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THE EFFECTS OF ANTICHOLINESTERASES UPON PERIPHERAL VASCULAR RESISTANCE IN THE DOG

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A number of organic phosphorus compounds known to be potent inhibitors of cholinesterase have been extensively studied for their effects on the heart rate and systemic blood pressure. The earlier literature concerning the pharmacological actions of these agents has been reviewed by Koelle & Gilman (1949) and by Holmstedt (1951).

Whereas there is general agreement that large doses of anticholinesterases often cause a fall in blood pressure in most species, it is not clear to what extent this is due to a reduction in cardiac output or to an alteration in peripheral vascular resistance or both. Verbeke (1949) showed in the dog that tetraethylpyrophosphate (TEPP) caused a fall in blood pressure and attributed this to peripheral vasodilatation. K. M. Wilson (personal communication) reached a similar conclusion with respect to *iso*propylmethylphosphonofluoridate (sarin) in the cat. On the other hand, information supplied by Holmstedt (1951) would indicate that, at least in the initial stages of poisoning with an infusion of ethyl *NN*-dimethylphosphoroamidocyanidate (tabun), an increase in calculated total peripheral resistance occurs. He measured changes in cardiac output by the direct Fick method in rabbits and found that a reduction in output occurred before there was any change in blood pressure. In the rat, intravenous injections of various anticholinesterases cause a rise in blood pressure, and the evidence presented by Dirnhuber & Cullumbine (1955) and by Varagić (1955) indicates that this is due to peripheral vasoconstriction. Dirnhuber & Cullumbine (1955) further suggest that peripheral vasodilator effects predominate in other species.

No direct measurements under controlled conditions appear to have been made of the changes in resistance in the peripheral circulation during poisoning with inhibitors of cholinesterase. Moreover, there is little information as to the vascular territories participating in these responses, although the observations

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of Dirnhuber & Cullumbine (1955) would indicate that skin vessels are to a large extent responsible. This paper deals with an investigation of these problems carried out on the dog. For the most part sarin has been used in this study, but in a few experiments TEPP was used for comparison. The effects of sarin on heart rate and blood pressure in the dog have been described recently by de Candole, Douglas, Evans, Holmes, Spencer, Torrance & Wilson (1953), Krop & Kunkel (1954) and by Heymans, Pochet & van Houtte (1956).

METHODS

Dogs, varying in weight from 7·0 to 16·8 kg, were anaesthetized with either chloralose (0·1 g/kg body weight, intravenously), sometimes preceded by morphine hydrochloride (1–2 mg/kg subcutaneously), or pentobarbitone sodium (Nembutal, 40 mg/kg intravenously).

The systemic blood pressure was measured from the femoral artery by means of a mercury manometer, and the heart rate by the method described by Daly & Schweitzer (1950) using a Thorp impulse counter (Thorp, 1948).

The tidal air volume of the animal breathing spontaneously was recorded by means of a bell spirometer connected to a conventional closed circuit respiratory system. Carbon dioxide was absorbed in two soda-lime towers and the rate of oxygen flow into the system was adjusted initially so as to maintain the limiting lines of the tidal air tracing horizontal. An upward or downward trend of the tracing after injection of a drug indicates, therefore, an increase or decrease respectively in oxygen uptake by the animal so long as there is no change in the functional residual capacity of the lungs.

When necessary, artificial positive pressure respiration was applied by means of a Starling 'Ideal' pump.

Measurement of changes in peripheral vascular resistance

Changes in peripheral vascular resistance were measured by perfusing various vascular territories through their main artery at constant blood volume inflow by means of a Dale & Schuster (1928) pump. Three types of experiment were performed.

(1) *Autoperfusion experiments.* In these experiments, the vascular territories were perfused with blood from the same animal (Fig. 1A). The lower limb was isolated from the general circulation, except for any blood passing through the femur itself, by applying an ecraseur de Chassaignac about 4 cm below the level of the inguinal ligament. The femoral and sciatic nerves were preserved. Perfusion was carried out through a cannula inserted into the distal end of the ligated femoral artery.

In some experiments the upper limb was perfused by the same method. The limb was skinned and the paw was severed at the level of the carpal joint to obtain vascular responses occurring in muscle only.

The same type of experiment was used for perfusion of parts of the splanchnic vascular bed through either the coeliac or superior mesenteric artery. In all these experiments the perfused blood returned to the right heart through the normal venous channels.

(2) *Cross-perfusion experiments.* The upper limb was *completely* isolated from the general circulation by ligating all the muscle masses and dividing the humerus. The innervation of the limb was preserved. The limb was perfused at constant volume inflow through the brachial artery with blood from the femoral artery of a donor dog to which the venous blood was returned from the cannulated brachial vein of the recipient's perfused limb (Fig. 1B).

In one experiment, the limb was perfused at constant pressure instead of at constant blood volume inflow. In this experiment, the brachial artery was connected directly with the femoral artery of the donor animal, and blood flow was measured by means of a rotameter of the type described by Shipley & Wilson (1951) but modified according to Bell (1954).

(3) *Responses of skin vessels* were investigated by two methods. In the first, the isolated paw of the upper limb was perfused at constant blood volume inflow by the method described in (1) above. Although the paw does contain a small amount of muscle, it is predominantly skin. In the other experiments, the technique of perfusion of the isolated innervated skin flap was used (Feldberg & Paton, 1951; Feldberg & Schachter, 1952). The skin was perfused with Locke's solution at constant head of pressure and the arterial inflow volume was measured with an

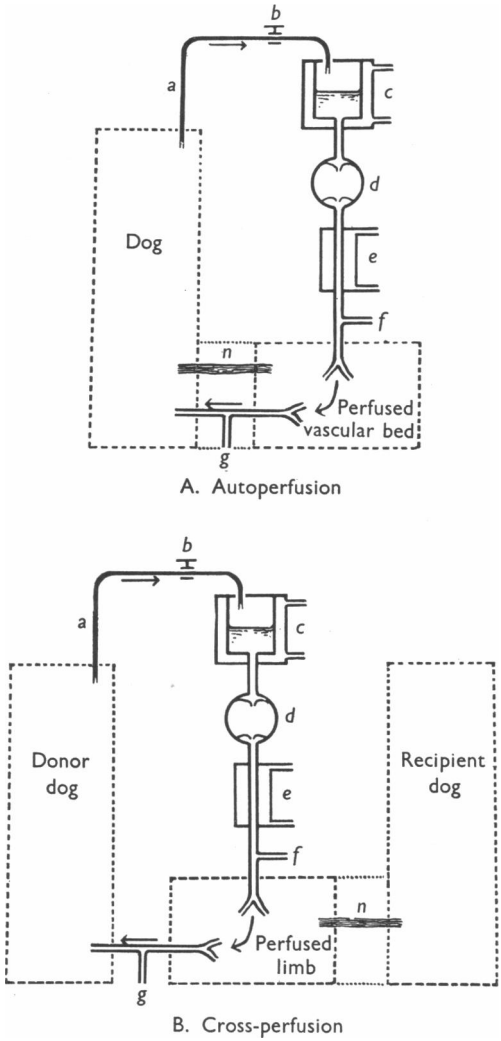

Fig. 1. Diagram illustrating autoperfusion of a partly isolated vascular bed (A) and cross-perfusion of the completely isolated limb (B). *a*, artery supplying reservoir; *b*, screw-clamp controlling volume of blood flow; *c*, water-jacketed reservoir; *d*, Dale–Schuster pump; *e*, warming coil; *f* and *g*, side-arms for measuring arterial perfusion pressure (Hg manometer) and venous pressure (saline manometer) respectively; *n*, nerve supplying vascular bed. For details, see text.

enclosed drop recorder. Each drop was recorded on the kymograph by means of a Thorp (1948) impulse counter. The vein draining the skin was opened to the atmosphere to exclude the effects of changes in venous pressure. The innervation of the skin flap was carefully preserved.

In all autoperfusion and cross-perfusion experiments the perfusion pressure was measured from a side arm of the inflow cannula by means of a mercury manometer and the venous pressure was recorded from the central end of a branch of the brachial or femoral vein by means of a saline manometer, the open end of which was connected to a small volume recorder. The portal pressure was recorded from the central end of the splenic vein when the splanchnic vascular bed was perfused. To prevent loss of blood into the spleen, the splenic artery was ligated.

In all autoperfusion and cross-perfusion experiments, the input side of the pump was connected to a small water-jacketed reservoir which was supplied with blood from the cannulated central end of the femoral artery (Fig. 1A, B). The level of the blood in the reservoir was controlled by means of an adjustable screw clamp on the inflow tube. Water from a water-bath maintained at 37° C was circulated through the jacket of the reservoir and through a warming coil situated between the pump and the arterial cannula.

The insertion of a blood reservoir in the perfusion circuit between the cannula in the central end of the femoral artery and the input side of the pump was a safeguard against alterations in pump output which might otherwise occur as a result of the wide fluctuations in femoral blood pressure produced by injection of the drug. Nevertheless, changes in the level of the blood in the reservoir did occur from time to time but they did not amount to more than 3 cm. The output of the pump was not influenced by changes in inflow pressure of this order of magnitude. The volume of blood in the extracorporeal circulation (reservoir, pump, warming coil and connecting tubes) was about 60 ml.

The top of the reservoir was open to the atmosphere via a small tube to keep the air pressure constant when variations in reservoir blood volume occurred. It seems unlikely that any significant loss of carbon dioxide from the blood occurred as substitution of a mixture of 95% O_2 and 5% CO_2 for the air in the reservoir had no effect on the perfusion pressure.

Calculation of changes in vascular resistance

The blood flow was not measured in every perfusion experiment. However, the output of the perfusion pump remained constant within 8% when tested over the whole range of pressures found in this investigation. As a close approximation, therefore, the change in vascular resistance can be taken as being proportional to the change in pressure drop across the perfused vascular bed, i.e. (mean arterial perfusion pressure minus mean venous B.P.) mm Hg. The change in vascular resistance was expressed as a percentage change in pressure drop across the vascular bed.

All animals were given heparin (Liquemin, Roche Products, Ltd.) (7·6 mg/kg) to render the blood incoagulable. *iso*Propylmethylphosphonofluoridate (sarin) was given in a 1 in 1000 aqueous solution into the femoral vein. In other experiments, tetraethylpyrophosphate (TEPP) was used in a 1 in 100 aqueous solution. Other drugs used were atropine sulphate (B.D.H.), acetylcholine (Roche Products, Ltd.), 2-benzyl-imidazoline hydrochloride (Priscol, Ciba Laboratories, Ltd.), and hexamethonium bromide (Vegolysen, May and Baker, Ltd.).

RESULTS

In most preparations, sarin, in doses of 25–35 μg/kg, and TEPP, 0·1–0·3 mg/kg, caused a gradual diminution in respiratory minute volume leading finally to complete cessation of respiratory movements. Occasionally there was an initial increase in minute volume. There was always a gradual reduction in heart rate and the blood pressure usually fell initially but subsequently recovered to its original level within a few minutes of injection, despite persistence of the bradycardia. Typical effects on respiration, heart rate and

blood pressure are shown in Figs. 2–4 and 5B, C. These results are similar to those described by other workers, using these and other anticholinesterases in the dog (see reviews by Koelle & Gilman, 1949, and by Holmstedt, 1951; also de Candole *et al.* 1953; Krop & Kunkel, 1954; Heymans *et al.* 1956).

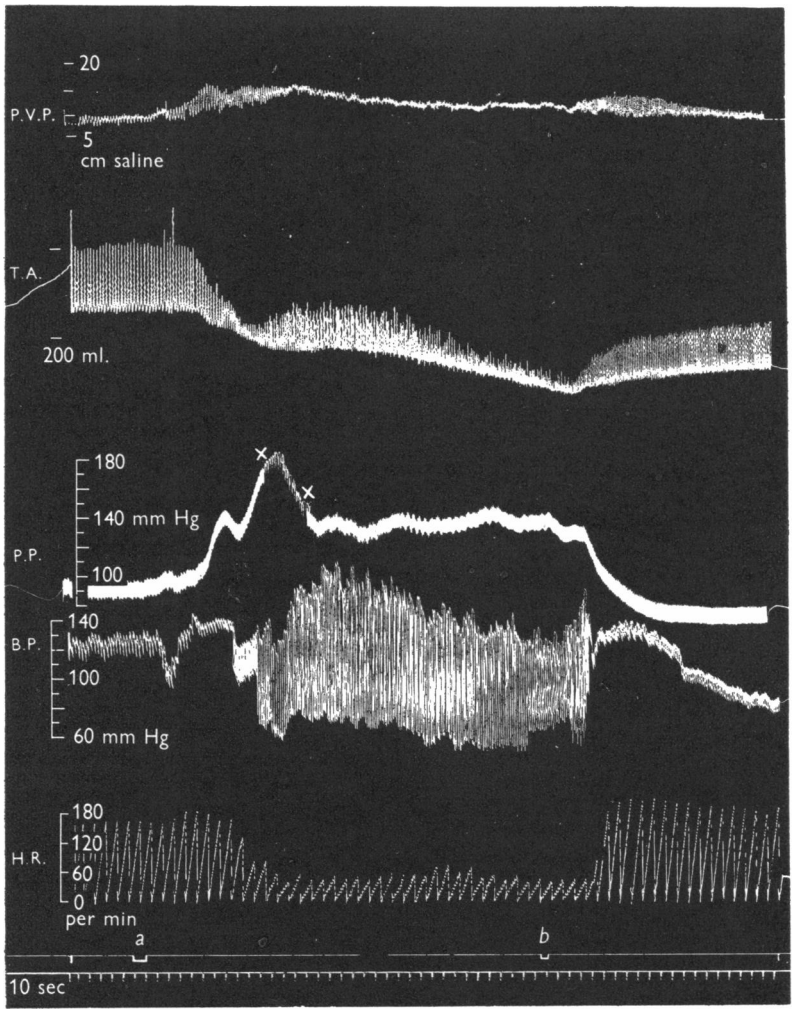

Fig. 2. Dog, ♀, 15·75 kg. Nembutal. Spontaneous respiration. Autoperfusion of the splanchnic vascular bed through the superior mesenteric artery. At *a*, sarin 25 μg/kg; at *b*, atropine 2 mg. intravenously. (Between marks X–X, the perfusion pressure manometer flag was sticking, but the trace is a true record of the changes in mean pressure.) In this and in subsequent figures: P.V.P. = portal venous pressure; F.V.P. = femoral venous pressure; R.A.P. = right atrial pressure; T.A. = tidal air volume (inspiration upwards); P.P. = perfusion pressure; B.P. = systemic blood pressure; and H.R. = heart rate; time marker, 10 sec.

The effect of sarin and TEPP on the peripheral vascular resistance

Autoperfusion experiments

By perfusing an isolated innervated organ with arterial blood from the same animal, the perfused blood vessels are subjected both to alterations in vasomotor tone and to changes in blood composition which may be brought about by the injection of a drug into the animal. The perfused organ is therefore subjected to practically the same conditions as other naturally perfused territories, but owing to the dead space in the reservoir, pump and connexions, there is delay in blood-borne agents reaching the organ, amounting to ½–1½ min in different experiments. There is, in addition, some dilution of the agents entering the organ.

TABLE 1. Autoperfusion experiments. The effect of sarin and TEPP upon the vascular resistance of the isolated innervated limb, splanchnic vascular bed, skin and muscle

Expt. no.	Perfused vascular bed	Agent	Arterial perfusion pressure (mm Hg)		Venous pressure* (mm Hg)		Right atrial pressure (mm Hg)		Change in vascular resistance (%)
			Before	After	Before	After	Before	After	
1	Right femoral artery	Sarin	155	180	—	—	—	—	+16
2		Sarin	135	190	5	19	—	—	+32
3		Sarin	115	125	4·5	7·5	—	—	+6
4	Right brachial artery	TEPP	130	254	—	—	−1	+10·5	+86
5	Coeliac artery	Sarin	70	210	7·5	17·5	—	—	+210
6	Superior mesenteric artery	Sarin	90	180	6	11	—	—	+100
7	Right radial artery (skin only)	TEPP	100	135	—	—	0	4	+31
8	Right brachial artery (muscle only)	TEPP	144	184	—	—	−1	5	+23·5
9		TEPP	118	220	—	—	0	16	+73

* Venous pressure was measured in the femoral vein in Expts. 2 and 3, and in the portal vein in Expts. 5 and 6.

In six experiments which involved autoperfusion of the upper or lower limb or part of the splanchnic vascular bed, sarin, in doses of 25 μg/kg, and TEPP, 0·3 mg/kg, caused an increase in perfusion pressure. The results are summarized in Table 1 and show that the increase in vascular resistance varied from 6 to 210%. In all cases, the perfusion pressure started to rise within 1½ min of injection and occasionally before there was any fall in systemic blood pressure or in heart rate. In one experiment in which the lower limb was perfused, the perfusion pressure fell below the control value after an initial rise. This fall was accompanied by a rise in systemic blood pressure which was larger than usual and may have caused the fall in perfusion pressure reflexly. Thereafter, the blood pressure fell and the perfusion pressure increased again. In all

experiments the increase in vascular resistance was maintained throughout the period of observation of the drug's action, although occasionally it showed some fluctuations. A typical response is seen in the experiment from which Fig. 2 is taken. The responses were found to be the same whether chloralose or Nembutal was the anaesthetic used. In five of the six experiments in which venous pressure was measured, a rise occurred, but it was small compared with the increase in arterial perfusion pressure (Table 1).

Further tests on this type of preparation suggested that the observed increase in vascular resistance is the result of an increase in sympathetic tone. This was shown as follows. In two experiments, the nerves innervating the limb were divided when the perfusion pressure reached its maximum height. A precipitous fall in pressure occurred. An intravenous injection of hexamethonium was given in one other experiment, and it caused a similar fall in perfusion pressure, although the final level attained in this instance was slightly higher than the control value.

Vascular responses in skin and muscle. The results of autoperfusion experiments of one paw (skin) and of two skinned limbs are summarized in Table 1, and show that TEPP, in doses of 0·2–0·4 mg/kg, causes vasoconstriction in both skin and muscle. The typical response in the skinned limb is illustrated in Fig. 3. There was a rise in perfusion pressure from 118 to 220 mm Hg which represents an increase in vascular resistance of 73%. Again, this increase in vascular resistance was immediately abolished by section of the nerves to the limb (Fig. 3*b*) suggesting that it was of sympathetic origin. The same increase in vascular resistance was observed in the innervated skin flap preparation perfused with Locke's solution when TEPP was injected intravenously into the dog (Table 2).

TABLE 2. The effect of TEPP upon the vascular resistance in the skin flap preparations perfused with Locke's solution

Expt. no.	Arterial perfusion pressure (mm Hg)		Arterial inflow (drops/min)		Change in vascular resistance (%)
	Before	After	Before	After	
10	80	80	150	120	+20
11	110	110	114	96	+16

Cross-perfusion experiments

Experiments in which the completely isolated innervated limb is perfused from a donor animal serve two purposes. First, when the recipient is poisoned, changes in perfusion pressure of the isolated limb reflect variations in sympathetic tone so long as the composition of the blood perfusing it remains constant. Secondly, when the donor is poisoned, changes in perfusion pressure indicate effects due to agents carried by the blood since the sympathetic discharge to the blood vessels remains constant.

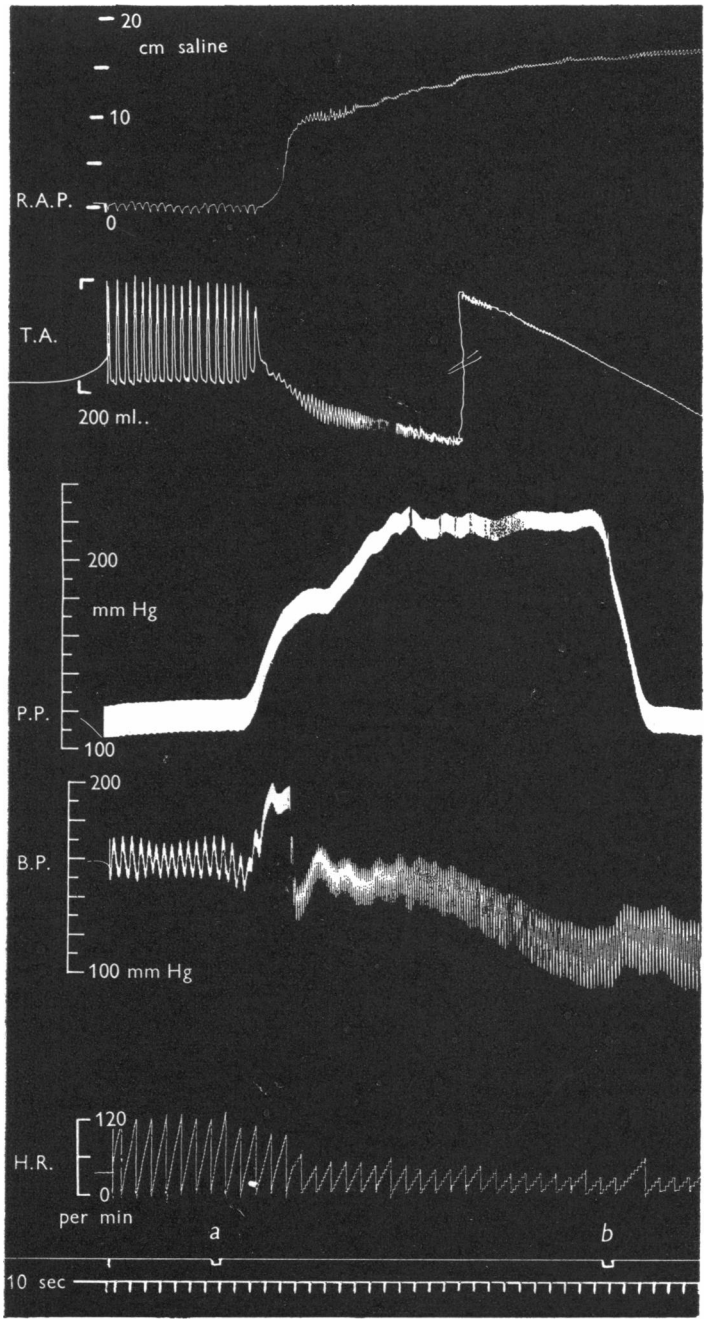

Fig. 3. Dog, ♀, 14·2 kg. Nembutal. Autoperfusion of the isolated innervated skinned fore-limb. At *a*, intravenous injection of TEPP 0·4 mg/kg. At *b* the nerves to the limb were cut.

Poisoning of recipient. The results of three experiments in which the recipient animal was injected with sarin, 25 μg/kg intravenously, are summarized in Table 3. In all three experiments there occurred a considerable increase in vascular resistance which must have been brought about by an increase in sympathetic tone. This is illustrated in Fig. 4. The experiment of Fig. 5C in which the donor was adrenalectomized shows that the recovery of

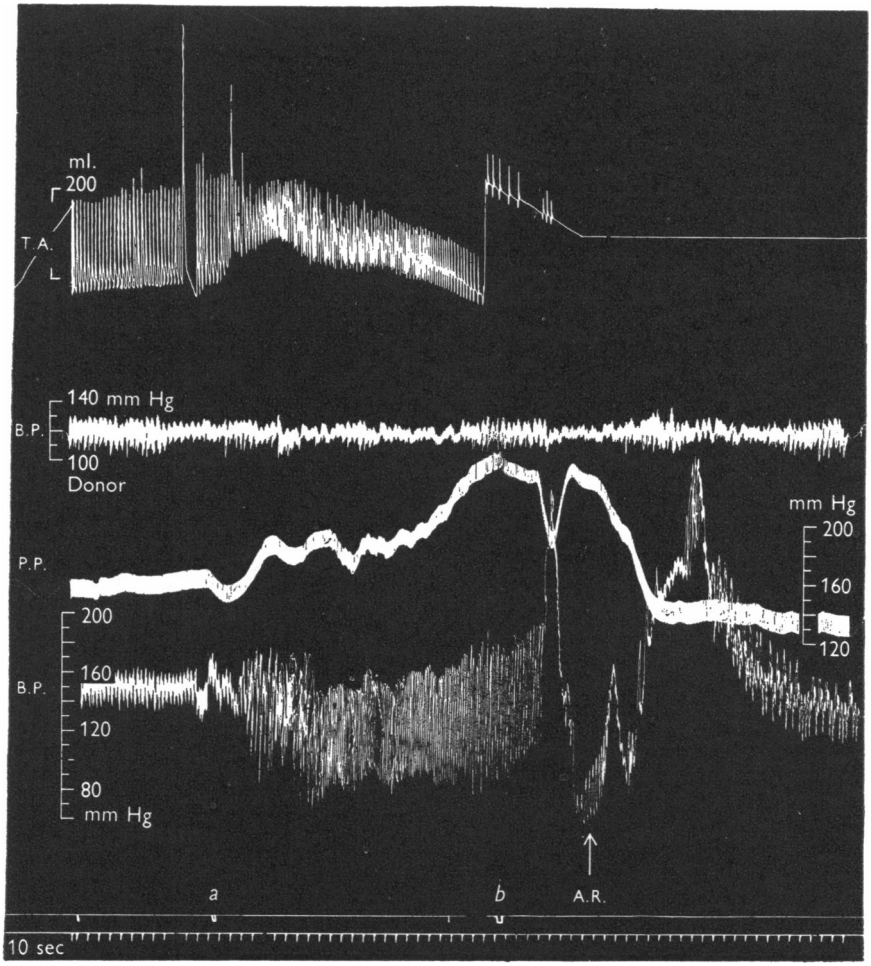

Fig. 4. Dog, ♂, 13·4 kg. Chloralose. Cross-perfusion experiment. Right lower limb isolated from the general circulation and perfused with a Dale–Schuster pump from donor dog. Records from above downwards: tidal air volume of recipient, blood pressure of donor, limb perfusion pressure and blood pressure of recipient. The recipient was injected intravenously with sarin 30 μg/kg at *a*, and with atropine 5 mg at *b*. Artificial respiration (A.R.) was begun at arrow ↑.

the arterial blood pressure after poisoning is accompanied by a considerable increase in sympathetic tone of the perfused limb and that this tone diminishes again as the blood pressure begins to fall.

Poisoning of donor. An intravenous injection of sarin (25μg/kg) into the donor dog caused a considerable increase in the perfusion pressure as shown from the results of the Expts. 12*b* and 15 in Table 3. This effect also occurs when the limb is denervated. In one of the two experiments, the sympatholytic drug Priscol (1·25 mg) was injected into the arterial inflow tubing to the perfused denervated limb at the height of the vasoconstrictor response. An immediate fall in perfusion pressure occurred to a level only slightly above its

TABLE 3. Cross-perfusion experiments. The effect of sarin upon the arterial pressure of the isolated innervated limb perfused from a donor animal at constant blood-volume inflow. Sarin was injected intravenously into the recipient and into the donor

Expt. no.	Arterial perfusion pressure (mm Hg) Before	After	Femoral venous pressure (mm Hg) Before	After	Change in vascular resistance (%)	Remarks
Recipient injected						
12*a*	165	240	—	—	+45	Recipient's right cervical vagosympathetic nerve cut
13	100	185	8	8	+92	—
14*a*	108	310	7·5	5	+190	Donor atropinized and bilaterally adrenalectomized
Donor injected						
12*b*	145	232	—	—	+60	Recipient atropinized
15	88	245	10	10	+202	Recipient atropinized, limb denervated
16	114	92	—	—	−19·5	Donor bilaterally adrenalectomized
14*b*	85	74	9	12	−18	
17	165	152	6	13	−12·5	

control value. These findings suggest that sarin causes the secretion of a sympathomimetic substance from the suprarenal glands, and recalls the early observation of Stewart & Rogoff (1921) who found that eserine causes the secretion of adrenaline from the suprarenal gland. This was proved by repeating these experiments using bilaterally adrenalectomized donors. The injection of sarin no longer caused a rise in perfusion pressure, but on the contrary, a small vasodilator response appeared as shown in the last three experiments of Table 3 and in the experiment illustrated in Fig. 5B. This vasodilator response could be due to accumulation of endogenous acetylcholine, or to alteration in the pO_2 and pCO_2 of the blood perfusing the limb brought about by respiratory failure, or to both, since it was found in these experiments that both acetylcholine given intra-arterially to the perfused limb and asphyxia of the donor produced by occlusion of its trachea caused a vasodilator response (Fig. 5A). Attempts to dissociate these two possible causes of the vasodilator response to sarin were not completely successful, but in two experiments intra-arterial

injection of atropine after poisoning the donor caused an immediate rise in perfusion pressure suggesting that accumulated acetylcholine was at least partly responsible (Fig. 5B).

In conclusion, therefore, these experiments show that the increase in peripheral vascular resistance resulting from the injection of sarin is due to an increase in sympathetic tone and also to a blood-borne vasoconstrictor substance. These effects mask a blood-borne vasodilator component acting directly on the blood vessels themselves.

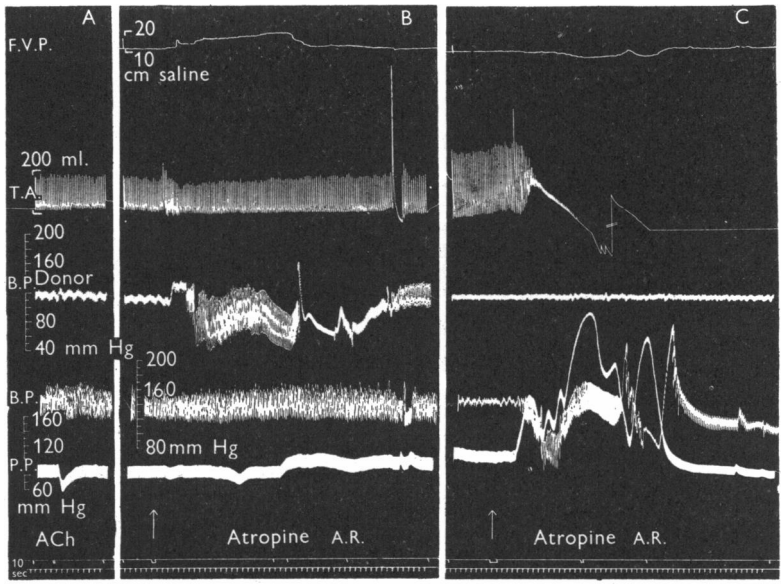

Fig. 5. Dog, ♂, 16·2 kg. Chloralose. Cross-perfusion experiment. Right upper limb perfused with a Dale–Schuster pump from an adrenalectomized donor dog. A, injection of acetylcholine 1 μg into arterial inflow tube. B, donor animal injected with sarin 25 μg/kg at arrow ↑. Atropine was then injected 0·5 mg into the arterial inflow tubing, followed 25 sec later by 4·5 mg intravenously. A.R., artificial respiration begun. C, donor animal breathing spontaneously. Recipient was injected with sarin 35 μg/kg at arrow ↑, followed by atropine 5 mg intravenously. A.R., artificial respiration to recipient begun.

The effect of sarin after denervation of the carotid sinuses and arch of the aorta

One possible explanation of the increase in sympathetic vasomotor tone occurring on injection of anticholinesterase agents is a reflex from the baro- and chemoreceptors of the carotid bifurcation region and arch of the aorta. Our experiments showed that a similar vasoconstrictor response occurred, although it was often less pronounced, when preparations were used in which

the carotid sinuses and arch of the aorta were denervated by dividing both carotid sinus, cervical vagosympathetic and recurrent laryngeal nerves.

A typical experiment is shown in Fig. 6 in which the upper limb was autoperfused. Sarin caused an immediate vasoconstriction followed 1½ min later

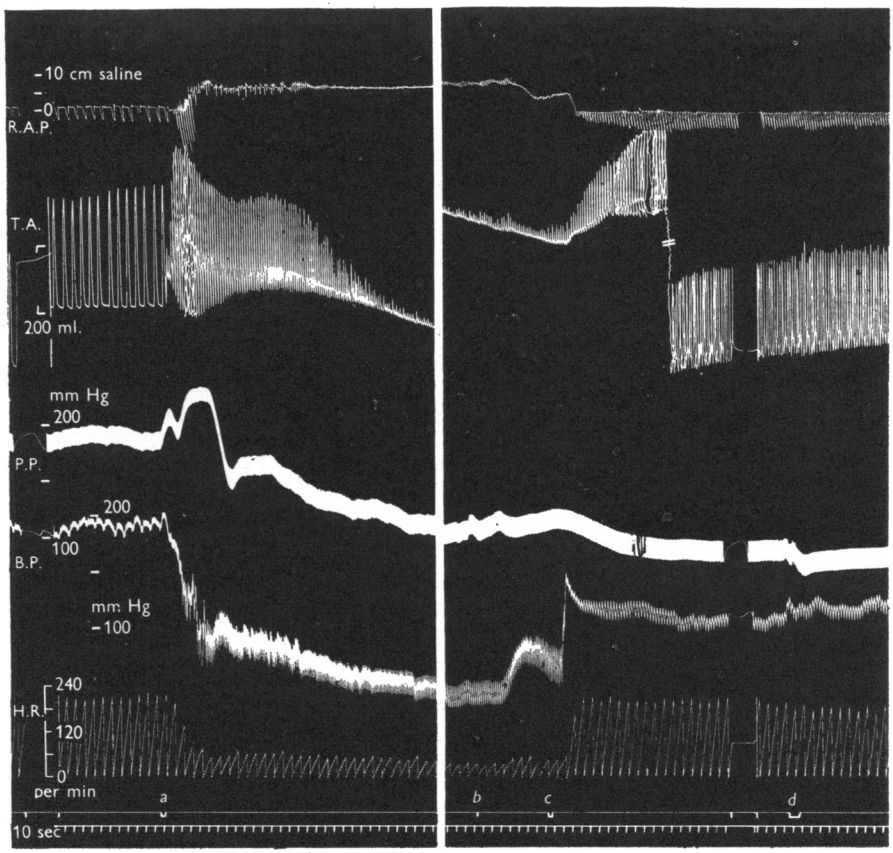

Fig. 6. Dog, ♂, 11·5 kg. Chloralose, autoperfusion of the upper limb. Both cervical vagosympathetic, carotid sinus and recurrent laryngeal nerves cut. At *a*, sarin 100 μg/kg injected intravenously; at *b*, atropine 0·5 mg injected into the arterial inflow tubing followed by 4·5 mg intravenously at *c*; at *d* the nerves innervating the limb were cut. An interval of 2½ min elapsed between the two records.

by a gradual vasodilatation. In the experiment of Fig. 6 the systemic blood pressure fell precipitously, but in some others the fall was preceded by a slight rise in pressure. In all experiments there was bradycardia, but never cardiac arrest; nor was there any conspicuous 'escape' of the heart such as occurred in preparations with innervated vasosensory zones.

In some experiments with denervated vasosensory zones, the vasoconstriction was not maintained and was followed by a permanent vasodilatation (Fig. 6). In others, such as in the experiment of Fig. 7, the vasoconstriction was well maintained. When a permanent vasodilatation occurred and the

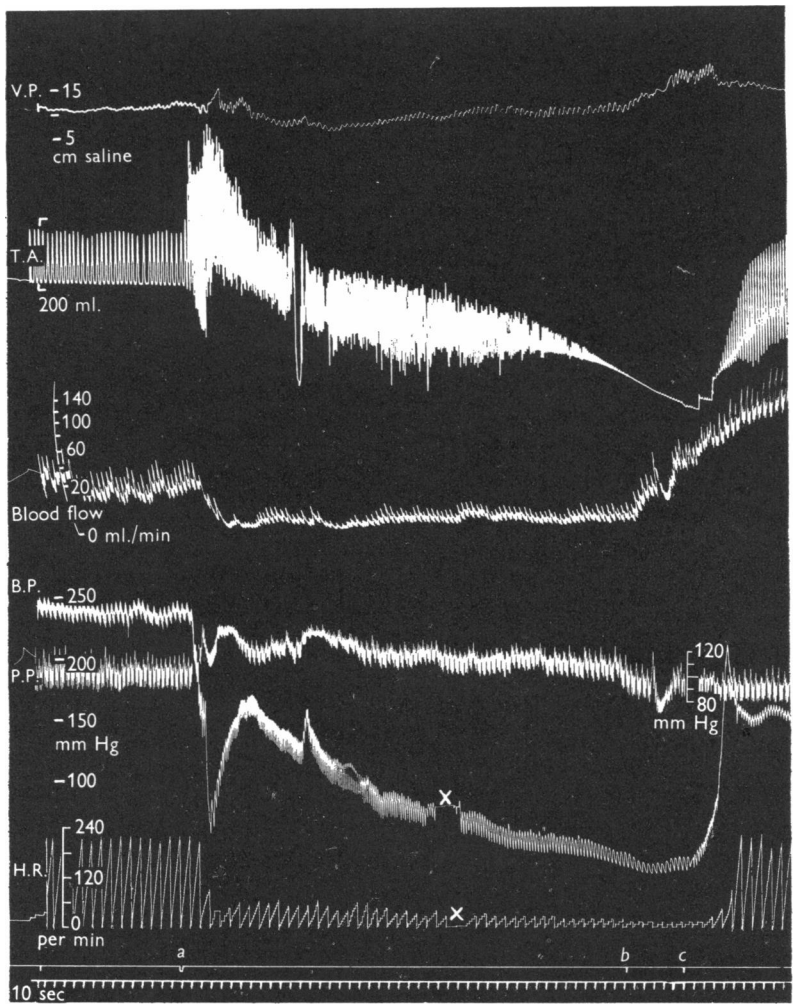

Fig. 7. Dog, ♂, 11·25, kg. Chloralose. Direct perfusion of the isolated innervated upper limb from the femoral artery of a donor dog at constant pressure. Arterial blood flow measured by means of a rotameter. Both cervical vagosympathetic, carotid sinus and recurrent laryngea nerves cut. Donor animal breathing spontaneously. At *a*, sarin 100 μg/kg injected intravenously into the recipient animal; at *b*, the nerves innervating the limb were cut; at *c* atropine 2 mg was injected intravenously. X, artifact. (Note the B.P. and P.P. records cross 15 sec after *a*.)

nerves to the limb were cut there was only a slight fall in perfusion pressure. When the nerves were cut during a maintained vasoconstriction, nerve section caused a pronounced vasodilatation. This is shown in Fig. 7 by an increase in blood flow to the limb. Whatever may be the role of the carotid sinuses and arch of the aorta in the initiation and maintenance of the increased sympathetic vasoconstrictor tone caused by sarin, these experiments with denervated vasosensory zones indicate that sarin has an action on the nervous system other than by reflexes from these zones.

Effect of atropine

The effects of atropine (2–5 mg) injected intravenously after sarin or TEPP were tested in experiments in which either the limb, skinned limb or splanchnic vascular bed were autoperfused. In the trunk of the animal it caused an increase in heart rate and a precipitous rise in blood pressure which then fell to its original level or slightly below it. The respiratory minute volume was often wholly or partly restored. In the perfused vascular bed the perfusion pressure returned to normal (Fig. 2). These effects occurred while the inflow to the reservoir was stopped momentarily to prevent atropine reaching the autoperfused vascular bed.

Atropine also caused a fall in perfusion pressure in preparations in which the vasosensory zones had been denervated. The size of the fall was, however, usually smaller, particularly when the initial vasoconstriction after sarin had not been maintained. Such an experiment is shown in Fig. 6*b*, *c*.

The effect of atropine on the vascular resistance is at least partly mediated through the sympathetic nervous system. Evidence for this was provided by cross-perfusion experiments of the limb, because in these atropine injected after sarin caused a diminution of perfusion pressure, the final level being slightly lower than the control value (Fig. 4 and 5C). Before reaching this final level, however, the perfusion pressure occasionally showed wide fluctuations. In the experiments in which atropine restored respiration, the perfusion pressure fell to a steady level which was maintained (Fig. 2). In those in which respiration remained depressed, the momentary rise in systemic blood pressure, accompanied by a fall in perfusion pressure, was followed by failure of the heart, a fall in systemic blood pressure and a rise in perfusion pressure. Artificial respiration then restored the heart and blood pressure whilst the perfusion pressure fell to a level which was maintained. These effects are shown in Figs. 4 and 5C.

These results suggest that in advanced poisoning with sarin asphyxia is partly responsible for the increase in sympathetic tone. Whether the immediate fall in perfusion pressure accompanying the momentary rise in blood pressure on giving atropine is due to a baroreceptor reflex, to an increase in cerebral blood flow and hence improved oxygenation of the medullary 'centres',

or to abolition of a possible central action of sarin is not possible to say. Some sympathetic tone was still present after injection of atropine, as was shown from the effect of dividing the nerves innervating the limb. Nerve section caused a fall in perfusion pressure.

Further doses of sarin or TEPP after atropine were without effect on the vascular resistance until respiration became depressed and then an increase in resistance occurred. Such an effect is shown in Fig. 8. The rise in perfusion

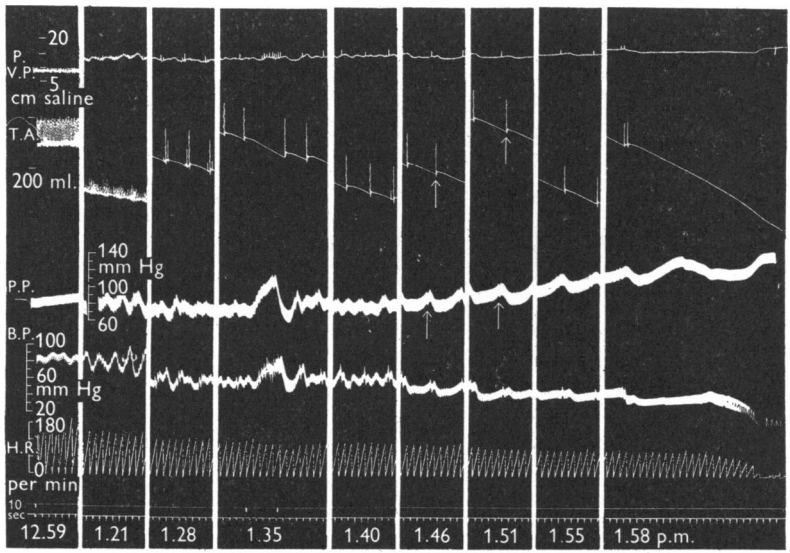

Fig. 8. Dog, ♀, 15·75 kg. Nembutal. Autoperfusion of the splanchnic vascular bed through the superior mesenteric artery. Sarin 25 μg/kg was given intravenously at 12.26 p.m. and atropine 2 mg at 12.32. The figure shows the effects of subsequent intravenous injections of sarin. Between 1.02 and 1.17 p.m. 1250 μg/kg was given in divided doses; at 1.42 p.m. a further single dose of 2000 μg/kg was given; at 1.35 p.m. the response to temporary occlusion of the trachea was tested. The arrows refer to the ordinates on each trace.

pressure is the result of asphyxia causing an increase in sympathetic tone and the secretion of a vasoconstrictor substance from the suprarenals. When the trachea was occluded for a period of 50 sec there resulted an immediate rise in systemic blood pressure and in perfusion pressure. Such a transient occlusion was carried out at 1.35 p.m. in the experiment illustrated in Fig. 8. The effect was probably due to an increase in sympathetic tone rather than to the secretion of vasoconstrictor substance: the response occurred too quickly to be accounted for by the passage of the hormone through the perfusion circuit. When in the experiment of Fig. 8 respiration became very slow, each breath was preceded by a gradual rise in blood pressure and perfusion pressure, and

after each breath there was a delay of about 5 sec before the pressures fell suddenly. These effects are evident in Fig. 8 from an examination of their time relationships and are probably due to an alteration in sympathetic tone.

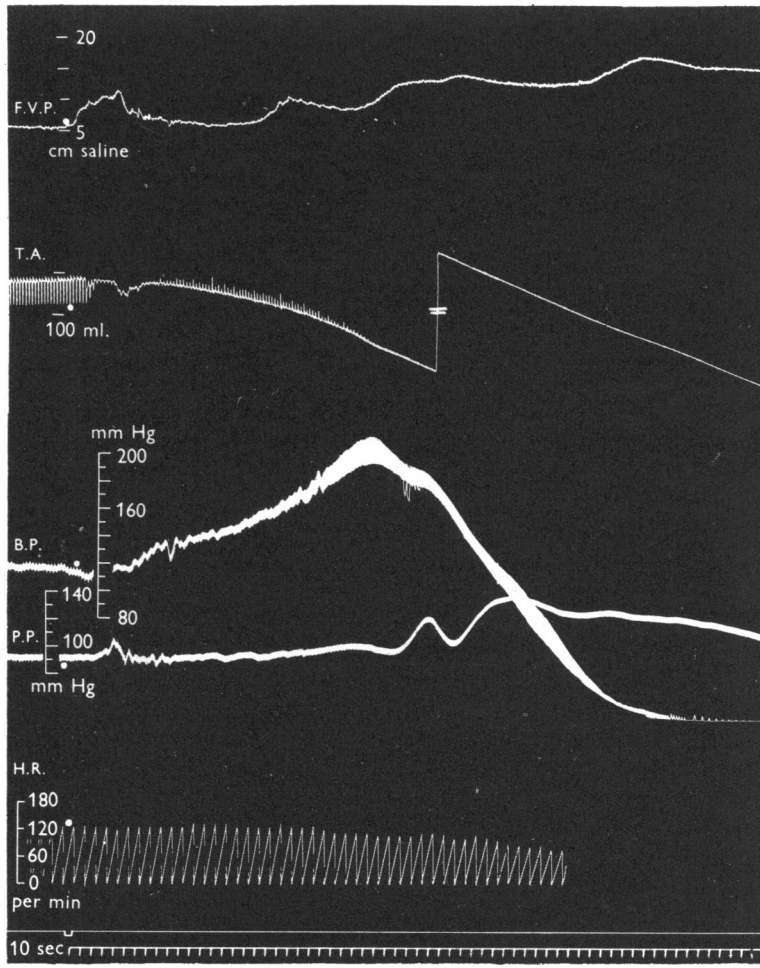

Fig. 9. Dog, ♀, 12·4 kg. Nembutal. Spontaneous respiration. Autoperfusion of the isolated *denervated* lower limb. Sarin 25 μg/kg given at 12.52 p.m. followed by atropine 2 mg at $\frac{1}{2}$ min past 1.00 p.m. At signal (1.25 p.m.), sarin 2000 μg/kg injected intravenously.

Moreover, the more gradual rise in perfusion pressure seen to occur during the last 10 min of the experiment illustrated in Fig. 8 may well be partly due to an output of the vasoconstrictor substance, since such a gradual vasoconstriction also occurred when limbs had been denervated. Such an experiment is illustrated in Fig. 9. After the injection of sarin (2000 μg/kg) in the atropinized

preparation, there developed a gradual rise in systemic blood pressure. The perfusion pressure rose too but the maximum rise occurred later, probably because the volume of blood in the dead space in the reservoir and pump delayed the arrival of the hormone in the perfused limb.

DISCUSSION

Our experiments have shown that, in the dog, the anticholinesterase agents sarin and TEPP cause an increase in resistance to blood flow through the limbs and splanchnic vascular bed due to an increased discharge in the sympathetic nervous system. This would appear to be at variance with the results of other workers. K. M. Wilson (personal communication) observed in the cat that intravenous injections of sarin increased limb volume, which he interpreted as due to vasodilatation. However, it is likely that the considerable rise in right atrial and peripheral venous pressures which occur under these conditions can by passive distension of the vascular bed account for the increase in volume which he observed. For instance, after injection of TEPP we have observed an increase in vascular resistance of one limb perfused at constant volume inflow, whilst the other in a plethysmograph showed a volume increase. Such an increase in limb volume cannot, therefore, be attributed to vasodilatation. In this connexion, it is interesting to note that Mendez & Ravin (1941) found in the cat a decrease in limb volume on injection of prostigmine in three out of four experiments.

Furthermore, there are observations of Verbeke & Votava (1949) and of Verbeke (1949) who obtained after injection of anticholinesterases a fall in arterial blood pressure which they attributed to vasodilatation. Their observations using large doses of hexaethyltetraphosphate (HETP, 0·5 mg/kg) and TEPP (0·1–0·3 mg/kg) were made on preparations in which changes in heart rate were excluded by previous injection of Parpanit (Geigy), a ganglionic blocking agent which according to Heymans & de Vleeschhouwer (1948) causes block of vagal ganglia only in doses of 0·5–3·0 mg/kg. However, it is not certain that the dose of Parpanit used did not also block sympathetic ganglia, for in the papers of Verbeke (1949) and Verbeke & Votava (1949) it is not clear what doses of parpanit were used. In one of their experiments (see Fig. 7 of the paper by Verbeke & Votava, 1949) a dose of 20 mg/kg was given, which is sufficient to paralyse not only vagal ganglia but sympathetic ganglia as well (Heymans & de Vleeschhouwer, 1948). In that case any sympathetic vasoconstriction produced by these anticholinesterases would be abolished. The observed fall in arterial blood pressure in their experiments might well be a peripheral effect of accumulated acetylcholine. In any case the effects observed on blood vessels after injection of anticholinesterases into preparations pretreated with Parpanit cannot be considered to be their usual response. Nevertheless, Verbeke (1949) did show that small doses of TEPP

(0·002–0·05 mg/kg) caused a fall in blood pressure, accompanied by no change or by an increase in heart rate, in the normal anaesthetized dog.

In our experiments the observed increase in perfusion pressure which occurs after the injection of an anticholinesterase could be produced by several mechanisms, such as an increase in haematocrit concentration, muscular fasciculations in the case of perfused limbs, increased sympathetic vasoconstrictor tone or the release of hormones from the suprarenal medulla.

An increase in haematocrit concentration actually occurs after the injection of anticholinesterase, probably as a result of emptying of the blood stores in the spleen (Daly, to be published). However, an examination of the time relationship of the onset of the rise in perfusion pressure and increase in haematocrit concentration, and also the immediate effects on the perfusion pressure of atropine and of dividing the nerves innervating the limb, have ruled out the possibility that an increase in viscosity of the blood is a major cause of the rise in pressure. Further, in the cross-perfusion experiments involving poisoning of the recipient animal, this possibility did not arise.

In a few experiments there was some suggestion that a change in perfusion pressure could be produced by contraction of the muscles such as occurs during muscular twitching. This usually consisted of a rise in pressure but was small compared with the maximum effects observed, and moreover no correlation was found between the general trend of the perfusion pressure and the onset or severity of muscular twitching.

Changes in sympathetic vasoconstrictor tone

In cross-perfusion experiments the injection of sarin or TEPP into the recipient animal caused a rise in limb perfusion pressure which was reduced by nerve section or by blocking autonomic ganglia with hexamethonium. This vasoconstriction did not occur if the nerves to the limb were previously divided. The most likely cause for such vasoconstriction is an increase in sympathetic tone.

The increased vasoconstrictor discharge after sarin or TEPP could be brought about by reflexes from the vasosensory zones of the carotid sinuses and arch of the aorta, by central asphyxia, by a central action of the anticholinesterases or by an action on the sympathetic ganglia.

Whilst it is probable that reflexes from the vasosensory zones would contribute to the increase in vasoconstrictor tone on injection of an anticholinesterase, the results of experiments in which these zones were denervated show that this effect is dependent on other factors as well. First, asphyxia occurs after injection of an anticholinesterase as a result of a reduction of pulmonary ventilation and could, therefore, produce peripheral vasoconstriction through a direct action of the changes in arterial blood gas tensions on the vasomotor centre (Traube, 1863; Dastre & Morat, 1884; Mathison, 1911) or on the spinal

cord (Kaya & Starling, 1909; Mathison, 1910). Furthermore, if the cerebral metabolism undergoes no change, the tensions of oxygen and carbon dioxide in the brain will be altered by changes in its blood flow. To what extent this varies during anticholinesterase poisoning is, as far as we are aware, unknown.

Secondly, the vasoconstrictor discharge may be due to a central excitatory action of anticholinesterases *per se* on some part of the central sympathetic pathway as these drugs are reported to have excitatory and paralytic central actions. Varying effects on the heart rate and blood pressure have been reported as a result of intracisternal, intraventricular or intracarotid injections of anticholinesterases. However, such a possible action of the anticholinesterases has to be proved experimentally beyond doubt before this mechanism can be taken as a cause for the sympathetic discharge.

Lastly, the anticholinesterases may facilitate synaptic transmission across sympathetic ganglia by prevention of hydrolysis of acetylcholine released locally. Such an effect of anticholinesterases has been observed in perfused sympathetic ganglia (Feldberg & Vartiainen, 1935; Marazzi & Jarvik, 1947; Holaday, Kamijo & Koelle, 1954). Another possible mechanism by which anticholinesterases may facilitate synaptic transmission is by an increase in the concentration of circulating adrenaline (Bülbring & Burn, 1942; Bülbring, 1944). However, in experiments on the entire animal, an action of anticholinesterases on sympathetic ganglia has been denied (Heymans & Jacob, 1947; Verbeke, 1949; Verbeke & Votava, 1949). In the absence of information on the preganglionic sympathetic activity in the entire animal during poisoning with anticholinesterases, it is not clear to what extent facilitation of synaptic transmission in sympathetic ganglia contributes to the increased peripheral resistance.

The effect of atropine in reversing the increase in sympathetic tone produced by anticholinesterases is of interest with regard to the mechanisms by which this increase in tone is produced. In the rat, both Dirnhuber & Cullumbine (1955) and Varagić (1955) observed that atropine had an antagonistic effect to anticholinesterases. Dirnhuber & Cullumbine (1955) found that pretreatment with atropine in doses of 10 mg/kg reduced the pressor effect of anticholinesterases. However, the effect of such a large dose of atropine need not only result from a competitive action in the central nervous system; the atropine could also act in the sympathetic ganglia, since in cats and rabbits intravenous injection of atropine in doses as small as 1–2 mg/kg are known to produce partial or complete block of synaptic transmission (Marrazzi, 1939; Fink & Cervoni, 1953; Acheson & Remolina, 1955). However, since some species of animals, especially rodents, can tolerate large doses of belladonna alkaloids owing to the presence of atropinase in the blood and liver, we consider this point requires further investigation. There is one other mechanism by which atropine could produce effects antagonistic to anticholinesterases. In instances

where injection of anticholinesterases causes a fall in arterial blood pressure, atropine could act by increasing cerebral blood flow through recovery of the blood pressure. The resulting reduction in cerebral tissue pCO_2 and increase in pO_2 might diminish the central stimulant action of asphyxia produced by the initial injection of the anticholinesterase. It is apparent, therefore, that the antagonistic effect of atropine does not allow us to differentiate between the various mechanisms by which anticholinesterases might increase sympathetic discharge.

Role of adrenaline

Our results strongly suggest that in addition to an increased sympathetic vasoconstrictor tone, a blood-borne sympathomimetic substance originating from the suprarenal glands, probably adrenaline or a mixture of adrenaline and noradrenaline, is responsible for the vasoconstriction. In this connexion, it is of interest to note that Stewart & Rogoff (1921) showed that, in cats under ether anaesthesia, eserine caused an increase in the output of adrenaline.

The following results provide the main evidence that a blood-borne sympathomimetic substance contributes to the vasoconstriction. In the autoperfusion experiments denervation of a limb did not completely abolish the rise in perfusion pressure. In the cross-perfusion experiments, intravenous injection of sarin in the donor animal led to increased vascular resistance in the isolated limb of the recipient and, in denervated limb preparations, this effect was abolished by intra-arterial injection of the sympatholytic drug, Priscol. But the vasoconstriction did not occur when the donor animal had been adrenalectomized. In view of the considerable increase in vascular resistance which occurred in denervated limbs, the secretion of hormones from the suprarenal medulla must play an important role in maintaining the arterial blood pressure in anticholinesterase poisoning.

The output of suprarenal hormones might be the result of an increased sympathetic discharge in the splanchnic nerves as already suggested by Stewart & Rogoff (1921) for the adrenaline secretory effect of eserine. The same mechanisms as were discussed for sympathetic vasoconstrictor discharge might then be responsible for the discharge to the suprarenals. On the other hand, the anticholinesterases might have a peripheral secretory effect on the medullary cells of the suprarenals or again arterial anoxia or a deficient circulation through the suprarenals might be the cause for the stimulation of the glands. Bülbring, Burn & de Elio (1948) have obtained an increased secretion of adrenaline from the suprarenals during arterial anoxia and when the circulation through the gland was diminished.

The question arises as to whether any other vasoconstrictor substance, such as 5-hydroxytryptamine, is liberated into the blood stream on poisoning with anticholinesterases. The results obtained in cross-perfusion experiments in

which the adrenalectomized donor was poisoned with an anticholinesterase speak against this possibility. In these experiments the anticholinesterase produced not vasoconstriction but vasodilatation. Although this result does not rule out the release of vasoconstrictor substances such as 5-hydroxytryptamine, it suggests that their contribution to the overall vasoconstriction could only be very small.

Peripheral vasodilator component

We found that injection of sarin causes vasodilatation in cross-perfusion experiments when the limb is perfused from an adrenalectomized donor. This is either due to a direct peripheral vasodilator action of the anticholinesterase, to accumulation of endogenous acetylcholine, or to asphyxia resulting from severe bronchoconstriction and depression of respiration, as the response can only have been brought about by agents carried in the blood stream. Acetylcholine is known to cause vasodilatation (Dale, 1914) as does local asphyxia (Cohnheim, 1872; Roy & Brown, 1879). Further, Paulet (1954) found that small doses of TEPP injected into the femoral artery caused vasodilatation. This may be a direct vasodilator action of TEPP but is more likely due to accumulation of acetylcholine.

Our results may have an important bearing on the treatment of anticholinesterase poisoning. In reported cases of accidental poisoning with anticholinesterases in man (Grob, Garlick & Harvey, 1950) the blood pressure usually increases and it is only just before death that it falls. If the intense vasoconstriction in both skin and muscle which we have observed in animal experiments occurs in man as well, it will result in a considerable reduction in the peripheral blood flow. In the treatment of poisoning, therefore, this possibility should be borne in mind.

SUMMARY

1. The effects of two potent inhibitors of cholinesterase, *iso*propylmethylphosphonofluoridate (sarin) and tetraethylpyrophosphate (TEPP), administered intravenously, have been investigated upon the cardiovascular system in anaesthetized dogs. Changes in peripheral vascular resistance were indicated by alterations in arterial pressure in isolated innervated organ preparations perfused at constant blood volume inflow. Perfusion was carried out either from the same animal or from a donor dog.

2. Sarin and TEPP, in doses of 25–35 μg/kg and 0·1–0·3 mg/kg respectively, invariably cause slowing of the heart, a fall in blood pressure and vasoconstriction in the limbs and splanchnic area.

3. In the limbs the vasoconstriction occurs in both skin and muscle.

4. The vasoconstriction in the limbs is due to an increase in sympathetic

vasoconstrictor tone and to liberation of hormones from the suprarenal gland. The possible causes of the increase in sympathetic tone are discussed.

5. When the vasoconstrictor mechanisms in the limb are excluded by division of the nerves to the limb and by removal of the suprarenals, a vasodilator effect of anticholinesterases is unmasked.

6. In animals poisoned with anticholinesterases atropine causes acceleration of the heart and a momentary rise in blood pressure to a level exceeding its initial value. A fall in peripheral vascular resistance also occurs provided pulmonary ventilation is adequate. If respiration remains depressed, the blood pressure falls, the heart fails and an increase in peripheral resistance occurs. These effects are probably the result of asphyxia, because they are reversed by applying artificial respiration.

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REFERENCES

ACHESON, G. H. & REMOLINA, J. (1955). The temporal course of the effects of postganglionic axotomy on the inferior mesenteric ganglion of the cat. *J. Physiol.* **127**, 603–616.

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

BÜLBRING, E. (1944). The action of adrenaline on transmission in the superior cervical ganglion. *J. Physiol.* **103**, 55–67.

BÜLBRING, E. & BURN, J. H. (1942). An action of adrenaline on transmission in sympathetic ganglia, which may play a part in shock. *J. Physiol.* **101**, 289–303.

BÜLBRING, E., BURN, J. H. & DE ELIO, F. J. (1948). The secretion of adrenaline from the perfused suprarenal gland. *J. Physiol.* **107**, 222–232.

COHNHEIM, J. (1872). *Untersuchungen über die embolischen Processe.* Berlin: Hirschwald.

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

DALE, H. H. & SCHUSTER, E. H. J. (1928). A double perfusion pump. *J. Physiol.* **64**, 356–364.

DALY, M. DE BURGH & SCHWEITZER, A. (1950). A method of recording heart-rate on the kymograph. *J. Physiol.* **111**, 50–52*P*.

DASTRE, A. & MORAT, J.-P. (1884). *Recherches expérimentales sur le système nerveux vaso-moteur.* Paris: G. Masson.

DE CANDOLE, C. A., DOUGLAS, W. W., EVANS, C. L., HOLMES, R., SPENCER, K. E. V., TORRANCE, R. W. & WILSON, K. M. (1953). The failure of respiration in death by anticholinesterase poisoning. *Brit. J. Pharmacol.* **8**, 466–475.

DIRNHUBER, P. & CULLUMBINE, H. (1955). The effect of anticholinesterase agents on the rat's blood pressure. *Brit. J. Pharmacol.* **10**, 12–15.

FELDBERG, W. & PATON, W. D. M. (1951). Release of histamine from skin and muscle in the cat by opium alkaloids and other histamine liberators. *J. Physiol.* **114**, 490–509.

FELDBERG, W. & SCHACHTER, M. (1952). Histamine release by horse serum from skin of the sensitized dog and non-sensitized cat. *J. Physiol.* **118**, 124–134.

FELDBERG, W. & VARTIAINEN, A. (1935). Further observations on the physiology and pharmacology of a sympathetic ganglion. *J. Physiol.* **83**, 103–128.

FINK, L. D. & CERVONI, P. (1953). Ganglionic blocking action of atropine and methylatropine. *J. Pharmacol.* **109**, 372–376.

GROB, D., GARLICK, W. L. & HARVEY, A. M. (1950). The toxic effects in man of the anticholinesterase insecticide Parathion (p-nitrophenyldiethylthionophosphate). *Johns Hopk. Hosp. Bull.* **87**, 106–129.

HEYMANS, C. & JACOB, J. (1947). Sur la pharmacologie du di-isopropylfluorophosphonate (DFP) et le rôle des cholinesterases. *Arch. int. Pharmacodyn.* **74**, 233–252.

HEYMANS, C., POCHET, A. & VAN HOUTTE, H. (1956). Contributions à la pharmacologie du Sarin et du Tabun. *Arch. int. Pharmacodyn.* **104**, 293–332.

HEYMANS, C. & DE VLEESCHHOUWER, G. (1948). Actions pharmacologiques de l'ester diethylaminoethylique de l'acide phenyl-cyclopentane-carboxylique (parpanit). *Arch. int. Pharmacodyn.* **75**, 307–324.

HOLADAY, D. A., KAMIJO, K. & KOELLE, G. B. (1954). Facilitation of ganglionic transmission following inhibition of cholinesterase by DFP. *J. Pharmacol.* **111**, 241–254.

HOLMSTEDT, B. (1951). Synthesis and pharmacology of dimethylamidoethoxyphosphoryl cyanide (Tabun) together with a description of some allied anticholinesterase compounds containing the N—P bond. *Acta physiol. scand.* **25**, Suppl. 90.

KAYA, R. & STARLING, E. H. (1909). Note on asphyxia in the spinal animal. *J. Physiol.* **39**, 346–353.

KOELLE, G. & GILMAN, A. (1949). Anticholinesterase drugs. *J. Pharmacol.* **95**, 166–216.

KROP, S. & KUNKEL, A. M. (1954). Observations on pharmacology of the anticholinesterases sarin and tabun. *Proc. Soc. exp. Biol., N.Y.*, **86**, 530–533.

MARRAZZI, A. S. (1939). Electrical studies on the pharmacology of autonomic synapses. I. The action of parasympathomimetic drugs on sympathetic ganglia. *J. Pharmacol.* **65**, 18–35.

MARRAZZI, A. S. & JARVIK, N. E. (1947). The differential effects on synaptic transmission and nerve conduction of di-isopropylfluorophosphate (DFP) and atropine. *Fed. Proc.* **6**, 354.

MATHISON, G. C. (1910). The action of asphyxia upon the spinal animal. *J. Physiol.* **41**, 416–448.

MATHISON, G. C. (1911). The effects of asphyxia upon medullary centres. Part I. The vasomotor centre. *J. Physiol.* **42**, 283–300.

MENDEZ, R. & RAVIN, A. (1941). On the action of prostigmine on the circulatory system. *J. Pharmacol.* **72**, 80–89.

PAULET, G. (1954). Nouvelle contribution à l'étude de l'action pharmacologique du tétraethylpyrophosphate (TEPP). *Arch. int. Pharmacodyn.* **97**, 157–185.

ROY, C. S. & BROWN, J. G. (1879). The blood pressure and its variations in the arterioles, capillaries and smaller veins. *J. Physiol.* **2**, 323–359.

SHIPLEY, R. E. & WILSON, C. (1951). An improved recording rotameter. *Proc. Soc. exp. Biol., N.Y.*, **78**, 727–728.

STEWART, G. N. & ROGOFF, J. M. (1921). The action of drugs upon the output of epinephrin from the adrenals. VII. Physostigmine. *J. Pharmacol.* **17**, 227–248.

THORP, R. H. (1948). A simple recording impulse counter. *Brit. J. Pharmacol.* **3**, 271–272.

TRAUBE, L. (1863). *Cited in* Bemerkungen zu den experimentellen Beitragen des Hrn. Landois. *Med. Zbl.* **32**, 785–790.

VARAGIĆ, V. (1955). The action of eserine on the blood pressure of the rat. *Brit. J. Pharmacol.* **10**, 349–353.

VERBEKE, R. (1949). On the pharmacology of tetraethylpyrophosphate (TEPP). *Arch. int. Pharmacodyn.* **80**, 19–27.

VERBEKE, R. & VOTAVA, Z. (1949). Contribution à la pharmacologie du hexaethyltetraphosphate (HETP). *Arch. int. Pharmacodyn.* **79**, 367–380.