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AN ANALYSIS OF THE REFLEX SYSTEMIC VASODILATOR RESPONSE ELICITED BY LUNG INFLATION IN THE DOG

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SUMMARY

1. A maintained inflation of the lungs caused a reflex reduction in total systemic vascular resistance in anaesthetized dogs under conditions in which the systemic circulation was perfused at constant blood flow and the arterial blood P_{O_2} and P_{CO_2} were maintained constant.

2. The fall in systemic arterial perfusion pressure evoked by inflation of the lungs was accompanied by an increase in blood flow to the lower limbs and a reduction in their calculated vascular resistance. Since the fall in resistance occurred when the limb was perfused either at constant pressure or at constant blood flow, it must be due to vasodilatation.

3. Lung inflation caused vasodilatation in skin, muscle, and in the splanchnic vascular bed. The responses in vertebral circulation were, however, small and variable.

4. The vasodilator responses in the vascular territories studied were reflex in nature, being abolished by cutting the cervical vagosympathetic nerves, in which run the afferent fibres, or by interrupting the sympathetic pathways to the blood vessels.

5. In the intact limb, muscle, skin and splanchnic vascular bed, the vasodilator responses to lung inflation were unaffected by atropine or propranolol, but were abolished by hexamethonium, dibenyline and bretylium tosylate, indicating that they were due predominantly to a reduction in the activity in sympathetic adrenergic vasoconstrictor fibres.

INTRODUCTION

It has been shown previously that inflation of the lungs in the anaesthetized dog causes a reflex reduction in systemic vascular resistance (Salis-

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bury, Galletti, Lewin & Rieben, 1959; Daly, Hazzledine & Ungar, 1967). Daly *et al.* (1967) presented evidence that the receptors initiating the response are situated within the lungs and that the afferent fibres run largely in the vagus nerves although a few pass through the stellate ganglia. The systemic vascular response was due to a reduction in sympathetic vasoconstrictor tone and its size varied directly with the pressure and volume of gas used to inflate the lungs.

Since the arterial blood flow was maintained constant in these experiments, the reduction in total systemic vascular resistance occurring on inflation of the lungs must indicate a predominance of vasodilatation. The possibility that an increase in vascular resistance occurs in some territories cannot, however, be ruled out. The present experiments were therefore undertaken to establish some of the vascular territories participating in the responses, and to gain further information concerning the mechanisms by which they are brought about.

METHODS

The preparation used in this study has been fully described in previous papers (Daly, Hazzledine & Howe, 1965; Daly & Ungar, 1966) and therefore only the essential details of the technique and modifications will be given here.

Two dogs were used for each experiment and after premedication with morphine hydrochloride (2 mg/kg subcutaneously) were anaesthetized with a mixture of α-chloralose (0·055 g/kg) and urethane (0·55 g/kg) intravenously.

The recipient (test) dogs varied in weight from 13·8 to 17·2 kg. The systemic circulation was perfused at constant volume flow by means of a Dale–Schuster pump through cannulae inserted into the femoral and vertebral arteries (Daly *et al.* 1965). The ventricles were tied tightly with tape immediately below the atrioventricular groove. The systemic venous blood returning to the right atrium was oxygenated in the isolated perfused lungs of the second dog, before being returned to the systemic circulation of the recipient animal. There was therefore no blood flowing through the pulmonary circulation of the recipient dog, but the blood supply to its lungs was maintained by the bronchial circulation.

The carotid sinuses and aortic arch were isolated from the circulation and perfused with blood, the pressures being maintained constant to prevent reflex compensatory changes in systemic vascular resistance when alterations in systemic arterial blood pressure were evoked. The method of perfusion, however, differed from that described by Daly & Ungar (1966) in that the carotid sinuses and aortic arch were perfused together by one pump instead of separately by two pumps. As shown in Fig. 1 the two regions were perfused through a T-cannula in the right common carotid artery. Blood from the carotid sinuses and aortic arch was returned to the main reservoir in the perfusion system via the cannulated external carotid arteries and a Starling-type resistance by which the perfusion pressure could be controlled.

Systemic vascular resistance

At constant systemic blood flow, a change in vascular resistance can be taken as being proportional to the change in the pressure difference across the perfused vascular bed, i.e. mean arterial perfusion pressure minus mean right atrial pressure. The right atrial pressure was maintained at approximately zero pressure, and the change in vascular resistance could therefore be expressed as a percentage change in arterial perfusion pressure.

Ventilation of the recipient lungs

With a widely open chest, the lungs were ventilated with room air by means of a Starling 'Ideal' pump. The lungs collapsed against a resistance of about 3 cm H_2O. To carry out tests of the effects of lung inflation, artificial respiration was temporarily stopped, and then the lungs were inflated from an expiratory position by injecting through the tracheal cannula known quantities of air for a syringe. The volume was measured at atmospheric pressure and was corrected for the pressure at which the lungs were inflated, taking into account the initial volume of air in the syringe and in the connecting tube between the syringe and tracheal cannula. The inflation pressure was measured from a catheter (bore 2 mm) inserted into the tracheal cannula. To the catheter was attached a Statham strain gauge (Model P23Gb) and after amplification by means of a carrier amplifier (S. E. Laboratories, Feltham, Middlesex) the pressure was recorded on a direct-writing ultra-violet light recorder (S.E. Laboratories).

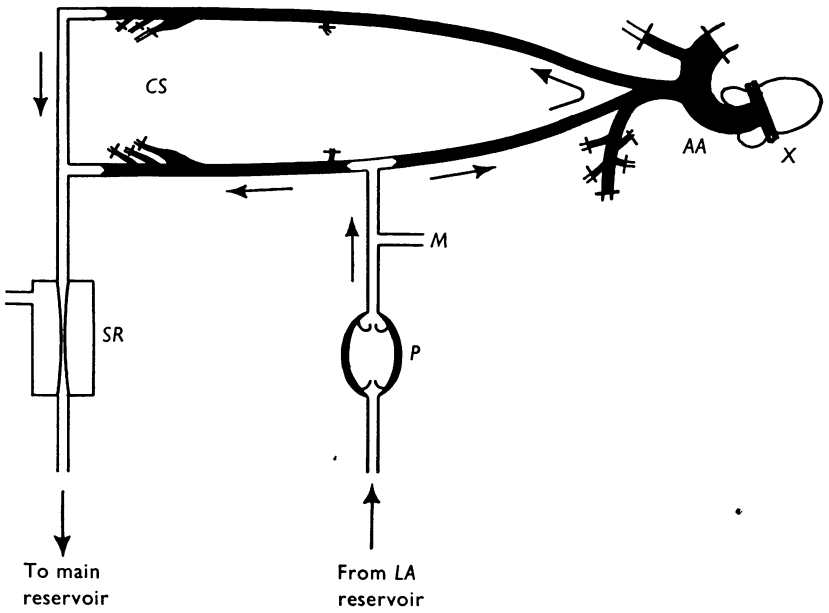

Fig. 1. Diagram showing the method of combined perfusion of the isolated carotid sinuses (*CS*) and aortic arch (*AA*) with a Dale–Schuster pump (*P*). Oxygenated blood for the pump was drawn from the left atrial (*LA*) reservoir and after perfusing the carotid sinuses and aortic arch was returned to the main reservoir via a Starling-type resistance (*SR*) by which the perfusion pressure was controlled. *X*, tape tied round ventricles below atrioventricular groove. *M*, connexion to manometer. For further details, see text.

Measurement of regional changes in vascular resistance

As already stated the systemic arterial blood flow was maintained constant by means of a pump. The blood flow to different vascular territories was continuously recorded so that changes in regional vascular resistance could be calculated from the systemic arterial per-

fusion pressure and regional blood flow. The vascular responses occurring in the intact limb muscle, skin, splanchnic vascular bed and vertebral circulation were studied as follows:

(*a*) *Intact lower limb.* A lower limb was perfused with blood from the systemic pump through a cannula inserted into the distal end of a ligated femoral artery. The blood flow to the limb was continuously recorded.

(*b*) *Muscle.* A lower limb was skinned and the paw severed at the level of the ankle joint to obtain vascular responses occurring in muscle. The femoral arterial blood flow was measured.

(*c*) *Skin.* Blood flow to a hind-limb paw, which comprises predominantly skin, was measured in the anterior tibial artery and plantar branch of the saphenous artery. Other small arteries in the region of the ankle joint were ligated.

(*d*) *Splanchnic vascular bed.* The responses of part of the splanchnic vascular bed were measured by recording blood flow through a cannula inserted into the distal end of the ligated superior mesenteric artery. Sympathetic fibres to the region embrace the artery and in dissecting it for cannulation care was taken to cause the minimum of disturbance to these nerve fibres.

(*e*) *Vertebral circulation.* Blood flow was measured through the cannulated vertebral arteries.

In all these experiments, the perfused blood returned to the right side of the heart through the normal venous channels.

The mean systemic arterial perfusion pressure was measured with a mercury manometer. The mean blood flow to all the vascular territories listed above, except the paw, was measured by means of a rotameter flowmeter of the type described by Shipley & Wilson (1951) but modified according to Bell (1954), and was recorded by a direct-writing milliammeter. The rotameter was calibrated at the end of each experiment as described by Daly (1957). Arterial blood flow to the paw was measured using a photo-electric drop counter (Lindgren, 1958) and recorded by means of a drop timer (Gaddum & Kwiatkowski, 1938).

Regional vascular resistance

Regional vascular resistance was calculated according to the formula: perfusion pressure (mm Hg)/blood flow (ml./min or drops/min) and was expressed in convenient units representing the pressure necessary to force 1 ml. of blood/min (or 1 drop/min) through the vascular bed.

Perfusion at constant arterial pressure of blood flow

In some experiments the above vascular territories were perfused with blood at constant blood-flow by means of a Dale-Schuster pump or a roller pump (type MHRE, Watson-Marlow, Ltd.). The change in vascular resistance can be taken as being directly proportional to the change in arterial pressure. In others, the perfusion pressure was maintained constant by adjusting the pump output, and in these blood flow was measured by a rotameter. The change in vascular resistance can then be taken as being inversely proportional to the change in arterial blood flow. In both types of experiment the right atrial pressure remained constant at approximately zero pressure.

In one experiment an upper limb was perfused through the brachial artery at constant blood flow by means of a separate pump. The method differed from that used for the lower limb in that the upper limb was isolated from the trunk of the animal by ligating all the muscle masses and dividing the humerus to exclude all collateral blood flow; its innervation was preserved.

Blood gas analysis

Samples of arterial blood withdrawn anaerobically from a systemic artery were transferred to electrode systems for measuring P_{O_2}, P_{CO_2} and pH. Methods for determining these parameters have been described previously (Daly & Ungar, 1966).

Drugs

The following drugs were used: acetylcholine chloride (Roche Products, Ltd.), atropine sulphate (British Drug Houses, Ltd.), hexamethonium bromide (May & Baker, Ltd.), bretylium tosylate (Burroughs Wellcome, Ltd.), phenoxybenzamine ('Dibenyline', Smith, Kline & French Laboratories, Ltd.), propranolol ('Inderal', Imperial Chemical Industries, Ltd.) and isoprenaline sulphate (Martindale Samoore, Ltd.). In all experiments coagulation of the blood was prevented by heparin ('Pularin', Evans Medical Ltd., 25 mg/kg) which was given when the operative procedures had been completed.

RESULTS

Table 1 shows the initial control values for the systemic arterial perfusion pressure, the carotid sinus and aortic arch perfusion pressure and the composition of the systemic arterial blood in twelve consecutive experiments. The values are those observed after the operative procedures

TABLE 1. Initial values for systemic arterial perfusion pressure, carotid sinus and aortic arch perfusion pressure, and for the composition of the blood perfusing the systemic circulation, carotid bifurcation regions and aortic arch

Dog wt. (kg)		15·2 ± 0·33 (13·8–17·2)
Systemic arterial perfusion pressure (mm Hg)		131·1 ± 2·9 (115–144)
Carotid sinus/aortic arch perfusion pressure (mm Hg)		120·8 ± 2·2 (102–132)
Systemic, carotid and aortic perfusate	P_{O_2} (mm Hg)	196·2 ± 30·8 (114–350)
	P_{CO_2} (mm Hg)	38·3 ± 1·3 (32–42)
	pH	7·42 ± 0·016 (7·35–7·51)

The open values are the means ± S.E.M.; those in parentheses the range.

had been completed and immediately before making observations on the effects of inflation of the lungs. In all experiments, the arterial blood P_{O_2} was greater than 113 mm Hg; the P_{CO_2} was within the range 32–42 mm Hg, and the pH 7·35–7·51.

Effects of lung inflation

Total systemic vascular resistance. In confirmation of the results of Salisbury *et al.* (1959) and of Daly *et al.* (1967), inflation of the lungs caused a reduction in systemic arterial perfusion pressure. Since the systemic blood flow was maintained constant, this indicates a reduction in total systemic vascular resistance. In twelve experiments, fifty-nine tests of lung inflation were made with volumes of 400–600 ml. (mean 495·0; S.E.M. ± 13·3), corresponding to inflation pressures of 7–16 cm H_2O (mean 11·4 ± 0·3). These caused falls in perfusion pressure of 12–48 mm Hg (mean 26·2 ± 1·3) corresponding to reductions in systemic vascular resistance of 9–36 % (mean 17·6 ± 0·9). Typical responses are shown in Figs. 2*A*, 3*A*, 4*B* and 5*A*.

Intact lower limb. In six experiments in which blood flow in a femoral artery was measured, inflation of the lungs invariably caused a striking increase in flow. This occurred in spite of the fact that the arterial per-

TABLE 2. The effects of inflation of the lungs on systemic arterial perfusion pressure, and blood flow and calculated vascular resistance in intact lower limb, skinned lower limb and lower limb paw, splanchnic and vertebral vascular territories. *C*, control state; *E*, experimental state, taken as the maximum change in vascular resistance

Perfused vascular territory	Nos. of expts.	Nos. of tests	Lung inflation volume (ml.)	Arterial perfusion pressure (mm Hg)			Blood flow (ml./min)			Vascular resistance (mm Hg/ml./min)		
				C	*E*	Diff.	*C*	*E*	Diff.	*C*	*E*	Change (%)
Intact lower limb	6	14	492·8 ± 30·4 (400–600)	148·0 ± 3·9 (126–170)	128·3 ± 3·2 (106–148)	−19·6 ± 1·4 (−14 to −32)	51·6 ± 4·6 (25–80)	67·6 ± 5·8 (30–104)	+16·1 ± 2·1 (5–28)	3·26 ± 0·51 (1·70–6·00)	2·16 ± 0·26 (1·06–4·54)	−33·6 ± 2·2 (−22 to −47)
Skinned limb (muscle)	6	24	525·0 ± 28·1 (400–800)	155·2 ± 2·4 (136–166)	126·8 ± 2·6 (102–145)	−28·0 ± 2·4 (−14 to −48)	42·9 ± 2·7 (23–66)	64·4 ± 3·7 (40–96)	+21·4 ± 3·0 (4–51)	3·94 ± 2·4 (2·32–6·27)	2·14 ± 0·14 (1·31–3·46)	−43·2 ± 3·6 (−13 to −75)
Lower limb paw (skin)	3	11	495·0 ± 19·6 (450–600)	144·4 ± 2·2 (130–158)	122·5 ± 3·4 (98–130)	−21·7 ± 2·9 (−8 to −42)	82·4 ± 8·9* (47–126)	96·2 ± 8·4* (70–140)	+13·5 ± 3·7 (5–46)	1·94 ± 0·19* (1·25–2·98)	1·36 ± 0·11* (0·92–1·85)	−27·1 ± 4·2 (−15 to −65)
Splanchnic	3	13	454·0 ± 14·4 (400–500)	140·2 ± 2·8 (128–154)	123·2 ± 3·9 (100–136)	−16·8 ± 2·3 (−2 to −32)	135·5 ± 9·6 (102–200)	140·2 ± 9·3 (102–200)	+4·8 ± 2·4 (0–21)	1·12 ± 0·11 (0·70–2·26)	0·94 ± 0·03 (0·63–1·85)	−15·6 ± 1·5 (−9 to −24)
Vertebral	4	12	487·5 ± 20·6 (400–600)	145·0 ± 3·5 (130–164)	116·5 ± 6·3 (86–152)	−28·4 ± 4·0 (−12 to −48)	131·8 ± 10·3 (82–185)	118·2 ± 10·2 (70–170)	−13·1 ± 4·9 (−55 to +20)	1·16 ± 0·07 (0·86–1·59)	1·04 ± 0·07 (0·86–1·49)	−10·1 ± 2·7 (−25 to +1)

The open values are the means ± S.E.M.; those in parentheses the range.
* Values for paw blood flow in drops/min, vascular resistance in mm Hg/drop/min.

fusion pressure fell. The results are summarized in Table 2 and show that in response to an average lung inflation of 492·8 ml. the systemic arterial perfusion pressure fell by 14–32 mm Hg (mean 19·6 ± 1·4), while the blood flow increased by 5–28 ml./min (mean 16·1 ± 2·1) or by 13–73%. The calculated vascular resistance decreased by 22–47% (mean 33·6 ± 2·2). The arterial perfusion pressure attained its lowest values at the same time as the limb blood flow reached its maximum, but both the pressure and flow showed some compensation when the stimulus was maintained. The values depicted in Table 2 are therefore the peak values before compensation had occurred.

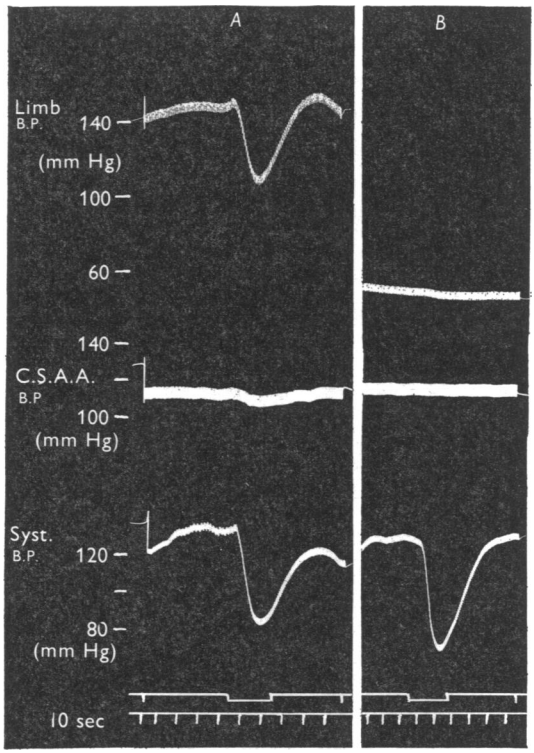

Fig. 2. Dog, female, 17·2 kg. Perfusion of the systemic circulation at constant blood volume inflow. No pulmonary circulation or ventilation. Lungs collapsed against pressure of 3 cm H_2O. Systemic venous blood oxygenated in isolated perfused lungs of a donor animal. Left upper limb of recipient dog vascularly isolated (including division of the humerus) and perfused by a separate pump at constant blood flow. *A* and *B* show the effects of inflation of the lungs of the recipient animal with 500 ml. air. Between *A* and *B*, transmission through the left stellate ganglion was blocked by procaine hydrochloride. Note the slower paper speed in *B*. Time marker, 10 sec.

In this and in subsequent figures: Syst. B.P., systemic arterial perfusion pressure; C.S.A.A. B.P., carotid sinus and aortic arch perfusion pressure.

It might be argued that the observed change in vascular resistance was the result of an alteration in the volume of blood flowing through the collateral circulation to the limb, which in these experiments remained intact. An experiment was therefore carried out on the upper limb which was isolated from the body except for the nerves which were left intact. Perfusion was carried out at constant blood flow by a separate pump. It was found that inflation of the lungs invariably caused a reduction in brachial arterial perfusion pressure. The typical response is shown in Fig. 2*A* and the results of all tests are summarized in Table 3. It is concluded therefore that the reduction in vascular resistance is due to dilatation of 'resistance' vessels in the limb.

The reduction in vascular resistance occurring under conditions of controlled regional perfusion at constant flow, as illustrated by Fig. 2*A*, was also observed in three further experiments, carried out at constant arterial pressure perfusion. The results are summarized in Table 3. In seven tests, lung inflation caused an increase in blood flow of 20–42 ml./min (mean 29·3 ± 3·0), representing a reduction in vascular resistance of 27–42% (mean 33·3 ± 1·7).

In an attempt to localize still further the vascular territories participating in the responses to lung inflation, experiments were carried out on the skinned limb and on the paw as representative of effects occurring in muscle and skin respectively.

Skinned limb. Responses in the skinned lower limb were obtained in all six experiments designed to study the responses of muscle blood vessels. Twenty-four inflations of the lungs with an average of 525 ml. air resulted in a fall in systemic arterial perfusion pressure of 14–48 mm Hg (mean 28·0 ± 2·4), an increase in muscle blood flow of 4–51 ml./min (mean 21·4 ± 3·0) and a reduction in calculated vascular resistance of 13–75% (mean 43·2 ± 3·6). Typical responses are shown in Figs. 3*A* and 4*B* and the results are summarized in Table 2.

Similar effects were observed in three experiments carried out at constant arterial perfusion pressure (Table 3). In six inflations of the lungs with an average of 441·8 ml. air, the muscle blood flow rose by 20–43 ml./min (mean 37·1 ± 3·7) corresponding to a reduction in vascular resistance of 37–63% (mean 45·3 ± 4·2).

Paw. The blood flow to four lower limb paws was measured in three dogs. It was found that inflation of the lungs invariably caused changes in flow but the pattern differed somewhat from that observed in the intact limb or in muscle. Typical responses are shown in Figs. 4*B* and 5*A* from which it may be seen that there was an initial reduction in blood flow as the arterial perfusion pressure fell and a small reduction in calculated vascular resistance. This was followed by an increase in blood flow even

TABLE 3. The effects of lung inflation on the resistance of various vascular territories under conditions in which either the arterial perfusion pressure or the blood flow was maintained constant. *C*, control state: *E*, experimental state, taken as the maximum change in vascular resistance

Perfused vascular territory	Nos. of expts.	Nos. of tests	Lung inflation volume (ml.)	Arterial perfusion pressure (mm Hg)		Blood flow (ml./min)			Vascular resistance (mm Hg/ml./min)		
				C	*E*	*C*	*E*	Diff.	*C*	*E*	Change (%)
Intact lower limb	3	7	442·8 ± 29·7 (400–600)	132·0 ± 5·3 (120–162)	Constant	58·6 ± 5·6 (36–80)	87·9 ± 8·2 (62–122)	+ 29·3 ± 3·0 (20–42)	2·44 ± 0·36 (1·50–4·50)	1·60 ± 0·19 (0·99–2·61)	− 33·3 ± 1·7 (− 27 to − 42)
Isolated upper limb	1	3	500	144·5 ± 5·1 (142–156)	112·0 ± 2·3 (108–116)	Constant		0	—	—	− 23·3 ± 1·5 (− 21 to − 26)
Lower limb (muscle)	3	6	441·8 ± 20·0 (400–500)	132·3 ± 4·1 (125–150)	Constant	40·1 ± 6·6 (25–68)	75·6 ± 8·6 (48–110)	+ 37·1 ± 3·7 (20–43)	3·66 ± 0·5 (2·21–5·05)	1·93 ± 0·26 (1·36–2·71)	− 45·3 ± 4·2 (− 37 to − 63)
Lower limb paw (skin)	3	8	450	137·2 ± 2·7 (126–148)	Constant	79·1 ± 4·2* (70–98)	121·7 ± 6·0* (95–140)	+ 43·9 ± 5·5 (25–68)	1·76 ± 0·07* (1·43–2·00)	1·15 ± 0·06* (0·92–1·37)	− 34·5 ± 2·8 (− 26 to − 49)
Splanchnic	2	9	455·5 ± 16·7 (400–500)	135·7 ± 2·6 (128–153)	116·2 ± 3·0 (100–126)	142·4 ± 10·3 (102–200)	Constant	0†	1·00 ± 0·09 (0·70–1·50)	0·86 ± 0·07 (0·58–1·23)	− 14·2 ± 1·8 (− 6 to − 19)
Vertebra	3	9	455·5 ± 10·0 (450–500)	136·6 ± 3·1 (125–152)	Constant	148·8 ± 8·8 (120–185)	165·8 ± 4·6 (140–185)	− 17·0 ± 6·6 (− 5 to + 47)	0·93 ± 0·04 (0·79–1·12)	0·84 ± 0·02 (0·76–0·95)	− 10·8 ± 3·5 (0 to − 27)

The open values are the means: those in parentheses the range.

* Values for paw blood flow in drops/min, vascular resistance in mm Hg/drop/min.

† Blood flow measured with rotameter maintained constant.

though the perfusion pressure was still below the control level and a larger reduction in vascular resistance. The results are summarized in Table 2 and refer to the average values for arterial perfusion pressure and blood flow at a time when the reduction in vascular resistance was maximal. In eleven tests inflation of the lungs with 495 ml. air caused a fall in arterial perfusion pressure of 8–42 mm Hg (mean 21·7 ± 2·9) and an increase in

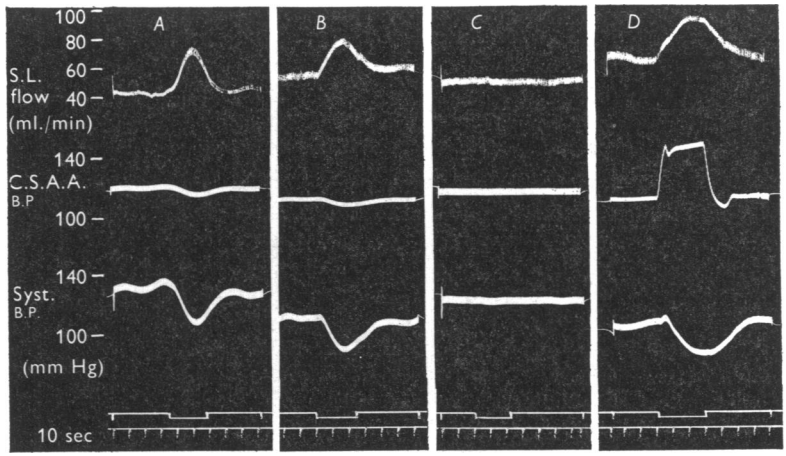

Fig. 3. Dog, male, 14·7 kg. Perfusion of the systemic circulation at constant volume inflow. Separate perfusion of the isolated carotid sinuses and aortic arch. No pulmonary circulation or ventilation. Lungs collapsed against pressure of 2·5 cm H_2O. Systemic venous blood oxygenated in isolated perfused lungs of a donor animal. Measurement of femoral arterial blood flow to the skinned left lower limb. *A–C*, inflation of the lungs of the recipient animal with 500 ml. air. Between *A* and *B*, atropine, 2 mg, into the main reservoir. Between *B* and *C*, both cervical vagosympathetic nerves divided. In *D*, effects of raising the pressure in the isolated carotid sinuses and aortic arch. Time marker, 10 sec. In this and subsequent figures: S.L., skinned limb.

blood flow through the paw of 5–46 drops/min (mean 13·5 ± 3·7). This corresponds to a reduction in calculated vascular resistance of 15–65% (mean 27·1 ± 4·2).

Comparison of the responses occurring in the skinned limb and paw in the same animal is shown in Fig. 4*B*. Apart from the differences in flow pattern brought about by inflation of the lungs, the changes in vascular resistance occurring in the skinned limb were significantly larger than those in the paw ($P < 0{\cdot}01$).

Figure 4 also compares the responses occurring in skin and muscle as a result of inflation of the lungs (*B*) with those evoked by stimulation of the arterial baroreceptors by raising the perfusion pressure in the isolated perfused carotid sinuses and aortic arch (*A*). It will be observed that the

pattern of the changes in blood flow in skin and muscle are the same in the two instances.

In three experiments the vascular responses in the paw were observed while the systemic arterial perfusion pressure was maintained constant. The results of eight tests are summarized in Table 3 and show that inflation of the lungs with 450 ml. air caused a reduction in vascular resis-

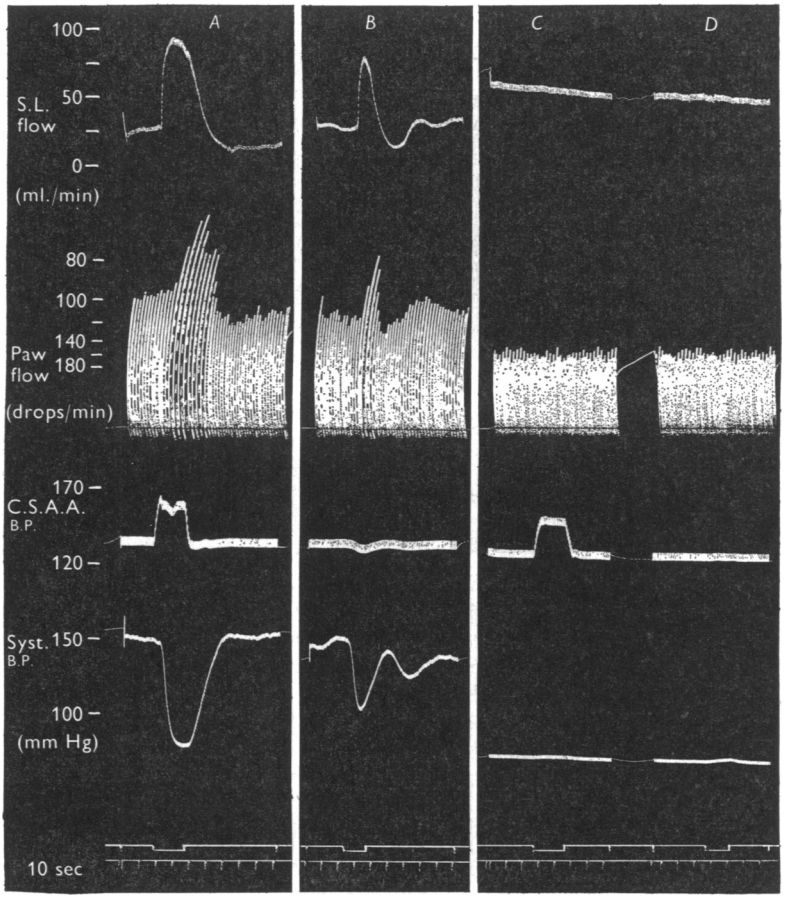

Fig. 4. Dog, female, 14·9 kg. Perfusion of the systemic circulation at constant volume inflow. Separate perfusion of the isolated carotid sinuses and aortic arch. No pulmonary circulation or ventilation. Lungs collapsed against pressure of 2 cm H_2O. Systemic venous blood oxygenated in isolated perfused lungs of a donor animal. Measurement of femoral arterial blood flow to the skinned left lower limb and of the anterior tibial arterial blood flow to right hind paw. *A* and *C*, temporary increase in pressure in the isolated perfused carotid sinuses and aortic arch. *B* and *D*, inflation of the lungs with 500 ml. air. Between *B* and *C*, hexamethonium, 250 mg, into the main reservoir. Time marker, 10 sec.

tance of 26–49% (mean 34·5 ± 2·8). The typical response is shown in Figs. 5*C* and 6*A*.

These experiments indicate therefore that both skin and muscle blood vessels contribute to the reduction in vascular resistance occurring in the intact limb on inflation of the lungs.

Splanchnic vascular bed. The pattern of the response of the superior mesenteric arterial blood flow was similar to that of the paw. Inflation of the lungs caused an initial reduction in flow as the systemic arterial pressure fell. The blood flow then either returned to its control level while the

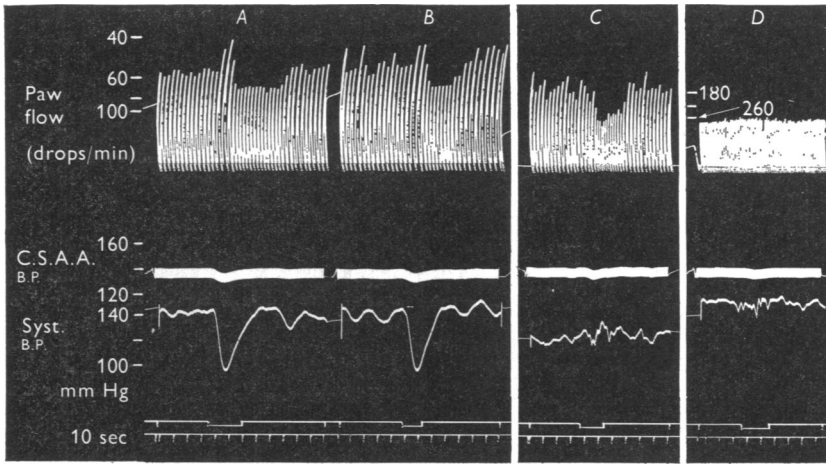

Fig. 5. Dog, female, 13·9 kg. Perfusion of the systemic circulation at constant blood flow. Separate perfusion of the isolated carotid sinuses and aortic arch. No pulmonary circulation or ventilation. Lungs collapsed against pressure of 2·5 cm H_2O. Systemic venous blood oxygenated in isolated perfused lungs of a donor animal. Measurement of arterial blood flow to the paw of the right hind limb. *A–D* show the effects of inflation of the lungs with 450 ml. air. In *C* and *D* the systemic arterial perfusion pressure was maintained constant during inflation of the lungs by altering the stroke volume of the systemic pump. Between *A* and *B*, propranolol, 15 mg, into blood reservoir. Between *C* and *D*, right paw denervated by infiltration with procaine. Time marker, 10 sec.

lungs were still inflated, or increased slightly. The results of thirteen tests in three experiments are shown in Table 2 and indicate that whereas the arterial perfusion pressure fell by 2–32 mm Hg (mean 16·8 ± 2·3), the blood flow remained unchanged in nine tests, and increased in the remaining four, the mean change being an increase of 4·8 ± 2·4 ml./min or 4·4%. The calculated vascular resistance decreased in all tests by 9–24% (mean 15·6 ± 1·5).

In two experiments, perfusion was carried out at constant blood flow and in these inflation of the lungs caused a fall in superior mesenteric

arterial perfusion pressure indicating a reduction in vascular resistance. The average reduction in the nine tests was 14·2% (S.E.M. ± 1·8; range 6–19) (Table 3).

Vertebral vascular bed. Changes in blood flow through the vertebral arteries in response to lung inflation were observed in twelve tests in four experiments. In two of the experiments the flow fell *pari passu* with the systemic arterial perfusion pressure and the calculated vascular resistance in each of the six tests altered by less than 6%. In the remaining two experiments the blood flow decreased in five tests and increased in one; the calculated vascular resistance decreased by an average of 17%. The results of the four experiments are summarized in Table 2 from which it may be seen that lung inflation caused an average reduction in vascular resistance of 10·1% (S.E.M. ± 2·7).

In nine tests in three experiments carried out at constant pressure perfusion, the average reduction in vascular resistance was 10·8% (S.E.M. ± 3·5) (Table 3).

It is concluded from these experiments that the fall in total systemic vascular resistance evoked by lung inflation is due to a reduction in vascular resistance occurring in muscle, skin and in the splanchnic region; the responses of the vertebral vascular bed were variable and the possible reasons for this are discussed below.

Reflex nature of the vascular responses

Afferent nerve pathways. It has been shown previously that the reduction in total systemic vascular resistance produced by inflation of the lungs is due to a reflex arising from the lungs, the afferent pathway running in the vagus nerves and through the stellate ganglia (Daly *et al.* 1967). Thus, division of the cervical vagosympathetic nerves either reduced the response (Daly *et al.* 1967) or abolished it (Salisbury *et al.* 1959; Daly *et al.* 1967). Denervation of the lungs by cutting the pulmonary branches of the vagosympathetic nerves, however, invariably abolished the systemic vasodepressor response (Brodie & Russell, 1900; Daly *et al.* 1967).

In the present study also evidence was obtained that the observed changes in regional vascular resistance are reflex in nature. Division of the cervical vagosympathetic nerves or denervation of the lungs by cutting the thoracic vagosympathetic nerves immediately above the lungs (Daly & Scott, 1958) was carried out in four experiments to interrupt the afferent pathway. It was found that these procedures abolished the fall in systemic arterial perfusion pressure and the vascular responses occurring in the intact limb, skinned limb, paw and splanchnic vascular bed. One such experiment is illustrated by Fig. 3 in which the blood flow to the skinned left limb was measured. Lung inflation produced a fall in systemic arterial

perfusion pressure and an increase in limb blood flow (*B*) which were abolished after division of the cervical vagosympathetic nerves (*C*). The reflex activity of the preparation was then tested by stimulating the baroreceptors in the carotid sinuses by raising the pressure in the perfused carotid sinuses (*D*). This resulted in a fall in systemic arterial perfusion pressure from 115 to 90 mm Hg, an increase in blood flow from 62 to 90 ml./min, and a reduction in calculated vascular resistance of 46%.

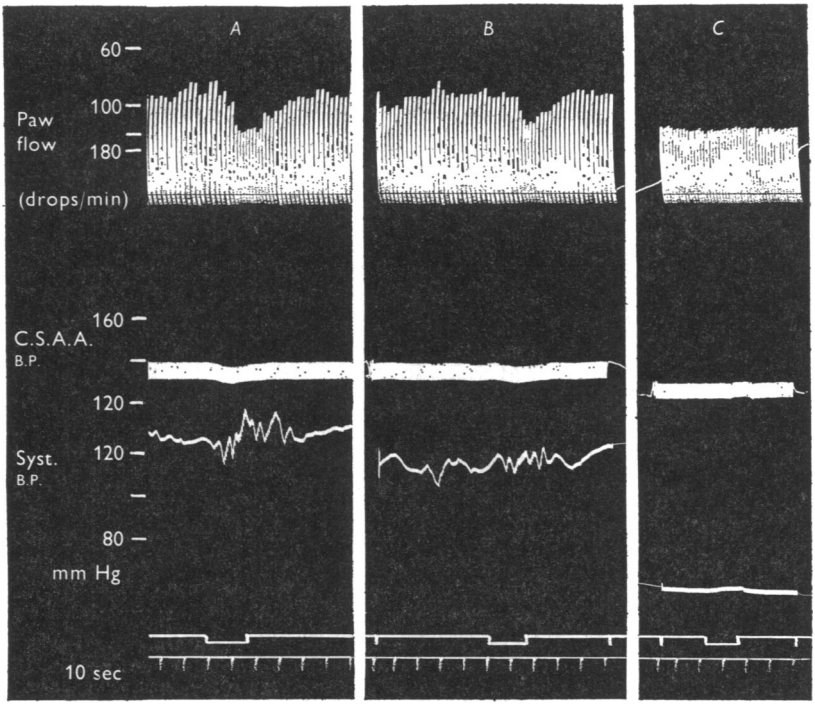

Fig. 6. Dog, female, 13·9 kg. Perfusion of the systemic circulation at constant blood pressure. Separate perfusion of the isolated carotid sinuses and aortic arch. No pulmonary circulation or ventilation. Lungs collapsed against pressure of 2·5 cm. H_2O. Systemic venous blood oxygenated in isolated perfused lungs of a donor animal. Measurement of arterial blood flow to the paw of the left hind limb. Propranolol, 15 mg, injected before recording began. *A*, *B* and *C* show the effects of inflation of the lungs with 450 ml. air. Between *A* and *B*, atropine 2 mg, and between *B* and *C* hexamethonium 200 mg, both drugs being added to the blood reservoir. Time marker, 10 sec.

Although the pressure in the aortic arch was raised as well in this test, no contribution from the aortic arch baroreceptors would be expected because the cervical vagosympathetic nerves had been cut.

Efferent nerve pathways. In five dogs the responses in different vascular

territories to lung inflation were observed before and after division of the efferent nerve supply.

As shown in Fig. 7 (Expt. nos. 9, 12 and 10*a*), cutting the femoral and sciatic nerves to the intact limb and skinned limb considerably modified the vascular responses. Whereas the fall in systemic arterial pressure was not appreciably affected owing to a reduction in vascular resistance taking place in other territories, the striking increase in limb blood flow observed before denervation was now converted to a fall. The calculated vascular

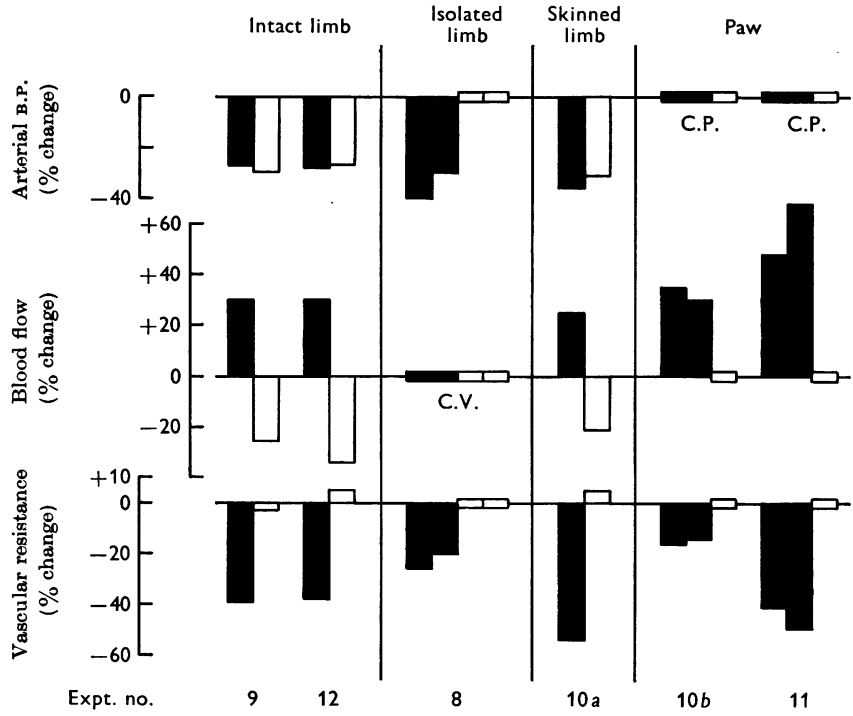

Fig. 7. The effects of denervation of the intact limb, isolated limb, skinned limb and paw on the changes in arterial perfusion pressure, blood flow and vascular resistance in response to inflation of the lungs with 500 ml. air. Closed rectangles, control observations; open rectangles, observations after denervation. The isolated limb was perfused at constant volume (C.V.) blood flow by a separate pump. The paws were perfused at constant pressure (C.P.). For denervation procedures, see text.

resistance in the control observations fell by 39, 38 and 54% respectively; after denervation it fell by 3% in Expt. no. 9, and rose by 5% in Expt. nos. 12 and 10*a*.

In one further experiment in which the left upper limb was isolated and perfused by a separate pump at constant blood flow the effects of interruption of the sympathetic supply on the response to lung inflation were

studied. The results are shown in Figs. 2 and 7 (Expt. no. 8). In Fig. 2*A* inflation of the lungs with 500 ml. air caused a reduction in systemic arterial perfusion pressure and in limb arterial perfusion pressure. Between *A* and *B*, transmission of nerve impulses through the left stellate ganglion was blocked by infiltration with procaine hydrochloride and this resulted in a fall of limb perfusion pressure indicating vasodilatation. Inflation of the lungs in *B* then had no effect on the vascular resistance of the limb though still affected the systemic arterial perfusion pressure due to a reduction in resistance in other vascular territories.

Figure 7 shows the results of two experiments in which the paw was perfused at constant pressure. Whereas before denervation by infiltration of the tissues round the ankle joint with procaine hydrochloride (1 %), lung inflation caused an increase in blood flow and a reduction in vascular resistance, no change in either parameter occurred when the test was repeated after denervation.

These results indicate that the reduction in vascular resistance occurring in the intact limb, skinned limb and paw are dependent on the integrity of the nerves to these vascular territories. Further information concerning the nature of the fibres mediating these responses was obtained by studying the action of certain drugs.

Action of drugs

Atropine. In ten experiments, atropine was added to the main reservoir in doses of 2–3 mg, sufficient to abolish the vasodilator action of acetylcholine (5–15 μg) when injected into the arterial supply to the vascular territories under study. It was found that the reduction in vascular resistance occurring in the intact limb, skinned limb, paw, splanchnic and vertebral vascular territories in response to inflation of the lungs was not appreciably affected by atropine. Responses illustrating this are shown in Figs. 3*B* and 6*B*.

In confirmation of the results of Daly *et al.* (1967), atropine also had no effect on the reduction in total systemic vascular resistance.

Propranolol. This β-receptor adrenergic blocking agent was added to the main reservoir in a dose of 1·0–1·25 mg/kg body wt. of recipient animal, which was sufficient to block the vasodilator action of 5 μg isoprenaline injected into the arterial supply to the vascular territories under study. It was found that propranolol had no effect on the reduction in total systemic vascular resistance produced by lung inflation or on the reduction in vascular resistance occurring in the intact limb, skinned limb, paw, splanchnic vascular bed (two experiments each), or in the vertebral circulation (one experiment). The responses occurring in the perfused paw are shown in Figs. 5*A*, *B* and 6*A*.

Hexamethonium, phenoxybenzamine, bretylium tosylate. Daly *et al.* (1967) showed that the reduction in total systemic vascular resistance evoked by inflation of the lungs was abolished by the ganglionic blocking agent hexamethonium and by the adrenergic blocking drugs guanethidine and bretylium tosylate. In the present experiments hexamethonium, 100–250 mg, abolished the systemic vascular reflex (five experiments) (Figs. 4*D* and 6*C*), as did phenoxybenzamine, 100 mg (three experiments) and bretylium tosylate, 150–250 mg (three experiments).

The effects of hexamethonium, phenoxybenzamine and bretylium tosylate were also tested on the vascular responses elicited in the intact limb, skinned limb and paw, and in the splanchnic and vertebral vascular beds. It was found that all three substances abolished the reduction in vascular resistance evoked in each of these territories by inflation of the lungs. The effects of hexamethonium on the responses occurring in the skinned limb and paw are illustrated by Figs. 4 and 6.

DISCUSSION

These results confirm and extend the previous observations of Salisbury *et al.* (1959) and of Daly *et al.* (1967) who showed that inflation of the lungs caused a reflex reduction in total systemic vascular resistance. Since the blood flow to the systemic circulation was maintained constant, the reduction in vascular resistance must be due to a predominance of vasodilatation.

The present experiments have shown that this systemic vasodilatation occurring on inflation of the lungs takes place in a number of vascular territories, namely, the intact limb, skin, muscle and the splanchnic vascular bed. Evidence for this was obtained from two types of experiment. In the first, the whole of the systemic circulation was perfused at constant flow, and the blood flow to different regions was measured simultaneously thereby enabling the regional vascular resistance to be calculated from the two parameters. In the second type of experiment, observations were made under conditions in which either the perfusion pressure or the blood flow to the vascular bed under study was maintained constant.

Observations were also made on the changes in resistance in the vertebral vascular bed, but these were found to be relatively small and variable. In the preparation used, the external and internal carotid arteries were ligated so that the carotid sinuses could be separately perfused at constant pressure to maintain the activity of the baroreceptors at a steady level. It is likely therefore that vertebral blood found its way into the vascular bed of the external carotid artery through anastomotic channels with the

circle of Willis (Ellenberger & Baum, 1891; Bouckaert & Heymans, 1935; Jewell, 1952; Chungcharoen, Daly, Neil & Schweitzer, 1952). The changes in vertebral vascular resistance observed in two of the four preparations may, therefore, be a reflexion of alterations in calibre of the extracerebral rather than the intracerebral vessels. Further studies are required to substantiate this view.

Daly *et al.* (1967) demonstrated that the afferent pathway for the reflex reduction in total systemic vascular resistance evoked by lung inflation ran in the cervical vagosympathetic nerves, though in two experiments it was found that some fibres travelled by way of the stellate ganglia. In the present experiments the regional vasodilator responses were always abolished by division of the cervical vagosympathetic nerves, as they were by specific denervation of the vascular beds, thereby demonstrating their reflex nature.

Comparison of the responses to lung inflation in skin, muscle and splanchnic vascular beds revealed clear cut differences. For instance, in experiments in which the systemic circulation was perfused at constant flow, inflation of the lungs invariably caused a fall in systemic arterial perfusion pressure. But whereas the muscle blood flow increased, that to skin and the splanchnic vascular bed decreased owing to the significantly larger reduction in vascular resistance occurring in muscle. Thus excitation of the lung inflation reflex causes a redistribution of blood flow towards muscle.

This finding may have a bearing on the functional role of the reflex. Evidence presented elsewhere strongly suggests that it is initiated by stimulation of pulmonary stretch receptors (Daly *et al.* 1967). Bearing in mind the close relationship which exists between the volume of gas used to inflate the lungs and the size of the systemic vasodilator response (Daly *et al.* 1967), it is possible that under conditions in which hyperventilation occurs, such as during muscular exercise, this reflex is elicited and redistributes blood flow towards muscle. In doing so it would contribute to the mechanisms which ensure an adequate blood supply to exercising muscle.

Mechanism of vasodilatation. In their studies on the mechanism of the reduction in total systemic vascular resistance evoked by inflation of the lungs, Daly *et al.* (1967) concluded that it was due to decreased activity in adrenergic sympathetic vasoconstrictor fibres. This view is supported by the results of the present experiments in which it was shown that the vasodilatation occurring in skin, muscle and in the splanchnic vascular bed was unaffected by atropine or propranolol, but was abolished by hexamethonium, bretylium tosylate and by phenoxybenzamine.

The question arises whether reflex activation of true vasodilator nerves contributes to the responses observed on inflation of the lungs. Although

our experiments were not specifically designed to evaluate the role, if any, of such vasodilator fibres, some of our results are pertinent. It was found that the vasodilatation in muscle occurring on inflation of the lungs was unaffected by atropine, a finding which suggests that the atropine-sensitive cholinergic sympathetic vasodilator outflow (see Uvnäs, 1966) makes no appreciable contribution to the response, although its participation cannot be excluded.

Evidence has been presented elsewhere for a second type of sympathetic vasodilator fibre which is histaminergic in nature and which is activated reflexly by stimulation of arterial baroreceptors (see Beck, 1965; Brody, 1966; Tuttle, 1966). Strong stimulation of arterial baroreceptors causes reflex vasodilatation the size of which is often in excess of that which results from loss of sympathetic vasoconstrictor tone brought about surgically or by pharmacological agents (Binet & Burstein, 1947*a*, *b*; Beck, 1961; Sakuma & Beck, 1961), and this is cited as evidence in favour of an active vasodilator component. In the present experiments, on the other hand, the lung inflation vasodilator response was never greater than the vasodilatation produced by cutting the sympathetic pathways or by administration of hexamethonium, phenoxybenzamine or bretylium tosylate. It must be pointed out, however, that the vasodilator responses observed in the present paper were obtained with lung inflation volumes of about 500 ml. and from our previous observations on dogs of similar weight (see Fig. 4 of Daly *et al.* 1967) were therefore almost certainly submaximal. No tests were carried out to determine the size of the maximum vasodilator responses for comparison with those produced by abolishing vasoconstrictor tone because of the risk of overdistending the lungs and permanently damaging them. Our experiments therefore do not provide evidence for or against the participation of these vasodilator fibres in mediating the lung inflation reflex.

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