J. Physiol. (1961), **156**, *pp.* 246–259
With 6 text-figures
Printed in Great Britain

THE EFFECTS OF ACETYLCHOLINE ON THE VOLUME AND VASCULAR RESISTANCE OF THE DOG'S SPLEEN

BY M. DE BURGH DALY* AND MARY J. SCOTT†
From the Department of Physiology, University College London

(*Received* 7 *October* 1960)

In a previous study of the effects of anticholinesterases on the spleen, Scott (1957) found that tetraethylpyrophosphate (TEPP) caused a small increase in volume after denervation of the spleen combined with bilateral adrenalectomy. Whether this response was the result of accumulation of acetylcholine or of a direct action of TEPP was not ascertained because passive effects due to alterations in arterial and venous pressures could not be ruled out.

Although the effects of acetylcholine on the spleen have been investigated previously by numerous workers, the results have been variable. In the dog, cat and rabbit intravenous injection of acetylcholine caused either a decrease in volume of the spleen (Ferguson, Ivy & Greengard, 1936), a decrease followed by an increase (Hunt, 1918; Gotsev, 1936) or only an increase in volume (Bacq & Fredericq, 1935). Hunt (1918) considered that the initial diminution in splenic volume produced by acetylcholine was a passive vascular effect due to the fall in systemic blood pressure, and that the subsequent increase in volume was due to a direct action of the drug on the muscular capsule of the spleen. In the view of Bacq & Fredericq (1935) the increase in volume of the spleen was due to vasodilatation within the organ. Studies on isolated strips of spleen *in vitro* indicate that acetylcholine causes contraction, not relaxation (Fredericq, 1929; Vairel, 1933; Saad, 1935; Ferguson *et al.* 1936).

The interpretation of changes in volume of the spleen occurring as a result of *intravenous* injections of a drug is often difficult because, apart from a direct effect on the organ, there are a number of other mechanisms by which the response may be brought about. First, the observed change in volume may be passive through an alteration in either arterial or portal venous pressure. Secondly, it may be the result of nervous influences on the spleen either by an action of the drug on the nervous system or reflexly through a change in arterial blood pressure. Thus Farber (1936) showed

* Present address: Department of Physiology, St Bartholomew's Hospital Medical College, London, E.C. 1.
† Present address: Department of Physiology, Marischal College, Aberdeen.

that the intravenous injection of acetylcholine in a dog whose spleen was isolated from the circulation and perfused by a second dog caused a diminution in volume of the organ, which could only have been brought about through the nerves to the spleen. Thirdly, the response of the spleen may be due to increased secretion of suprarenal medullary hormones.

The present study was undertaken first to discover the direct effect of acetylcholine on the spleen and secondly to investigate the mechanisms by which the responses were brought about. To exclude nervous and humoral effects the spleen was isolated and perfused with blood and injections were made into the splenic artery. This technique had the advantage that it enabled changes in vascular resistance to be measured simultaneously with changes in volume of the organ.

METHODS

Dogs, varying in weight from 10·2 to 18·6 kg, were anaesthetized with 0·25 ml./kg intravenously of a 1:1 mixture of Dial Compound (Ciba Laboratories Ltd, diallylbarbituric acid 0·1 g and urethane 0·4 g/ml.) and pentobarbitone sodium solution (Nembutal, Abbott Laboratories, Ltd, 60 mg/ml.) preceded by morphine hydrochloride (3 mg/kg subcutaneously). One animal was given chloralose (0·1 g/kg intravenously) after premedication with morphine (3 mg/kg subcutaneously).

In most experiments the spleen was freed from its nervous connexions by removal from the abdomen; it was perfused from a femoral artery of the same animal. The vascular connexions of the spleen with the omentum, pancreas and stomach were divided between ligatures. The splenic artery was tied close to its origin from the coeliac artery and cannulated towards the spleen; the splenic vein was likewise cannulated. The remaining tissue in the splenic pedicle was then divided between ligatures, and the spleen was removed from the abdomen and placed in a Perspex plethysmograph. The method used for perfusion of the spleen is shown in Fig. 1. Blood from a femoral artery passed through the flowmeter *a* into the splenic artery *b*. The blood was returned to the animal from the splenic vein *c* via a femoral vein. The arterial inflow pressure to the spleen was measured from a T-piece in the arterial tubing by a mercury manometer. The splenic vein pressure was measured from the venous tubing with a vertical saline manometer, the open end of which was connected to a small volume recorder. The flowmeter used for measuring blood flow was a rotameter of the Shipley & Wilson (1951) type, in which were incorporated the modifications described by Bell (1954). Blood flow was recorded continuously on the kymograph by means of a direct recording milliammeter. The instrument was calibrated at the end of each experiment as described by Daly (1957).

In all these experiments coagulation of the blood was prevented by heparin (Liquemin, Roche Products, Ltd, 7–21 mg/kg). Unless otherwise stated, injections of drugs were made into the splenic arterial tubing distal to the rotameter. Control injections were made in nearly every experiment with a solution of sodium chloride, 0·9 g/100 ml., and were without effect. The volume of the injected fluid did not exceed 0·2 ml.

The plethysmograph was filled with liquid paraffin and rested on an electric heating pad to maintain the temperature of the fluid at 37–39° C. Changes in volume of the spleen were measured by means of a Krogh type of volume recorder connected to the plethysmograph.

In a few experiments the splenic nerve was placed on platinum wire electrodes inside the plethysmograph. An Attree (1950) electronic stimulator giving independent control of the voltage, frequency and duration of the rectangular pulses was used.

In one experiment changes in volume of the naturally perfused spleen with the splenic nerve intact were measured using the plethysmograph. To mobilize the spleen sufficiently to enable it to be put into the plethysmograph its vascular connexions with the stomach were severed. In this experiment no heparin was given. Drugs were injected via an indwelling needle in the splenic artery.

In three experiments the animals were treated with reserpine (Serpasil, Ciba) to deplete the stores of catechol amines. Two doses of 0·1–0·2 mg/kg, intravenously or intraperitoneally, were given on successive days, the acute experiment being performed the day after the second injection.

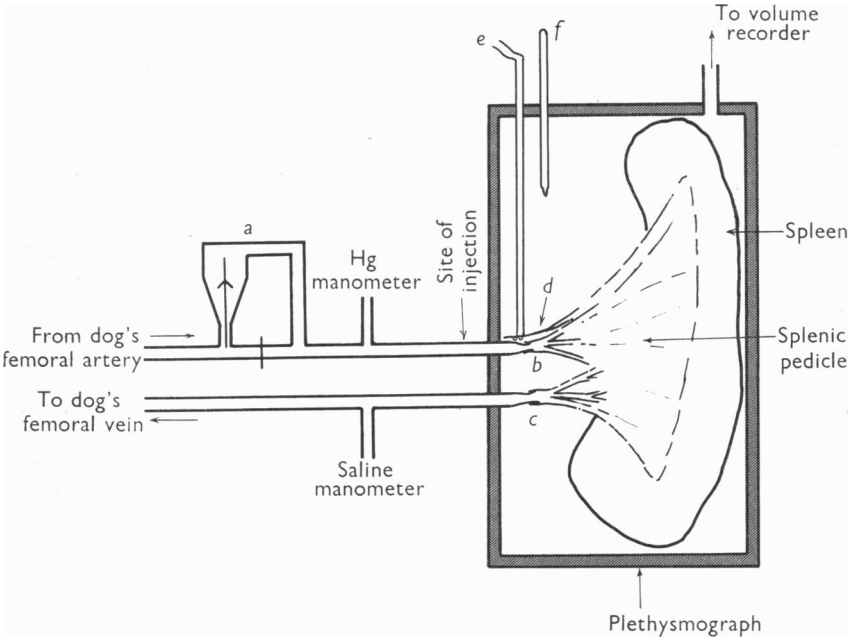

Fig. 1. Diagram showing the method of perfusing the spleen at a constant head of pressure. *a*, rotameter; *b*, splenic artery; *c*, splenic vein; *d*, splenic nerve; *e*, stimulating electrodes; *f*, thermometer. For further details see text.

The following drugs were used: acetylcholine chloride (Roche Products, Ltd), atropine sulphate (British Drug Houses, Ltd), hexamethonium bromide ('Vegolysen', May and Baker, Ltd), eserine sulphate (British Drug Houses, Ltd), tetraethylpyrophosphate (Albright and Wilson), dibenzyline hydrochloride (Smith, Kline and French), dibenamine (Light and Co. Ltd), nicotine acid tartrate (British Drug Houses, Ltd) and 1: 1-dimethyl-4-phenylpiperazinium iodide (DMPP, Pharmacia).

Degenerative section of the nerves to the spleen

At a preliminary operation carried out under pentobarbitone anaesthesia with full aseptic precautions, the spleen was denervated by cutting all the attachments to neighbouring organs and by dividing the splenic nerve at the level of the origin of the splenic artery. Thus the spleen was only connected by the splenic artery and vein, which were stripped of the surrounding nerve plexus as far as possible. The acute experiment was performed 8–10 days later.

RESULTS

Effects of acetylcholine

Splenic volume. The response of the isolated perfused spleen to acetylcholine injected into the splenic arterial tubing depended on the dose. In twelve experiments the smallest effective dose, which varied from 0·01 to 5 μg in different experiments, always caused a small increase in splenic volume, which rarely exceeded 5 ml. This response occurred within 2–3 sec of injection and usually lasted not more than 2 min, although occasionally a response lasting up to 5 min was observed. The same response was produced in the naturally perfused innervated spleen. Larger doses of acetylcholine, 5–25 μg, produced an increase in splenic volume which was followed by a decrease; a second increase in volume then sometimes occurred. With still larger doses, only a decrease in volume took place. Compared with the increases in volume evoked by small doses of acetylcholine, the decreases were large, frequently 20–30 ml. Typical responses are shown in Figs. 2 and 3*A*.

All these responses occurred in the adrenalectomized preparation and without change in splenic arterial pressure. A rise in venous pressure of 1–2 cm saline sometimes occurred during contraction of the spleen. This was probably due to a temporary increase in splenic venous blood flow resulting from emptying of the blood stores of the organ.

Vascular resistance. In eleven experiments acetylcholine invariably caused an increase in splenic arterial blood flow which was sometimes preceded by a momentary decrease. Since the splenic arterial and venous pressures remained constant, these changes in blood flow indicate a similar directional change in splenic vascular resistance. The vasodilator response usually lasted up to 2 min, although occasionally the increase in blood flow was prolonged, as in the experiment illustrated by Fig. 3*A*. These vascular responses occurred with doses of acetylcholine varying from 0·05 to 100 μg and independently of the changes in volume of the spleen. It was noted, however, that in those tests in which the acetylcholine vasodilator response was prolonged, the increase in volume of the spleen also persisted (Fig. 3*A*).

Atropinized spleens. Farber (1936) found that acetylcholine in doses of 0·5–4 mg injected into the splenic artery of the atropinized dog caused contraction of the spleen. In our experiments atropine, in doses of 2–5 mg (0·16–0·3 mg/kg) intravenously, invariably abolished the increase in splenic volume evoked by the small doses of acetylcholine. When the response to acetylcholine was diphasic, atropine also reduced or abolished the decrease in volume (Fig. 3*A*, *B*). If the dose of acetylcholine was now increased a reduction in volume invariably reappeared at a dose level 1·5–10 times that required to evoke the same response before atropine

(eight experiments). This effect, occurring in atropinized preparations, did not appear to be caused by the larger doses of acetylcholine overcoming the atropine block, because it could still be obtained after a second dose of atropine, although occasionally it was then slightly reduced (Fig. 3*B*, *C*).

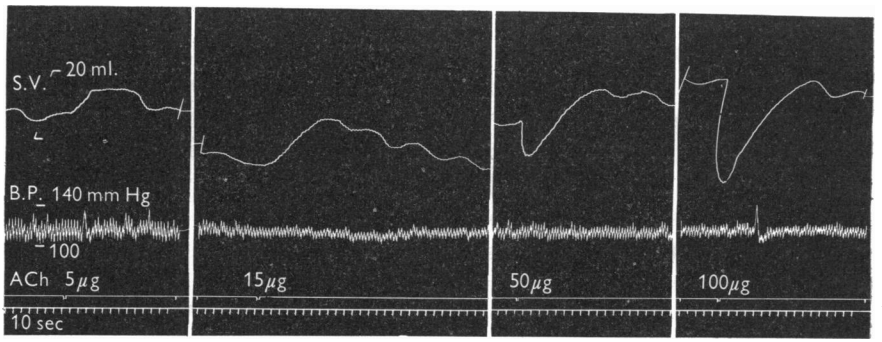

Fig. 2. Dog, *m*, 14·9 kg. Dial-Nembutal. Innervated spleen with natural blood supply. The effects of acetylcholine in doses of 5, 15, 50 and 100 μg injected into the splenic artery. In this and in subsequent figures: S.V. = splenic volume (increase in volume upwards); S.V.P. = splenic venous pressure; S.A. flow = splenic arterial blood flow; B.P. = femoral arterial blood pressure.

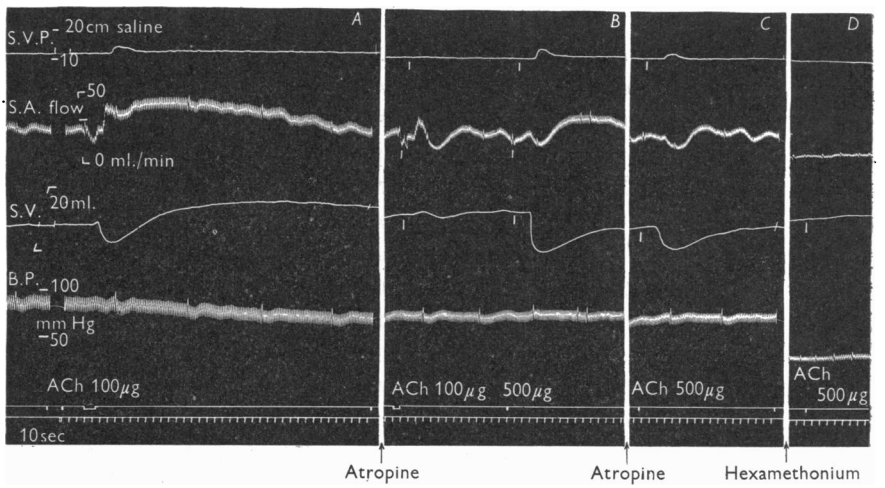

Fig. 3. Dog, *f*, 12·0 kg. Dial-Nembutal. Perfusion of the isolated spleen. *A–D* show the effects of close arterial injections of acetylcholine into the spleen. Between *A* and *B*, atropine 2 mg injected intravenously. Between *B* and *C*, a further 2 mg atropine. Between *C* and *D* hexamethonium, 10 mg/kg, intravenously.

The effect of atropine on the acetylcholine vasodilator response was variable. Usually the response was abolished, but occasionally acetylcholine initiated rhythmic variations in blood flow (Fig. 3*B*, *C*). In other experiments acetylcholine caused vasoconstriction in atropinized preparations. In this connexion, Hunt (1918) found that the increase in the venous outflow caused by acetylcholine in the isolated cat's spleen perfused at a constant head of pressure was abolished by atropine; large doses of acetylcholine after atropine caused a reduction in outflow.

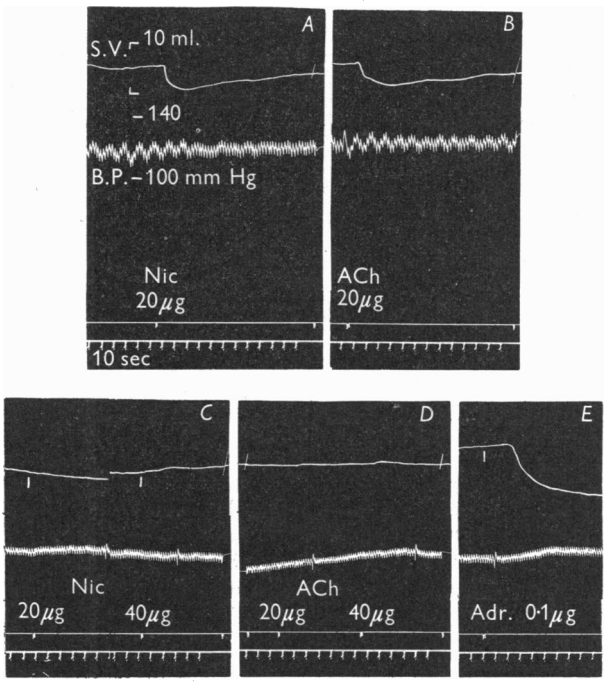

Fig. 4. Dog, *f*, 18·0 kg. Dial-Nembutal. Bilaterally adrenalectomized. Perfusion of the isolated spleen. Atropine 4 mg before recording begun. *A*, nicotine, 20 μg. *B*, acetylcholine, 20 μg. Between *B* and *C*, hexamethonium, 10 mg/kg, intravenously. *C*, nicotine, 20 and 40 μg. *D*, acetylcholine, 20 and 40 μg. *E*, adrenaline, 0·1 μg. All injections not specified given into the splenic arterial tubing.

For reasons which will be discussed below, we attribute the small increases in volume of the non-atropinized spleen evoked by acetylcholine, not to an action on the capsule, but to a passive effect of vasodilatation in the organ. Our finding that acetylcholine evoked large reductions in volume is in keeping with the results of previous workers who found that acetylcholine caused contraction of isolated strips of the capsule. Our results suggest that there are two mechanisms by which acetylcholine

causes contraction of the spleen; one of these is abolished by atropine and is therefore a muscarine-like action, the other is a nicotine-like effect because it is practically unaffected by atropine. This latter response can be mimicked by nicotine, 15–20 μg, and by DMPP, 2–10 μg (Figs. 4*A*, 5*C*).

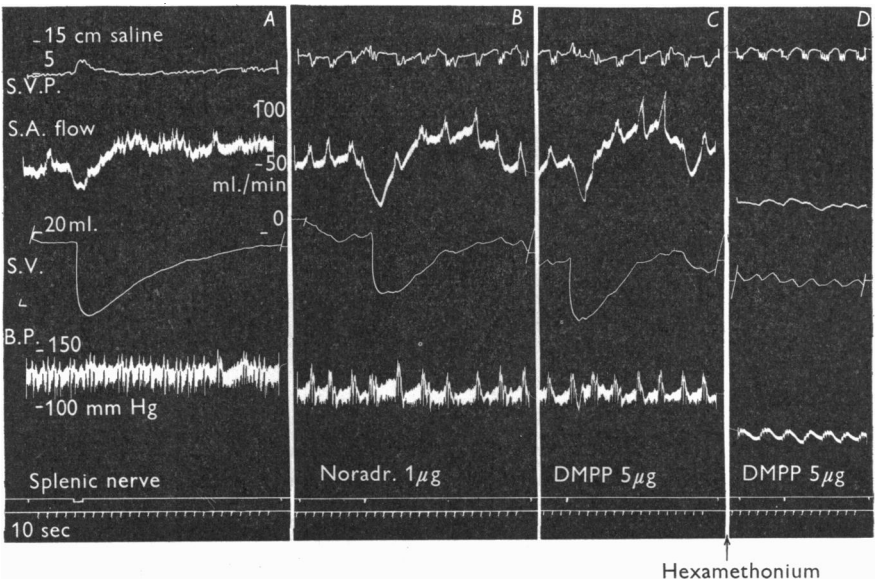

Fig. 5. Dog, *m*, 11·2 kg. Morphine-chloralose. Perfusion of the isolated spleen. Atropine, 6 mg, before recording begun. *A*, electrical stimulation of the splenic nerve, 8 V, 0·2 msec, 100 c/s. *B*, noradrenaline, 1 μg. Between *C* and *D*, hexamethonium, 10 mg/kg, intravenously. *C* and *D*, DMPP, 5 μg. All injections not specified given into splenic arterial tubing. Initial volume of spleen about 150 ml.

The effect of a ganglionic-blocking agent

It was reported by Fotino & Stoiculesco (1956) that the acetylcholine contraction of the spleen was unaffected by hexamethonium. Our results indicate that the effect is dependent on whether or not atropine was given previously.

Non-atropinized spleens. The effect of hexamethonium, 10 mg/kg intravenously, was tested in three preparations. It was found that the increase in volume of the spleen and the vasodilatation produced by threshold doses of acetylcholine were unaffected by hexamethonium. The contraction of the spleen with larger doses also persisted. Since these effects were abolished by a subsequent injection of atropine, these findings provide further evidence that the contraction of the spleen is in part due to a muscarine-like effect of acetylcholine, whose site of action must be peripheral to the ganglia.

Atropinized spleens. Hexamethonium invariably abolished the contraction of the spleen evoked by acetylcholine, nicotine, and by DMPP. The effects are illustrated by Figs. 3*C*, *D*, 4 and 5*C*, *D*. The increase in splenic vascular resistance produced by these substances was also abolished (Fig. 5*C*, *D*). On the other hand, hexamethonium had no appreciable effect on the contraction caused by splenic nerve stimulation.

Effect of anticholinesterases

In two atropinized preparations the contraction of the spleen produced by acetylcholine was potentiated by eserine in doses of 0·06 and 0·07 mg/kg intravenously respectively. A similar result was obtained in two other atropinized preparations with TEPP, 0·1 mg/kg intravenously.

Effect of anti-adrenaline drugs

In two atropinized preparations the effect of acetylcholine and nicotine was tested before and after intravenous injection of dibenzyline in doses of 10 and 15 mg/kg respectively. In both experiments the contraction of the spleen was considerably reduced. The small residual effect which occurred may be due to dibenzyline incompletely blocking transmission at sympathetic post-ganglionic nerve endings. This view is supported by the fact that these doses of dibenzyline did not completely abolish the contraction of the spleen evoked by close arterial injection of noradrenaline or by electrical stimulation of the splenic nerve. The normal responses to noradrenaline, and splenic nerve stimulation are shown in Fig. 5*A*, *B*. In one further experiment in which the spleen was naturally perfused, thereby obviating the need to give heparin, dibenamine abolished the contraction of the atropinized spleen produced by 150 μg acetylcholine injected into the splenic artery (Fig. 6). These results indicate therefore that the effect of nicotine and the nicotine-like effect of acetylcholine are reduced or abolished by agents opposing the effects of adrenaline and noradrenaline.

Effect of chronic denervation of the spleen

Since the majority of sympathetic preganglionic fibres to the spleen relay in the coeliac plexus, division of the splenic nerve at the level of the origin of the splenic artery would result in degeneration of post-ganglionic fibres with their cells in this plexus. Aberrant ganglion cells situated along the course of the splenic artery would not, however, be affected (Kuntz & Jacobs, 1955).

In three atropinized spleens which had been denervated at a previous operation (see Methods), neither acetylcholine, nicotine nor DMPP in doses up to 50 μg had any effect on the volume of the spleen. In one

experiment large doses of acetylcholine of 0·5 and 1 mg were tested and these caused small reductions of about 2 ml. in the volume of the spleen followed by an increase. The absence of any response to injection of these drugs was not due to the preparation being unresponsive to drugs, because 0·5 μg doses of noradrenaline had profound effects.

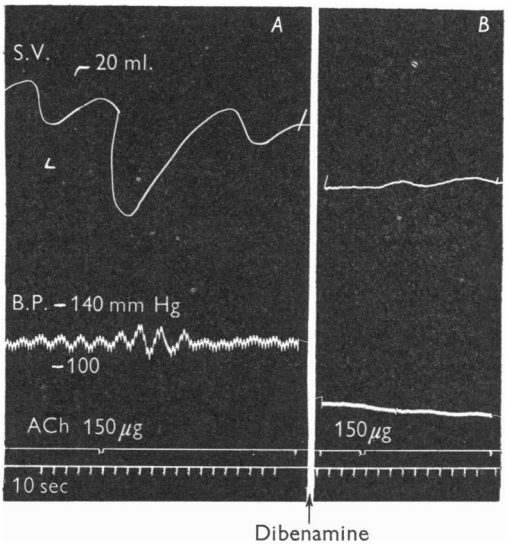

Fig. 6. Dog, *m*, 14·9 kg. Dial-Nembutal. Innervated spleen with natural blood supply. Atropine 4 mg before recording begun. *A* and *B* show the effects of close arterial injection of acetylcholine, 150 μg, into the spleen. Between *A* and *B*, dibenamine, 400 mg, intravenously. A period of 1 hr 15 min elapsed between the injections of dibenamine and acetylcholine in *B*.

Effect of reserpine

The response of the atropinized spleen to acetylcholine, in doses of 25–150 μg, was reduced but not abolished by reserpine. In two of these experiments the spleen failed to respond to nerve stimulation, but in the third experiment stimulation of the splenic nerve caused a small diminution in volume, indicating incomplete depletion of the stores of catechol amines. It is of interest to note that Burn & Rand (1960) were rarely able to abolish the contraction of the spleen of the cat in response to stimulation of the splenic nerve by treatment with reserpine.

DISCUSSION

Our results have shown that the smallest effective dose of acetylcholine almost invariably causes an increase in volume of the spleen; with larger doses this is followed by contraction, and with still larger doses only con-

traction takes place. These effects occur not only in acutely denervated spleens, but also in the naturally perfused, innervated spleen. Since all injections were made into the splenic artery the responses must be the result of a direct effect on the spleen or on structures in the splenic pedicle.

The increase in splenic volume with small doses of acetylcholine may be due to a passive distension of the organ through dilatation of its blood vessels, since the increases in volume were always accompanied by vasodilatation, and the two effects occurred simultaneously. Moreover, both the vasodilator response and the increase in splenic volume caused by acetylcholine were unaffected by hexamethonium but were abolished by atropine. Finally, previous workers have shown that in isolated strips of spleen, acetylcholine causes contraction, not relaxation (Fredericq, 1929; Vairel, 1933; Saad, 1935; Ferguson *et al.* 1936; Brandon & Rand, 1961).

Bacq & Fredericq (1935) were also of the opinion that the increase in volume of the spleen evoked by acetylcholine was due to vasodilatation in the organ. Grindlay, Herrick & Baldes (1939) made simultaneous measurements of splenic arterial and venous blood flow and of splenic volume in dogs, and presented evidence which suggested that the rhythmic volume changes were caused, not by alterations in the tone of the capsule, but by variations in blood flow through the spleen.

The contraction of the spleen produced by larger doses of acetylcholine is due to two different actions: (1) a muscarine-like action on the capsule of the spleen, which is abolished by atropine (Dale, 1914) but unaffected by hexamethonium; (2) a nicotine-like effect seen in the atropinized spleen, which can be mimicked by nicotine and DMPP, and is abolished by hexamethonium. These nicotine-like substances also mimic the effects produced by splenic nerve stimulation and by adrenaline and noradrenaline, and since they become ineffective after chronic denervation of the spleen, they must act somewhere on the nerve supply to the organ. It seems unlikely that the vagus plays any part, because there appears to be no evidence for any definite action of the vagus nerve on the spleen (Schäfer & Moore, 1896; Masuda, 1927). Furthermore, Utterback (1944) found no change in the number of myelinated and non-myelinated fibres in the splenic nerve after degenerative section of both vagus nerves below the level of the diaphragm. Presumably, therefore, these substances act through the sympathetic nervous system.

There are four possible mechanisms by which nicotine-like substances may cause contraction of the spleen, namely stimulation of (1) sympathetic ganglion cells; (2) sympathetic post-ganglionic nerve fibres or endings in continuity; (3) sensory nerve endings, with the initiation of an axon reflex; and (4) a release of adrenaline or noradrenaline from stores beyond the nerve endings.

There is some evidence in favour of the view that the nicotine-like action of acetylcholine on the spleen is the result of stimulation of peripheral ganglion cells situated in the vascular bed of the splenic artery. Ambache (1951) has suggested that the inhibitory action of nicotine on the intestine might be due to stimulation of local adrenergic ganglion cells, since it was abolished by hexamethonium and by large doses of nicotine. We have shown that, in atropinized spleen preparations, (1) the acetylcholine contraction of the spleen is potentiated by anticholinesterases and is abolished by hexamethonium; (2) ganglion-stimulating drugs such as nicotine and DMPP cause contraction of the spleen, which is also abolished by hexamethonium, and by anti-adrenaline drugs such as dibenzyline or dibenamine. On the other hand, this view which would ascribe the nicotine-like effect of acetylcholine on the spleen to an action on ganglia situated in either the spleen or splenic pedicle is contrary to the generally held concepts of the organization of the sympathetic nervous system, which assume that all preganglionic sympathetic fibres traversing the coeliac plexus relay in this plexus; the post-ganglionic fibres issuing from it then proceed to the organ which they innervate. With regard to the spleen, this view is supported by the work of Glaser (1928), Riegele (1929) and of Utterback (1944). Langley (1896), on the other hand, found in the cat small ganglia along the course of nerve strands from the solar ganglia. He states, 'I have no doubt that some of the splanchnic fibres run on past the solar ganglia to be connected with more peripheral ganglia, just as some fibres run through the inferior mesenteric ganglia on their way to more peripheral ganglia'. Evidence for sympathetic nerve fibres passing through the coeliac ganglion and relaying more peripherally was also obtained by Babkin, Hebb & Sergeyeva (1939). More recently, Kuntz & Jacobs (1955) have demonstrated by histological methods the presence of ganglia along the course of the splenic artery in the cat and rat, although not in man. At our request, the late Dr A. Kuntz very kindly examined the nerves accompanying the splenic artery in the dog for ganglion cells. He reported as follows:

> The first one examined showed a small ganglion in the splenic plexus in which we counted forty-seven ganglion cells. This seemed rather meagre, so we examined two more without finding any ganglion cells. Our findings in the first dog show that ganglia may occur in the nerves along the splenic artery. Finding none in the next two dogs was rather disappointing, but I do not take this to mean that ganglion cells occur only very rarely in the splenic nerves in the dog.

We regard it as most unlikely, however, that the mechanism of action of nicotine-like substances is on ganglion cells in the spleen, for two reasons. First, although the histological evidence suggests that ganglion cells are not to be found consistently in the dog's splenic nerve, nicotine-like substances regularly caused contraction of the spleen. Secondly, this

contraction could not be evoked after degeneration of the nerves to the spleen, a procedure which these ganglion cells would be expected to survive.

The second possibility, that nicotine-like substances may act on post-ganglionic nerves in continuity, is also unlikely because acetylcholine does not stimulate isolated frog's nerve (Lorente de Nó, 1944), and Brown & MacIntosh (1939) found that the intra-arterial injection of acetylcholine fails to set up action potentials in nerves.

The third possibility is suggested by the fact that acetylcholine and nicotine-like substances can excite sensory nerve endings (see Gray & Diamond, 1957). The centripetal discharge of impulses evoked by acetylcholine is prevented by hexamethonium, and the sensory ending in general shows many of the pharmacological properties of a sympathetic ganglion cell. The acetylcholine contraction of the spleen might be due to such an axon reflex set up by the excitation of sensory nerve endings, but there are, of course, difficulties in accepting the existence of such reflexes in the spleen. It implies either that sensory receptors form an integral part of the peripheral sympathetic system or that there exist in the spleen sensory axons, a collateral branch of which supplies the capsule of the organ with adrenergic fibres. The only means of deciding between these two possibilities would be to perform a chronic section of posterior nerve roots of such an extent that the spleen is deprived of all afferent connexions.

Axon reflexes involving post-ganglionic (non-sensory) branching collateral fibres have been postulated previously to explain the action of certain nicotine-like substances (Coon & Rothman, 1940; Ambache & Robertson, 1953). Their effects occur in atropinized preparations and are abolished by hexamethonium and by chronic denervation of the organ.

The fourth possibility is that acetylcholine causes contraction of the atropinized spleen through the release of adrenaline and noradrenaline from stores surrounding the sympathetic nerve endings (see Burn & Rand, 1959*b*, 1960). Our results have shown that the contraction produced by acetylcholine is reduced or abolished after degeneration of the splenic nerve and after treatment with reserpine. These procedures also considerably reduce the noradrenaline content of the spleen (von Euler & Purkhold, 1951; Burn & Rand, 1959*a*; Brandon & Rand, 1961). Our finding that the response to acetylcholine is abolished by dibenamine and by dibenzyline is also in keeping with the view that the nicotine-like effects of acetylcholine on the spleen are due to the release of noradrenaline. Brandon & Rand (1961), using the cat's spleen, have obtained similar results. The site of action of acetylcholine and nicotine is at present uncertain. Gillespie & Mackenna (1960) have tentatively suggested an action on some form of terminal sympathetic nerve network intervening between the sympathetic

nerves and the smooth muscle. Our own experiments provide no further information, and clearly further work is required to establish this point.

SUMMARY

1. Close arterial injections have been made into the dog's spleen isolated and perfused with blood at a constant head of pressure. Splenic arterial blood flow was measured continuously by means of a rotameter.

2. The smallest effective doses (0·01–5 μg) of acetylcholine caused a small increase in volume of the spleen and a decrease in vascular resistance. These effects were abolished by atropine. The increase in volume of the spleen is attributed to a passive effect of dilatation of the splenic blood vessels.

3. Larger doses (5–100 μg) of acetylcholine caused contraction of the spleen and this response occurred in atropinized preparations. Since nicotine had a similar effect, this action of acetylcholine is due to its nicotine-like properties. It was abolished by hexamethonium.

4. When the nicotine-like action of acetylcholine was prevented by hexamethonium in non-atropinized preparations, injection of acetylcholine still caused contraction of the spleen and this response was now abolished by atropine. This suggests that acetylcholine also exerts a muscarine-like action directly on the capsule of the spleen.

5. The contraction of the atropinized spleen by acetylcholine or nicotine is abolished by sympatholytic drugs and by degenerative section of the nerves to the spleen, and is reduced by treatment with reserpine.

6. The possibility that the nicotine-like actions of acetylcholine are due to the release of noradrenaline is discussed.

We wish to express our thanks to Mr D. R. Bacon for technical assistance and to Drs Brandon and Rand for allowing us to read the manuscript of their paper before publication. This work was supported in part by a grant from the Royal Society to one of us (M. de B. D.).

REFERENCES

AMBACHE, N. (1951). Unmasking, after cholinergic paralysis by botulinum toxin, of a reversed action of nicotine on the mammalian intestine, revealing the probable presence of local inhibitory ganglion cells in the enteric plexuses. *Brit. J. Pharmacol.* **6**, 51–67.

AMBACHE, N. & ROBERTSON, P. A. (1953). The nicotine-like actions of the 3-bromo- and 3:5-dibromo-phenyl ethers of choline (MBF and DBF). *Brit. J. Pharmacol.* **8**, 147–155.

ATTREE, V. H. (1950). An electronic stimulator for biological research. *J. sci. Instrum.* **27**, 43–47.

BABKIN, B. P., HEBB, C. O. & SERGEYEVA, M. A. (1939). The parasympathetic-like effect of splanchnic nerve stimulation on pancreatic secretion. *Quart. J. exp. Physiol.* **29**, 217–237.

BACQ, Z. M. & FREDERICQ, H. (1935). Sur la nature de l'innervation de la rate. Action de divers agents pharmacodynamiques. *Arch. int. Physiol.* **41**, 322–333.

BELL, P. M. G. (1954). A modified recording rotameter for measuring blood flow. *J. Physiol.* **125**, 9–10*P*.

BRANDON, K. W. & RAND, M. J. (1961). Acetylcholine and the sympathetic innervation of the spleen. *J. Physiol.* (In the Press.)

BROWN, G. L. & MACINTOSH, F. C. (1939). Discharges in nerve fibres produced by potassium ions. *J. Physiol.* **96**, 10–11*P*.

BURN, J. H. & RAND, M. J. (1959*a*). The cause of the supersensitivity of smooth muscle to noradrenaline after sympathetic degeneration. *J. Physiol.* **147**, 135–143.

BURN, J. H. & RAND, M. J. (1959*b*). The sympathetic postganglionic mechanism. *Nature, Lond.*, **184**, 163–165.

BURN, J. H. & RAND, M. J. (1960). Sympathetic postganglionic cholinergic fibres. *Brit. J. Pharmacol.* **15**, 56–66.

COON, J. M. & ROTHMAN, S. (1940). The nature of the pilomotor response to acetylcholine; some observations on the pharmaco-dynamics of the skin. *J. Pharmacol.* **68**, 301–311.

DALE, H. H. (1914). The action of certain esters and ethers of choline, and their relationship to muscarine. *J. Pharmacol.* **6**, 147–190.

DALY, M. DE B. (1957). The cardiovascular effects of anticholinesterases in the dog with special reference to haemodynamic changes in the pulmonary circulation. *J. Physiol.* **139**, 250–272.

FARBER, S. (1936). The action of acetylcholine on the volume of the spleen. *Arch. int. Pharmacodyn.* **53**, 367–376.

FERGUSON, J., IVY, A. C. & GREENGARD, H. (1936). Observations on the response of the spleen to the intravenous injection of certain secretin preparations, acetylcholine and histamine. *Amer. J. Physiol.* **117**, 701–707.

FOTINO, S. & STOICULESCO, P. (1956). Action des médiateurs nerveux sur la rate. Possibilité d'existence de 'substances receptrices' bivalentes au niveau des cellules effectrices spléniques. *J. Physiol., Paris*, **48**, 1011–1034.

FREDERICQ, H. (1929). Le nature parasympathique probable de l'innervation motrice de la rate du chien. *C.R. Soc. Biol., Paris*, **101**, 1164–1165.

GILLESPIE, J. S. & MACKENNA, B. R. (1960). The inhibitory action of nicotine on the rabbit colon. *J. Physiol.* **152**, 191–205.

GLASER, W. (1928). Über die vasomotorische Innervierung der Blutgefässe der Milz nebst Bemerkungen zur intramuralen Nervenversorgung der Blutgefässe im Knochenmark. *Z. ges. Anat.* **87**, 741–745.

GOTSEV, T. (1936). Über die Wirkung des Acetylcholins auf Blutgefässe, Blutdruck, Herz und Vasomotorenzentren. *Arch. exp. Path. Pharmak.* **181**, 207–214.

GRAY, J. A. B. & DIAMOND, J. (1957). Pharmacological properties of sensory receptors and their relation to those of the autonomic system. *Brit. med. Bull.* **13**, 185–188.

GRINDLAY, J. H., HERRICK, J. F. & BALDES, E. J. (1939). Rhythmicity of the spleen in relation to blood flow. *Amer. J. Physiol.* **127**, 119–126.

HUNT, R. (1918). Vasodilator reactions. I. *Amer. J. Physiol.* **45**, 197–230.

KUNTZ, A. & JACOBS, M. W. (1955). Components of periarterial extensions of celiac and mesenteric plexuses. *Anat. Rec.* **123**, 509–520.

LANGLEY, J. N. (1896). On the nerve cell connection of the splanchnic nerve fibres. *J. Physiol.* **20**, 223–246.

LORENTE DE NÓ, R. (1944). Effects of choline and acetylcholine chloride upon peripheral nerve fibres. *J. cell. comp. Physiol.* **24**, 85–97.

MASUDA, T. (1927). The action of the vagus on the spleen. *J. Physiol.* **62**, 289–300.

RIEGELE, L. (1929). Über die mikroscopische Innervation der Milz. *Z. Zellforsch.* **9**, 511–533.

SAAD, K. (1935). The effects of drugs on the isolated splenic capsule of man and other animals. *Quart. J. Pharm.* **8**, 31–38.

SCHÄFER, E. A. & MOORE, B. (1896). On the contractility and innervation of the spleen. *J. Physiol.* **20**, 1–50.

SCOTT, M. J. (1957). The effects of anticholinesterases upon the spleen of the dog. *J. Physiol.* **139**, 489–496.

SHIPLEY, R. E. & WILSON, C. (1951). An improved recording rotameter. *Proc. Soc. exp. Biol., N.Y.*, **78**, 724–728.

UTTERBACK, R. A. (1944). The innervation of the spleen. *J. comp. Neurol.* **81**, 55–68.

VAIREL, J. (1933). Action de l'adrénaline et de l'acétylcholine sur la rate. *J. Physiol. Path. gén.* **31**, 42–52.

VON EULER, U. S. & PURKHOLD, H. (1951). Sympathetic denervation and nor-adrenaline and adrenaline content of spleen, kidney and salivary glands. *Acta physiol. scand.* **24**, 212–217.