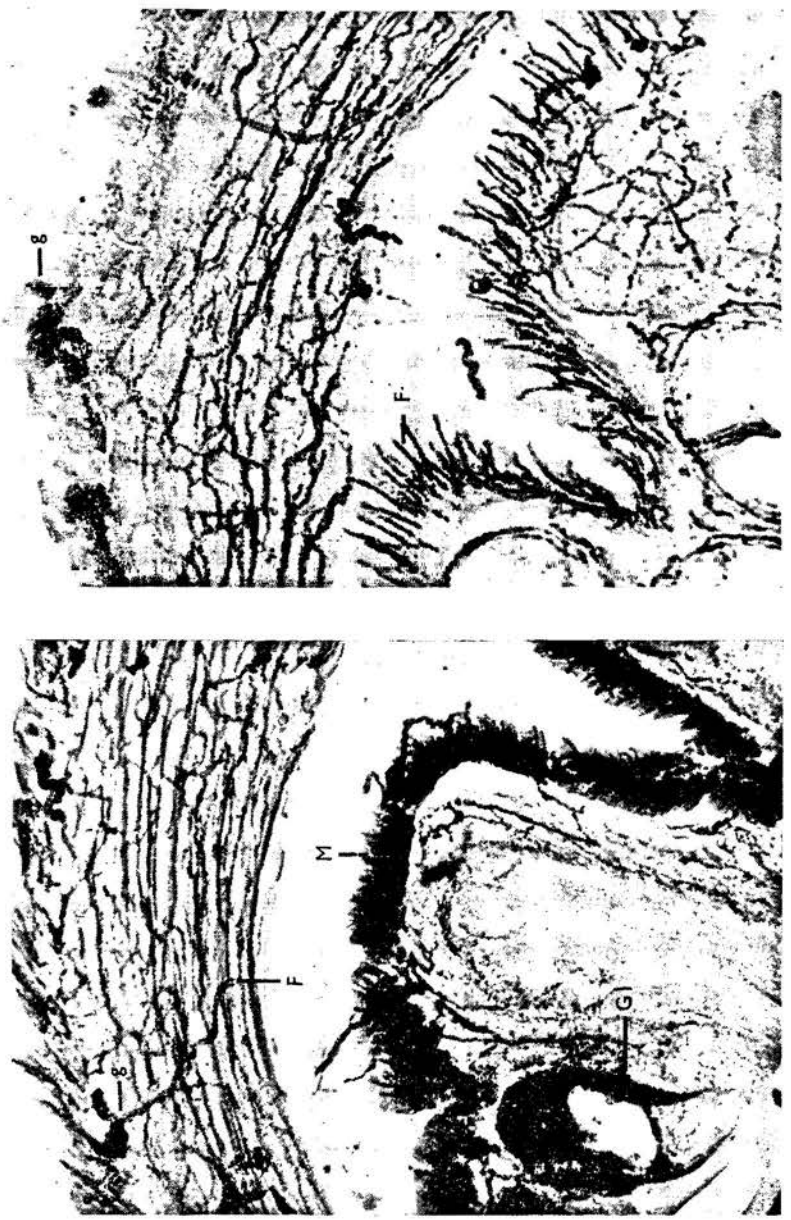


Fig. 2. Transverse section of post-crop oesophagus (×75). Black staining depicts butyrylcholinesterase activity. Dense staining was present in the muscularis mucosae (M) and mucosal glands (GI).



(a) (b)

Fig. 3. Transverse sections of post-crop oesophagus incubated with (a) acetylthiocholine and (b) acetyl-thiocholine and DFP (10 μg/ml.) (×100). Staining depicts cholinesterase activity in ganglia (g), nerve fibres (F), muscularis mucosae (M) and mucosal glands (Gl). In (a), acetylcholinesterase and butyryl-cholinesterase contribute to the stain, and in (b) acetylcholinesterase only is responsible for it.

Cholinesterase staining

Sections made from different parts of the oesophagus exhibited a similar pattern of staining for cholinesterases, and in sections incubated in the presence of physostigmine staining was absent.

In sections incubated with butyrylthiocholine, butyrylcholinesterase activity was demonstrated in the muscularis mucosae and mucosal glands. The stain was usually black (Fig. 2), but in some sections the staining was lighter and brown in appearance. The external longitudinal muscle was usually stained faintly but the circular muscle, ganglia and nerve fibres were not stained. Butyrylcholinesterase activity, and thus staining, was inhibited by pretreatment of the sections with DFP (10 μg/ml.).

In sections incubated with acetylthiocholine and DFP (10 μg/ml.) acetylcholinesterase activity was demonstrated in ganglia and nerve fibres (Fig. 3b). In sections incubated with acetylthiocholine alone, however, some additional staining attributable to butyrylcholinesterase was found in the muscularis mucosae and mucosal glands, and in these sections the staining attributable to mucosal acetylcholinesterase was masked by butyrylcholinesterase being present at the same site (Fig. 3a). Acetylcholinesterase activity was found in ganglia between the external longitudinal and circular muscle layers, in a few ganglia in the muscularis mucosae and in nervous networks associated with the external muscle, the muscularis mucosae and the mucosal glands.

DISCUSSION

The contraction of the isolated chicken oesophagus produced by physostigmine was blocked by hyoscine, and cocaine, in a concentration sufficient to block vagal stimulation, also antagonized it. This suggests that when physostigmine inhibits cholinesterases, an acetylcholine-like substance accumulates and stimulates neural structures. Cocaine reduced the height of the physostigmine-induced contraction of the whole oesophagus to a greater extent than the contractions of the external muscle and muscularis mucosae preparations. This suggests that separation of the external muscle from the mucosa damaged neural structures stimulated by acetylcholine when cholinesterases are inhibited.

The small residual contraction obtained with physostigmine in the presence of cocaine was blocked by hyoscine, which suggests that an acetylcholine-like substance was produced. Such an acetylcholine-like substance could have originated at nerve terminals or at a non-neural structure. According to Cuthbert (1963), an acetylcholine-like substance is present in the nerve-free plain muscle of the chick amnion.

A high level of butyrylcholinesterase activity was found in the muscularis mucosae and mucosal glands and not in the external muscle. Because physostigmine produced similar contractions of anaesthetized preparations of the external muscle and muscularis mucosae, butyrylcholinesterase does not seem to play any part in the metabolism of endogenous acetylcholine. Staining in sections incubated with acetylthiocholine depicted the location of acetylcholinesterase and butyrylcholinesterase, but the activity of the latter enzyme can be selectively inhibited by DFP (10 μg/ml.). In sections incubated with acetylthiocholine and DFP (10 μg/ml.) acetylcholinesterase activity was confined to neural structures. Thus the distribution of acetylcholinesterase does not support the

hypothesis that physostigmine causes an accumulation of acetylcholine at a non-neural structure, and we assume that in the chick oesophageal preparations small amounts of acetylcholine were released from anaesthetized nerves.

SUMMARY

1. Physostigmine salicylate (5 μg/ml.) produced contractions of the isolated whole oesophagus, and external muscle and muscularis mucosae preparations. These contractions were blocked by hyoscine.

2. In the presence of cocaine, at a concentration sufficient to block the effect of vagal stimulation, the contractions produced by physostigmine became smaller whereas those produced by acetylcholine were potentiated.

3. Acetylcholinesterase activity was demonstrated histochemically in ganglia and nerve fibres. Butyrylcholinesterase activity was found only in the muscle and glands of the mucosa.

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