J. Physiol. (1969), **205**, *pp.* 317–327
With 5 text-figures
Printed in Great Britain

INDIRECT CHOLINERGIC VASOMOTOR CONTROL OF INTESTINAL BLOOD FLOW IN THE DOMESTIC CHICKEN

BY CHRISTOPHER BELL*

From the Department of Zoology, University of Melbourne, and the Department of Physiology, A.R.C. Institute of Animal Physiology, Babraham, Cambridge†

(*Received* 21 *April* 1969)

SUMMARY

1. A histochemical and pharmacological study has been made of the relationship between the medial circular and adventitial longitudinal smooth muscle coats of the anterior mesenteric artery of the domestic chicken.

2. The longitudinal (LM) coat was found to be innervated by excitatory cholinergic and inhibitory adrenergic nerves, confirming previous reports.

3. The circular (CM) coat was found to be innervated by adrenergic vasoconstrictor nerves. There was no evidence for the existence of cholinergic nerves supplying the circular muscle.

4. Contraction of the LM had little effect on the resting perfusion pressure of the isolated artery, but caused potentiation of perfusion pressure responses to vasoconstrictor stimuli directly affecting the CM.

5. It is suggested that the function of the LM is to modulate the reactivity of the anterior mesenteric artery to vasomotor stimuli, and that this may be particularly implicated in the cardiovascular response to sudden stress.

INTRODUCTION

In general, the smooth muscle coats of arteries are arranged with the muscle cells running in a circular or helical direction. Longitudinally orientated muscle is rare, although it does occur, for example, in some of the larger conducting and distributing arteries, where it is present mixed with circularly arranged muscle within the media or in scattered bundles outside the circular muscle (Benninghoff, 1930; Schafer, 1949).

In the anterior mesenteric artery of certain species of fowl, however, a

* National Heart Foundation of Australia Overseas Research Fellow.
† Present address.

discrete coat of longitudinal muscle is present external to the circular medial muscle (Ball, Sautter & Katter, 1963). Recently Bolton (1968*a*, *b*) has demonstrated that in the domestic chicken this longitudinal muscle is innervated by excitatory cholinergic and by inhibitory adrenergic nerves, is spontaneously active, and responds to neurohumoral stimuli by large changes in length. Thus it resembles gastrointestinal smooth muscle rather than vascular smooth muscle in its properties.

The close association of this longitudinal muscle coat with the anterior mesenteric artery suggests that it may serve some function in controlling visceral blood flow. However, in the absence of evidence regarding the innervation of the circular muscle coat of the artery, and the actual effect of longitudinal muscle activity on the calibre of the arterial lumen, such a function remains obscure. In the present paper, a histochemical and pharmacological examination of the innervation of both circular and longitudinal muscle coats has been performed, and simultaneous recording of intraluminal perfusion pressure and longitudinal tension of the artery has enabled some conclusions to be drawn regarding possible mechanisms of vasomotor control of the vessel.

METHODS

White Leghorn chickens aged between 12 and 16 weeks were killed by dislocation of the neck. The abdominal cavity was opened and the anterior mesenteric artery removed. The mesenteric investment and vein accompanying the artery were cleared away, a polyethylene cannula (PE 90) was tied into the proximal end of the vessel, and the preparation was mounted in a bath of physiological saline (Bolton, 1968*a*) maintained at 41° C. The same solution was perfused through the artery via a Watson–Marlow roller pump delivering a constant flow of 9 ml./min, and intraluminal pressure was recorded with a Statham transducer (P 23BC) coupled into the perfusion system distal to the pump. Longitudinal contractile activity was recorded via a thread attached to the distal end of the artery and an isotonic lever system coupled by a weak spring to a Grass transducer (FT 05). The fixed weight of the lever system, in terms of load on the tissue, was 2 g. The perfusion and recording set up is illustrated in Fig. 1. Pressure and length changes were registered on a Grass Model 5 polygraph. Periarterial nerve stimulation was elicited by means of shielded platinum ring electrodes placed around the proximal end of the preparation, and square wave pulses of 1 msec duration and supramaximal voltage were delivered from a Grass S 5 stimulator in 20 sec bursts at intervals of not less than 7 min. Neurohumoral drugs were injected into the perfusion system just proximal to the preparation, or into the bath. Other drugs were delivered from the reservoir of perfusion fluid or were applied directly to the bath. Drugs used were acetylcholine perchlorate, cinchocaine hydrochloride (Nupercaine), hyoscine hydrobromide, mepyramine maleate (Anthisan), methysergide, noradrenaline bitartrate, phentolamine hydrochloride (Regitine) and propranolol (Inderal). All doses and concentrations cited refer to the above salts or bases.

Fluorescent histochemistry was performed on freeze-dried tissue by the method of Falck (1962). The specific fluorescence visualized in nervous elements was as intense

after formaldehyde treatment for 1 hr as it was after treatment for 3 hr. It can therefore be assumed that the fluorescence was due at least mainly to the presence of noradrenaline rather than adrenaline (Falck, 1962).

Localization of acetylcholinesterase (AChE) was performed on tissue fixed in formol-sucrose and stained by the method of Karnovsky & Roots (1964), as described in a previous paper (Bell, 1968). The AChE-positive nervous elements visualized were of a similar intensity of staining as that seen in known cholinergic systems. It has therefore been assumed that such nerves were cholinergic in nature (Bell & McLean, 1967).

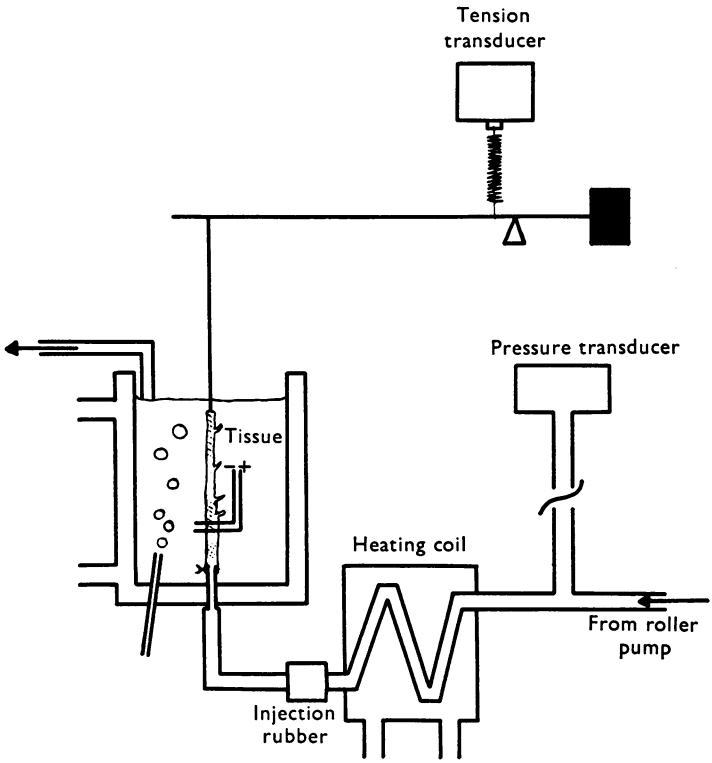

Fig. 1. The experimental set-up for simultaneous recording of perfusion pressure and longitudinal tension of the anterior mesenteric artery.

RESULTS

Histochemical localization of adrenergic and cholinergic nerves

The outer adventitia of the artery contained numerous large nerve trunks which included both NA-positive and AChE-positive elements (Fig. 2). These trunks presumably represented perivascular visceral nerves supplying the intestine. The longitudinal muscle coat (LM) contained scattered NA-positive fibres, and more numerous AChE-positive

fibres. Medial to the LM lay the circular muscle coat (CM), which was surrounded by a layer of connective tissue. The junction between connective tissue and CM contained a plexus of NA-positive fibres, but was devoid of AChE-positive elements (Fig. 2).

Pharmacology of the LM

Responses of the LM to nervous and chemical stimuli were as reported by Bolton (1968*a*). Under resting conditions, periarterial electrical stimulation produced contraction of the LM (Fig. 3*a*) which was maximal in response to stimulation at 5–10 Hz, and was mimicked by ACh (10^{-8}–10^{-7} g/ml.). The maximal contractions elicited by these stimuli corresponded to shortening of the LM by 50–60% of its resting length. Responses to ACh and to nervous stimulation were abolished by hyoscine (10^{-7} g/ml.). If the LM was first contracted with $BaCl_2$ (5×10^{-5}–10^{-4} g/ml.) or ACh, the cholinergic excitatory response to nervous stimulation was followed by a relaxation (Fig. 3*b*) which was maximal at stimulation frequencies of 40–50 Hz, was mimicked by NA (10^{-7} g/ml.) and was abolished by propranolol (5×10^{-7} g/ml.).

Responses of the CM

Under the conditions employed the perfusion pressure of the artery was 20–30 mm Hg. The CM appeared to be maximally relaxed as the perfusion pressure was not reduced by intraluminal injection of vasodilator substances such as papaverine. Periarterial electrical stimulation produced vasoconstrictor responses which were blocked by infusion of phentolamine (5×10^{-6} g/ml.) (Fig. 4*a*), and which were mimicked by intraluminal NA (10^{-7}–10^{-6} g/ml.). These vasoconstrictor responses varied in magnitude between preparations, but were, considering the muscular nature of the artery, generally small (Table 1). In five out of twenty-one experiments, the adrenergic vasoconstrictor response to nervous stimulation was preceded by a more rapid constriction which was not affected by phentolamine, hyoscine, methysergide or mepyramine, but was abolished by cinchocaine (5×10^{-6} g/ml.) (Fig. 4*a*). This concentration of cinchocaine did not significantly affect the responsiveness of the CM to NA, indicating that the non-adrenergic response to electrical stimulation was due to excitation of nervous elements.

If the CM was constricted by perfusion with $BaCl_2$ (5×10^{-4} g/ml.) or NA (10^{-7} g/ml.), ACh (10^{-7} g) caused a vasodilatation (Fig. 4*b*), which was abolished by hyoscine (10^{-7} g/ml.). Under these conditions, however, no dilatory response to nervous stimulation was observed (Fig. 4*b*).

In an attempt to determine whether the LM might serve some function in regulation of intra-arterial flow, observations were made on the changes

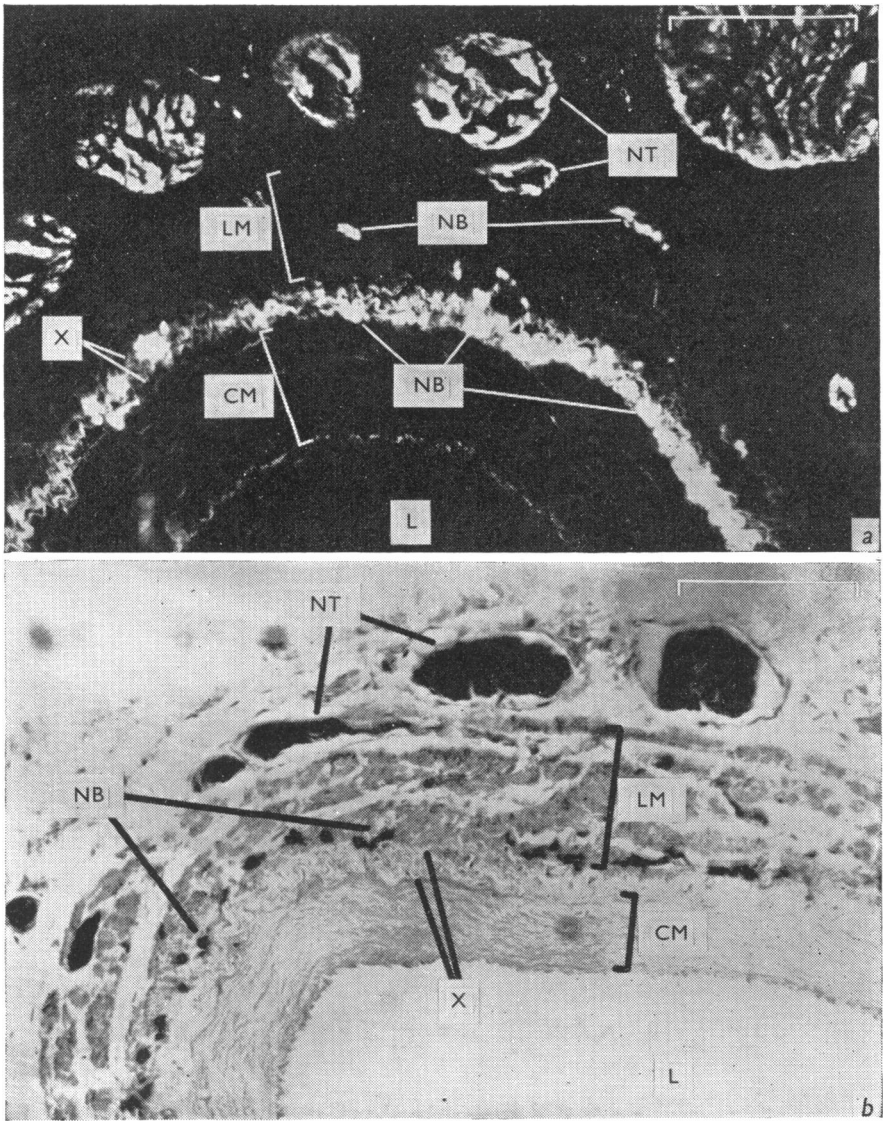

Fig. 2. Transverse sections (10 μ) of the anterior mesenteric artery, showing the patterns of (*a*) catecholamine fluorescence, and (*b*) AChE staining. LM, longitudinal muscle coat; CM, circular muscle coat; NT, large nerve trunks; NB, smaller nerve bundles; X, connective tissue layer separating LM and CM; L, lumen. Note that although both those nerve bundles containing catecholamines and those staining for AChE are close to the junction between muscle layers, the bundles staining for AChE are restricted to the LM layer outside the layers of connective tissue, while those containing catecholamines lie mainly at the boundary between connective tissue and CM. Calibrations: 100 μ.

in perfusion pressure of the artery during ACh-induced changes in length of the LM. Shortening of the LM by 30 % or more of its maximum resting length produced a rise in perfusion pressure. This rise, which varied in magnitude between different preparations (Table 1), was in any one

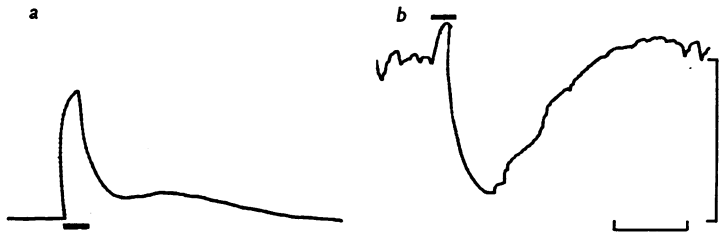

Fig. 3. Contractile responses of the LM to periarterial stimulation (20 sec: 5 Hz) (*a*) at maximum resting length, and (*b*) after shortening the muscle by 50 % with $BaCl_2$ (10^{-4} g/ml.). Calibrations: 1 min and 50 % resting length change.

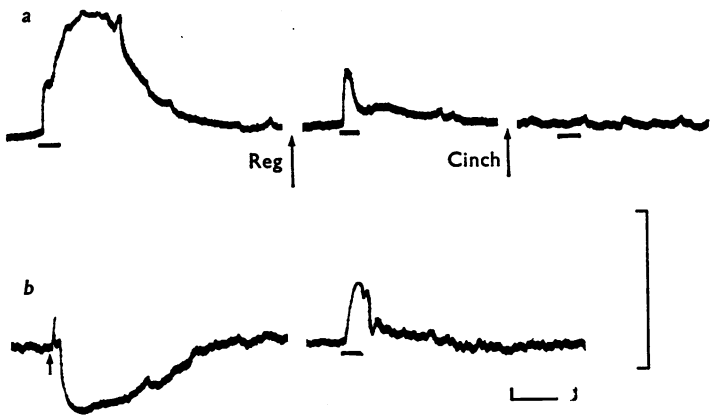

Fig. 4. Perfusion pressure recording from two anterior mesentery arteries. (*a*) A typical vasoconstrictor response to periarterial nerve stimulation (20 sec: 10 Hz), revealing after phentolamine (Reg, 5×10^{-6} g/ml.) a second component of the response which was blocked by cinchocaine (Cinch, 5×10^{-6} g/ml.). Resting perfusion pressure: 23 mm Hg.
(*b*) Following perfusion with $BaCl_2$ (5×10^{-4} g/ml.) which resulted in constriction of the CM, ACh (10^{-7} g/ml.) at arrow, caused vasodilatation. However no vasodilator component of the response to periarterial nerve stimulation was observed. Resting perfusion pressure: 45 mm Hg. Calibrations: 1 min and 50 mm Hg.

preparation dependent on the amount of LM shortening, and had a time course which paralleled the presence of the LM contraction (Fig. 5*a*). This perfusion pressure increase was not pronounced, being in general small even in comparison to the relatively weak response elicited by vasomotor nerve stimulation (Fig. 5*a*, Table 1). However, during the period of

LM shortening there was a pronounced potentiation of the response of the CM to vasoconstrictor nerve stimulation (Fig. 5*b*, Table 1). The response of the CM to intraluminal NA was also potentiated, although less consistently.

TABLE 1. Comparison of the effect of ACh-induced contractions of the LM on pressor response to vasoconstrictor nervous stimulation (20 Hz, 20 sec) in ten experiments

Expt.	% shortening LM in response to ACh	Resting perfusion pressure rise (mm Hg)	Response to vasoconstrictor nerve stimulation (mm Hg)		
			(*a*) Control	(*b*) During LM shortening	Ratio *a*:*b*
1	20	1			
	60	5	12	28	2·3
2	40	1	10	45	4·5
3	20	0			
	50	10	11	26	2·4
4	45	1	3	11	3·7
5	40	3			
	60	5	13	34	2·6
6	50	5	6	15	2·5
7	30	12	30	51	1·7
8	40	5			
	50	10	15	32	2·1
9	35	10	12	16	1·3
10	20	0			
	50	8			
	60	18	55	55	1·0

DISCUSSION

The present pharmacological results support those of Bolton (1968 *a*, *b*) regarding a dual innervation of the LM of the anterior mesenteric artery of the domestic chicken. Further confirmatory evidence has been obtained by the demonstration that the LM contains nerve fibres which stain heavily for AChE, and others which exhibit fluorescence characteristic of NA. The fact that these fibres are distributed through the LM layer is another point of similarity of the LM to visceral smooth muscle, as opposed to vascular smooth muscle, where the innervation is generally restricted to the outer margin of the muscle coat (Ehinger, Falck & Sporrong, 1967; Fillenz, 1967; Bell, 1969).

The present results have also demonstrated that the CM coat of the anterior mesenteric artery responds to periarterial nervous stimulation by a constriction which is mimicked by NA and abolished by α-adrenergic blockade. Histochemical examination revealed that a plexus of fine nerve

fibres containing catecholamine fluorescence is closely applied to the outer surface of the CM, although such fibres are absent from the interior of the muscle layer. Thus the CM agrees with the classical concept of arterial smooth muscle in its arrangement and innervation. No evidence has been obtained to suggest the existence of a dilator innervation to the CM, although inhibitory receptors for ACh appear to be present. However, in some experiments a small, non-adrenergic constrictor response to nervous stimulation was recorded. As this response was abolished by cinchocaine in concentrations which did not affect the response of the CM to direct stimulation by NA, it would seem to be neurogenic. However it was not

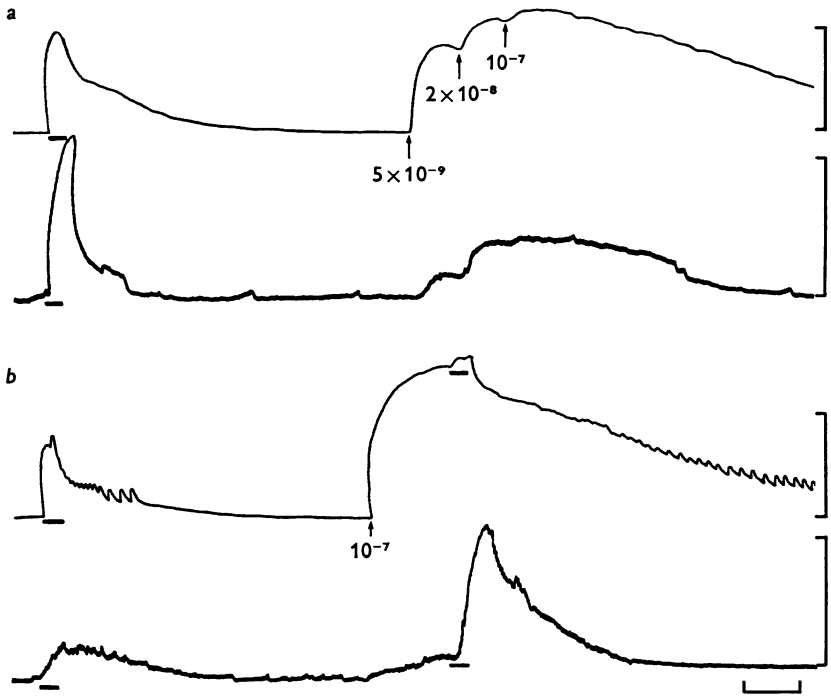

Fig. 5. Simultaneous recording of LM tension (upper traces) and perfusion pressure (lower traces) in two experiments (preparations treated with propranol (5×10^{-7} g/ml.)). Resting perfusion pressures: (*a*) 25 mm Hg, (*b*), 23 mm Hg. The initial resting length of the LM was maximal in each experiment.

(*a*) Showing that maximal tone changes of the LM in response to ACh (at arrows) have relatively little effect on perfusion pressure, compared to the response to periarterial nerve stimulation (black bars, 20 sec: 20 Hz).

(*b*) Demonstrating the potentiation of the vasoconstrictor response to periarterial nerve stimulation (black bars, 20 sec: 20 Hz) by contraction of the LM in response to ACh (at arrows), despite the minimal effect of this LM contraction on resting perfusion pressure. Calibrations: 1 min, 50 % length change, 50 mm Hg.

abolished by antagonists of ACh, 5-HT or histamine, and its nature remains obscure.

The close anatomical association of the LM with the anterior mesenteric artery would seem to suggest that this muscle layer serves some function in control of blood flow through the vessel. However, near-maximal length changes of the LM produced changes in perfusion pressure which were in general small compared to those elicited by stimuli affecting the CM. This suggests that the LM does not have a direct vasomotor function. Such a suggestion is supported by the fact that the CM itself is innervated by vasomotor fibres. However, shortening of the LM did strongly potentiate the response of the CM to vasomotor nerve stimulation. Folkow & Sivertsson (1964) have produced evidence to suggest that, at least in some cutaneous vessels, the wall–lumen ratio can dramatically affect the reactivity of the vessel to vasoconstrictor stimuli. An increase in this ratio due to LM contraction therefore seems a likely basis for the potentiation of vasoconstrictor responses seen in the anterior mesenteric artery. However, there are two other possible mechanisms of action which must be considered. It has been demonstrated that the responses to excitatory stimuli of both vascular and visceral smooth muscles can be potentiated by agents which cause partial muscle membrane depolarization (de la Lande, Cannell & Waterson, 1966; Bell, 1967), and it may be that LM contraction can also cause depolarization of the CM cell membranes by mechanical distortion of the tissue (Bayliss, 1902; Bülbring, 1955; Mellander & Johansson, 1968). In addition it must be remembered that ACh can cause the release of noradrenaline from adrenergic nerve terminals (Brandon & Boyd, 1961; Angelakos & Bloomquist, 1965; Löffelholz, 1967, and others), and that therefore the potentiation observed could be due partly to such an effect, both by facilitating noradrenaline release during nervous stimulation and by causing noradrenaline release in the absence of nervous stimulation sufficient to produce partial muscle membrane depolarization.

Attempts have been made to resolve this situation with electrophysiological techniques. However, to date, although excitatory junctional potentials have been recorded from the cells of the CM during adrenergic nerve stimulation, any effects of LM contraction on this transmission process have been obliterated by contraction artifacts from the LM (C. Bell, unpublished observations).

The effect of LM shortening on the responses of the CM suggests that, *in vivo*, the LM may serve to modulate the response of the artery to vasomotor stimuli. While no evidence is available to indicate the significance of such a mechanism, the fact that the anterior mesenteric artery consti-

tutes the blood supply of virtually the entire intestine indicates that it may be involved in the restriction of visceral blood flow during sudden stress.

This work was supported by the National Heart Foundation of Australia.

I should like to thank Professor G. Burnstock, in whose department the experimental section of this work was performed, for his kind provision of facilities, and Mr T. Bennett for assistance with the fluorescent histochemistry. I am also grateful to Dr M. Vogt for her present hospitality, and for her comments on the manuscript.

REFERENCES

ANGELAKOS, E. T. & BLOOMQUIST, E. (1965). Release of norepinephrine from isolated hearts by ACh. *Archs int. Physiol.* **73**, 397–402.

BAYLISS, W. M. (1902). On the local reactions of the arterial wall to changes in internal pressure. *J. Physiol.* **28**, 220–231.

BALL, R. A., SAUTTER, J. H. & KATTER, M. S. (1963). Morphological characteristics of the anterior mesenteric artery of fowl. *Anat. Rec.* **146**, 251–255.

BELL, C. (1967). Effects of exogenous choline on adrenergic responses of the guinea-pig vas deferens. *Br. J. Pharmac. Chemother* **30**, 436–444.

BELL, C. (1968). Dual vasoconstrictor and vasodilator innervation of the uterine arterial supply in the guinea-pig. *Circulation Res.* **23**, 279–289.

BELL, C. (1969). Fine structural localization of acetylcholinesterase at a cholinergic vasodilator nerve-arterial smooth muscle synapse. *Circulation Res.* **24**, 61–70.

BELL, C. & MCLEAN, J. R. (1967). Localization of norepinephrine and acetylcholinesterase in separate neurons supplying the guinea-pig vas deferens. *J. Pharmac. exp. Ther.* **157**, 69–73.

BENNINGHOFF, A. (1930). Blutgefässe und Herz. In *Handbuch der Mikroskopischen Anatomie des Menschen*, vol. 7. Berlin: Springer.

BOLTON, T. B. (1968*a*). Studies on the longitudinal muscle of the anterior mesenteric artery of the domestic fowl. *J. Physiol.* **196**, 273–282.

BOLTON, T. B. (1968*b*). Electrical and mechanical activity of the longitudinal muscle of the anterior mesenteric artery of the domestic fowl. *J. Physiol.* **196**, 283–292.

BRANDON, K. W. & BOYD, H. (1961). Release of noradrenaline from the spleen of the cat by acetylcholine. *Nature, Lond.* **192**, 880–881.

BÜLBRING, E. (1955). Correlation between membrane potential, spike discharge and tension in smooth muscle. *J. Physiol.* **128**, 200–221.

DE LA LANDE, I. S., CANNELL, V. A. & WATERSON, J. G. (1966). The interaction of serotonin and noradrenaline on the perfused artery. *Br. J. Pharmac. Chemother.* **28**, 255–272.

EHINGER, B., FALCK, B. & SPORRONG, B. (1967). Adrenergic fibres to the heart and to peripheral vessels. *Biblthca anat.* **8**, 35–45.

FALCK, B. (1962). Observations on the possibilities of the cellular localization of monoamines by a fluorescence method. *Acta physiol. scand.* **56**, suppl. 197.

FILLENZ, M. (1967). Innervation of blood vessels of lung and spleen. *Biblthca anat.* **8**, 56–59.

FOLKOW, B. & SIVERTSSON, R. (1964). Aspects of the difference in vascular 'reactivity' between cutaneous resistance vessels and A–V anastomoses. *Angiologica* **1**, 338–345.

KARNOVSKY, M. J. & ROOTS, L. (1964). A 'direct-coloring' thiocholine method for cholinesterases. *J. Histochem. Cytochem.* **12**, 219–221.

LÖFFELHOLZ, K. (1967). Untersuchungen über die Noradrenalinfreisetzung durch Acetylcholin am perfundierten Kaninchenherzen. *Arch. exp. Path. Pharmak.* **258**, 108–122.

MELLANDER, S. & JOHANSSON, B. (1968). Control of resistance, exchange and capacitance functions in the peripheral circulation. *Pharmac. Rev.* **20**, 117–196.

SCHAFER, E. S. (1949). *Essentials of Histology*, ed. CARLETON, H. M. & LEACH, E. H., 15th edn., pp. 220–222. London: Longmans, Green and Co.