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THE EFFECTS OF ANTICHOLINESTERASES ON THE BRONCHIOLES AND PULMONARY BLOOD VESSELS IN ISOLATED PERFUSED LUNGS OF THE DOG

BY

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The effects of two potent inhibitors of cholinesterase, *iso*propylmethylphosphonofluoridate (sarin) and ethyl pyrophosphate (TEPP), were investigated on the bronchioles and pulmonary blood vessels in isolated dog lung preparations perfused with heparinized blood and ventilated artificially. These anticholinesterases caused gradual bronchoconstriction as indicated by a diminution in tidal air volume, a reduction in compliance and an increase in expiratory resistance. These effects were antagonized by atropine. An increase in pulmonary vascular resistance occurred which was enhanced by the addition of small quantities of acetylcholine to the perfusing blood. The pulmonary vascular effects of acetylcholine, sarin and TEPP were prevented and abolished by atropine. Evidence is presented which suggests that the site of action of these anticholinesterases is, at least in part, peripheral to the autonomic ganglia.

The effects of anticholinesterases on the pulmonary circulation in the dog have been studied recently by Daly (1957) and by Daly and Wright (1957). Under conditions in which respiration, pulmonary blood flow and left atrial pressure were controlled to exclude passive effects on the pulmonary circulation, the intravenous administration of anticholinesterases caused an increase in pulmonary vascular resistance (Daly and Wright, 1957). It was suggested that several mechanisms probably contributed to this response, and evidence was presented that one of these was an alteration in alveolar gas tensions. Owing to the difficulty of controlling other variables it was not possible in these experiments to obtain evidence of a possible direct action of anticholinesterases on the pulmonary blood vessels. This problem has now been investigated and the results are presented in this paper. For the most part, *iso*-propylmethylphosphonofluoridate (sarin) was used, but a few experiments were made with ethyl pyrophosphate (TEPP).

METHODS

Dogs, varying in weight from 6 to 14.7 kg., were given morphine hydrochloride (1 to 2 mg./kg. body weight, subcutaneously). Under local anaesthesia, a femoral artery was cannulated, and after intravenous injection of heparin ("Liquemin," Roche Products, 7 to 8 mg./kg.) to prevent intravascular clotting in later stages of making the preparation, the animal was bled to death. By means of a Dale-Schuster pump, the lungs were perfused *in situ* through the pulmonary artery at a constant blood volume inflow. Blood from the cannulated left atrium was collected in a reservoir, the bottom of which was connected to the input side of the pump. To prevent blood escaping from the pulmonary circulation by way of the bronchial vascular system, the superior and inferior venae cavae, the vena azygos, the aorta just above the level of the diaphragm and the oesophagus above and below the lung hilus were ligated. The ventricles were compressed by tying them tightly with tape just below the atrio-ventricular junction.

Both the pump and reservoir were placed in a thermostatically controlled water-bath at 37°. The pulmonary arterial pressure was measured with a small Marey tambour whose displacement was 0.25 ml./cm. saline change in pressure. Changes in the volume of blood in the reservoir were recorded by means of a piston recorder; these indicate inverse changes in lung blood volume provided allowances are made for capacity changes in the manometer (I. de B. Daly, 1928).

In other experiments the lungs were perfused at constant head of pressure. A side-arm in the pulmonary arterial tubing was connected to a small reservoir the blood level in which was maintained constant by an overflow pipe which returned the excess of blood back to the main reservoir. The output of the pump was initially set so that a small

volume of blood overflowed from the small reservoir. The level of the blood in this reservoir varied between 15 and 23 cm. above the pulmonary artery in different experiments. The left atrial blood outflow was measured by means of a recorder similar to that described by Gaddum (1939).

The volume of blood in the reservoir, pump and connecting tubes was 300 to 450 ml.

Lung Ventilation.—In some experiments the lungs were ventilated artificially by negative pressure ventilation. The lungs were enclosed in a reasonably airtight chamber from which the air was continuously exhausted by means of an electric vacuum pump. The negative pressure thereby created was then practically abolished by means of a cam-operated valve admitting atmospheric air to the chamber 12 times/min. The tidal air volume was measured with a small spirometer.

In other experiments the lungs were ventilated at a constant peak inflationary pressure which varied between 5 and 12.5 cm. water in different experiments (Konzett and 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 piston recorder. Since anticholinesterases usually produced large reductions in tidal air volume, it was found more convenient to collect the ventilation overflow volume continuously in a balanced 5-litre spirometer. Thus, an increase in slope of the spirometer trace indicates a diminution in tidal air volume. The ventilation overflow volume (V.O.V.)/respiratory cycle can be determined at any instant from the slope of the trace and the speed of the respiratory pump:

$$\text{V.O.V. (ml./respiration cycle)} = \frac{\text{V.O.V. (ml./min.)}}{\text{Respiratory pump rate (rev./min.)}}$$

The tidal air volume can then be calculated by subtracting the ventilation overflow volume from the corrected stroke volume of the respiratory pump. The stroke volume of the pump was measured at atmospheric pressure and was corrected for the pressure at which the lungs were inflated, taking into account the volume of air in the pump at the beginning of its stroke and in the connecting tubes between the pump and the lungs. This latter volume was approximately 800 ml. A change of 3 ml. in ventilation overflow volume/respiratory cycle is detectable by this method.

In a few experiments the compliance and expiratory resistance of the lungs were measured by a modification of the method used by Dixon and Brodie (1903) and by Comroe, Nisell and Nims (1954). The lungs were ventilated by negative pressure as described above and the tidal air volume was recorded on a fast-moving paper by means of a spirometer. On inspiration a volume plateau was reached, and then, by allowing the lungs to collapse passively by means of their own elastic recoil, the expiratory volume curve was obtained. The respiratory rate which was determined by the expiratory valve was 12 cycles/min., and this was found to be too fast when bronchial obstruction became severe, resulting in expiration occurring before the inspiratory volume plateau was reached. It was more convenient therefore to operate the valve by hand during the test periods.

In all preparations complete collapse of the lungs during expiration was prevented by immersing a tube connecting the expiratory side of the Starling respiration pump 2 to 3 cm. under water; in the case of lungs ventilated by negative pressure, the extrapulmonary pressure during expiration varied from -1.5 to -3 cm. water in different experiments.

In three preparations the lungs were not ventilated; the changes in intrapulmonary volume were measured at ambient pressure by means of a small volume recorder connected directly to the trachea.

A 1:1000 solution of *iso*propylmethylphosphonofluoridate (sarin) or a 1:100 solution of ethyl pyrophosphate (TEPP) was usually injected into the pulmonary arterial inflow tubing. Other drugs used were: acetylcholine (Roche), atropine sulphate (B.D.H.), hexamethonium bromide ("Vegolysen," May & Baker), adrenaline chloride, 0.1% solution with 0.5% chloretone (Parke, Davis), noradrenaline ("Levophed," Bayer Products) and isoprenaline (Burroughs Wellcome).

Results

In the experiments to be described, the range of single doses of sarin injected into the pulmonary arterial tubing or into the venous reservoir was 90 to 500 μg., and for TEPP 3 to 5 mg. No differences in the responses to these two anticholinesterases were found and the results with each of these compounds will therefore be presented together.

The Effect of Sarin and TEPP on Tidal Air Volume

The effect of these anticholinesterases on the tidal air volume was tested in 14 experiments in which artificial respiration was carried out by positive or negative pressure ventilation. The results are summarized in Table I. In 11 of the experiments the initial dose of sarin or TEPP caused a mean reduction in tidal air volume of 55.4% (range 2 to 100%). Such an effect is illustrated by Fig. 1 and is indicated by the increase in the slope of the ventilation overflow volume trace. In experiment Nos. 1 and 18 (Table I) the injection of the anticholinesterase was made during an infusion of acetylcholine. In three experiments there was no effect (Table I).

In one additional experiment (No. 14 of Table I) the lungs were ventilated at constant volume instead of at constant pressure, and TEPP caused an increase in peak intrapulmonary pressure from 12 to 23 cm. water (Fig. 2).

TABLE I

THE EFFECTS OF SARIN AND OF TEPP ON ISOLATED LUNGS PERFUSED AT CONSTANT BLOOD VOLUME INFLOW OR AT CONSTANT HEAD OF PRESSURE. THE LUNGS WERE VENTILATED WITH EITHER CONSTANT POSITIVE OR NEGATIVE PRESSURE

S=Sarin. T=TEPP. * Intrapulmonary pressure increased from 12 to 23 cm. water when ventilation was carried out with an intermittent constant volume of air.

Expt. No.	Drug	Tidal Air Volume % Change	Pulmonary Arterial Pressure (cm. Saline)		Left Atrial Outflow (ml./min.)		Vascular Resistance % Change	Remarks
			Before	After	Before	After		
1	S	−94			240	220	+8	Infusion of acetylcholine (20 μg./min.)
2	S	0			645	645	0	
3	S	No ventilation			325	325	0	
4	S	,,			415	415	0	Hexamethonium
5	S	,,			420	420	0	,,
6	S	−3			425	425	0	
7	S	0	14	15			+7	
8	S	−62	21	22·5			+7	
9	S	−7	14	14			0	
10	S	−33	6·5	6·5			0	
11	S	0	13	13			0	Hexamethonium
12	S	−2	8	8			0	,,
13	T	−60	12·5	14			+12	,,
14	T	*	18	20·5			+14	
15	T	−81	18·5	18·5			0	Injection of acetylcholine (160 μg.)
16	S	−100	17	18·5			+9	
17	T	−96	16	19			+19	
18	T	−71	12	15·5			+29	Infusion of acetylcholine (20 μg./min.)

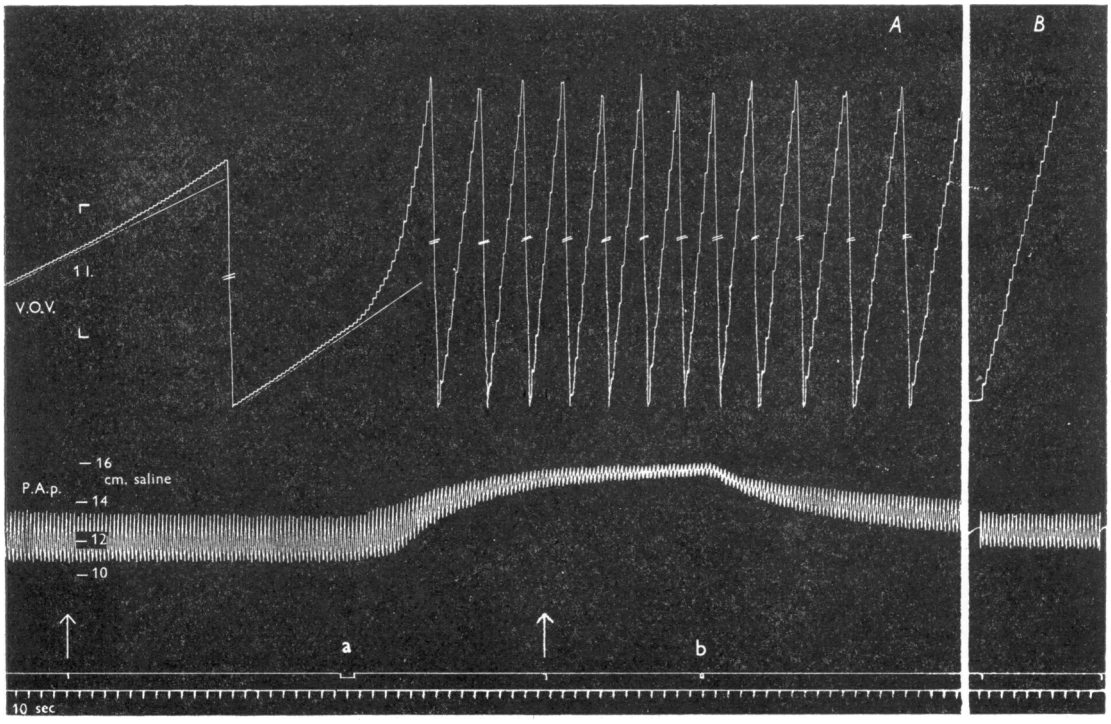

FIG. 1.—Dog, male, 14.0 kg. Isolated lungs perfused at constant blood volume inflow. Lungs ventilated at constant peak inflationary pressure. Respiratory pressure, 10 cm. water. Respiration pump stroke, 410 ml. An infusion of acetylcholine, 20 μg./min., was given into the pulmonary arterial inflow tubing between the arrows. At a, TEPP, 3 mg., and at b, atropine, 1 mg., were given. Between *A* and *B* there was an interval of 6 min. during which a further dose of atropine, 1 mg., was given. V.O.V.=ventilation overflow volume; P.A.p.=pulmonary arterial pressure.

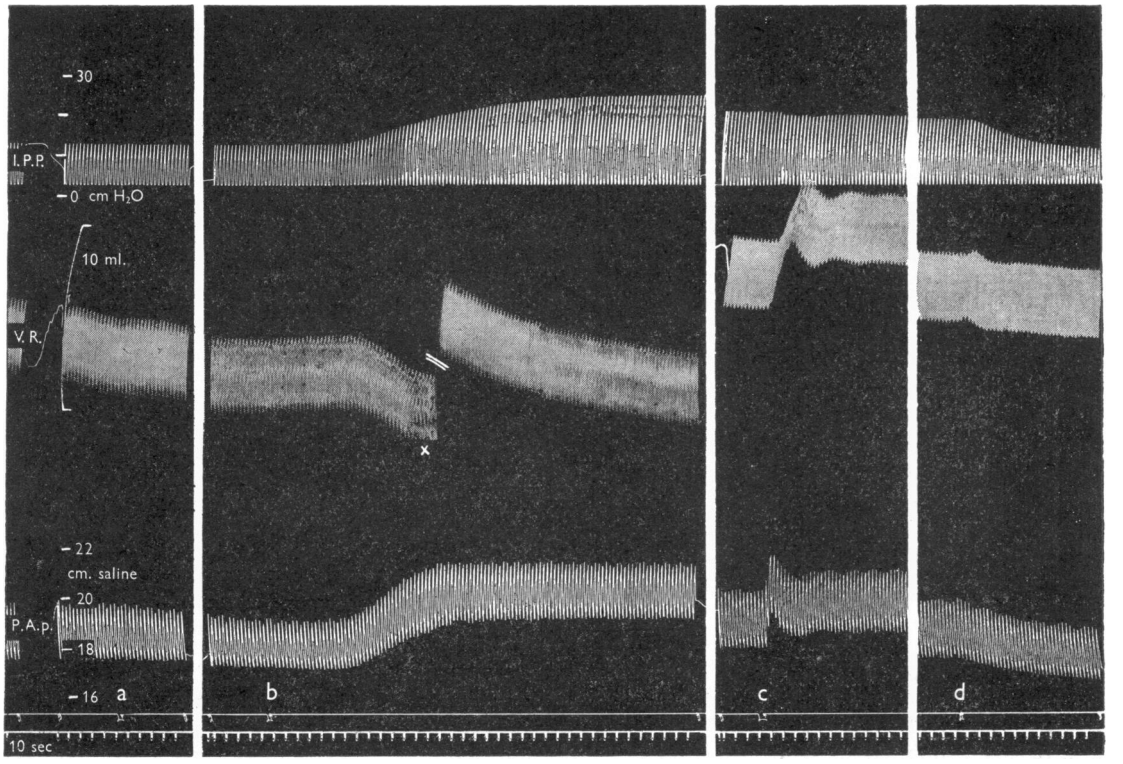

FIG. 2.—Dog, male, 11.0 kg. Isolated lungs perfused at constant blood volume inflow. Positive pressure ventilation with a Starling "Ideal" pump (constant volume). Records from above downwards: intrapulmonary pressure (I.P.P.); changes in volume of the venous reservoir (V.R.) in which an upward trend of the trace indicates an increase in volume of blood in reservoir; and pulmonary arterial perfusion pressure (P.A.p.). a, 0.2 ml. sodium chloride solution, 0.9% w/v; b, TEPP, 5 mg.; c, adrenaline, 10 μg.; d, atropine, 1 mg. All injections were made into the pulmonary arterial tubing. The downward excursion of the recorder was limited at X.

The response of the tidal air volume differed considerably from that observed in the entire animal using comparable methods of artificial respiration (Daly, 1957). In isolated lungs there was a longer latency before the effect came on, and the full response usually took several minutes to develop. A similar effect was observed by Alcock, Berry and I. de B. Daly (1935) in isolated perfused dog lungs in response to eserine. In the present experiments, however, it was found that the effect of the anticholinesterase appeared more quickly if it was given either immediately after an injection of acetylcholine or during a continuous infusion of acetylcholine. It had been found previously that the acetylcholine contraction of the isolated tracheal muscle of the guinea-pig was potentiated by sarin and by TEPP (de Candole, Douglas, Evans, Holmes, Spencer, Torrance and Wilson, 1953). In the present experiments acetylcholine in doses which were just effective in the untreated preparation caused a considerable reduction in tidal air volume after sarin or TEPP.

Three experiments were carried out in which the compliance and resistance of the lungs were measured. Both sarin and TEPP caused a reduction in compliance and an increase in inspiratory and expiratory resistances. Although the static pressure-volume relationship of the lungs was determined before the injection of the anticholinesterase, it was not possible to repeat the measurements after injection owing to the rapidity of the change in the visco-elastic properties of the lungs. Thus no accurate values can be given for the changes in expiratory resistance which depend upon knowing this relationship. It is clear, however, from Fig. 3 that a considerable obstruction to expiration occurred.

In order to gain some information on the site of action of the anticholinesterases, three preparations (expt. Nos. 11, 12, and 13 of Table I) were pretreated with hexamethonium to block intrapulmonary autonomic ganglia. Hexamethonium itself, in doses of 5 to 100 mg., had no effect on the tidal air volume. In two of the experiments,

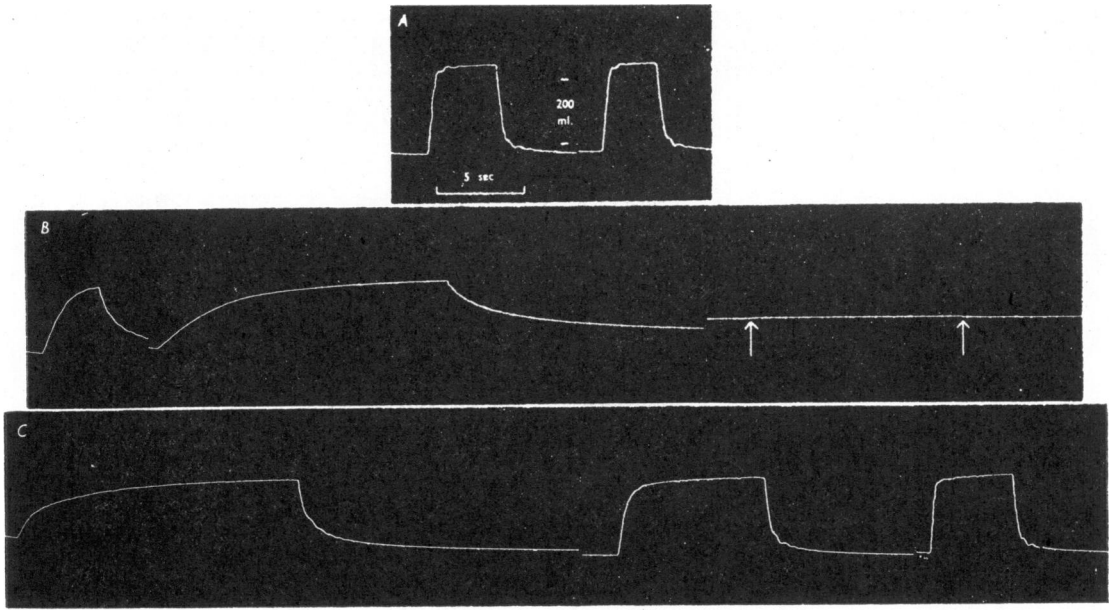

FIG. 3.—Dog, male, 10.3 kg. Isolated lungs perfused at constant head of pressure (21 cm. saline). Negative pressure ventilation. Inspiratory pressure, −15 cm. H_2O; expiratory pressure, −1.5 cm. H_2O. Record is that of the tidal air volume (inspiration upwards) showing changes in compliance and resistance of the lungs. *A*, control. *B* shows three respiratory cycles after injection of sarin, 600 μg., into the pulmonary arterial tubing. In the third cycle the negative inspiratory pressure applied to the outside of the lungs (between the arrows) caused no change in tidal air volume. *C*, effect of atropine, 1 mg., which was injected immediately after the tidal air volume had been reduced to zero as a result of injection of sarin.

sarin or TEPP injected subsequently still caused a reduction in tidal air volume; in the other, sarin had no effect. In one further experiment in which hexamethonium was injected at the height of the response produced by sarin, the tidal air volume remained unaffected. These results indicate that the site of action of these anticholinesterases is peripheral to the ganglia.

Effect of Adrenaline, Noradrenaline, and Isoprenaline.—In a few experiments, these drugs were injected into the pulmonary arterial tubing to test their efficacy in relieving bronchospasm produced by anticholinesterases. In three of four experiments, adrenaline 5 to 25 μg. and noradrenaline 2 to 20 μg. caused a small increase in tidal air volume or a decrease in intrapulmonary pressure after poisoning with sarin or TEPP (Fig. 2c); in the fourth experiment, they had no effect. On the other hand, isoprenaline 3 to 30 μg. invariably caused increases in tidal air which were larger than those produced by either adrenaline or noradrenaline when equal doses of the drugs were administered. Owing to the variability of the responses to repeated injections of the same drug, however, it was not possible to arrive at an accurate value for their relative antispasmodic potencies. In this connexion, Hebb and Konzett (1949) found that a dose of isoprenaline only one-tenth that of adrenaline was usually required to produce identical responses on the tidal air volume.

Effect of Atropine.—In doses of 1 to 10 mg., atropine reversed the effects of sarin and TEPP in 14 of 15 experiments, although in only six of these did the tidal air volume or intrapulmonary pressure return to its original value. In one experiment atropine was without effect. These responses are illustrated by Figs. 1 (at b) and 2d. In the experiments shown in Figs. 3*C* and 4*B* the expiratory resistance returned approximately to normal, although the compliance remained reduced. In experiments in which after poisoning with an anticholinesterase atropine failed to increase the tidal air volume to its original value, it was found that the most effective way of restoring it was to hyperinflate the lungs once or twice by an increased positive pressure in the trachea (Fig. 4*C*) or by an increased negative pressure applied to the outside of the lungs.

Subsequent injections of acetylcholine, sarin or TEPP in five atropinized preparations were without effect on the tidal air volume. In one preparation pretreated with atropine (2.5 mg.) neither sarin nor acetylcholine had any effect.

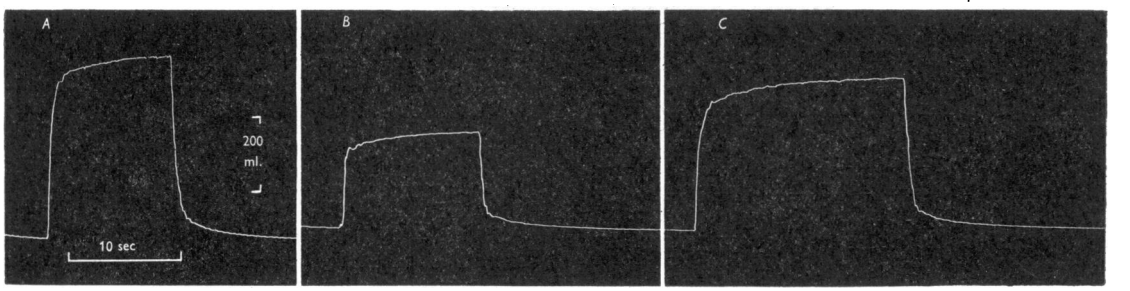

FIG. 4.—Dog, male, 14.0 kg. Isolated lungs perfused at constant blood volume inflow. Negative pressure ventilation. Inspiratory pressure, −10 cm. water; expiratory pressure, −1.5 cm. water. Record of tidal air volume. *A*, control. TEPP 3 mg. was then injected which resulted in a reduction in tidal air volume to 115 ml., and was followed by 2 mg. atropine. *B* shows maximum recovery of the tidal air after atropine. *C* was taken using the same respiratory pressure but after the lungs had been inflated with an increased negative pressure.

Pulmonary Vascular Effects of Sarin and TEPP

The effects of the initial dose of sarin and TEPP upon the pulmonary vascular resistance are shown in Table I. An increase in vascular resistance occurred in eight experiments as indicated by an increase in pulmonary arterial pressure when the lungs were perfused at constant blood volume inflow or by a decrease in left atrial outflow at constant head of pressure. In 10 experiments there was no effect.

In 4 of 7 experiments, changes in the volume of blood in the reservoir were observed on injection of anticholinesterases, but they were small and may have been due to capacity changes in the manometer rather than to alterations in lung blood volume.

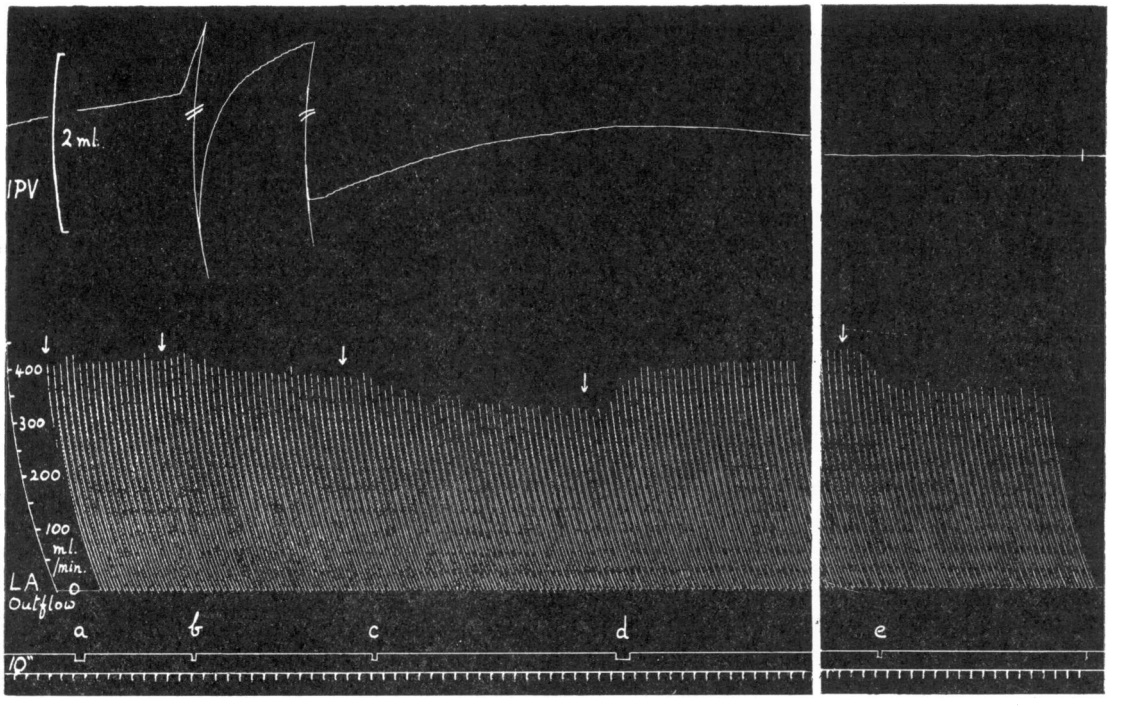

FIG. 5.—Dog, male, 10.2 kg. Isolated lungs perfused at constant head of pressure. No pulmonary ventilation. The upper record shows the *changes* in intrapulmonary air volume (I.P.V.) measured by a small volume recorder connected to the tracheal cannula (an upward trend of lever denotes decrease in intrapulmonary air volume). The lower record is of the left atrial outflow (LA outflow). Hexamethonium, 100 mg. before recording began. At a, sarin, 325 μg. At b and c, acetylcholine, 40 μg. At d, atropine 5 mg. In e, adrenaline, 50 μg.

It was found that anticholinesterases produced larger changes in pulmonary vascular resistance if they were injected on a " background " of acetylcholine. For instance, in experiment Nos. 1 and 18 of Table I an infusion of 20 μg./min. acetylcholine was begun about 3 min. before injecting sarin or TEPP and was continued for 3 min. afterwards. This rate of infusion was not sufficient in itself to alter the pulmonary vascular resistance although it did cause a slight reduction in tidal air volume in both cases. The injection of 360 μg. sarin during the infusion caused a fall in left atrial outflow from 240 to 220 ml./min. (8%); in the second experiment 3 mg. TEPP caused a rise in pulmonary arterial pressure from 12 to 15.5 cm. saline (29%). This effect is illustrated by Fig. 1. A second dose of sarin or TEPP nearly always caused a further increase in pulmonary vascular resistance. These responses were observed in preparations ventilated by positive or negative pressure ventilation and in those in which no ventilation was carried out. Furthermore, they occurred in preparations pretreated with hexamethonium (5 to 100 mg.).

In three normal preparations the smallest effective dose of acetylcholine caused an increase in pulmonary vascular resistance and this effect was potentiated by sarin or TEPP. Reductions in left atrial outflow up to 30% were observed on injection of acetylcholine in preparations treated with these anticholinesterases. The typical effect is shown in Fig. 5b and c. An increase in volume of blood in the reservoir also occurred, indicating a diminution in pulmonary blood volume. A similar effect was observed by Alcock *et al.* (1935).

Effect of Adrenaline, Noradrenaline, and Isoprenaline.—After injection of sarin or TEPP, adrenaline, 5 to 25 μg., and noradrenaline, 2 to 20 μg., caused an increase in pulmonary vascular resistance and an increase in volume of the blood in the reservoir, indicating a diminution in lung blood volume. These effects are shown in Figs. 2c and 5e. On the other hand, isoprenaline, 3 to 30 μg., invariably caused a small but definite decrease in pulmonary vascular resistance. In confirmation of the results of Hebb and Konzett (1949), this drug also produced a fall in pulmonary vascular resistance in the normal isolated perfused lung preparation.

Effect of Atropine.—In doses varying from 1 to 10 mg., atropine almost invariably reversed the pulmonary vasopressor response of sarin and TEPP. These effects are illustrated by Figs 1b, 2d, and 5d. Further doses of these anticholinesterases or of acetylcholine injected after atropine were without effect. In one preparation pretreated with atropine (2.5 mg.), neither sarin, total dose 870 μg., nor acetylcholine had any effect on the pulmonary arterial pressure.

Discussion

Bronchomotor Effects.—Our experiments have shown that both sarin and TEPP cause bronchoconstriction as indicated by a diminution in compliance of the lungs and an increase in expiratory resistance. These results are in keeping with those obtained by other workers using bronchoconstrictor drugs such as acetylcholine and pilocarpine (Bayliss and Robertson, 1939; Mount, 1956).

Anticholinesterases have been shown previously to cause bronchoconstriction in isolated perfused lungs (Alcock *et al.*, 1935; Green, McKay, and Krop, 1947: Bhattacharya and Pochet, 1956). They also cause contraction of muscle in isolated trachea or bronchial chain preparations (Macht and Ting, 1921; Hawkins and Schild, 1951; de Candole *et al.*, 1953) and diminish the rate of flow in perfused bronchial tree preparations (Swanson and Webster, 1930; Cotui, Burstein and Wright, 1936). Sollmann and Gilbert (1937) made microscopic observations on thin sections of lung and found that application of eserine caused bronchiolar contraction.

Comparison of the changes in tidal air volume occurring in the present experiments and in those on the entire animal reported by M. de B. Daly (1957) shows that in the perfused lung preparations the responses were slower in onset and were on the whole smaller in size even though the concentrations of the injected drugs were often higher, assuming even distribution throughout the animal. There are several plausible explanations for this. It may be that, in the entire animal, the concentration of acetylcholine in the lung after poisoning is higher because of the amount formed locally at vagus nerve endings by the tonic activity of the vagus on the bronchioles (Roy and Brown, 1885; Chauveau, 1889; M. de B. Daly and Schweitzer, 1952) and also because considerable quantities of acetylcholine may be conveyed to the lungs from other sites such as the bowel (Douglas and Paton, 1954). Furthermore, there is the possibility suggested by M. de B. Daly (1957) that an increase in vagal bronchomotor tone may occur in anticholinesterase poisoning as a result of a central action of asphyxia consequent upon depression of pulmonary ventilation. Such a mechanism cannot operate in the isolated perfused lung preparation.

In the entire animal ventilated artificially at constant peak inflationary pressure atropine

occasionally did not completely restore the tidal air volume to its original value after poisoning with an anticholinesterase (Holmstedt, 1951; M. de B. Daly, 1957; M. de B. Daly and Wright, 1957). In the present experiments this well-known antagonistic effect of atropine was confirmed, but again there were several instances in which the tidal air volume failed to return to normal; the compliance of the lungs remained reduced although the expiratory resistance returned to normal, suggesting that parts of the lung were not now being ventilated. Whether this was due to failure of some of the obstructed bronchioles to relax or to some other effect preventing the return of lung movements is not altogether clear. The bronchial circulation which supplies arterial blood to the bronchial tree was not perfused in these experiments, so that pulmonary arterial blood would supply not only tissues in and beyond the respiratory bronchioles but probably some of the bronchi and bronchioles as well (Ghoreyeb and Karsner, 1913). Pulmonary artery blood at relatively low pressure would reach these structures by back-flow through communicating vessels between the bronchial and pulmonary vascular systems situated in the region of the respiratory bronchioles (Küttner, 1878; Miller, 1925). Since the blood vessels supplying the bronchial tree pass freely through the bronchial musculature, constriction of the bronchial muscles by anticholinesterases might conceivably cut off their blood supply and so prevent access to them of drugs subsequently injected. An alternative explanation is that contraction of the bronchioles and increased glandular secretion produced by anticholinesterases renders complete closure of airways more likely, with the result that, when atropine is given and the bronchial muscles relax, these airways fail to open again owing to the effect of surface tension. Much greater forces would then be necessary to reopen these airways (Radford and Lefcoe, 1955). In our preparations in which atropine only partially restored respiratory movements, it was found that inflation of the lungs once or twice at increased pressure caused the compliance to return to normal, or very nearly so, on establishing the original respiratory pressure.

Pulmonary Vascular Effects.—Our results have shown that both sarin and TEPP cause an increase in pulmonary vascular resistance. Alcock *et al.* (1935) observed a similar effect on injection of eserine in isolated perfused dog lungs, and Bhattacharya and Pochet (1956) have reported recently that sarin caused a slowing of perfusion through the isolated lung of the guinea-pig.

In the present experiments the increase in pulmonary vascular resistance was nearly always accompanied by bronchoconstriction, and this raises the question as to how far the vascular response is the result of a direct action of the drugs on the pulmonary blood vessels or of a passive effect of a change in intrapulmonary pressure. Our findings would suggest that the observed vascular and bronchomotor effects are, at least in part, independent of each other, because in one experiment (No. 7 of Table I) an increase in pulmonary arterial pressure occurred in response to injection of sarin with no alteration in tidal air volume. Examination of the records of other experiments showed that there was no consistent relationship between the changes in tidal air volume and in pulmonary arterial pressure or left atrial outflow. Furthermore, similar changes in vascular resistance were obtained in unventilated lungs. Our experiments, however, provide no evidence that would enable us to state which parts of the pulmonary vascular bed are responsible for these effects.

A systematic study of the effects of acetylcholine on the pulmonary vascular bed has not been made. From the literature, small doses of acetylcholine in the dog usually produce pulmonary vasodilatation and large doses vasoconstriction, either response being enhanced by eserine and abolished by atropine (I. de B. Daly and Euler, 1932; Gaddum and Holtz, 1933; Alcock *et al.*, 1935). A reduction in pulmonary outflow in response to large doses of acetylcholine was also found by Tronci (1934). Franklin (1932) showed, in isolated rings of blood vessels taken from the dog, that acetylcholine caused relaxation of extrapulmonary arteries but contraction of the veins. Responses of intrapulmonary vessels were variable, but contraction predominated in both types. In a small number of experiments reported here the smallest effective dose of acetylcholine always produced pulmonary vasoconstriction which was enhanced by both sarin and TEPP.

Our experiments suggest that the site of action of sarin and TEPP is peripheral to the ganglia because these agents caused bronchoconstriction and an increase in pulmonary vascular resistance in preparations pretreated with hexamethonium. This peripheral effect could be the result of a direct action of the anticholinesterases or to accumulation of endogenous acetylcholine. In this connexion, the earlier work of Thornton (1934) and of Kordik, Bülbring and Burn (1952) suggested a slow synthesis of acetylcholine is present in the lung. Thornton (1934) found on

stimulation of the cervical vagus nerve a substance in the effluent from the perfused guinea-pig lung with the properties of acetylcholine, and Kordik *et al.* (1952) presented evidence that acetylcholine was synthesized by non-nervous elements in the mucous membrane of the rabbit trachea. The possibility that bronchoconstriction occurring in response to anticholinesterases is in part the result of an action on intrapulmonary vagal ganglia (Heymans, 1949) is not ruled out by our experiments.

The bronchoconstrictor and pulmonary vasoconstrictor responses occurring on injection of anticholinesterases are antagonized by atropine. In a few experiments the efficacy of adrenaline, noradrenaline and isoprenaline in reversing the bronchoconstriction produced by sarin and TEPP was tested. These drugs were found to have smaller and more evanescent effects than atropine. Furthermore, whereas atropine and isoprenaline caused pulmonary vasodilatation after poisoning, the injection of adrenaline and noradrenaline, in confirmation of the findings of other workers (see I. de B. Daly, 1933; also Konzett and Hebb, 1949), led to a rise in pulmonary arterial pressure and a diminution in lung blood volume. It must be pointed out, however, that an assessment of the value of a drug antagonizing the pulmonary circulatory effects of anticholinesterases cannot be made solely from observations obtained in the isolated organ, but must take into consideration passive cardiomotor and respiratory effects influencing the vascular bed. These points are considered more fully elsewhere (M. de B. Daly, 1957).

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