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ACETYLCHOLINE AND THE SYMPATHETIC INNERVATION OF THE SPLEEN

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This paper is concerned with the role of acetylcholine-like substances in the sympathetic innervation of the spleen. The presence of cholinergic fibres in the sympathetic nerve supply to the cat's spleen was deduced by Burn & Rand (1960*c*) from observations on the response of the spleen of reserpine-treated cats to splenic nerve stimulation. Stimulation of the splenic nerve in a normal cat releases noradrenaline (Peart, 1949) and causes contraction of the spleen. But when the spleen is depleted of noradrenaline by reserpine (Burn & Rand, 1959*a*), splenic nerve stimulation produces either a smaller contraction than usual or a relaxation which is enhanced by eserine and abolished by atropine.

The innervation of the cat's spleen has been studied by Utterback (1944), who concluded that there was no parasympathetic innervation and that there were no ganglion cells in the spleen. Sympathetic denervation leads to a decrease in the noradrenaline content of the spleen (von Euler & Purkhold, 1951; Burn & Rand, 1959*a*), and von Euler (1956) concluded that the loss of noradrenaline on denervation could be explained if it were normally contained in the nerves. However, in another sympathetically innervated structure, the vessels of the rabbit's ear, denervation has been shown to cause a fall in acetylcholine content (Armin, Grant, Thompson & Tickner, 1953). It is well known that the spleen contains acetylcholine. This was first observed by Dale & Dudley (1929), although as yet no explanation has been given for its presence. In order to investigate the possibility that the acetylcholine might be associated with the splenic nerves, we have studied the effect of sympathetic denervation on the acetylcholine content of the cat's spleen.

Acetylcholine has been detected in the venous effluents after the stimulation of the sympathetic nerves to a number of structures: the sweat glands (Dale & Feldberg, 1934), the blood vessels of the dog's hind legs (Bülbring & Burn, 1935), and the vessels of the rabbit's ear (Burn & Rand,

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1960*c*). We have now shown that an acetylcholine-like substance appears in perfusates of cats' spleens after stimulation of the splenic nerves.

The drug hemicholinium causes failure of the response to cholinergic nerve stimulation (MacIntosh, Birks & Sastry, 1956; Reitzel & Long, 1959*a*, *b*; Wilson & Long, 1959) by inhibiting the synthesis of acetylcholine (MacIntosh *et al.* 1956; Gardiner, 1957). The effect of hemicholinium on responses to splenic nerve stimulation has been investigated. Observations have been made on the actions of acetylcholine in causing contractions of the spleen, to determine to what extent its effects mimicked those of splenic nerve stimulation.

METHODS

Determination of acetylcholine and noradrenaline contents of spleen. Cats were anaesthetized with ether and the spleens were removed. A segment of the spleen weighing 2 g was cut from its middle third. The portion was finely divided with scissors and ground with sand under 8 ml. of ice-cold 10% trichloroacetic acid. The mixture was transferred to a centrifuge tube, allowed to stand for 20 min, and then centrifuged at 4200 rev/min for 5 min. The supernatant was decanted and washed three or four times with 50 ml. ether. The remaining ether was removed by bubbling air through the extract. Acetylcholine was precipitated as the reineckate by the method of Shaw (1938) as modified by Bentley & Shaw (1952). The extract was placed in a 10 ml. centrifuge tube and 1 mg of choline chloride and 1·5 ml. of a freshly prepared saturated solution of ammonium reineckate in absolute alcohol were added for each 1 ml. of extract. The mixture was kept at −10° C for 2 hr, and then centrifuged at 4200 rev/min for 2 min. The supernatant was discarded and the residue was dissolved in 16 ml. of 60% ethanol. Dilutions of the alcoholic extract (1:5 to 1:10) with distilled water were assayed against acetylcholine chloride on isolated segments of guinea-pig ileum suspended in a 10 ml. bath of Tyrode solution maintained at 32° C. No correction has been made for any losses of acetylcholine which may have occurred during the extraction procedure. The method was also used on ox spleen, which was found to contain 16 μg/g of acetylcholine, which was within the range of values of 4–30 μg/g found by Chang & Gaddum (1933).

The noradrenaline content of the spleen was determined in extracts prepared by the method of Burn & Rand (1959*a*), which were assayed on the blood pressure of the pithed rat, using a Statham strain-gauge manometer (Pennefather & Rand, 1960).

For experiments in which both acetylcholine and noradrenaline were determined, the central portion of the spleen was divided in a chequer-board fashion and alternating pieces were recombined for the separate extraction and estimation of each substance.

Sympathetic denervation of spleen. In cats under ether anaesthesia the abdomen was opened aseptically in the mid line and all the nerve fibres accompanying the coeliac axis were divided. Utterback (1944) has shown that the cat's spleen is completely denervated by dividing the coeliac plexus.

Isolated perfused spleen. Cats were anaesthetized with ether. The abdomen was opened and the main splenic artery, vein and nerve were dissected free. All other vessels running between the spleen and the greater curvature of the stomach, the omentum and the pancreas were doubly ligated and divided. The main splenic artery and vein were cannulated and the spleen was removed. A mixture of 95% O_2 and 5% CO_2 was bubbled through the perfusion fluid in the reservoir which was approximately 1 m above the spleen. The fluid was passed through coils immersed in a bath at 32° C before it reached the spleen. In some experiments in which the spleen was perfused with Tyrode solution the venous effluent was arranged to

superfuse a strip of guinea-pig ileum (Gaddum, 1953). In other experiments in which we wished to observe the effect of splenic nerve stimulation in contracting the spleen, we perfused with McEwen's (1956) solution and suspended the perfused spleen in a 50 ml. isolated organ bath so that its longitudinal contractions could be measured with a writing lever on a kymograph. In other experiments the venous outflow from the spleen was measured by counting drops with a Thorp (1948) impulse counter.

The splenic nerve was stimulated by one of two procedures: (*a*) a length of nerve was dissected and passed through a tube containing bipolar platinum electrodes, continually irrigated with the solution used to perfuse the spleen (Burn & Rand, 1960*a*); (*b*) a length of silver wire (0·008 in. (0·2 mm) diam.) was passed down the arterial cannula so that one end was near the tip of the cannula to form one electrode and a second electrode was attached to the tissue adjacent to the cannula. The nerve was thus stimulated by current traversing the splenic nerves which surround the artery. Square-wave stimuli of 2 msec duration from a constant voltage source at 5–50 V were used. Details of the frequency and the period of stimulation are given in Results.

Drugs were injected into the arterial cannula of the perfused spleen or into a 10 ml. bath containing a strip of spleen suspended in McEwen's solution at 32° C.

Cats were treated with reserpine by giving an intraperitoneal injection of reserpine 1·5–2·5 mg/kg dissolved in a 20% solution of ascorbic acid on each of the 2 days before the experiments.

Doses of noradrenaline are expressed as the base, acetylcholine as the chloride, bretylium as the tosylate (Darenthin), hemicholinium (α, α′ dimethylaminoethanol 4,4′ biacetophenone) as the dibromide and neostigmine as the methylsulphate.

RESULTS

Acetylcholine equivalent of normal and denervated cat spleen

The mean acetylcholine equivalent of spleens from 18 cats was 0·472 μg/g (S.E. = standard error of mean ± 0·0517). The spleens from 11 cats in which the splenic nerves had been divided at the coeliac axis had a mean acetylcholine equivalent of 0·105 μg/g (S.E. ± 0·0259); the individual values are given in Table 1. The difference between the mean value of the acetylcholine equivalent in normal and denervated spleens was found to be very highly significant when tested by Student's *t* test ($t = 16{\cdot}7$; $P < 0{\cdot}001$). The assays of the reineckate-precipitated material from extracts of the spleens were performed on strips of guinea-pig ileum. Since the method of purification by reineckate-precipitation is specific for choline esters (Bentley & Shaw, 1952), it is unlikely that contractions of the ileum were produced by other pharmacologically active substances. The activity on the ileum has been expressed in terms of acetylcholine, although Banister, Whittaker & Wijesundera (1953) have shown that propionylcholine and possibly another choline ester are present in spleens of another species, the ox.

The noradrenaline content was determined in separate portions of the denervated spleens (Table 1). The mean noradrenaline content of 11 denervated spleens in these experiments was 0·205 μg/g, which is the same as the mean value obtained by Burn & Rand (1959*a*) for 8 denervated

spleens. Normal cat spleens contain 1·13 μg of noradrenaline/g (Burn & Rand, 1959*a*).

After degeneration of the sympathetic fibres the percentage fall in acetylcholine content of denervated spleens was 22 % of the value obtained for the normal spleens; the noradrenaline content of denervated cat spleen was 18 % of normal.

Reserpine treatment causes a depletion of noradrenaline in the spleen (Burn & Rand, 1959*a*), but the acetylcholine content of spleen was not affected. The mean value for acetylcholine content in spleens from four reserpine-treated cats was 0·43 μg/g, which was not significantly different from the mean value of 0·47 μg/g found in the intact spleens from normal cats.

TABLE 1. Acetylcholine and noradrenaline contents of denervated cat spleens

	Acetylcholine (μg/g)	Noradrenaline (μg/g)
	0·02	0·12
	0·02	0·06
	0·04	0·037
	0·04	0·65
	0·08	0·07
	0·08	0·12
	0·10	0·10
	0·12	0·10
	0·15	0·10
	0·20	0·50
	0·30	0·06
Mean	0·105	0·205

In two experiments designed to test whether operation *per se* affected the spleen, the nerves accompanying the superior mesenteric artery were divided. Ten days after this operation the spleens were removed and the noradrenaline and acetylcholine contents were determined. The noradrenaline contents of these two spleens were 0·75 and 1·0 μg/g, and the acetylcholine contents 0·4 and 1·0 μg/g, showing that neither was affected.

The release of acetylcholine on stimulating the splenic nerve

In order to test whether an acetylcholine-like substance was released by splenic nerve stimulation we used spleens from reserpine-treated cats to eliminate interference caused by the release of noradrenaline. The release of a substance which resembles acetylcholine into the splenic perfusate after splenic nerve stimulation was successfully demonstrated in each of five experiments.

In order to reduce the time required for handling the samples the venous effluent was allowed to act directly on an acetylcholine-sensitive preparation by arranging it to superfuse a strip of guinea-pig ileum. Stimulation of the splenic nerve in the perfused reserpinized spleen was without effect

on the superfused ileum in the absence of an anticholinesterase. When neostigmine (1 μg/ml.) was added to the fluid perfusing the spleen the ileum contracted, but subsequently relaxed. After neostigmine had been present in the perfusion fluid for 30–60 min, splenic nerve stimulation produced a contraction of the superfused ileum, which started 1–3 min after the beginning of stimulation. Thus, in the experiment shown in Fig. 1, the splenic nerve was stimulated for 1 min at 50/sec; during the period of stimulation there was an increase in the rhythm of the ileum and then a gradual increase in tone leading to a contraction which began about 90 sec

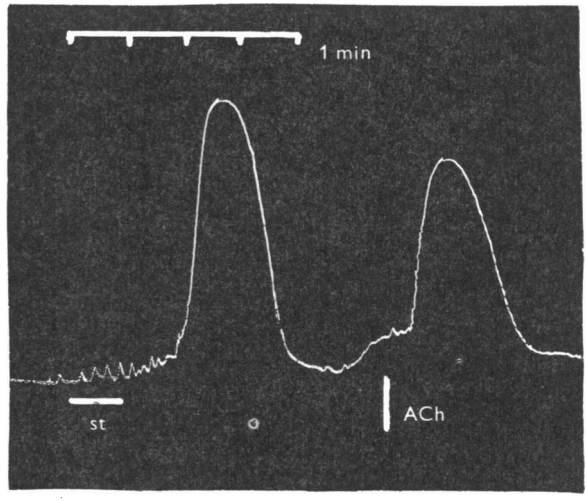

Fig. 1. Contractions of guinea-pig ileum superfused with outflow from the isolated spleen of a reserpinized cat. The spleen was perfused with Tyrode solution containing neostigmine (1 μg/ml.). The splenic nerve was stimulated with 2 msec pulses at 50/sec for 1 min at st. At ACh, 0·2 μg of acetylcholine was injected into the splenic arterial cannula.

after the end of stimulation and was followed by a relaxation which was complete 3½ min after stimulation. The contraction and subsequent relaxation of the ileum were similar in time course to the effects of 0·2 μg acetylcholine injected into the splenic artery cannula. The superfused ileum was contracted by 0·01 ng of acetylcholine when this was injected directly into the superfusion stream as it emerged from the spleen. The relatively weak action of a much larger amount of acetylcholine administered to the ileum via the perfused spleen could be explained either by an incomplete neostigmine block of splenic cholinesterase, with consequent destruction of acetylcholine, or by dilution of the acetylcholine when injected into the spleen. Consequently it is possible that the contraction of the ileum produced by splenic nerve stimulation was due only to a small fraction of the choline esters liberated by these nerves in the spleen.

The contractions of the superfused ileum produced by splenic nerve stimulation or by acetylcholine were abolished by atropine (0·1 μg/ml.) added to the perfusion fluid, as shown in Fig. 2. In this figure four consecutive contractions of the superfused ileum produced by repeated splenic nerve stimulations were followed by a contraction caused by 0·5 μg of acetylcholine injected into the splenic arterial cannula; when 0·1 μg/ml. of atropine was added to the perfusion fluid splenic nerve stimulation was without effect on the ileum.

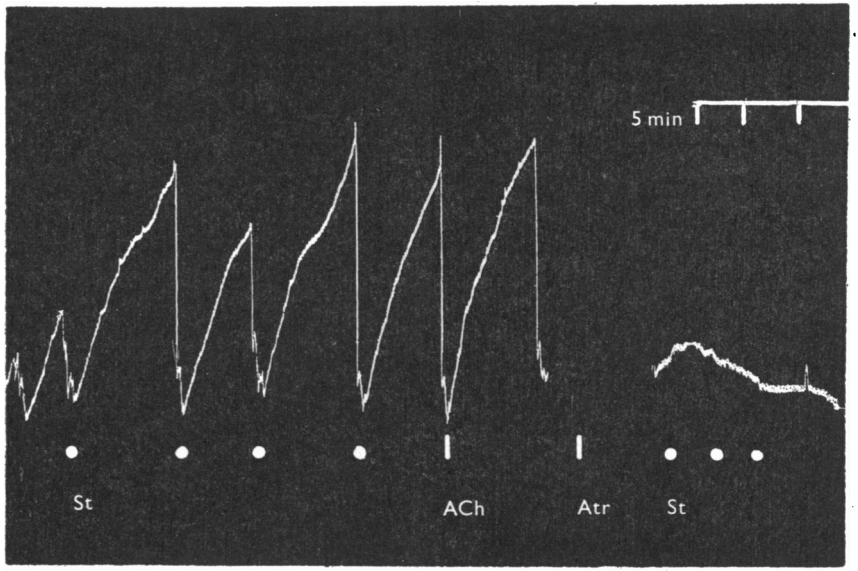

Fig. 2. Contractions of guinea-pig ileum superfused with outflow from a reserpinized cat spleen perfused with Tyrode solution containing neostigmine (1 μg/ml.). The splenic nerves were stimulated with 2 msec pulses at 25/sec for 1 min at the white dots (St). Acetylcholine (0·5 μg) was injected into the arterial cannula at Ach. At Atr, atropine (0·1 μg/ml.) added to the perfusion fluid abolished the contractions of the ileum following splenic nerve stimulation.

Reduction of the response to sympathetic nerve stimulation by hemicholinium

Hemicholinium (50 μg/ml.) added to the perfusion fluid reduced the contractions of the normal spleen in response to splenic nerve stimulation. Thus, in an experiment in which we recorded longitudinal contractions of the isolated perfused spleen produced by sympathetic nerve stimulation for 1 min periods in every 10 min, the contractions observed after perfusing with hemicholinium gradually decreased in size. After 75 min the contraction was 65 % of the original, after 145 min it was 56 % and after 215 min it was 35 %. At this stage choline chloride (500 μg/ml.) added to the perfusion fluid restored the response to 75 % of its original height.

In other experiments the contractions of the spleen produced by sympathetic nerve stimulation were observed by recording the outflow from the spleen, measured with a Thorp drop counter (Fig. 3). When the

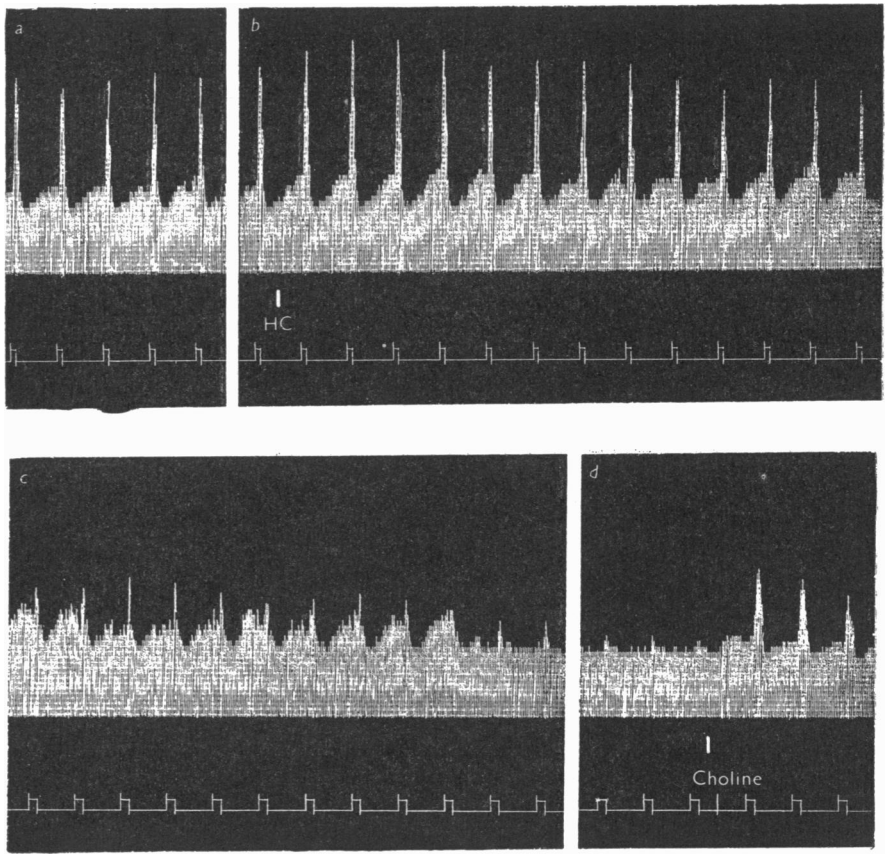

Fig. 3. Contractions of cat spleen in response to splenic nerve stimulation measured by recording the outflow with a drop counter, returning to the base line every 10 sec. Lower signal, stimulation of splenic nerve with 2 msec pulses at 50/sec for 30 sec periods every 4·5 min in *a* and *b*, and for 50 sec periods in *c* and *d*. Initially, in *a*, responses were constant. In *b*, hemicholinium (50 μg/ml.) was added to the perfusion fluid at HC. The interval between *b* and *c* was 200 min, and between *c* and *d* 63 min. In *d* choline chloride (500 μg/ml.) was added to the perfusion fluid and restored contractions.

splenic nerves were stimulated the outflow increased from 16 to 40 drops/10 sec. It was observed that the increase in outflow was due to a visible contraction of the spleen. After the period of stimulation there was a reduction in outflow which accompanied relaxation of the spleen. The addition of hemicholinium (Fig. 3*b*) to the perfusion fluid produced a

transient increase in the responses to sympathetic stimulation and then a gradual decrease leading to complete block (Fig. 3*c*). After the addition of choline to the perfusion fluid there was a partial restoration of the response to stimulation (Fig. 3*d*).

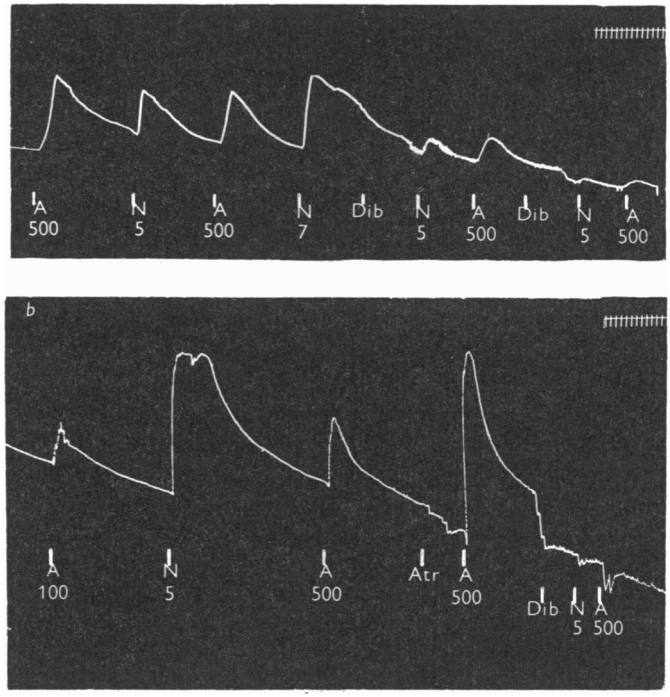

Fig. 4. Longitudinal contractions of isolated perfused cat spleen. Drugs were injected into the arterial cannula. At A (acetylcholine), and at N (noradrenaline), the numerals refer to doses in μg. At Dib, 0·5 mg of dibenzyline (phenoxybenzamine), and at Atr, 0·1 mg of atropine. Records *a* and *b* are from different experiments. Time marker, 30 sec.

Effect of acetylcholine on the spleen

Observations were made on the effect of injections of acetylcholine into the arterial cannula in experiments on the isolated perfused spleen. Figure 4 shows that acetylcholine had about 1/100 of the potency of noradrenaline in producing contractions of the spleen. After the injection of the antiadrenaline drug phenoxybenzamine (dibenzyline) into the cannula the contractions produced by acetylcholine and the contractions produced by noradrenaline were both abolished (Fig. 4). After atropine the injection of acetylcholine caused a bigger contraction than it had before (Fig. 4*b*). Burn & Rand (1960*c*) found that acetylcholine caused a relaxation of the spleen of a reserpine-treated cat and this effect was blocked by

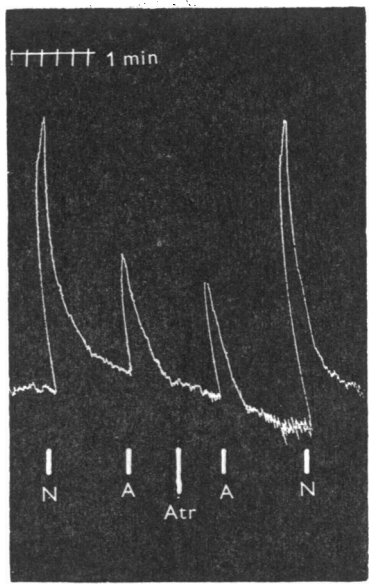

Fig. 5. Contractions of isolated strip of cat spleen in 10 ml. bath. At N, 2 μg noradrenaline, and at A, 200 μg acetylcholine, both washed out after 30 sec. After Atr the bath fluid contained 0·5 μg/ml. of atropine, which did not affect responses to acetylcholine or to noradrenaline.

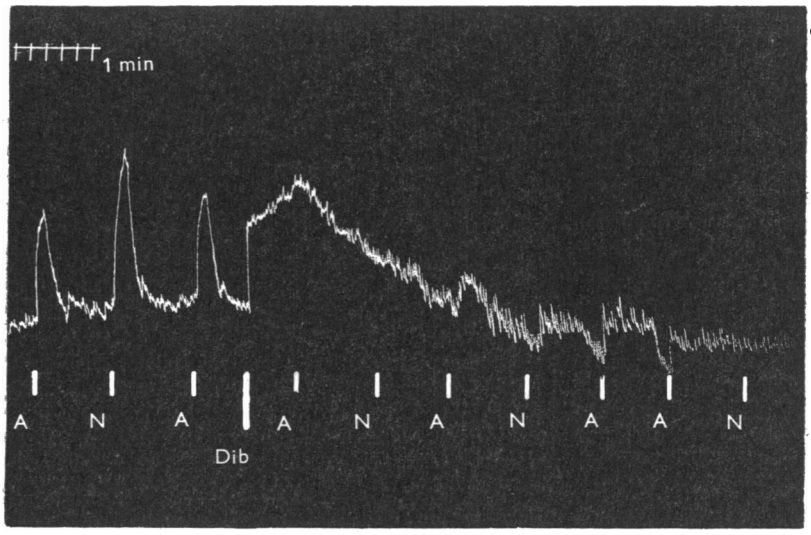

Fig. 6. Isolated cat spleen strip; 10 ml. bath. At A, 300 μg acetylcholine and at N, 20 μg noradrenaline in contact for 30 sec. The spleen strip was exposed to dibenzyline (phenoxybenzamine) 0·1 mg/ml. at Dib for 10 min, which blocked responses to acetylcholine and to noradrenaline.

atropine, which suggests that the larger contraction produced by acetylcholine after atropine in Fig. 4*b* may have been a result of a block of a possible relaxing action of acetylcholine.

Further experiments were carried out on strips cut from spleens and suspended in the organ bath to record contractions. The contractions caused by acetylcholine were not affected by atropine (Fig. 5), but they were abolished by phenoxybenzamine (Fig. 6) and by another anti-adrenaline drug piperoxan (0·2 mg/ml.), as were the contractions produced

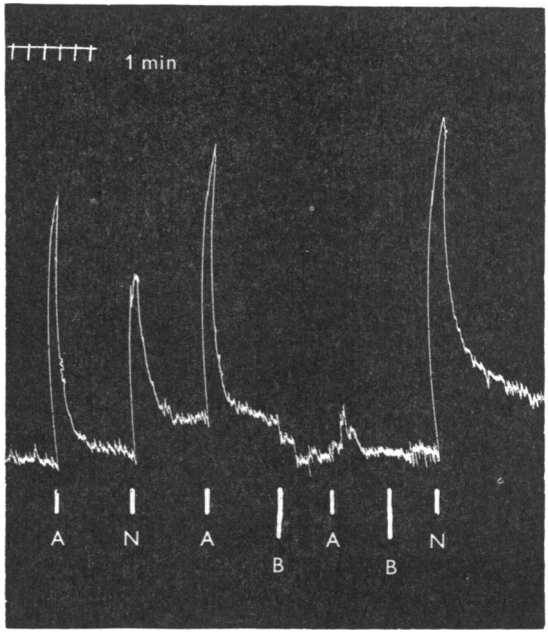

Fig. 7. Isolated cat spleen strip. A, 100 μg acetylcholine; N, 30 μg noradrenaline. At B, 10 μg/ml. of bretylium was added to the 10 ml. bath and washed out after the subsequent responses to acetylcholine and noradrenaline. The response to acetylcholine was blocked by bretylium, whereas the response to noradrenaline was potentiated.

by noradrenaline itself. Bretylium (10 μg/ml.), which abolishes the response to sympathetic nerve stimulation, blocked the contraction of the spleen strip to acetylcholine but the response to noradrenaline was potentiated (Fig. 7).

Spleen strips taken from reserpine-treated cats contracted to a less extent to acetylcholine than did strips from normal cats, whereas the response to noradrenaline was enhanced. After the spleen was exposed to noradrenaline, the response to acetylcholine was increased, whereas the response to noradrenaline was decreased.

DISCUSSION

Acetylcholine has been found in the spleen of the ox and of the horse (Dale & Dudley, 1929; Chang & Gaddum, 1933). Bannister *et al.* (1953) found that three choline esters were present in ox spleen, namely acetylcholine, propionylcholine and a third unidentified ester. We have now shown that cat's spleen contains an acetylcholine-like substance the amount of which is decreased after degeneration of the sympathetic nerves. This decrease could be accounted for if the acetylcholine-like substance were normally contained in the splenic nerve fibres. Acetylcholine has been demonstrated in sympathetic post-ganglionic nerves by Loewi & Hellauer (1938), who found 0·6–2·5 μg/g of acetylcholine in the post-ganglionic fibres from the superior cervical ganglion of cattle. Moreover, the enzymes concerned with acetylcholine metabolism are also present in sympathetic post-ganglionic neurones; Bullock, Grundfest, Nachmansohn & Rothenberg (1947) found that the post-ganglionic fibres from the superior cervical ganglion of the cat contained cholinesterase, and Koelle (1955) has reported that all 'adrenergic' neurones contained variable concentrations of specific acetylcholinesterase. However, there is the possibility that the acetylcholine which disappeared from the spleen on denervation may have originated from preganglionic sympathetic fibres relaying in aberrant sympathetic ganglion cells within or outside the spleen. The histological evidence relevant to this is that Utterback (1944) could not find ganglion cells in the cat's spleen, but Kuntz & Jacobs (1955) have found that some preganglionic fibres were present in the splenic nerve trunk.

Peart (1949) showed that noradrenaline was released from the spleen when the splenic nerves were stimulated. Our experiments demonstrate that in the reserpinized spleen stimulation of the splenic nerves releases an acetylcholine-like substance which contracts the guinea-pig ileum. This substance appeared only after the inhibition of cholinesterase by neostigmine, and its action on the ileum was blocked by atropine; thus it had the properties of a choline ester. If the presence of cholinergic sympathetic fibres to the spleen be accepted, the question must arise, to what extent do the responses to nerve stimulation depend on such fibres? Hemicholinium abolishes contractions of the spleen to nerve stimulation, and previous work has shown that it produces block at cholinergic junctions. For example, hemicholinium can abolish the responses to motor nerve stimulation (Wilson & Long, 1959; Reitzel & Long, 1959*b*), to parasympathetic nerve stimulation (Wilson & Long, 1959) and to preganglionic sympathetic stimulation (MacIntosh *et al.* 1956). MacIntosh *et al.* (1956) showed that hemicholinium inhibited the synthesis of acetylcholine by nervous tissue and they considered that its neuronal blocking action was

due to a gradual disappearance of stored acetylcholine. Choline restored the synthesis of acetylcholine (MacIntosh *et al.* 1956) and thus reversed the failure of transmission at cholinergic junctions (MacIntosh *et al.* 1956; Reitzel & Long, 1959*a*). We have now found that choline restores contractions of the spleen to nerve stimulation after they are blocked by hemicholinium. This suggests that there is a cholinergic junction in the splenic nerves.

Burn & Rand (1959*b*, 1960*c*, *d*) suggested that the role of acetylcholine liberated from post-ganglionic sympathetic fibres may be to release noradrenaline from stores in the tissue, thus rendering the action of these fibres ultimately adrenergic. Acetylcholine, like splenic nerve stimulation, contracts the spleen of the dog (Daly & Scott, 1961) and of the cat. Actions of acetylcholine resembling the adrenergic effects of sympathetic nerve stimulation have frequently been observed, and in many cases it is unlikely that these effects were due to stimulation of sympathetic ganglion cells (Burn, Leach, Rand & Thompson, 1959; Lee & Shideman, 1959). Utterback (1944) has shown that ganglion cells are not present in the cat spleen, although Kuntz & Jacobs (1955) found a few small ganglia in the plexus of the splenic nerve about the splenic artery. A store of noradrenaline in the tissues is necessary for acetylcholine to exert a sympathomimetic action (Burn *et al.* 1959); when the store of noradrenaline is depleted by reserpine this action is lost. We have found that reserpine treatment considerably reduced the action of acetylcholine on the spleen. The stores of noradrenaline in reserpine-treated tissues can be replenished by an infusion of noradrenaline (Pennefather & Rand, 1960). Burn & Rand (1960*c*) found that an infusion of noradrenaline produced an increase in the contractions of the spleen in reserpine-treated cats in response to splenic nerve stimulation, and we have now found that after strips of reserpine-treated spleen were soaked in noradrenaline there was an increase in the response to acetylcholine. Bretylium blocks the response of the spleen to splenic nerve stimulation (Boura & Green, 1959) and we have found that it also blocked the contractions produced by acetylcholine, although the response to noradrenaline was enhanced. The locus of action of bretylium is still uncertain; Boura & Green have concluded that it paralyses sympathetic nerve endings, whereas Burn & Rand (1960*c*) have suggested that it blocks the release of noradrenaline from its peripheral stores by acetylcholine. The adrenergic blocking drugs dibenzyline and piperoxan also blocked the contractions produced by acetylcholine and by noradrenaline. Their action is exerted on the receptors for noradrenaline and the block of the acetylcholine effect is further evidence that acetylcholine was acting through noradrenaline release (cf. Burn & Dutta, 1948). Daly & Scott (1961) have studied the effect of acetylcholine on the isolated blood-perfused spleen

of the dog. They found that large doses of acetylcholine produced contraction of the dog's spleen and, as in our experiments, this response was not blocked by atropine, but was abolished by dibenamine and dibenzyline, and by chronic denervation, and was reduced by reserpine treatment.

Dale (1933) proposed the terms 'adrenergic' and 'cholinergic' to describe those nerves which released (nor)adrenaline and acetylcholine after stimulation. Von Euler (1956) has summarized the evidence that led to the hypothesis that sympathetic adrenergic nerve fibres contain noradrenaline; this evidence is based principally on the findings that sympathetically innervated organs contain extractable noradrenaline, but after section and degeneration of the post-ganglionic sympathetic nerves the noradrenaline content of these organs is diminished. Our findings indicate that sympathetic denervation leads to a loss of the acetylcholine content of the spleen, that sympathetic stimulation releases acetylcholine from the spleen, and that acetylcholine can produce a sympathomimetic effect on the spleen provided that the noradrenaline store is present. Recently Burn & Rand (1960*c*, *d*) have pointed out that there are many accounts in the literature of the occurrence of chromaffin cells in sympathetic nerve trunks and ganglia and in sympathetically innervated organs, although it is not known at present whether there are chromaffin cells in the spleen. These cells, rather than the nerve fibres themselves, could be the site of noradrenaline storage. The suggestion that the chromaffin cells are the site of the noradrenaline store is compatible with the loss of noradrenaline after nerve degeneration seen in some organs, because Burn *et al.* (1959) observed that the chromaffin cells in the nictitating membrane became pyknotic and were reduced in number after denervation. It may be that innervation has a trophic influence in maintaining the integrity of the chromaffin cells or of their noradrenaline content.

SUMMARY

1. The mean acetylcholine content of the cat's spleen was 0·47 μg/g. Ten days after section of the splenic nerve it was 0·10 μg/g. The percentage fall in the acetylcholine content of the spleen which was produced by denervation (78%) is approximately equal to the percentage fall in noradrenaline content after denervation (82%).

2. In reserpinized cats stimulation of the splenic nerves to the isolated spleen perfused with a neostigmine-containing solution led to the release of an acetylcholine-like substance into the venous effluent. This substance contracted the superfused guinea-pig ileum; its action was blocked by atropine.

3. Hemicholinium produced a failure of the contractions of the isolated

cat's spleen in response to nerve stimulation. This effect of hemicholinium was reversed by choline.

4. Acetylcholine had a sympathomimetic action in contracting the isolated spleen. This action of acetylcholine was not blocked by atropine; it was reduced by reserpine treatment, and was abolished by the anti-adrenaline drugs phenoxybenzamine and piperoxan and by the sympathetic nerve-blocking drug bretylium.

5. These findings are discussed in relation to the hypothesis that the sympathetic fibres are cholinergic, and that the acetylcholine then liberates noradrenaline.

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