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THE EFFECTS OF ANTICHOLINESTERASES UPON PULMONARY VASCULAR RESISTANCE IN THE DOG.

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In a previous study (Daly, 1957*a*) it was found that anticholinesterases caused variable changes in pulmonary vascular resistance (Δp/flow). It was not possible to establish with certainty their cause because they might have been brought about not only as a result of alterations in vasomotor tone but also passively through changes in pulmonary blood flow and in left atrial pressure (Laux, 1930; Edwards, 1951; Haddy & Campbell, 1953; Borst, McGregor, Whittenberger & Berglund, 1956). The object of the present experiments was to gain further information on the effects of anticholinesterases on pulmonary vascular resistance under conditions in which these passive mechanisms were excluded.

METHODS

Dogs, varying in weight from 6·5 to 26·3 kg, were anaesthetized with either chloralose (0·1 g/kg body weight, I.V.) preceded by morphine hydrochloride (1–2 mg/kg, subcutaneously), or pentobarbitone sodium (Nembutal, Abbott Laboratories, Ltd., 40–45 mg/kg, intraperitoneally). In a few experiments the dogs received premedication with morphine hydrochloride (3 mg/kg, subcutaneously) and about half an hour later, 0·25 ml./kg I.V. of a 1:1 mixture of Dial (Ciba Laboratories, Ltd., diallylbarbituric acid 0·1 g and urethane 0·4 g/ml.) and pentobarbitone. Two hours later another injection of morphine (1·5 mg/kg) was given.

The systemic blood pressure was measured from a 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).

Measurement of changes in pulmonary vascular resistance

Two types of experiment were performed in which part of the pulmonary vascular bed was perfused with blood at constant volume inflow.

(1) *Autoperfusion experiments.* Part of the pulmonary vascular bed, usually the whole of the left lung, was perfused by the method of I. de B. Daly & Duke (1948). The chest was opened in the third left intercostal space or by splitting the sternum in the mid line. By means of a Dale-Schuster pump blood was drawn from the right atrium or from a small reservoir connected to the

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right atrium and perfused through a cannula inserted into the distal portion of the left pulmonary artery ligated close to its origin. The whole of the right ventricular output therefore passed through the right lung. Pulmonary arterial perfusion pressure was measured from the side arm of the inflow cannula by means of a Marey tambour. A cannula was inserted into the left atrium through the auricular appendix, and the pressure measured with a vertical saline manometer, the open end of which was connected to a small volume recorder. In other experiments the left diaphragmatic lobe only was perfused, the cannula being inserted into its lobar artery.

In some of these experiments the pulmonary venous pressure was maintained constant by one of two methods. In the first the left atrial cannula was connected to a large diameter glass reservoir which was immersed in a water-bath at 37° C. The level of blood in the reservoir was adjusted initially to the same height as that registered on the saline manometer measuring left atrial pressure. Thus, a tendency towards a rise or fall in the left atrial pressure was compensated by the transference of blood between the left atrium and the reservoir. Since large and sudden changes in pressure usually occurred, it was found necessary, to ensure complete compensation, to alter the height of the reservoir in the appropriate direction while the changes in left atrial pressure were taking place. A second method was adopted in those experiments in which the left diaphragmatic lobe only was perfused. The pulmonary veins draining this lobe usually converge to form only one vein before entering the left atrium and this vein was therefore cannulated towards the lobe. Since the vein was sometimes very short, it was found more convenient to cannulate it through the appendix of the left atrium; the cannula was then tied into the vein extrapericardially. The blood from the lung lobe drained into a small reservoir and was returned to the animal at a constant rate.

In all experiments, the values for pulmonary arterial and left atrial pressures were taken with reference to the level of the mitral valve as zero.

The lungs were artificially ventilated at a constant peak inflationary pressure which varied between 8 and 14 cm water in different experiments (Konzett & Rössler, 1940). The ventilation overflow volume, that is the volume of air not entering the lungs but spilling over the constant pressure device, was measured by means of a small Krogh spirometer or from the slope of the trace produced by collecting the air continuously in a 5 l. spirometer (Daly, 1957*b*). The lungs collapsed passively during the expiratory phase of respiration against a resistance of 2–3 cm water.

(2) *Cross-perfused lungs.* Experiments were carried out in which the whole of the left lung of a dog was isolated and perfused with venous blood from a donor animal. The method of perfusing the isolated lung was similar to that described by Daly (1957*b*) except that the cannula was inserted into the left pulmonary artery instead of into the main pulmonary trunk. The right lung was tied off at the hilum.

The method used is shown diagrammatically in Fig. 1. The water-jacketed reservoir *a* received blood from a cannula inserted through the right external jugular vein into the inferior vena cava of the donor dog. This reservoir was placed a few centimetres below the level of the inferior vena cava and so was kept filled with blood by gravity. When the blood rose to a certain level in the reservoir, determined by the position of two platinum wires, an electrical circuit was completed and this actuated an electromagnetic clamp *b* on the rubber tube feeding blood from the inferior vena cava. In this way the level of the blood in the reservoir and hence the pressure on the input side of the Dale-Schuster pump *c* was maintained constant. The blood was pumped through the warming coil *d* and then into the left pulmonary artery of the isolated lung. The pulmonary arterial perfusion pressure was measured from a side arm of the inflow cannula. Blood leaving the isolated lung through a cannula in the left atrium drained into the small reservoir *e* and thence into the cannulated left external jugular vein of the donor. In this way, blood returning from the isolated lung had to pass through the lungs of the donor dog before being perfused through the isolated lung again from the inferior vena cava. The height of the end of the outflow tube from the left atrium was maintained constant 2–5 cm above the level of the mitral valves. The volume of blood in reservoir *a*, pump and connecting tubes to the left pulmonary artery was about 120 ml.

Ventilation of the isolated lung was carried out artificially at a constant peak inflationary

pressure as described above. In other experiments ventilation was by means of an intermittent negative pressure applied to the outside of the lungs. The trachea cannula was connected to a conventional closed circuit respiratory system and tidal air volume was measured with a small spirometer. The carbon dioxide was absorbed in two soda-lime towers, and the flow of oxygen into the system was controlled by an adjustable needle valve, thus allowing the limiting lines of the tidal air tracing to be set horizontal before poisoning with an anticholinesterase. Changes in the slope of the tidal air tracing therefore indicate alterations in oxygen uptake by the lungs provided their functional residual capacity remains unchanged.

The donor animal breathed spontaneously, and its tidal air volume was measured using a similar closed circuit respiratory system. When necessary, artificial respiration was applied by means of a Starling 'Ideal' pump.

In some autoperfusion and cross-perfusion experiments, haematocrit estimations were performed on blood samples taken from the cannula in the left pulmonary artery. The blood was centrifuged in Wintrobe tubes at 3000 rev/min for 30 min.

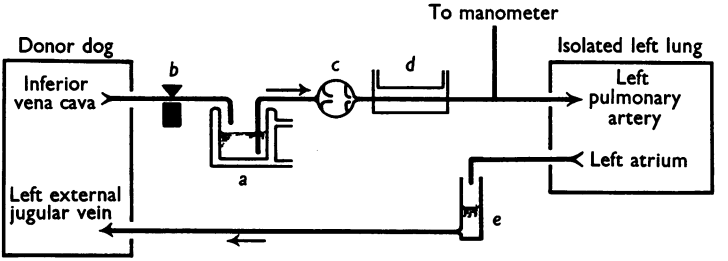

Fig. 1. Diagram showing the method of cross-perfusion of the isolated left lung with venous blood from the inferior vena cava of a donor dog. *a*, water-jacketed blood reservoir; *b*, electromagnetic clamp; *c*, Dale-Schuster pump; *d*, warming coil; *e*, reservoir. Water at 37° C was passed through the jacket of reservoir *a* and around the coils of *d*. For further details see text.

Calculation of changes in pulmonary vascular resistance. When the blood flow remains constant, the pressure gradient across the vascular bed, i.e. mean pulmonary arterial perfusion pressure minus mean left atrial pressure (mm Hg), varies with the vascular resistance. Since the blood flow was not measured in every experiment, the change in vascular resistance was expressed in terms of a percentage change of the initial pressure gradient across the lungs.

Pressure gradient–flow relationship of the pulmonary vascular bed

To establish more fully the mechanisms concerned in the changes in pulmonary vascular resistance produced by anticholinesterases, it was necessary to determine the pressure gradient–blood flow relationship of the pulmonary vascular bed at different levels of left atrial pressure. This was done in two experiments in which the left lung was isolated and perfused with heparinized blood. The pulmonary arterial perfusion pressure and flow were varied by altering the height of a small reservoir which was connected to the pulmonary arterial cannula. This reservoir was supplied with blood from a larger reservoir by means of a Dale-Schuster pump and an overflow device maintained the level in it constant. The pressure was, therefore, non-pulsatile. Blood from the left atrium was returned to the large reservoir. The left atrial pressure was varied by altering the height of the end of the outflow tube in relation to the left atrium. With each change of pressure, 1–2 min was allowed for the pressures and flow to stabilize. Left atrial blood flow was measured with a graduated cylinder and stopwatch.

In all experiments the blood was rendered incoagulable with heparin (Liquemin, Roche Products, Ltd., 7–8 mg/kg). Half this dose was repeated every 30 min. *iso*Propylmethylphos-

phonofluoridate (sarin) in a 1:1000 aqueous solution, and tetraethylpyrophosphate (TEPP), 1% aqueous solution, were given into a femoral vein. Other drugs used were: atropine sulphate (British Drug Houses), hexamethonium bromide (Vegolysen, May and Baker, Ltd.), adrenaline chloride solution, 0·1% with 0·5% chloretone (Parke, Davis and Co.) and L-noradrenaline (Levophed, Bayer Products, Ltd.).

Special precautions

In experiments involving autoperfusion of the whole or part of the left lung it was necessary to take certain precautions to exclude mechanical effects as causes of the changes in pulmonary arterial perfusion pressure produced by anticholinesterases.

First, anticholinesterases often caused muscular twitching and sometimes generalized convulsions, particularly under chloralose anaesthesia. It was necessary to make certain, therefore, that the pulmonary arterial cannula did not become displaced and so kink the left pulmonary artery. Lateral movement of the thoracic cage was prevented by firmly clamping either the two ribs exposed by the thoracotomy or alternatively the cut edges of the sternum. The pulmonary arterial cannula was also supported by a clamp. Secondly, anticholinesterases invariably cause a considerable increase in right atrial pressure (Holmstedt, 1951; Daly & Wright, 1956). If the input side of the pump perfusing the lung lobes is connected directly to the right atrium, the resulting rise in inflow pressure to the pump might increase its output and so lead to a spurious interpretation of events taking place in the pulmonary circulation. Repeated tests showed, however, that no change in output of the pump occurred when the input pressure was varied over the range of pressures found in the right atrium in these experiments. In half the experiments the technique was modified slightly so as to obviate this rise in pressure on the input side of the pump by allowing it to draw its blood from a small reservoir connected by rubber tube to the right atrium. The method used was similar to that described above for cross-perfusion experiments. Thirdly, in experiments in which changes in heart volume were measured by means of a Henderson (1906) type of cardiometer, sarin, in doses similar to those used in the present investigation, invariably caused an increase in diastolic volume of the heart (M. de B. Daly & P. G. Wright, unpublished). It is conceivable, therefore, that in experiments involving autoperfusion of the left lung changes in pulmonary arterial perfusion pressure might result from compression of the lung through an increase in weight of the heart. In some experiments this was prevented by opening the thorax widely and taking the weight of the heart off the perfused lung with the aid of threads tied to the intact pericardium. To minimize the mechanical effects of movements of the diaphragm, both phrenic nerves were crushed. Final proof that the observed changes in pulmonary arterial perfusion pressure occurring in autoperfusion experiments were not the result of extrinsic mechanical effects was obtained in the cross-perfused isolated lung preparations.

RESULTS

The effect of anticholinesterases on tidal air volume

In all experiments, sarin, in doses of 25–40 μg/kg, or TEPP, 0·1–0·3 mg/kg, was injected into a femoral vein. These anticholinesterases invariably caused a diminution in tidal air volume as indicated by an increase in ventilation overflow volume. Occasionally there was a temporary recovery of the tidal air volume about 3½ min after poisoning. These effects are similar to those described by Daly (1957*a*) and are illustrated by Fig. 2.

The percentage diminution in tidal air volume occurring in cross-perfused lung preparations was nearly always much smaller than that occurring in autoperfusion experiments. This is illustrated by Fig. 5, which shows the effect of 30 μg/kg sarin on the tidal air volume measured directly.

Atropine, in doses of 0·2–0·4 mg/kg intravenously, almost invariably restored the tidal air volume to normal. Such an effect is illustrated by Figs. 2 and 5.

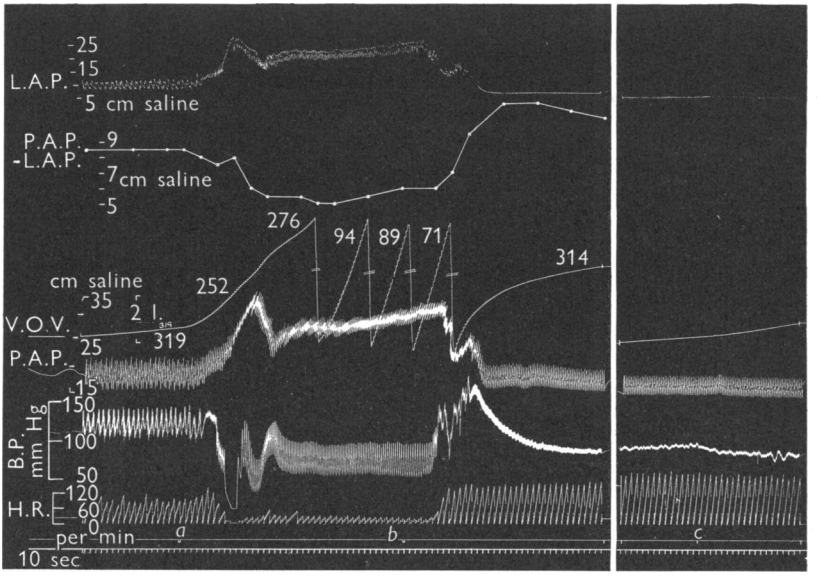

Fig. 2. Dog, ♂, 14·4 kg. Morphine-chloralose. Autoperfusion of the left lung. Constant peak inflationary pressure respiration. Respiratory pressure, 11·5 cm H_2O. Respiration pump stroke volume, 340 ml. Values for the pressure gradient across the lungs (mean pulmonary arterial pressure minus mean left atrial pressure) have been plotted separately. The figures on the ventilation overflow volume record are those for the calculated tidal air volume (in ml.). Time marker, 10 sec. At *a*, TEPP, 0·15 mg/kg. At *b*, atropine, 2 mg. In *c*, TEPP, 10 mg/kg. Both drugs were given intravenously. In this and in subsequent figures: T.A. = tidal air volume; V.O.V. = ventilation overflow volume; L.A.P. = left atrial pressure; P.A.P. = pulmonary arterial perfusion pressure; B.P. = systemic blood pressure; H.R. = heart rate.

Effects of sarin and TEPP on pulmonary vascular resistance

Autoperfusion experiments

The results of four experiments in which the whole of the left lung was perfused at constant blood volume inflow are summarized in Table 1*A*. Both sarin and TEPP caused slowing of the heart, a fall in systemic blood pressure and, in all instances, a considerable rise in pulmonary arterial perfusion pressure. The response occurring in one experiment is shown in Fig. 2*a*. It had been found previously (Daly, 1957*a*) that anticholinesterases cause a considerable rise in left atrial pressure. In the present experiments the pressure in the left atrium invariably showed a larger rise than that in the left pulmonary artery, so the pressure gradient across the perfused lung, the mean pulmonary arterial perfusion pressure minus the mean left atrial pressure, diminished. This is illustrated by Fig. 2 in which the pressure gradient across the pulmonary

vascular bed of the perfused lung has been plotted separately. Since the blood flow through the lung remained constant, the pulmonary vascular resistance decreased. The average reduction in vascular resistance in the four experiments was 30·1% (range 25–37·5%) (Table 1*A*).

Two observations suggested that the increase in left atrial pressure was, at least in part, responsible for the rise in pulmonary arterial perfusion pressure and also for the fall in vascular resistance by passive distension of the vascular

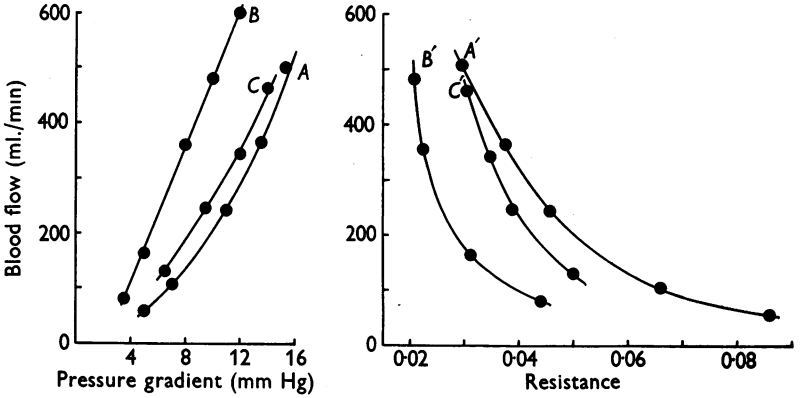

Fig. 3. Dog, ♂, 15·6 kg. Isolated left lung perfused through the pulmonary artery with its own heparinized blood. No ventilation. The effect of raising the left atrial pressure on the pressure gradient–blood flow relationship. (*Left*) The relation between pulmonary artery blood flow and pressure gradient across the pulmonary blood vessels (pulmonary arterial pressure minus left atrial pressure). Curve *A* was obtained with the left atrial pressure maintained constant at 2·5 mm Hg, curve *B* at 11 mm Hg and curve *C* at 2·5 mm Hg, in that order. (*Right*) The relation between pulmonary arterial blood flow and pulmonary vascular resistance $\frac{\Delta P \text{ (mm Hg)}}{\text{Flow (ml./mm)}}$. The data used to construct curves *A′*, *B′* and *C′* were the same as for curves *A*, *B* and *C* respectively.

bed. First, the general trend of the changes in pulmonary arterial perfusion pressure closely followed those of the left atrial pressure. This can be seen in the experiment illustrated by Fig. 2. Secondly, evidence obtained from experiments on isolated perfused lungs may be cited. In these, the relationship between the pressure gradient across the pulmonary vascular bed and blood flow was determined at various levels of left atrial pressure. The results of the typical experiment are shown in Fig. 3. Control curve *A* was obtained with the left atrial pressure 2·5 mm Hg and shows a convexity towards the pressure axis, confirming the results of Laux (1930), Edwards (1951), Hall (1953), Williams (1954) and of Borst *et al.* (1956). Curve *B* was obtained with the left atrial pressure maintained constant at a higher level (11 mm Hg). The left atrial pressure was then lowered to 2·5 mm Hg and curve *C* was obtained. It will be noted that curve *B* falls to the left of control curves *A* and *C*, indicating that at elevated levels of left atrial pressure the pulmonary

TABLE 1. Autoperfusion experiments. The effect of intravenous sarin and TEPP upon the pulmonary arterial perfusion pressure in preparations in which part of the pulmonary vascular bed was perfused with venous blood at constant volume inflow. The left atrial or pulmonary venous pressure was uncompensated in *A*, but compensated in *B*

Expt. no.	Drug	Systemic B.P. (mm Hg)		Pulm. art. perfusion pressure (mm Hg)		Left atrial pressure (mm Hg)		Pulm. art. perfusion pressure minus left atrial pressure (mm Hg)		Pulmonary vascular resistance (as % change of pressure gradient)
		Before	After	Before	After	Before	After	Before	After	
A										
1	Sarin	60	60	13·5	16·5	3	9	10·5	7·5	−28·5
2	Sarin	105	55	14	25	2	17·5	12	7·5	−37·5
3	TEPP	120	145	12	21·5	3·5	15·5	8·5	6	−29·5
4	TEPP	125	77	13·5	22	7·5	17·5	6	4·5	−25
	Mean	102	84	13·25	21·25	4·0	14·9	9·25	6·4	−30·1
B										
5	Sarin	115	45	15·5	23·5	0	0	15·5	23·5	+51·5*
6	Sarin	110	60	14·5	16	0	0	14·5	16	+10·5*
7	Sarin	105	55	15	18·5	4·5	4·5	10·5	14	+33·5†
8	TEPP	158	120	16	19	6·5	6·5	9·5	12·5	+32†
	Mean	122	70	15·3	19·3	2·8	2·8	12·5	16·5	+31·9
9	TEPP	102	65	14·5	17	3·5	6	11	11	0‡

* Pulmonary venous pressure constant. † Left atrial pressure compensated. ‡ Left atrial pressure partly compensated.

vascular resistance decreases. In Fig. 3 the relationship between blood flow and vascular resistance is also shown. Curve B', obtained with a raised left atrial pressure, is shifted to the left of the control curves A' and C', so that at any given blood flow the pulmonary vascular resistance is diminished by an increased left atrial pressure. The data presented by Edwards (1951) and by Williams (1954) showed that, with normal left atrial pressure, the pulmonary vascular resistance increased with decreasing flow rates and pressures. This is also true of lungs in which the left atrial pressure is maintained at a higher level than normal (Fig. 3) as found by Borst *et al.* (1956).

The results indicate, therefore, that in anticholinesterase poisoning the observed decrease in pulmonary vascular resistance occurring in autoperfusion experiments can be attributed to passive distension of the pulmonary blood vessels through the rise in left atrial pressure. To what extent changes in calibre of the vessels through active vasomotor effects contribute to this response cannot be assessed from these experiments. This question can only be resolved when the conditions are such that the pulmonary blood flow is maintained constant and, in addition, the left atrial or pulmonary venous pressure is compensated.

Five such experiments were performed and the results are summarized in Table 1 *B*. In all these experiments, sarin or TEPP caused a rise in perfusion pressure. The average rise in pressure was approximately half that occurring in autoperfusion experiments in which the pulmonary venous pressure was not compensated. This suggests that in the latter experiments, the rise in pulmonary arterial perfusion pressure is due, in part, to back pressure from the increase in left atrial pressure. Since in four of the five experiments (nos. 5–8) shown in Table 1 *B* there was no change in left atrial or pulmonary venous pressure, the rise in pulmonary arterial perfusion pressure must be the result of an increase in pulmonary vascular resistance. The average increase in the four experiments was 31·9% (range 10·5–51·5%). In the fifth experiment, no. 9 of Table 1 *B*, the left atrial pressure was not fully compensated, and this experiment was not included therefore in the series from which mean values for changes in pulmonary vascular resistance were calculated. The response occurring in one of these experiments is shown in Fig. 4. It may be noted in this experiment that, as occurred in the others, there was a delay in the onset of the rise in perfusion pressure and then it was a gradual one reaching a maximum about 3 min after injection of sarin.

Cross-perfusion experiments

The results of three experiments in which the left lung was isolated and perfused with venous blood from the inferior vena cava of a donor dog are summarized in Table 2 (Expts. nos. 10–12). Sarin or TEPP was injected into a femoral vein of the donor dog and a rise in pulmonary arterial perfusion

pressure, indicating an increase in vascular resistance, occurred in all three experiments (Fig. 5*a*). In one of these experiments (no. 12 of Table 2), the donor animal was bilaterally adrenalectomized so that the rise in pulmonary arterial perfusion pressure cannot be the result of the secretion of hormones from the suprarenal medulla (Stewart & Rogoff, 1921; Daly & Wright, 1956).

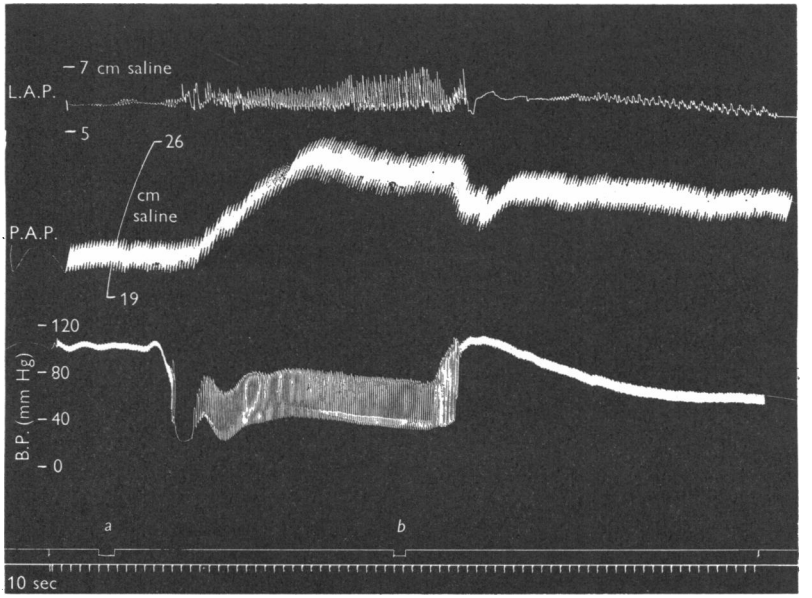

Fig. 4. Dog, ♂, 11 kg. Nembutal; autoperfusion of the left lung; constant peak inflationary pressure ventilation; left atrial pressure maintained constant with a compensator. Time marker, 10 sec. At *a*, sarin, 30 μg/kg intravenously. At *b*, atropine, 5 mg intravenously.

At the height of the pulmonary vascular response occurring in one of the experiments, hexamethonium (100 mg) was injected into the pulmonary arterial inflow tubing. This had no effect on the pulmonary arterial perfusion pressure, suggesting that the pressure rise was not due to an action of the anticholinesterase on intrapulmonary ganglia.

The observed increases in pulmonary arterial perfusion pressure were found to occur independently of changes in the haematocrit value of the pulmonary arterial blood. In a previous study it was shown that anticholinesterases caused an increase in the systemic arterial haematocrit value through emptying of the blood stores of the spleen (Daly, 1957*a*). In some of the present experiments blood samples drawn from the left pulmonary arterial cannula showed a similar increase in haematocrit value, but the time of onset of this effect and of the rise in pulmonary arterial perfusion pressure were different. Furthermore, in one experiment a rise in perfusion pressure occurred without change in the haematocrit value of the pulmonary arterial blood.

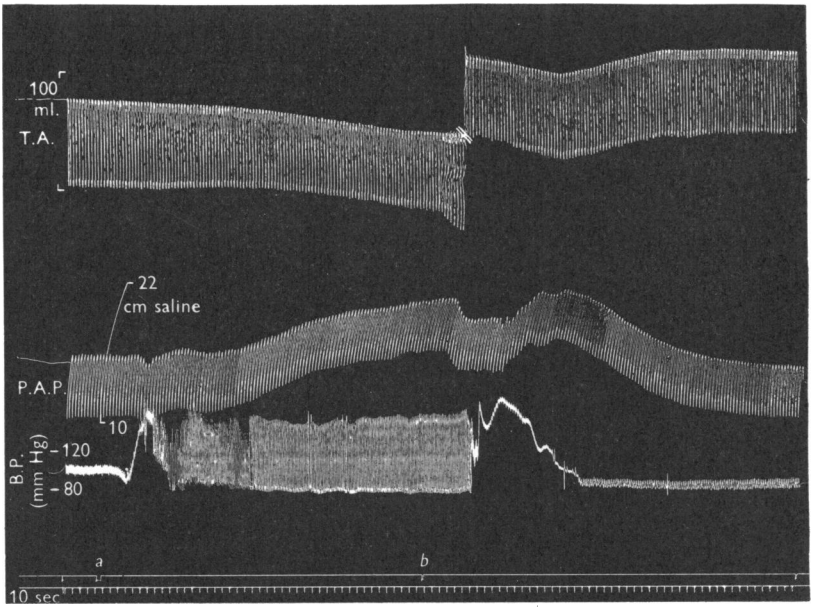

Fig. 5. Cross-perfused isolated lung preparation; recipient dog, ♂, 8·5 kg; negative pressure ventilation; left atrial pressure maintained constant. Donor dog (♂, 13·0 kg) was anaesthetized with Nembutal. Records from above downwards; tidal air volume (inspiration downwards) and pulmonary arterial pressure of the perfused lung, and systemic blood pressure of the donor dog. Time marker, 10 sec. At *a*, sarin, 30 μg/kg body weight of donor dog injected intravenously. At *b*, atropine, 5 mg, was injected into the input side of the pump and therefore passed through the perfused lung before reaching the donor animal.

TABLE 2. Cross-perfusion experiments. Changes in pulmonary vascular resistance and tidal air volume in the isolated left lung preparation perfused at constant volume inflow with venous blood from the inferior vena cava of a donor dog. Artificial respiration was carried out at either a constant positive or negative peak inflationary pressure. The left atrial pressure remained constant. Sarin or TEPP was injected via a femoral vein of the donor animal

Expt. no.	Drug	Tidal air volume (ml.)		Pulm. art. perfusion pressure (mm Hg)		Pulm. vascular resistance (as % change of pressure gradient)	Remarks
		Before	After	Before	After		
10	Sarin	95	60	12	13·5	+12·5	—
11	TEPP	146	142	17·5	20·0	+14·3	—
12	Sarin	76	64	10·5	13·5	+28·6	Donor adrenalectomized
13	TEPP	88	88	16·0	25·0	+56	Donor adrenalectomized and atropinized

Effect of atropine on pulmonary vascular resistance

The effect of atropine, in doses of 0·2–0·5 mg/kg intravenously, was tested after poisoning with an anticholinesterase. In autoperfusion experiments carried out without compensating the left atrial pressure, atropine caused an immediate fall in pulmonary arterial perfusion pressure and in left atrial pressure. The typical effect is shown in Fig. 2*b*. The pulmonary vascular resistance which was decreased by TEPP was now restored almost to its original value.

In autoperfusion experiments in which the pulmonary venous pressure of the perfused lobes was maintained constant, and also in cross-perfused isolated lung preparations, atropine injected after the anticholinesterase had a slightly different effect; it caused a fall in perfusion pressure in two stages. The first fall occurred almost immediately on injection of atropine and was usually smaller than the second fall which took several minutes to reach its final level. Examples of this type of response are shown in Figs. 4*b* and 5*b*. The delayed fall in pulmonary arterial perfusion pressure may have been the result of failure of some of the vascular channels to dilate again for mechanical reasons rather than persistence of some stimulus to the smooth muscle of the walls of the blood vessels. This possibility seems unlikely, however, because if after injecting atropine the vascular bed was distended by momentarily increasing the output of the perfusion pump, there still occurred this characteristic slow fall in perfusion pressure.

It is suggested that in the cross-perfused isolated lung preparation the fall in pulmonary arterial perfusion pressure on injection of atropine is the result of two different mechanisms: one of these is by antagonizing the action of the anticholinesterase on the pulmonary blood vessels (Daly, 1957*b*); the other is an effect resulting from improvement of pulmonary ventilation in the donor animal. The evidence for this is as follows. If after poisoning with an anticholinesterase atropine was injected into the input side of the perfusion pump, so as to reach the isolated lung before the donor animal, an immediate small fall in perfusion pressure occurred. Such an effect is shown in Fig. 5*b*, although in this case the pressure recovered again. It may be noted, however, that the initial pulmonary vascular effect of atropine took place 40 sec before there was any effect on the donor animal as indicated by the change in heart rate and systemic blood pressure. We interpret this initial fall in pulmonary arterial perfusion pressure as being due to abolition of the vasoconstrictor effect of the anticholinesterase on the pulmonary blood vessels.

The secondary more gradual fall in perfusion pressure only occurred when the donor dog's respiratory minute volume, which was reduced by injection of the anticholinesterase, was restored to its original value by atropine. If respiratory depression persisted after atropine, the pulmonary arterial

perfusion pressure remained elevated. But if artificial respiration was now applied to the donor dog the pressure was restored to its original level. These results suggest that in these preparations the increase in pulmonary vascular resistance caused by anticholinesterases is, in part, the result of asphyxia of the donor dog.

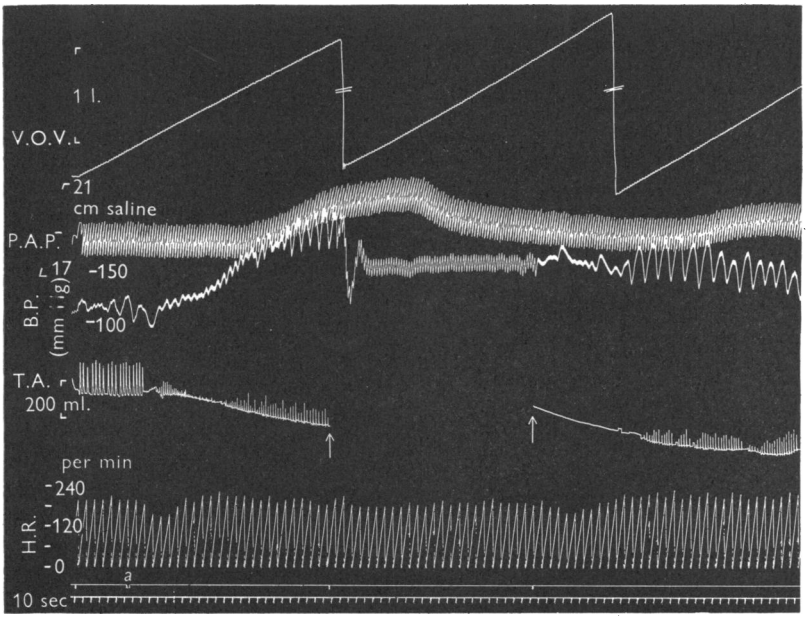

Fig. 6. Cross-perfused isolated lung preparation. Recipient dog, ♂, 11·4 kg; constant peak inflationary pressure ventilation; initial tidal air volume, 56 ml. Donor dog, ♂, 15·0 kg; morphine-Dial-Nembutal; spontaneous respiration. Records from above downwards: ventilation overflow volume and pulmonary arterial perfusion pressure of the isolated left lung, and systemic blood pressure, tidal air volume (inspiration upwards) and heart rate of the donor dog. Atropine, 5 mg. Time marker, 10 sec. TEPP, 10 mg/kg body weight of donor dog, was injected intravenously at *a*. Artificial respiration (constant volume) was applied to the donor dog between arrows ↑ ↑.

Further evidence in support of this view was obtained by injecting large doses of sarin or TEPP into the atropinized cross-perfused isolated lung preparation. In these atropinized preparations the direct effect of anticholinesterases on the pulmonary blood vessels was eliminated (Daly, 1957*b*). It was found that there was no effect on the pulmonary arterial perfusion pressure until respiration of the donor dog became depressed, and then a rise in pressure occurred. Such a response was obtained in the experiment illustrated by Fig. 6, in which 10 mg/kg TEPP was injected into the atropinized preparation. Application of artificial respiration to the donor dog 4 min after injection of TEPP restored the pressure almost to its original level. When artificial

respiration was stopped, spontaneous respiratory efforts were still absent and the pulmonary arterial perfusion pressure gradually increased again. It may be noted in this experiment that there was a delay of about 1½ min after applying artificial respiration before the pulmonary arterial perfusion pressure began to fall. This was probably due in part to the dead space in the reservoir, pump and connecting tubes. These changes in pulmonary vascular resistance occurred without any alteration in tidal air volume as indicated by the ventilation overflow volume (Fig. 6).

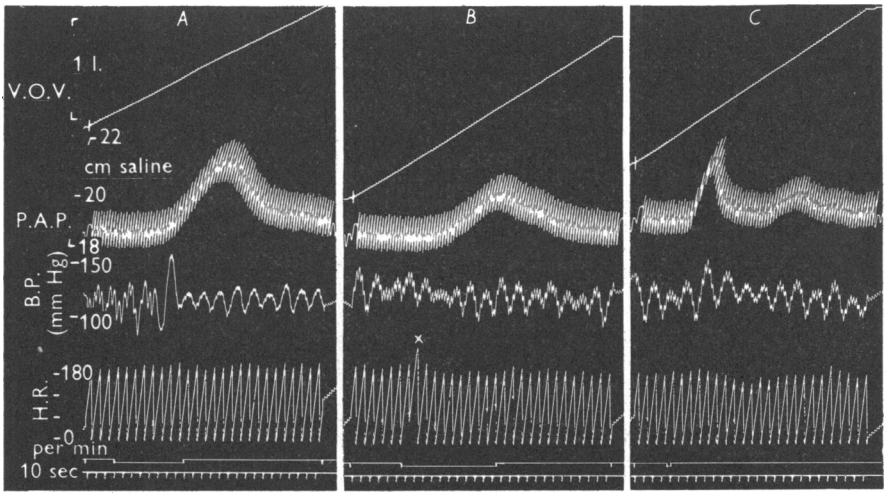

Fig. 7. Same experiment as in Fig. 6; initial tidal air volume of isolated left lung, 57 ml. Donor dog artificially ventilated at constant volume. Atropine, 5 mg. Time marker, 10 sec. The ventilating gas of the isolated left lung was changed temporarily from air to 5% O_2 in N_2 in *A*, and from air to 10% CO_2, 21% O_2 in N_2 in *B*. In *C*, adrenaline, 3 μg, was injected into the pulmonary arterial inflow tube to the isolated lung. ×, artifact.

Asphyxia of the donor dog occurring as a result of injection of an anticholinesterase could only produce its effect on the isolated lung by blood-borne agents. These are probably changes in pO_2 and pCO_2 of the venous blood perfusing the lung and increased secretions from the suprarenal medulla. In this connexion the increase in pulmonary vascular resistance of 56% occurring in Expt. no. 13 (Table 2), in which the donor dog was bilaterally adrenalectomized, cannot have been due to increased secretions of suprarenal medullary hormones.

In view of the importance of asphyxia as a cause of the increase in pulmonary vascular resistance in anticholinesterase poisoning, tests were made of the effects of altering the alveolar gas tensions and of adrenaline on pulmonary vascular resistance.

Effects of changes in alveolar gas tensions and of adrenaline

In two of three atropinized cross-perfused lung preparations, changing the ventilating gas of the isolated lung from air to 5% O_2 in N_2, or to 10% CO_2, 21% O_2 in N_2, or to 5% O_2, 10% CO_2 in N_2 resulted in a rise in pulmonary arterial perfusion pressure. The effects occurring in one of these experiments are shown in Fig. 7 *A* and *B*. In one experiment these gas mixtures had no effect.

In two similar preparations the effects of altering the oxygen and carbon dioxide contents of the inspired air of the *donor dog* were observed while the isolated lung was ventilated with air. It was found that changing the inspired gas from air to 5% O_2 in N_2 or to 10% CO_2, 21% O_2 in N_2 caused pulmonary vasoconstrictor responses in the isolated lung. A similar response occurred in another experiment on increasing the respiratory dead space of the donor dog which was bilaterally adrenalectomized (Fig. 8). In these tests there was no change in tidal air volume of the perfused left lung.

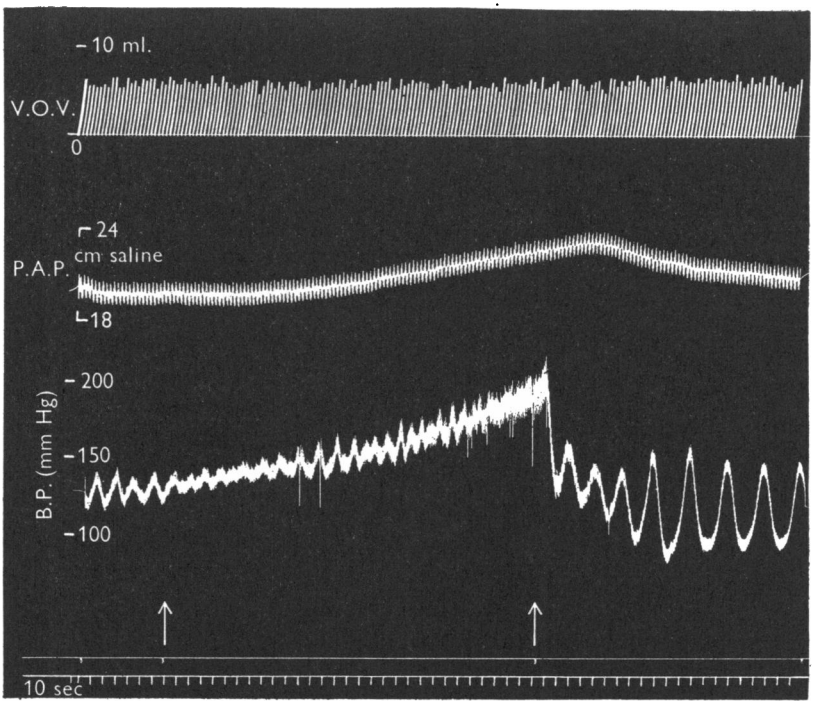

Fig. 8. Cross-perfused isolated lung preparation. Recipient dog, ♂, 19·3 kg; constant peak inflationary pressure ventilation (air); respiratory pump stroke volume, 100 ml. Donor dog, ♂, 20·6 kg; morphine-Dial-Nembutal; spontaneous respiration; bilaterally adrenalectomized; atropine 5 mg. Records from above downwards: ventilation overflow volume and pulmonary arterial perfusion pressure of the isolated perfused left lung, and systemic blood pressure of the donor dog. Time marker, 10 sec. Between arrows ↑ ↑, the donor dog was asphyxiated by increasing the respiratory dead space.

In confirmation of other workers (see I. de B. Daly, 1933; also Konzett & Hebb, 1949), adrenaline and noradrenaline injected into the pulmonary arterial inflow tubing caused pulmonary vasoconstrictor responses. The effect of an injection of 3 μg adrenaline is shown in Fig. 7*C*.

DISCUSSION

Our results have shown that in preparations in which part of the pulmonary bed is perfused at constant blood volume inflow, anticholinesterases cause an increase in pulmonary arterial perfusion pressure, indicating an increase in pulmonary arterial inflow resistance. This confirms the results of Daly (1957*a*) who showed in the entire animal that the pulmonary arterial pressure increased despite a considerable reduction in pulmonary blood flow. The present experiments show that this increased inflow resistance is the result of two effects: a back pressure effect of the increase in left atrial pressure, and an increase in pulmonary vascular resistance. This latter effect can only be demonstrated when the passive effect of the increased left atrial pressure is excluded. In this connexion Dirken & Heemstra (1948) observed that administration of an eserine aerosol to the right lung of the spontaneously breathing rabbit resulted in a reduction in the oxygen uptake by this lung relative to the total uptake by both lungs. They interpreted this as due to weak pulmonary vasoconstriction. Administration of an acetylcholine-eserine aerosol in the same way caused a pronounced vasoconstriction together with a diminution in tidal air volume of the lung. These effects were abolished by the addition of atropine to the aerosol.

Mechanism of increase in pulmonary vascular resistance

In the entire animal, changes in pulmonary vascular resistance (Δp/flow) may occur passively as a result of alterations in pulmonary blood flow, respiration and left atrial pressure. In some autoperfusion experiments and in all cross-perfused isolated lung preparations these passive mechanisms were excluded by controlled perfusion of part of the pulmonary vascular bed, by artificial ventilation of the lungs and by maintaining the left atrial or pulmonary venous pressure constant. In these preparations, therefore, the increase in pulmonary arterial perfusion pressure occurring on injection of an anticholinesterase must have been the result of an increase in pulmonary vascular resistance.

As will become evident from the discussion below, there are several mechanisms by which anticholinesterases may cause an increase in pulmonary vascular resistance. We have been unable to distinguish many of these because other variables could not be conveniently controlled in the present experiments. Neverthess, by a process of exclusion it has been possible to obtain suggestive evidence as to one definite cause for the increase in pulmonary

vascular resistance occurring in the cross-perfused lung preparation which was originally designed to exclude mechanical effects on the lungs (see Methods). We are, however, unable to express a definite opinion as to the part played by other mechanisms which were excluded in these experiments.

Mechanisms affecting pulmonary vascular resistance have been reviewed recently by I. de B. Daly (1956) and may be classified as passive, nervous, humoral and chemical. The possible contribution of each of these to the increase in pulmonary vascular resistance produced by anticholinesterases is summarized in Table 3 for the two types of preparation used in the present investigation. For comparison, information obtained from the isolated lung preparation perfused with its own blood (Daly, 1957*b*) has been included in Table 3.

TABLE 3. Summary of the possible mechanisms contributing to the increase in pulmonary vascular resistance occurring in response to injection of sarin or TEPP in the autoperfused lung preparation, the cross-perfused lung preparation, and in the isolated lung perfused with its own blood (Daly, 1957*b*). In all preparations the lungs were perfused at constant blood volume inflow and the left atrial pressure was maintained constant. For details, see text

	Mechanism	Auto-perfused lung preparation	Cross-perfused isolated lung preparation		Isolated perfused lung preparation
			Non-atro-prinized	Atro-pinized	
Passive	Bronchomotor	?+	?+	0	?+
	Bronchial circulation:				
	Changes in bronchial arterial pressure	+	0	0	0
	Changes in bronchial vasomotor tone	?	0	0	0
Nervous	Central and/or reflex	?	0	0	0
	Ganglionic	?	0	0	0
Humoral	Anticholinesterase Accumulation of acetylcholine	+	+	0	+
	Suprarenal medullary hormones	?	? or 0*	? or 0*	0
	Other hormones	?	?	?	0
Chemical	Changes in alveolar pO_2 and pCO_2	+	+	+	0

+ = Contributes to increase in pulmonary vascular resistance.
0 = No effect.
* Donor dog adrenalectomized.

Passive mechanisms. Passive increases in pulmonary vascular resistance may be brought about through an increase in intrapulmonary pressure by bronchoconstriction. Scrutiny of our records showed, however, that the time course of the changes in the tidal air volume and pulmonary arterial perfusion pressure were often different both on injection of an anticholinesterase and subsequently on injection of atropine, and this suggests that the increase in pulmonary vascular resistance is, at least in part, independent of bronchomotor phenomena. In atropinized cross-perfused lung preparations the increase in pulmonary vascular resistance occurred without any change in bronchomotor tone.

Another passive mechanism affecting pulmonary vascular resistance is an alteration in the volume of blood traversing the communicating channels between the bronchial and pulmonary vascular systems (Berry & I. de B. Daly, 1931). This could result from changes in systemic (bronchial) arterial pressure or from alterations in bronchial arteriolar tone and contribute to the observed changes in pulmonary vascular resistance in autoperfusion experiments. But in cross-perfused isolated lung preparations such a passive mechanism can be excluded because the bronchial circulation was not perfused. Thus, in atropinized preparations of this type the observed increase in pulmonary arterial perfusion pressure occurring in the absence of changes in bronchomotor tone must be attributed to active constriction of the pulmonary vascular bed proper.

Nervous effects. We are unable to say whether nervous influences on lung blood vessels contribute to the increase in pulmonary vascular resistance occurring on injection of sarin or TEPP. The upper thoracic sympathetic outflow contains pulmonary vasoconstrictor fibres (I. de B. Daly, Duke, Hebb & Weatherall, 1948) and such an effect would therefore be expected if these fibres were activated in anticholinesterase poisoning as are vasoconstrictor fibres to the peripheral vascular bed (Daly & Wright, 1956). In three atropinized autoperfusion experiments the functional activity of the sympathetic innervation to the lungs was tested by electrical stimulation of the left upper thoracic sympathetic chain, and this resulted in an increase in pulmonary vascular resistance in two of them;* there was no effect in the third experiment. In cross-perfused isolated lung preparations an increase in sympathetic discharge arising reflexly or centrally cannot be responsible for the pulmonary vasopressor effect of injected anticholinesterase. It is also unlikely that in this type of preparation the response is the result of an action of the anticholinesterases on intrapulmonary ganglia because it was not influenced by hexamethonium.

Humoral effects. It has been shown that in the isolated dog lung preparation perfused with its own blood, anticholinesterases cause an increase in pulmonary vascular resistance (Alcock, Berry & I. de B. Daly, 1935; Daly, 1957*b*), either by a direct action on the pulmonary blood vessels or through accumulation of endogenous acetylcholine. This effect is abolished by atropine.

Release of hormones from the suprarenal medulla by anticholinesterases has been described by Stewart & Rogoff (1921) and by Daly & Wright (1956) and may contribute to the pulmonary vascular response observed in the present

* In a paper by Lee, Matthews & Sharpey-Schafer (1954), reference is made on p. 311 to unpublished data of M. de B. Daly (1954) to the effect that pulmonary arterial constriction by electrical stimulation of the stellate ganglion and of the cervical and thoracic vago-sympathetic system is small in the intact animal. One of us (M. de B. D.) wishes to state that this reference to his unpublished work is incorrect and that it was made by Lee *et al.* without his authority.

experiments. In cross-perfused lung preparations, however, bilateral adrenalectomy of the donor animal did not prevent a rise in pulmonary arterial perfusion pressure on injection of sarin or TEPP. The possibility that anticholinesterases release hormones from other parts of the body which contribute to the pulmonary vascular response cannot be excluded, but in this connexion the evidence presented by Daly & Wright (1956) indicated that, at least in the peripheral circulation, the effects of such vasoconstrictor substances were either absent or very small.

Chemical changes. As a result of a diminution in pulmonary ventilation, anticholinesterases cause in the entire animal a fall in arterial oxygen content, a rise in arterial carbon dioxide content (Holmstedt, 1951), a decrease in alveolar pO_2 and an increase in alveolar pCO_2 (M. de B. Daly, unpublished). A similar change in alveolar gas tensions probably takes place in the isolated lung cross-perfused with venous blood because of the diminution in its alveolar ventilation resulting from the reduction in tidal air volume. These changes in alveolar gas tension, therefore, may be a cause of the observed increase in pulmonary vascular resistance. The literature on this subject has been reviewed recently by Lilienthal & Riley (1954) and by Rahn (1955). In this connexion, we have shown, in confirmation of others (see Lilienthal & Riley, 1954; Rahn, 1955), that increasing the carbon dioxide content of the inspired air causes pulmonary vasoconstriction. With regard to anoxia, Hall (1953) and Duke (1957) found in isolated perfused dog lung experiments that lowering the oxygen content of the inspired air caused pulmonary vasoconstriction. On the other hand, others have concluded that the local effect of anoxia is vasodilator (Aviado, Cerletti, Alanis, Bulle & Schmidt, 1952; Aviado, Ling, Quimby & Schmidt, 1954). In the present experiments, we had an opportunity of investigating the effect of anoxia in the cross-perfused isolated lung preparation. It was found that changing the ventilating gas of the perfused lung from air to 5% O_2 in N_2 invariably caused an increase in pulmonary vascular resistance. This response occurred in the absence of changes in tidal air volume and must be attributed to a direct action on the pulmonary blood vessels.

The evidence presented strongly suggests that changes in alveolar gas tensions occurring in anticholinesterase poisoning are an important mechanism contributing to the observed increase in pulmonary vascular resistance. Notwithstanding a possible action of circulating hormones other than those from the suprarenal medulla, the response occurring in the atropinized cross-perfused isolated lung preparation in which the donor animal was adrenalectomized must be attributed to this mechanism, because all other known causes had been excluded (Table 3). Although the tidal air volume and hence alveolar ventilation of the atropinized isolated lung remained unchanged after poisoning with an anticholinesterase, a decrease in alveolar pO_2 and an increase

in alveolar pCO_2 would be expected because the pulmonary arterial blood became more venous in composition owing to depression of pulmonary ventilation in the donor dog. This pulmonary vascular response is probably due to constriction of pulmonary capillaries and/or venules, although the possibility cannot be ruled out that at the same time dilatation of the pulmonary arteries or arterioles occurs (Nisell, 1951).

It is difficult to say to what extent our results are transferable to the entire animal breathing spontaneously, in which changes in pulmonary vascular resistance may be determined more by passive effects of cardiac and respiratory events than by direct effects on the lung blood vessels themselves. Our experiments do in fact provide evidence that the increase in vascular resistance through pulmonary vasoconstriction is masked by passive distension of the pulmonary vascular bed through the rise in left atrial pressure. Furthermore, events occurring in the pulmonary circulation of animals with closed thorax may be considerably modified by trapping of air in the lungs resulting from severe bronchoconstriction and the increase in intrapleural pressure fluctuations (de Candole, Douglas, Evans, Holmes, Spencer, Torrance & Wilson, 1953; Krop & Kunkel, 1954). Further studies on the circulation in closed chest preparations are therefore required before an assessment can be made of the extent to which such mechanisms as these modify pulmonary haemodynamics in anticholinesterase poisoning.

SUMMARY

1. The effects of two potent inhibitors of cholinesterase, *iso*propylmethylphosphonofluoridate (sarin) and tetraethylpyrophosphate (TEPP), administered intravenously, have been investigated upon the pulmonary vascular resistance in anaesthetized dogs. The whole or part of the left lung was perfused at constant volume inflow with blood either from the right atrium of the same animal artificially ventilated or from a donor dog breathing spontaneously.

2. Sarin and TEPP, in doses of 25–40 μg/kg and 0·1–0·3 mg/kg respectively, caused slowing of the heart, a fall in systemic blood pressure and a rise in left atrial pressure. The pulmonary arterial perfusion pressure rose, indicating an increase in pulmonary arterial inflow resistance.

3. When the passive effect of the increase in left atrial pressure was excluded, anticholinesterases still caused a rise in pulmonary arterial perfusion pressure, indicating an increase in pulmonary vascular resistance. The possible causes of this increased vascular resistance are discussed, and it is concluded that the response is due in part to constriction of the pulmonary vascular bed proper.

4. In animals poisoned with an anticholinesterase, atropine caused tachycardia, and restoration of the systemic blood pressure and left atrial pressure. The pulmonary arterial perfusion pressure fell to its control level provided pulmonary ventilation was restored to normal.

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