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THE ACTION OF CERTAIN DRUGS IN THE DETERMINATION
OF INTRINSIC PULMONARY VASCULAR MECHANISMS.

by

R. Patricia Alcock, B.Sc.

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INTRODUCTION

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INTRODUCTION.

The amount of blood contained in the pulmonary vascular bed has been shown by recent work to be affected to a marked degree by nervous stimulation, powerful vasoconstriction being brought about by stimulation of the pulmonary sympathetic fibres (Daly and Euler, 1932). The nature of the nervous supply to the lungs has been worked on by many investigators with somewhat differing results. Further work on this question appears to be necessary.

Elliott, in 1904, made the suggestion that sympathetic nerves acted by the peripheral liberation of adrenaline. This theory received support in 1921, by experimental evidence shown by Loewi for both parasympathetic and sympathetic nerve impulses. Further evidence has been accumulated by a large number of workers that post-ganglionic fibres of the autonomic system act by liberating a chemical transmitter; autonomic fibres have been called cholinergic or adrenergic, as the nature of the transmitters appears to be closely allied to acetyl choline and adrenaline (Dale, 1933).

Experiments were planned, therefore, in order to discover if possible the site of action of adrenaline and acetyl choline on the isolated perfused lung vessels of/

of the dog, with the object of throwing light on their autonomic control.

Also in the intact animal and on the isolated perfused lungs adrenaline causes a rise of pulmonary arterial pressure. Exercise, however, shows little or no such rise in spite of the increased cardiac output and the presence in the circulation of large quantities of adrenaline. The problem arises as to whether there are factors determining the nature of the response to adrenaline, or possibly factors which inhibit this response. The question also arises as to whether part of the adrenaline response is due to vasodilatation and part vasoconstriction; in which case it might be possible to alter conditions so that either the pressor or the depressor component would predominate.

The effect of histamine was tried on the preparation, with regard to its role in pathological conditions, particularly that of anaphylactic shock.

METHOD.

The method used was the same as that described by Alcock, Berry and Daly (1935). In all the experiments, the isolated lungs of the dog were perfused/

perfused with defibrinated blood taken from the same animal and the lungs were inflated by rhythmical negative pressure variations applied to their outer surface. The animals were for the most part bled from the femoral artery under local anaesthesia; in a few cases they were bled from the carotid artery under general anaesthesia (chloroform and ether).

The isolated lungs were perfused at constant blood inflow, through the pulmonary circulation alone. The changes in the volume of blood in the lungs were recorded by the difference method of measuring the blood content of the venous reservoir (Daly, 1928). The pulmonary arterial pressure was usually recorded by a Marey tambour, to avoid ^{the necessity for} ~~correcting for~~ ^{the} blood accommodated in the manometer.

When a constant adrenaline infusion was given to the preparation, a mechanically propelled syringe was used, which injected four cubic centimetres an hour into the venous reservoir.

All drugs were given by injection into the tube leading to the pulmonary artery except where otherwise stated.

ADRENALINE.

Introduction.

The most usual effects of injecting adrenaline on the isolated perfused lungs of the dog are a rise of/

of pulmonary arterial pressure and a fall in the blood volume of the lungs (Berry and Daly, 1931; Gaddum and Holtz, 1933). In interpreting this result the following possibilities arise: the rise of resistance might be due to vasoconstriction of the pulmonary arterioles, capillaries or veins. The fall in lung blood volume might be due to vasodilatation, the chief site of this effect being the veins; or this effect might be due to a capacity effect, blood being squeezed out by the constricting vessels. Also it was found by Gaddum and Holtz that adrenaline only gave a fall of lung blood volume or increase in outflow in five out of ten experiments. In two experiments they found that adrenaline gave a decrease in outflow. There seem to be two main possibilities in interpreting this effect; that of capillary dilatation and that of venous constriction. The problem arose as to whether it were possible to determine the conditions causing adrenaline to give an increase or a decrease in outflow. The following experiments were tried:-

1. The effect of ergotoxine on the adrenaline response. The adrenaline pressor response has been shown to be reversed by ergotoxine in the isolated perfused lungs of the dog (Daly and Euler, 1932). This was repeated to try to see if an adrenaline dilator effect was unmasked/

unmasked by inhibition of the pressor response, or whether the pressor response was itself reversed.

The action of cocaine in increasing the sensitivity of the preparation to adrenaline was tried both before and after ergotoxine.

A constant adrenaline infusion was given to the lungs in order to reproduce to some extent the adrenalaemia of exercise. The effect of giving adrenaline injections superimposed on this background was tried.

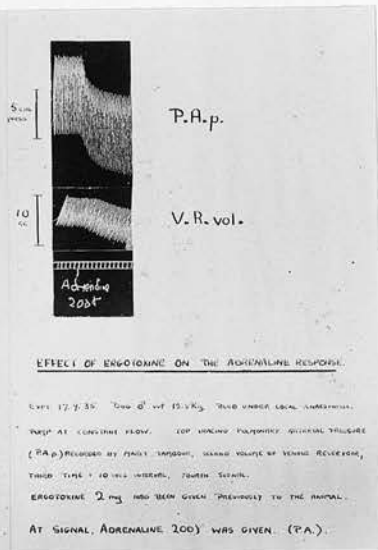
An attempt was made to determine the site of action of adrenaline in the lungs by giving adrenaline with a reversed circulation; that is the blood being driven in through the pulmonary auricle and thence to the veins by the pump and collected from the pulmonary artery into the venous reservoir.

The following experiments were done on the ^{adrenaline} outflow response:-

1. The effect of negative pressure ventilation.

If the outflow decrease seen by Gaddum and Holtz was due to capillary dilatation, then negative pressure ventilation by distending the capillaries mechanically might prevent dilatation of the capillaries from the adrenaline so in that case the outflow decrease should disappear.

2./



2. The effect of adrenaline with different phases of respiration.

3. The effect of ether on the adrenaline response.

Results.

1. The effect of single injections of adrenaline (crystalline, B.D.H.) on the isolated perfused lungs of the dog, with the pump arranged to give a constant inflow was as follows. The usual response was a rise of pulmonary arterial pressure and a rise in outflow. A fall in pulmonary arterial pressure was seen in one out of 23 experiments, and a fall in outflow in two experiments.

2. The effect of ergotoxine on the adrenaline response. The adrenaline pressor response was reversed in the ergotoxinised animal, confirming Daly and Euler. The effect on the outflow varied. Out of 20 experiments and 35 injections, 26 gave no change in outflow, 7 gave a fall in outflow and 2 gave an increase in outflow, one of these being very slight. When therefore a change in outflow was obtained after ergotoxine the effect was usually a decrease.

3. Action of cocaine on the adrenaline response.

Cocaine was given in order to enhance the pressor response to adrenaline in the isolated perfused lungs of/

See page 14.

See figure over.

of the dog, as has been shown in the whole animal (cat) by Fröhlich and Loewi (1910) and also by Burn and Tainter (1931) in isolated tissues. The enhancement, however, was slight and not always seen (4 out of 6 experiments); in the ergotoxinised animal the depressor response of adrenaline showed no significant change after administration of cocaine.

4. The action of adrenaline during constant adrenaline infusion.

The response to adrenaline injections under these conditions was qualitatively the same; that is, a rise of pulmonary arterial pressure and an increase in outflow. The animals, however, were much less sensitive to adrenaline; even with large doses (100Y to 1 mg.) very small effects were seen. Also relatively the outflow increase was larger in proportion to the rise of pulmonary arterial pressure.

5. Effect of adrenaline during reversed circulation.

An attempt was made to determine the site of action of adrenaline by trying the effect of adrenaline injections when the lungs were perfused backwards; that is, the pump sending blood through the pulmonary veins to be collected from the pulmonary artery into the venous reservoir. Injections of small doses of adrenaline gave the same response as normal, a rise of/

For interpretation

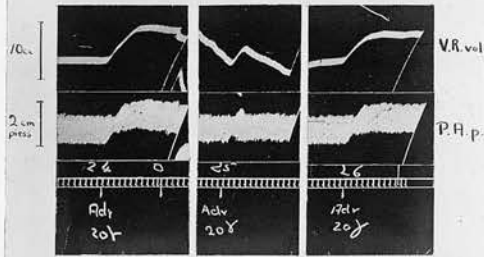
*See pages 10, 11
and 12*

of ^{in inflow} ~~pulmonary arterial~~ pressure and an increase in outflow. Two differences, however, were noted: the latent period ^{of the response} was considerably longer, and the effect itself more gradual in onset though approximately of the same magnitude. It was argued that the vasoconstriction responsible for the rise of pulmonary arterial pressure ^{in normal perfusions} might be due to constriction of arteries, capillaries or veins. ⁺ Constriction of the veins did not seem probable as in the ordinary circulation no dose of adrenaline, however large, gave a fall in outflow. Though a small constriction of widely dilated veins might result in blood being squeezed out of that area without a significant change in resistance, larger doses acting on partially constricted veins must result in a rise of resistance, and a damming back of blood in the lungs. But a series of increasing doses of adrenaline in "forward" circulation does not result in the appearance of a fall in outflow. It was argued at this point that in the "reversed" circulation, the fact that the response had a longer latent period and was more gradual in onset might indicate that the vessels constricting were further away from the veins than from the arteries; when coming from the veins the drug took longer/

+
The total pulm. resistance is very low and experiments have shown that arterial or venous constriction may affect the pulmonary arterial pressure.
J. S. D.

TRACING AND GRAPH

TO SHOW EFFECT OF RESPIRATION ON ADRENALINE RESPONSE



EXPT. 24. 1. 24. 200 g. Wt. 180. E.P. 4.50. ADRENALINE 20γ. LUNG. INSPIRED. EXPIRED.
 IN CONTROL. P.A. P. TOP. VOLUME. VOLUME OF VENTRICLE. INSPIRED. EXPIRED. ADRENALINE.
 ORIGINAL PRESSURE. TIME. TIME. TO GET. VENTRICLE. FOURTH. SIGNAL.

- 1st Signal. Adrenaline 20γ, with lungs. 'Expired'
- 2nd Signal. Adrenaline 20γ, with lungs. 'Inspired'
- 3rd Signal. Adrenaline 20γ, with lungs. 'Expired'

longer to arrive. Hence if increasing doses of adrenaline were given during a reversed circulation a point should be reached where the partly closed veins on constricting further would cause greater resistance to the flow of blood, and less squeezing out, with the net result that the response should show a fall in outflow. This experiment was carried out and a fall in outflow obtained with a large dose of adrenaline.

5. The effect of negative pressure ventilation on the response to adrenaline.

The lungs were kept inflated for a short time and adrenaline injections given (two experiments). Compared to the normal response, the effect was considerably smaller. The response was still a rise of pulmonary arterial pressure and an increase in outflow. The ratio between the pressure effect and the outflow effect changed, the outflow effect being larger than in the normal response.

The effect of adrenaline was then tried on the deflated lungs; the response was distinctly larger (one experiment). The ratio between the pulmonary arterial pressure effect and the outflow effect had also changed slightly, the outflow effect now being smaller in relation to the pressure effect.

The/

See p 12 for
interpretation

See figure over.

The effect of adrenaline on different phases of respiration was also tried during ordinary negative pressure ventilation by recording the outflow during one phase only by means of a magnet recorder, while the complete change in outflow was still recorded by the change in the venous reservoir. These experiments are not yet fully analysed, but adrenaline given in one phase, probably the inspiring phase, gave without exception (3 experiments) a fall in outflow, while the complete outflow response as shown by the venous reservoir record, was an increase.

6. In the majority of the experiments the animals did not have a general anaesthesia but were bled under local anaesthesia. The effect of the administration of ether was tried on the response to adrenaline.

Ether vapour was put into the lungs by introducing a bottle into the closed circuit between the trachea and the volume recorder. There did not appear to be any significant change in the response to adrenaline.

7. The effect of giving adrenaline not by single injections but by constant infusion into the venous reservoir, doses varying from 2γ per minute to 50γ per minute, was tried. The usual response of a rise in pulmonary arterial pressure and a rise in outflow was seen, the effects being more gradual than those from the single injections. The pulmonary arterial pressure/

These expts.
are too
inconclusive
to admit
of
discussion
see note
p. 13

B. EFFECT OF ADRENALINE GIVEN DURING

A. EFFECT OF ADRENALINE ON NORMAL ANIMAL

DATE	DOSE	P.A.P mm	V.R. vol. c.c.'s. corrected	P.A.P RECORDER	WHERE INJECTED	OUTFLOW P.A.P.
25635	2Y	+ 10	+ 1.5	MAREY	P.A	0.15
	2Y	+ 2	+ 1	TAMBOUR	P.A	0.5
	10Y	+ 6	+ 1.8		P.A	0.3
8735	50Y	+ 1	+ 1.4	WATER MANOMETER	P.A	1.4
	60	+ 3	+ 1.9		P.A	0.6
9735	2Y	+ 3	+ 0.9	WATER	P.A	0.3
	5Y	+ 2	+ 0.8	MANOMETER	P.A	0.4
	10Y	+ 3.5	+ 0.77		P.A	0.22
16735	100Y	+ 3.5	- 0.3	WATER	P.A	-0.4
		+ 1.5	- 0.3	MANOMETER	P.A	-0.6
13935	200Y	+ 17	+ 3.8	WATER	P.A	0.2
	50Y	+ 2.5	+ 1.6	MANOMETER	P.A	0.6
28635	100Y	+ 1.5	+ 1.2	WATER MANOMETER	P.A	0.8
3735	50Y	- 7	+ 1	WATER	P.A	
	50Y	+ 11	- 0.6	MANOMETER	P.A	-0.4
5.6.35	2Y	+ 1.5	0	MAREY TAMBOUR	P.A	0
13235	50Y	+ 20	0	WATER MANOMETER	P.A	0
	40Y	+ 14	0		P.A	0
15335	500Y	+ 40	+ 2.5	MAREY TAMBOUR	P.A	0.06

B. EFFECT OF ADRENALINE GIVEN DURING
CONSTANT ADRENALINE INFUSION.

DATE	DOSE	P.A.p. mm.	V.R. vA c.c.'s	P.A.P RECORDER	STRENGTH OF INFUSION	WHERE INJECTED	OUTFLOW P.A.p.
3.09.35	25Y	+ 1.5	+ 1.5	MAREY	20Y/min	P.A	1
	50Y	+ 1.5	+ 1.5	TAMBOUR		P.A	1
	250Y	+ 3	+ 2			P.A	0.66
1.10.35	25Y	+ 0.5	+ 0.25	MAREY	20Y/min	P.A	0.5
	100Y	+ 1.5	+ 0.25	TAMBOUR		P.A	0.16
	500Y	+ 2	+ 1			P.A	0.5
2.10.35	100Y	+ 2	+ 1	MAREY	5Y/min	P.A	0.5
	250Y	+ 1	+ 1.25	TAMBOUR		P.A	1.25
7.10.35	2Y	- 0.5	0	MAREY	5Y/min	P.A	
	10Y	+ 0.5	0	TAMBOUR		P.A	
	30Y	+ 1.5	+ 1			P.A.	0.66
9.10.35	200Y	+ 2	+ 1	MAREY	50Y/min	P.A	0.5
				TAMBOUR		P.A	0.66

NOTE I. ANIMAL IS LESS SENSITIVE TO ADRENALINE INJECTIONS

II. P.A.p. RESPONSE SMALLER IN RELATION TO OUTFLOW RESPONSE.

pressure and the outflow became usually however finally straight at a new level, although the infusion was still continued. The pressure effect appeared to vary with the dose, larger doses giving larger effects; but the outflow increase was greatest with a low infusion dose from 2γ to 5γ per minute.

*You
interpretation
see below*

Discussion.

The response to adrenaline in the isolated perfused lungs of the dog appeared in these experiments to be a rise in pulmonary arterial pressure and a decrease in lung blood volume, with only rare exceptions. Apart from the ergotoxinised animal, adrenaline did not cause a fall in pulmonary arterial pressure under any conditions in spite of the teleological advantages of such an effect. When single injections of adrenaline were superimposed on a constant adrenaline infusion, thus somewhat reproducing the adrenal-æmia of exercise, they still caused a rise of pulmonary arterial pressure; the whole effect, however, was very much diminished and it is possible therefore that the action of adrenaline in increasing pulmonary resistance is much lessened when there is already a large amount of adrenaline in the blood; a second hypothesis/



hypothesis is the supposition of an "exercise substance", not identical with adrenaline which dilates the pulmonary blood vessels and possibly alters or inhibits to some extent the action of adrenaline. It is possible this is the same substance as sympathin ~~in~~ I, which according to Cannon and Bacq (1931) is liberated at sympathetic nerve endings; the action of sympathin on the pulmonary blood vessels being not yet fully understood.

The action of adrenaline in raising the pulmonary arterial pressure might be due to three causes, namely vasoconstriction of the arteries, capillaries or veins. From the experiments on the reversed circulation it appeared that the main action of adrenaline was on the arterioles. It appeared from these experiments that the same vessels were responsible by their reduction in diameter for the rise in resistance and for the squeezing out of blood, resulting in the increase in outflow. This hypothesis would therefore suggest that there was no ^{pluvious} dilator component in the adrenaline response.

In these experiments under negative pressure ventilation, an increase in outflow was almost invariably seen with an injection of adrenaline; but when the different phases of respiration were recorded one/

one phase showed a fall in outflow while the whole response was still an increase. Also the results of Gaddum and Holtz on deflated lungs show a difference; out of 10 experiments, 5 gave a small increase in outflow, 3 no change and 2 a decrease in outflow. This decrease in outflow might be due either to venous constriction or to capillary dilatation.

The hypothesis that adrenaline may cause constriction of the pulmonary veins is supported by the (1932) results of Franklin who found this constriction taking place on isolated strips of pulmonary veins.

Against this contention, and in favour of the theory of capillary dilatation is the fact that the outflow decrease was shown in the magnet recorders experiments to take place in one phase of respiration only. It seems on the whole more likely that the vessels affected by ventilation are the capillaries. The results from the experiments on static inspiration and static expiration show that there is some augmentation in the outflow increase relative to the resistance change during inspiration, and some decrease relatively during static expiration. If it is supposed that the capillaries cannot dilate so much when held passively expanded by negative pressure but are dilating unhindered during expiration, this might explain/

See note on p 10

explain this change in ratio between the resistance and the outflow effects. Also in the experiments of Gaddum and Holtz the lungs were deflated, and the capillaries should have been free to dilate. This suggests an explanation for their smaller outflow increase responses and the occasional outflow decrease.

Also injections of adrenaline given during constant adrenaline infusion show this same change of ratio, this being a relatively larger outflow increase. Again it might be supposed that the capillaries, dilated by the constant infusion now offer less resistance to the squeezed out blood from the constricting arterioles.

Again the action of ergotoxine appears to favour the capillary dilatation theory. It was seen that the effect of adrenaline in the ergotoxinised animal was usually that of a fall in pulmonary arterial pressure, with no change in outflow. This effect might be interpreted to be dilatation of the arterioles. But in 7 experiments a fall of pulmonary arterial pressure combined with a fall of outflow was seen. Since the animal is ergotoxinised it appears more probable that this fall in outflow is due to capillary dilatation rather than to persistent venous constriction.

As/

As a hypothesis, therefore, it might be supposed that adrenaline constricts the arterioles and dilates the capillaries, the capillary response depending to some degree on the ventilation and condition of the lungs.

In defence of this theory, some workers have shown adrenaline to have some action in capillary dilatation. Krogh (1929) showed adrenaline caused active dilatation of the capillaries of the frog. Dale and Richards (1918) showed transient dilatation of capillaries to take place in the cat after intravenous injections of small doses of adrenaline.

ACETYL CHOLINE.

Introduction.

The action of acetyl choline on the isolated perfused lungs of the dog shows some interesting features. Both vasopressor and vasodilator effects may be obtained; the vasodilator response appears to be most readily obtained with small doses, and vasoconstriction with large. The question arises as to whether the large doses are having a toxic effect, but both vasodilator and vasoconstrictor effects are increased with eserine and abolished by atropine (Daly and Euler; Gaddum and Holtz).

It/

It appeared possible that one of these two actions of acetyl choline might be peripheral, and the other ganglionic. Chemical transmission occurs at the synapse of a sympathetic ganglion, the transmitter being indistinguishable from acetyl choline (Feldberg and Gaddum, 1933). This action of acetyl choline (or some closely allied substance) has been called "nicotine-like" (Dale, 1933). It was proposed to give injections of acetyl choline after massive doses of nicotine, so that with the ganglia paralysed, the peripheral action alone should be obtained.

It is seen that acetyl choline in large doses has an action similar to adrenaline on the isolated perfused lungs of the dog. It was planned to try the effect of ergotoxine on this adrenaline-like response.

It was also proposed to try the effect of adrenaline on the acetyl choline response. Adrenaline has been found in the isolated perfused lungs of the guinea-pig to increase the pressor response of acetyl choline (Dale and Narayana, 1935). It was proposed to repeat this on the dog in particular to see if adrenaline caused any change of the dilator response to acetyl choline.

It was also proposed to give acetyl choline with the/

the circulation reversed, but these experiments have not yet been done.

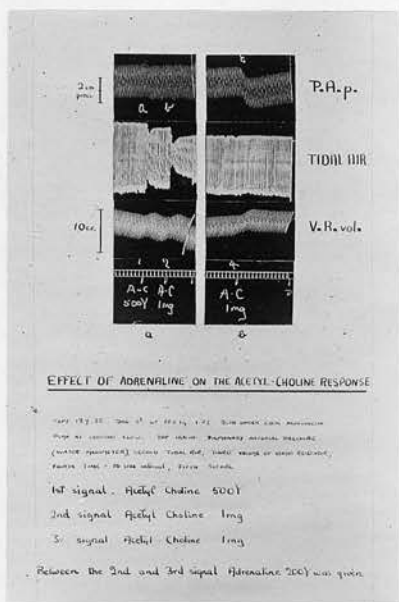
Results.

1. The effect of injections of acetyl choline (crystalline, B.D.H.) on the pulmonary arterial pressure is sometimes to cause a rise and sometimes a fall, thus confirming Daly and Euler, Gaddum and Holtz and others. The effect on the outflow was either an increase, no change or a decrease. Any one, therefore, of four effects may be seen (ignoring no change in outflow): one, a fall of pulmonary arterial pressure and a fall of outflow; two, a rise of pulmonary arterial pressure and a fall in outflow; three, a fall of pulmonary arterial pressure and a rise in outflow; four, a rise in pulmonary arterial pressure and a rise in outflow.

2. The effect of dosage.

As found by Gaddum and Holtz, the smaller doses tended to give a fall of pulmonary arterial pressure, and the larger a rise. It was also found (confirming Alcock, Berry and Daly) that smaller doses tended to give a fall in outflow, and larger doses a rise. The sensitivity of the preparation to acetyl choline was increased by eserine; after administration of atropine no effects from acetyl choline were seen.

3./



- A. EFFECT OF INCREASING ACETYL CHOLINE DOSE
IN NORMAL ANIMAL
3. The effect of single doses of adrenaline on the response to acetyl choline was tried. In 5 experiments the dilator response of acetyl choline, that is the fall in pressure, was increased by injection of adrenaline. In one experiment the pressor response of acetyl choline was converted to a depressor response.
 4. The effect of acetyl choline during a constant infusion of adrenaline.

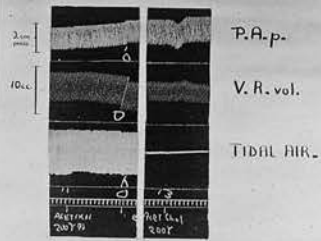
Out of 18 injections of acetyl choline given during constant adrenaline infusion, on 6 animals, 14 gave a fall of pulmonary arterial pressure, 2 no change and 2 gave a rise. The doses were large (from 100 μ to 1 mg., with 2 exceptions); eserine was present for 7 of the injections. Compared to the normal preparation without adrenaline, this showed a large preponderance of the dilator response. All doses large enough to cause a change in pulmonary arterial pressure (that is, except two) gave a rise in outflow. In one experiment one milligram of acetyl choline caused a rise before the adrenaline infusion and a fall during the infusion. In another experiment the same dose of acetyl choline caused no effect before the infusion and a fall during it. In a third experiment acetyl choline before the adrenaline infusion caused a fall of pulmonary arterial pressure and/

A. EFFECT OF INCREASING ACETYL CHOLINE DOSAGE IN NORMAL ANIMAL.

DATE	DOSE	P.A.p	V.R. vol	TIOAL AIR	P.A.P RECORDER	ESERINE	WHERE INJECTED
25635	50Y	-	0	0	MAREY	Abs.	P.A
	200Y	+	0	-	TAMBOUR	Abs.	P.A
28635	50Y	-	+	-	WATER MANOMETER	Pres.	V.R.
	100Y	+	0	cc		Pres	V.R.
	500Y	+	+ -	cc		Pres.	V.R.
12735	100Y	-	0	-	WATER MANOMETER	Pres	V.R
	200Y	+	-	-		Pres	V.R
19735	200Y	+	+	0	WATER MANOMETER	Abs.	P.A
	50Y	-	0	0		Abs.	P.A
30735	50Y	-	+	-	MAREY	Pres.	V.R.
	100Y	+	0	cc	TAMBOUR	Pres.	V.R.
13935	250Y	-	+	cc	WATER MANOMETER	Pres	P.A
	1mg	+	+	cc		Pres	P.A
6235	200Y	+ -	0	-	MAREY	Abs	P.A
	200Y	+	0	-	TAMBOUR	Abs	P.A
25735	500Y	- +	+	-	WATER MANOMETER	Abs.	P.A
	500Y	+	+	-		Abs	P.A

**B. EFFECT OF INCREASING ACETYL CHOLINE DOSAGE
DURING CONSTANT ADRENALINE INFUSION.**

DATE	DOSE	P.A.p.	V.R.vol (CORRECTED)	TIDAL AIR	WHERE INJECTED	P.A. P RECORDER	ESERINE	STRENGTH OF INFUSION PER MINUTE
30935	100γ	0	-	0	P.A.	MAREY	Abs.	20γ
	650γ	0	-	-	P.A.	TAMBOUR	Abs.	
	100γ	-	+	-	P.A.		Pres	
	500γ	+	+	cc	P.A.		Pres	
1.10.35	100γ	-	0	0	P.A.	MAREY	Abs	20γ
	500γ	-	+	-	P.A.	TAMBOUR	Abs.	
	1mg	-	+	-	P.A.		Abs	
	500γ	-	+	-	P.A.		Pres	
2.10.35	500γ	-	+	-	P.A.	MAREY	Abs	5γ
	1mg	-	+	-	P.A.	TAMBOUR	Abs.	
	750γ	-	+	cc	P.A.		Pres	
7.10.35	10γ	-	0	0	P.A.	MAREY	Abs	5γ
	50γ	-	0	-	P.A.	TAMBOUR	Abs	
	100γ	-	0	-	P.A.		Abs	
	500γ	-	+	cc	P.A.		Pres	
	1mg	-	+	cc	P.A.		Pres	
9.10.35	1mg	-	0	-	P.A.	MAREY TAMBOUR	Abs	50γ
16.10.35	500γ	+	+	cc	P.A.	MAREY TAMBOUR	Pres	5γ



EFFECT OF ERGOTAMINE ON THE AORTIC ORIGIN RESERVE

Case of J. H. ...
 First signal ...
 Second signal ...

1st signal. Acetyl Choline 200Y

2nd signal. Acetyl Choline 200Y

Between 1st and 2nd signal Ergotamine 2.5mg was given.

and a fall in outflow; the same dose during the infusion caused a fall of pulmonary arterial pressure but no change in outflow. Apparently, therefore, the presence of adrenaline increased the occurrence of this effect of acetyl choline, namely a fall of pulmonary arterial pressure and a rise in outflow and also augmented to some degree the response itself.

4. Action of ergotoxine on the acetyl choline response.

In three experiments the administration of ergotoxine converted a pressor response (rise of pulmonary arterial pressure) of acetyl choline to a depressor response; in one the response was already depressor and was not altered by the ergotoxine. Out of four experiments when the animal was nicotinated, one showed the pressor response of acetyl choline reversed by ergotoxine, two showed the pressor response suppressed, and in one the pressor response remained although reduced in size. In the two experiments in which the response was suppressed, increasing the dose to 3 mg. produced a small rise of pulmonary arterial pressure.

5. Effect of nicotine.

After massive doses of nicotine both effects of acetyl choline on the pulmonary arterial pressure were seen.

Discussion.

EFFECT OF ERGOTOXINE ON THE ACETYL CHOLINE RESPONSE

DATE	ACETYL CHOLINE BEFORE ERGOTOXINE			DOSE OF ERGOTOXINE	ACETYL CHOLINE AFTER ERGOTOXINE			WHERE INJECTED	PREVIOUS DRUGS			
	DOSE	P.A.p.	V.R.vol		DOSE	P.A.p.	V.R.vol		ESERINE	NICOTINE	OTHER DRUGS	
19735	200Y	+	0	2.5mg	200Y	-	0	P.A	Abs.	Abs.	None	
24735	500Y	+	+	3mg	500Y	-	+	P.A.	Abs.	Abs.	None	
					500Y ¹	-	+	P.A	Abs.	Abs.	None	
25735	500Y	+	+	2mg ²	500Y	-	+ -	P.A	Abs.	Abs.	None	
					500Y	-	+	P.A	Pres.	Abs.	None	
26735	50Y	-	+	3mg	50Y	-	0	V.R	Pres	Abs	None	
					750Y	-	+	V.R.	Pres.	Abs.	None	
12935	1mg	+	+	3mg	1mg	0	0	P.A	Pres.	Pres.	None	
					3mg	+	+	P.A	Pres.	Pres.	None	
					3mg	-	0	P.A	Pres.	Pres.	ADRENALINE	
17935	200Y	+	+	2mg	200Y	-	+	P.A.	Pres.	Pres.	None	
					3mg	200Y	-	+	P.A.	Pres.	Pres	None
					500Y	-	+	P.A.	Pres.	Pres.	None	
18935	100Y	+	+	3mg	100Y	+	0	P.A	Pres.	Pres.	None	
					400Y	+	+	P.A	Pres.	Pres.	None	
23935	500Y	+	+	5mg	500Y	0	0	P.A	Pres.	Pres.	None	
					3mg	+	-	P.A	Pres.	Pres.	None	

1. WITH PRESSURE RETURNED TO NORMAL AFTER THE ERGOTOXINE RISE

2. PRESSURE NOT ALLOWED TO RISE WITH THE ERGOTOXINE, BUT KEPT FLAT BY ALTERING PUMP.

Discussion.

Both pressor and depressor responses were obtained from acetyl choline, smaller doses giving the depressor, and larger doses the pressor, confirming Gaddum and Holtz.

Adrenaline appeared to increase the dilator response of acetyl choline. It is suggested as a hypothesis that adrenaline is giving tone to the arterioles so that they dilate more readily. Adrenaline may here be having a similar action to that shown by Dale and Richards, who found the presence of adrenaline to be necessary in obtaining vasodilator effects of histamine in the perfused vessels of the cat.

The acetyl choline response that appears to be increased by adrenaline in this preparation is that of a fall in pulmonary arterial pressure with a rise in outflow. If the hypothesis is ^{put forward} ~~made~~ that this effect is due to dilatation of arterioles together with constriction of capillaries, then it might be that adrenaline constricting the arterioles made them more ready to dilate, and dilating the capillaries made them more ready to constrict. Here then acetyl choline and adrenaline would be acting in opposition as is the case on most tissues of the body.

Larger/

Larger doses of acetyl choline, however, particularly if adrenaline is not present, cause a rise of pulmonary arterial pressure, with an increase in outflow. The capillary constriction may still be present but the question arises as to whether the arterioles are constricting, when with smaller doses they were dilating. The action of ergotoxine was to reverse this rise of pulmonary arterial pressure, but then giving a larger dose of acetyl choline ^{brought it} ~~got~~ back the rise. It might be possible that ergotoxine reverses the acetyl choline constriction of the arterioles, which appears to be a somewhat adrenaline-like action, but does not affect the capillary constriction, which however would require a large dose to cause sufficient wide-spread constriction to result in a rise of resistance.

Acetyl choline, therefore, according to this hypothesis, dilates arterioles and constricts capillaries; in large doses it has an adrenaline-like action in constricting arterioles, which action is reversed by ergotoxine.

It is seen that acetyl choline has little or no constrictor action during constant adrenaline infusion. It might be argued from this that parasympathetic stimuli at least would not give rise to an increase in/

in pulmonary vascular resistance during exercise. This supposition has the support that it would obviously be of advantage to the animal.

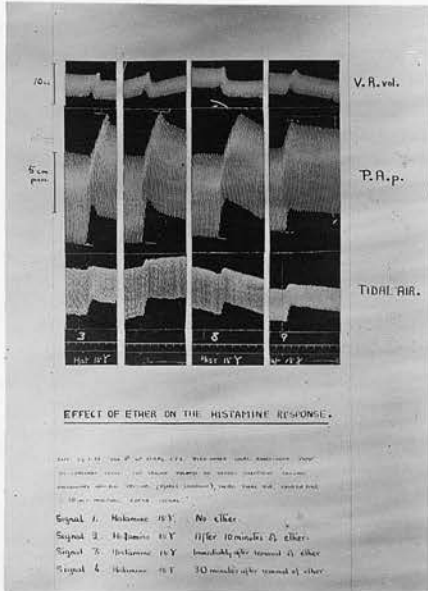
The effect of acetyl choline in the nicotised animal may be either pressor or depressor. It seems probable therefore that in these effects acetyl choline is peripheral in its site of action, and that the ganglionic action of acetyl choline does not play much part in this preparation.

HISTAMINE.

Results.

1. The action of injections of histamine on the pulmonary arterial pressure was pressor. No depressor response has been seen from histamine. The action of histamine was as follows: in the earlier experiments the response was a decrease in outflow (one exception, one injection only). These experiments were done during June and July. Experiments done during September, October, November, December and January showed sometimes an inflow increase and sometimes a decrease.
2. Effect of dosage. The preparation used was crystalline histamine acid phosphate (B.D.H.), dosage being given in terms of the base.

In/



In no experiment was a fall of pulmonary arterial pressure seen, the minimum effective dose of histamine giving a rise. In some experiments the smallest dose of histamine gave a fall in outflow, but in some a rise in outflow was changed to a fall by increasing the dose.

3. In the earlier experiments, those done during June and July, the typical outflow response was a fall. In the experiments done during the winter months the outflow response was usually a rise. This gave the impression of a possible seasonal variation, but it was found that in the earlier experiments the animals had been induced by a general anaesthetic (50/50 ether + chloroform). In the later experiments the animals were bled under local anaesthesia. Experiments were then planned to test the effect of anaesthetic ether on the histamine response of the lungs. Ether vapour was put into the lungs by introducing a bottle into the closed circuit between the trachea and the volume recorder. It was found that the pulmonary arterial pressure response was not much altered but the outflow response was changed from a rise to a fall. When the ether had been removed for $\frac{1}{2}$ hour, histamine again caused an increase in/

in outflow. It was noted that in the experiments where the animals were induced with a general anaesthetic, the preparations were less sensitive to histamine generally but gave a fall in outflow.

4. Effect of increasing the venous pressure.

It appeared over a series of experiments that a raised venous pressure increased the tendency of histamine to cause a rise in outflow. This was found to be the case on experiment, an increase in venous pressure causing the outflow decrease to become smaller or even to become an increase. Raising the venous pressure, however, holds back some blood in the lungs. There is, therefore, some change in dilution and further experiments are necessary to control this.

5. Effect of histamine in reversed circulation.

Histamine in normal circulation gave a rise of pulmonary arterial pressure and a fall in outflow; with the reversed circulation a rise in pulmonary arterial pressure with a rise in outflow was seen.

Discussion.

Two types of response have been seen from histamine, a rise of pulmonary arterial pressure with a rise of outflow, and a rise of pulmonary arterial pressure/

pressure with a fall of outflow.

In causing the rise of resistance, either arteries, capillaries or veins might be constricting; any of these might also account for the rise in outflow due to a "capacity effect", that is blood being squeezed out from the constricting vessels. The fall in outflow, however, seems most likely to be due either to capillary dilatation or to a venous constriction, marked enough to cause a rise in resistance and hold blood back in the lungs.

If the main site of action of histamine was on the arterioles or the capillaries, then the change from an outflow decrease ^{to an increase} would necessitate a complete change of action of histamine; but if it is supposed that the main action of histamine is on the veins then the following should occur. A small effect on widely dilated veins will result in a large capacity and a low resistance response; that is, a rise in pulmonary arterial pressure and a rise in outflow. But a stronger effect on partially constricted veins will result in a large resistance and a low capacity effect; blood will be held back in the lungs and the response will be a rise of pulmonary arterial pressure and a fall in outflow.

The action of ether itself when introduced to the lungs/

lungs was to cause a slight rise of pulmonary arterial pressure and a prolonged fall of lung blood volume. This effect was unexpected. Burn and Bhatia (1933) found isolated perfused vessels of the hind limb to be unaffected by ether, the sympathetic effects in the whole animal being abolished by nicotine. It is possible the pulmonary vascular bed has a particular sensitivity to anaesthetics.

If the ether which appears to be causing some vasoconstriction is actually partially closing the veins, then it is clear that the same dose of histamine will now cause a fall in outflow, the diameter of the vein having reached the point where further closing will cause resistance rather than capacity effects.

As far as can be judged from the present experiments, the raising of venous pressure has opposite effects. The blood is held back in the lungs, and the veins are distended. Histamine now shows a capacity rather than a resistance effect.

The effect of histamine during reversed circulation also bears this out as a fall in outflow becomes converted to a rise with the backwards circulation. Further experiments, however, are also necessary here.

GENERAL CONCLUSIONS.

It is seen that the actions of adrenaline and histamine on the isolated perfused lungs of the dog have a marked superficial resemblance. Both drugs cause a rise of pulmonary arterial pressure and both cause sometimes a rise in outflow and sometimes a fall. Some resemblance between the actions of adrenaline and histamine was observed by Dale and Richards (1918) in the hind limb vessels of the cat. To quote from their paper "... the conditions of vascular tone which favour the predominance of either vasoconstriction or vasodilatation in the normally enervated leg are the same for histamine as for adrenaline..." It appeared an attractive theory that adrenaline and histamine acted here on the pulmonary vessels at the same site, but most of the experimental evidence appears to favour the theory that the resemblance is chiefly superficial and entirely different vessels are contracting with the two drugs.

It is seen that with small doses of acetyl choline and adrenaline typically antagonistic actions are produced. But it appears in larger doses the two drugs here act together even to the possibility of reversal/

reversal by ergotoxine. The pressor response, however, given by acetyl choline shows this difference to the adrenaline rise in that it can be abolished by atropine.

SUMMARY.

The actions of adrenaline, acetyl choline and histamine on the isolated perfused lungs of the dog were found to be in general the same as those published by Alcock, Berry and Daly.

The pressor response of adrenaline was not greatly enhanced by cocaine.

The pressor responses of adrenaline and acetyl choline could be reversed by ergotoxine.

The effect of ether on adrenaline was negligible.

The effect of ether on histamine was to convert an increase in outflow to a decrease.

The effect of adrenaline, acetyl choline and histamine on the isolated perfused lungs of the dog were tried under varying conditions.

REFERENCES.

- Alcock, P., Berry, J.L. and Daly, I. de Burgh. (1935).
Quart. J. Exp. Physiol., 25, 369.
- Berry, J.L. and Daly, I. de Burgh. (1931). Proc. Roy.
Soc. B, 109, 319.
- Burn, J.H. and Bhatia, B.B. (1933). J. Physiol., 78,
257.

- Burn, J.H. and Tainter, M.L. (1931). *J. Physiol.*, 71, 169.
- Cannon, W.B. and Bacq, Z.M. (1931). *Amer. J. Physiol.*, 96, 392.
- Dale, A.S. and Narayana, B. (1935). *Quart. J. Exp. Physiol.*, 25, 85.
- Dale, H.H. (1933). *J. Physiol.*, 80, 10P.
- Dale, H.H. and Richards, A.N. (1918). *J. Physiol.*, 52, 110.
- Daly, I. de Burgh. (1928). *J. Physiol.*, 65, 422.
- Daly, I. de Burgh. (1933). *Physiol. Rev.*, 13, 149.
- Daly, I. de Burgh and Euler, U.S.v. (1932). *Proc. Roy. Soc. B*, 110, 92.
- Elliott, T.R. (1904). *J. Physiol.*, 31, 20P.
- Feldberg, W. and Gaddum, J.H. (1933). *J. Physiol.*, 80, 12P.
- Franklin, K.J. (1932). *J. Physiol.*, 75, 471.
- Fröhlich, A. and Loewi, O. (1910). *Arch. exp. Path. Pharmak.*, 62, 159.
- Gaddum, J.H. and Holtz, P. (1933). *J. Physiol.*, 77, 139.
- Krogh, A. (1929). *The Anatomy and Physiology of the Capillaries*. New Haven and London.
- Loewi, O. (1921). *Pflügers Arch.*, 189, 238.
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THE ACTION OF DRUGS ON THE PULMONARY CIRCULATION.
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the Physiology Departments, University of Birmingham and
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THE ACTION OF DRUGS ON THE PULMONARY CIRCULATION.

By P. ALCOCK, J. L. BERRY, and I. DE BURGH DALY. From the Physiology Departments, Universities of Birmingham and Edinburgh.

(Received for publication 30th October 1935.)

THE present-day conceptions of neuro-humoral transmitters and of the release of a histamine-like substance in certain organs during anaphylactic shock have given added significance to what is known of the physiological action of the three substances—acetylcholine, adrenaline, and histamine. The lungs are of particular interest in this respect, for there is already strong presumptive evidence that the local release of these substances plays an important part in controlling the air and to a lesser degree the blood entering the lungs under physiological or, in the case of histamine, pathological conditions. The demonstration of the release of a substance with the pharmacological properties of acetylcholine from the lungs during vagal stimulation [Thornton, 1934] and of the release of a histamine-like substance during anaphylactic shock [Bartosch, Feldberg, and Nagel, 1932 *a*, 1932 *b*, 1933; Spinelli, 1932; Daly, Peat, and Schild, 1935] falls into line on the one hand with the conception of neuro-transmitters extensively developed in the laboratories of Loewi and of Dale, and on the other with the "histamine-release" theory of anaphylactic shock [Dale, 1929]. With regard to the action of adrenaline on the lungs, attention of recent years has chiefly been directed towards its bronchodilator action, it being widely held that the sympathetic innervation of the lungs is too weak to exert an appreciable effect on the blood-vessels. It has, however, been shown by Daly and Euler [1932] that if precautions are taken to maintain the viability of the pulmonary nerves, stimulation of the pulmonary sympathetic fibres leads to powerful vasoconstriction. For this reason interest in the mode of action of adrenaline, adrenaline-like substances or sympathin [Cannon] on the pulmonary vascular bed should not be allowed to relax. That stimulation of the pulmonary sympathetic nerves and the exhibition of adrenaline cause an increase in pulmonary arterial pressure in isolated perfused lungs cannot be denied, yet such evidence as we possess shows that this pressure does not rise greatly during

exercise [Shaw Dunn, 1919]. If pulmonary constriction did occur during exercise because of sympathetic nerve activity and of an increase in the circulating adrenaline, the ensuing cardiac embarrassment with a reduction in the pulmonary circulation rate and in oxygen uptake would seriously impair the functional capacity of the individual to take exercise. In this connexion Barcroft [1934] aptly remarks that "the musculature of the pulmonary vessels has a significance from the point of view of constriction, surely its main significance must be to confer on these vessels the power of dilatation." One explanation of the discrepancy mentioned above may be that the condition of the pulmonary blood-vessels in isolated preparations is quite different from that in the normal animal, their response to sympathetic stimulation and to adrenaline being dilator rather than constrictor. It must be confessed that the available evidence obtained from experiments on anaesthetised animals lends little support to this suggestion since the pulmonary vessel response in respect of sympathetic nerve-stimulation and adrenaline injections in the entire animal appears to be the same as that obtained from perfused preparations. We have attempted to clarify the position by making extensive tests with minimal effective doses of adrenaline.

In a recent review [Daly, 1933] the results of some forty-three experiments on the action of adrenaline on isolated perfused lungs of twelve species were tabulated, from which there appeared good evidence in support of the original contention of Tribe [1914] that in some species the smaller doses tend to cause vasodilatation, the larger vasoconstriction. That this effect may in part be attributed to adrenaline producing constriction on one part of the pulmonary vascular bed and dilatation on another has been shown by Gaddum and Holtz [1933] who described a constrictor action of adrenaline on the pulmonary blood "inflow" and a dilator action on the "outflow." To what extent these effects take place in lungs under negative pressure ventilation forms part of our inquiry.

We have paid particular attention to the action of small doses of adrenaline and acetylcholine, because the release of physiological quantities of the chemical transmitters during activity of adrenergic or cholinergic fibres [Dale, 1933] is extremely minute. Although we did not expect to obtain vascular responses with such small physiological quantities when injected into the blood stream of a relatively insensitive preparation as compared with their natural and intimate release in the normal animal, we hoped by measuring the response to injection of minimal effective dose to obtain comparative information as to the effect of a physiological release of the transmitter and not of what might be its toxic action. The experimental conditions have been varied from time to time in order to eliminate the possibilities of technical error and these will be described in the appropriate places.

METHODS.

In all the experiments the pulmonary circulation of dogs was perfused with defibrinated blood taken from the same animal, and with the exception of one series of experiments the lungs were inflated by rhythmical negative pressure variations applied to their outer surface. The animals were bled from the carotid artery under chloroform and ether anæsthesia, or under nembutal (45 mg. per kg. body weight) injected intravenously under local anæsthesia, or, as in the majority of experiments, through a cannula introduced into the femoral artery under local anæsthesia in order to eliminate the action of a general anæsthetic.

We have used three different methods for determining the action of the substances under investigation. Their effect upon the pulmonary arterial pressure has been measured (1) in isolated lungs perfused at constant blood inflow through the pulmonary and bronchial circulations [Berry and Daly, 1931], and (2) in lungs perfused through the pulmonary circulation alone, either at constant pressure or constant inflow of blood. In some experiments the changes in the volume of blood in the lung were also recorded by the difference method of measuring the blood-content of the venous reservoir [Daly, 1928]. The drugs were injected into the pulmonary arterial tubing unless otherwise stated: the solutions were warmed, and control injections of Tyrode solution in which the drugs were usually dissolved were made from time to time.

If the pulmonary arterial pressure is recorded with a blood manometer connected to a volume-recording system, a rise in pressure in the manometer will deplete the venous reservoir by the extra amount of blood accommodated in the manometer. Although it is possible to correct for this error, we used a Marey tambour for measuring the pulmonary arterial pressure in many of the experiments: the capacity changes in this instrument were negligible during the pulmonary arterial pressure alterations.

Gaddum and Holtz [1933] showed that drugs may act in the opposite sense on the blood inflow and outflow of perfused lungs, and we use the terms "inflow" and "outflow" in their sense—accepting tentatively that effects on arterioles or capillaries produce an action on the inflow, and those on the veins or capillaries an action on the outflow. It is clear, however, that arteriolar effects may influence the outflow. Gaddum and Holtz used non-ventilated perfused lungs in which they recorded the lung volume and the pulmonary blood outflow. They pointed out that as regards the outflow effects it was not possible to distinguish between venous constriction and capillary dilatation or between venous dilatation and capillary constriction. The blood volume changes of the lungs of dogs produced by adrenaline, acetylcholine, or histamine in their experiments were surprisingly small, being in the experiments they describe less than 1.0 c.c., and the authors suggested that much larger changes might occur under more physiological

conditions when the lungs are being normally ventilated. In order to observe the effect upon the blood inflow and outflow in lung preparations perfused at constant pressure, we devised a third method, described in the next section, which is suitable for lungs under negative pressure ventilation. In the tracings the methods employed are easily distinguishable. The pulmonary arterial pressure records are taken at

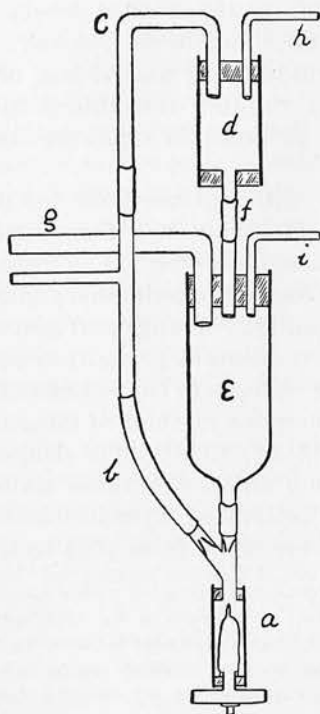


FIG. 1.—Perfusion apparatus for measuring blood inflow and outflow of lungs under negative pressure ventilation. For description see text.

constant inflow perfusion, and the inflow and outflow of blood records at constant pressure perfusion.

Perfusion Apparatus for Measuring Inflow and Outflow.—A Dale and Schuster [1928] pump, *a* (fig. 1), perfuses the lungs through the tube *b* at a constant pressure of 10 to 15 cm. of blood determined by the height of the wide bore overflow tube *c* above the level of the heart. The output of the pump is adjusted so that the blood just trickles over into the arterial overflow reservoir *d*, thence to the venous reservoir *e* by way of the connecting tube *f* which is fitted with a screw clip. The venous return from the lungs enters the venous reservoir through the wide bore tube *g*. The air spaces of the overflow (*d*) and venous (*e*) reservoirs are connected to small vertical volume recorders at *h* and *i* respectively; for convenience of comparison of the tracings recorders were made, the

calibration of which did not differ by more than 4 per cent. The apparatus depicted in fig. 1 is kept in the region of 37° to 40° C. by enclosure in a copper thermostat fitted with automatic temperature control. The whole animal with the lungs *in situ* is also maintained at approximately the same temperature in the negative pressure respiratory tank [for full details see Berry and Daly, 1931].

On closing the connecting tube between the overflow and venous reservoirs by adjustment of the screw clip *f*, on which is fitted an extension handle passing to the outside of the thermostat, the overflow reservoir gains as much blood as the venous reservoir loses. This is true on the assumption that the output of the pump is constant at the low pressures used and that the blood volume of the lungs remains unchanged. With pulmonary inflow equal to the outflow, the lever of the overflow reservoir recorder writes a gradually upward sloping tracing, the angle (from the horizontal) of which is equal to that of the downward sloping tracing obtained from the venous reservoir volume recorder. Under these conditions, therefore, the venous outflow tracing is a mirror image of the arterial overflow tracing, if the mirror is placed in a horizontal plane. Any departure from mirror imagery following the injection of drugs will denote an alteration in blood capacity of the lungs which may be due to effects on the blood-vessels controlling the inflow or the outflow. In fig. 10 *a* the effect of screwing up and then partially loosening a clamp placed on the pulmonary arterial tubing is shown. The temporary increase in the overflow record is accompanied by a temporary diminution in the venous outflow record.

The method has been tested by measuring the inflow to and outflow from a rubber bag in a plethysmograph in place of the lungs. Curves were constructed giving the relationship between the angle of slope of the recorder tracings and the flow in c.c./min. for smoked paper velocities of 1.6, 1.8, and 2.0 cm./min. which were within the limits of the paper velocity for all experiments. The scale was large enough to read off (from the angles measured on the tracings) the flows to the nearest c.c./min. Various combinations of inflow and outflow diminution or increase were imitated on this model by adjustments of the screw clips on the inflow and outflow tubes carried out simultaneously or repeated at short intervals of time (5 to 15 secs.). The rubber bag took rather a long time to reach its new volume after such manipulations (capacity effect) and in consequence the final equilibrium of inflow and outflow took some minutes. Nevertheless, the discrepancy between inflow and outflow after such manipulations was as a rule not more than 10 per cent. if care was taken to allow equilibrium to be established. Since the flows are proportional to the tangents of the angles of the tracings, the accuracy of measurement diminishes as the region of higher angles is approached, and when angles greater than 65° were involved, the measurements showed an error larger than 10 per cent. The kymograph paper therefore should be so adjusted that at the greatest flows the angle is not larger than 65° . In view of the fact that the base line of each recorder is initially at an angle to the horizontal and then changes to a new angle as a result of experimental procedures, it is necessary to measure the *initial* and *final* angles of each record and then read off on the graph the corresponding initial and final flows into each reservoir. The difference between the initial and final flow thus obtained for each recorder indicates the blood inflow and outflow changes to and from the lungs at constant pressure perfusion.

The method is sensitive, needs only simple recording instruments, and can be used for lungs under positive or negative pressure ventilation. It was devised some years ago and subsequently used by Macgregor [1933] for perfusion of the isolated lungs of the cat.

ACETYLCHOLINE.

Reid Hunt [1918] reported that large doses injected into the isolated perfused lungs of the cat, rabbit, and guinea-pig reduced the rate of outflow, an effect which was not abolished by atropine. Later work, however, has shown that acetylcholine in perfused preparations may cause pulmonary vaso-constriction or -dilatation depending upon the species of animal and the strength of dose used. In the dog vaso-constriction or -dilatation may take place, either response being enhanced by eserine and suppressed by atropine [Daly and Euler, 1932]. Large doses of acetylcholine cause inflow vasoconstriction and small doses vasodilatation, an effect also probably on the inflow [Gaddum and Holtz, 1933]. Tronci [1934] observed very large doses of acetylcholine to decrease the pulmonary outflow. In the cat a vasoconstrictor response was reported by Hirose [1932] and also by Gaddum and Holtz, who showed that inflow and outflow constriction occurred with the larger doses and vasodilatation, the site of which was not determined, with smaller doses. In the lungs of the rabbit perfused at constant inflow, acetylcholine leads to a rise in pulmonary arterial pressure, an effect which is enhanced by eserine and suppressed by atropine [Euler, 1932]. In the guinea-pig's lungs perfused at constant pressure the ester causes a diminution in outflow [Dale and Narayana, 1935].

Perhaps the most interesting features of the work quoted above are the pulmonary vasoconstrictor response to large doses and the vasodilator response to small doses of acetylcholine. In attempting to discover the significance of this effect we have confined our attention almost exclusively to dogs, because we had already made certain observations which appeared to shed light on the mechanism involved.

RESULTS.

In all experiments acetylcholine bromide (B.D.H.) was used. The response to a dose of acetylcholine may be vaso-constrictor or -dilator (figs. 2, 3, and 5), but we have been unable to determine with certainty all the conditions which govern the type of response, although in general our results confirm the contention of Gaddum and Holtz that the larger the dose the greater the tendency to inflow constriction or to a rise in pulmonary arterial pressure. The results of thirty-seven successive experiments are incorporated in a graph (fig. 4), in which the test

corresponding to each experiment are placed on the same abscissa. Further experiments in which the minimal effective dose of acetylcholine was determined are not incorporated in the Table. In each experiment tests were made starting with the smaller doses which were gradually increased, but after the administration of eserine the dose was again reduced and gradually raised with succeeding injections.

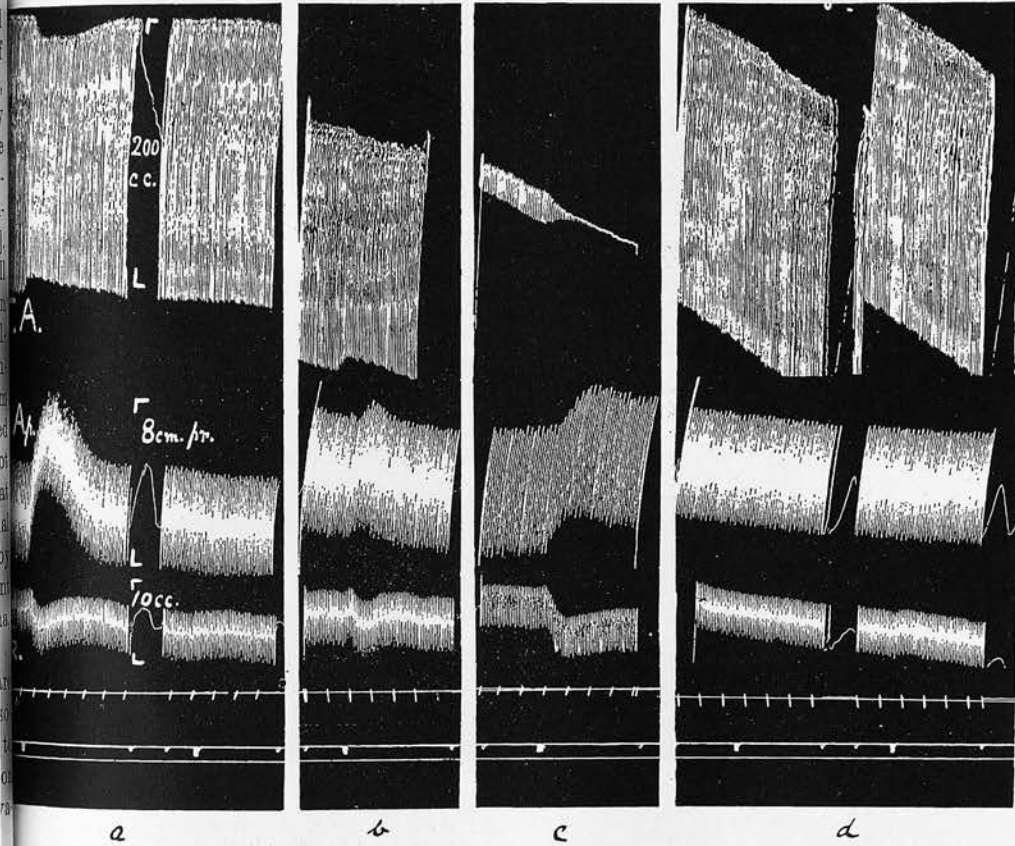


FIG. 2.—Dog. 11 kg. I.P.L. Constant inflow perfusion. Upper tracing =tidal air (T.A.); 2nd =pulmonary arterial pressure (P.A.p.); 3rd =volume of venous reservoir (V.R.); 5th =30 sec.
 a =two successive injections of nicotine 50 mg. each; b =acetylcholine (A.C.) 1.0 mg. Eserine 5 mg. given between b and c; c =A.C. 1.0 mg. Atropine 5 mg. given between c and d; d =A.C. 1.0 mg. followed by nicotine 50 mg.

The vasodilator response to acetylcholine is more evanescent than the vasoconstrictor, and on occasion has been seen to disappear and give place to a vasoconstriction or even to a diphasic change in the pulmonary arterial pressure on injection of a series of equal doses. The diphasic response may show as a fall in pressure followed by a rise, or more rarely a rise followed by a fall. Moreover, the sensitivity of preparations to

acetylcholine varies considerably as regards the vascular response, 20 to 30 per cent. showing 1.0 cm. or less blood-pressure change to initial doses of 1.0 mg. acetylcholine in eserinated preparations, although such doses produce as a rule well-marked bronchoconstriction in the non-eserinated preparation.

Effect of Perfusion Pressure on the Pulmonary Arterial Response.—From

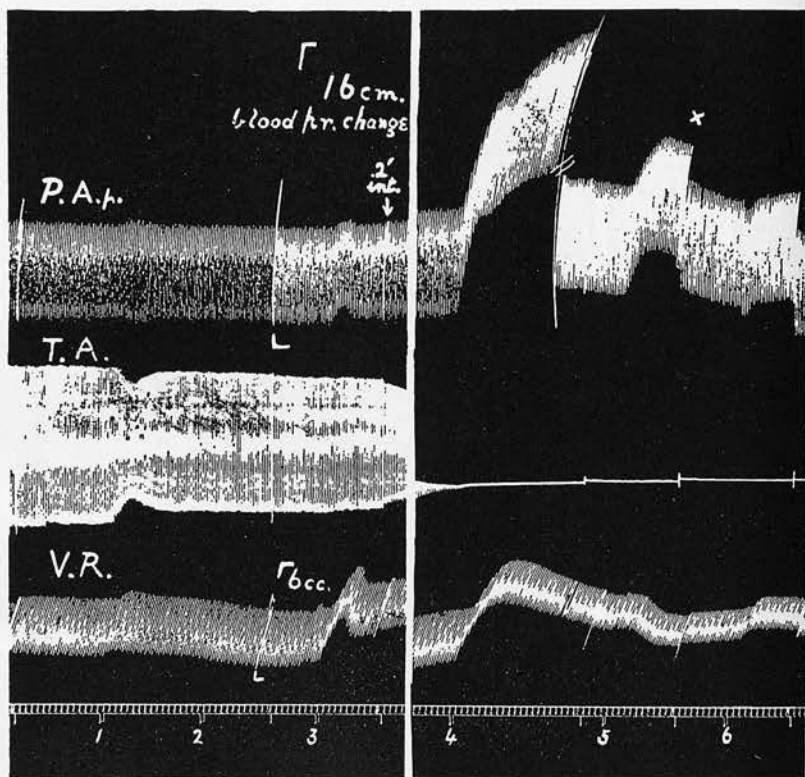


FIG. 3.—12.5 kg. I.P.L. Constant inflow perfusion. 1=nicotine 15 mg.; 2=nicotine 15 mg.; 3=eserine 3.0 mg.; 4=A.C. 200 γ ; 5=ergotamine 2 mg.; 6=A.C. 200 γ . At x the pump output was reduced to bring back the P.A.p. to same level as that previous to first dose of A.C.

time to time we have been impressed by the fact that a dose of acetylcholine injected into the pulmonary artery at a high perfusion pressure tends to cause a smaller vasoconstriction or a larger vasodilatation than one of equal strength given at a low perfusion pressure. We were inclined at first to consider that the phenomena might be due to a difference in the response of the blood-vessels in a stretched and in a relaxed condition, or to acetylcholine acting in an opposing sense upon two territories of the vascular bed, the raised perfusion pressure opening up more of the territory which responds by dilatation [Daly, 1933]. The fact that the vascular response is largely determined by the strength of dose of

acetylcholine exposed a new field of enquiry, for if acetylcholine is injected into the pulmonary artery its concentration in the blood reaching the lungs will depend upon the rate of injection, the velocity of circulation, and possibly upon the blood volume of the lungs.

Fig. 5, a, b, c, shows that an arterial injection of 1.0 mg. of acetylcholine at a perfusion pressure of 16 cm. blood causes a rise in pressure,

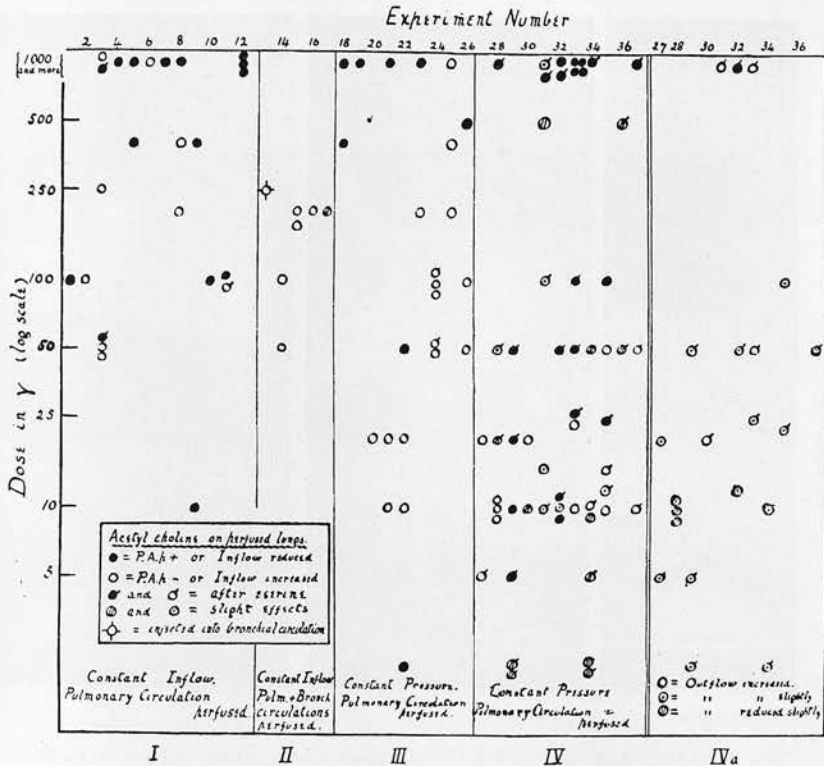


FIG. 4.—Action of acetylcholine on perfused lungs. P.A.p.=pulmonary arterial pressure. In experiments under sections I., II., and III., the lungs were under negative pressure ventilation. In experiments under IV. and IV.a the lungs were not respired. Sections IV. and IV.a show blood inflow and outflow changes of experiments 27-37 inclusive.

a second injection of the same amount given after the perfusion pressure had been raised to 36 cm. blood a fall in pressure, and finally a third similar injection after reducing the perfusion pressure once more to 16 cm. again a rise. It will be seen that a dilator response is obtained at the higher perfusion pressure. The time taken for injection and the quantity of solution injected are the same in each case, and we have obtained somewhat similar results on a number of occasions, although difficulties have arisen in selecting the best dose in some of the preparations. A not unusual effect encountered in some experiments was a diminution in the constrictor response at the higher perfusion pressures

as compared with the lower. In an endeavour to interpret these observations we had in mind that at the higher perfusion pressures the velocity of blood past the orifice of the hypodermic needle, which was used for injection through the rubber tubing, is greater than that at the lower pressures, which means that the amount of blood immediately entering the lungs and diluting the acetylcholine will also be greater;

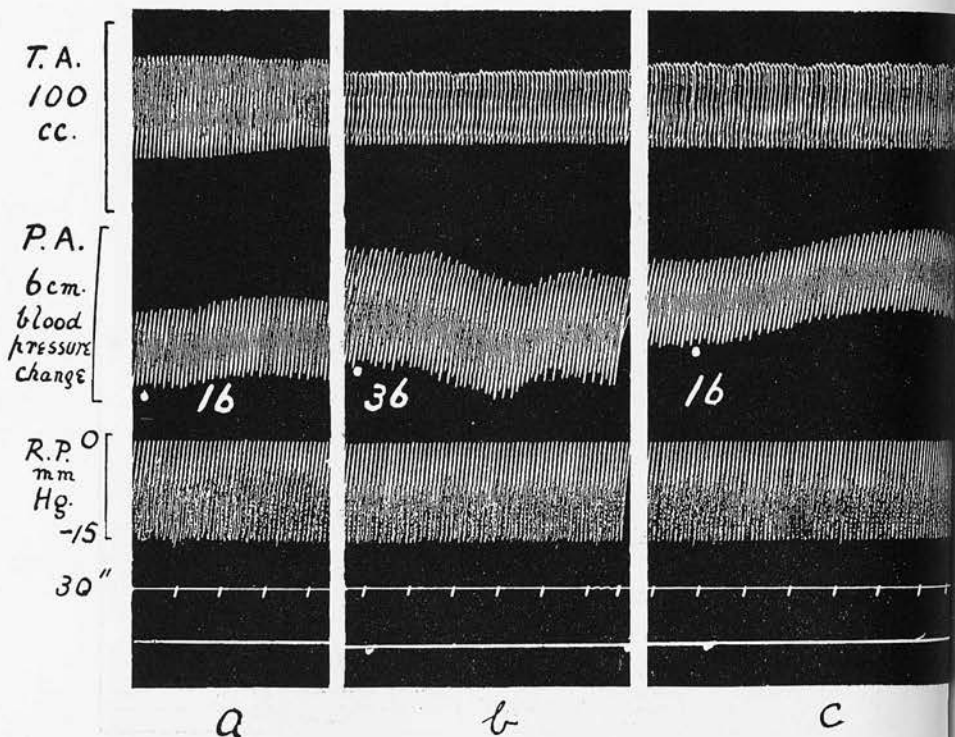


FIG. 5.—Dog, 11.6 kg. I.P.L. Eserinised preparation. Three injections of A.C. 1.0 mg. into the pulmonary artery. Perfusion pressure at (a) 16 cm. blood, (b) 36 cm. blood, (c) 16 cm. blood. R.P. = respiratory pressure.

thus, the concentration of acetylcholine in the blood reaching the pulmonary vascular bed will be smaller with high perfusion pressures than with low. Further, it has been shown that an increase in perfusion pressure gives rise to a considerable augmentation in lung blood volume [Daly, 1928], and if we assume that mixing of blood in the lungs takes place to any significant degree, the dilution of the incoming blood containing acetylcholine with the blood in the lungs will be greater with high perfusion pressures than with low.

In one experiment we determined the amount of blood in the lungs (estimated colorimetrically) after clamping simultaneously the inflow and outflow tubes while the pump was running. The quantity in the lungs was 30 c.c., and in the venous reservoir 55 c.c. The venous reservoir blood volume decreased by 15 c.c. when the perfusion pressure was raised, showing that the lung blood volume had increased by the same amount.

We would expect then, what was actually observed, that equal doses of acetylcholine injected into the pulmonary artery would give the typical response to weak doses (dilator or weak constrictor) at the higher perfusion pressures, and the typical response to stronger doses (constrictor) at the lower perfusion pressures; even so we were not entirely satisfied that the altered hæmodynamical conditions controlling the drug concentration were the only factors concerned. The arrangement of the apparatus for constant inflow perfusion enabled us to test the hypothesis further. The combined blood volume of the lungs and external apparatus being constant, a rise in perfusion pressure leads to a reduction in the venous reservoir blood owing to the extra filling of the lungs with blood. Within limits the higher the perfusion pressure, the smaller the quantity of blood in the reservoir, and therefore the greater the concentration of acetylcholine in the reservoir blood, if the same quantity is always introduced into the reservoir. The acetylcholine was injected into the venous stream just as it entered the venous reservoir in order to mix it with the reservoir blood. In two experiments it was found that the vasoconstriction was greater at the higher perfusion pressures than at the lower (fig. 6)—just the reverse of the effects obtained by injections into the pulmonary arterial tubing—and in two other experiments in which only a weak constrictor response was obtained no significant differences in the vascular effects at the high and low perfusion pressures were obtained. The procedure adopted in the experiment illustrated in fig. 6 was repeated with the blood volume of the reservoir maintained constant by the introduction of more blood at the high perfusion pressures and its withdrawal at the low: for this purpose a 20 c.c. syringe full of blood and armed with a wide bore needle piercing the rubber tube of the reservoir was kept in the same water bath as the venous reservoir. Under these conditions, which eliminated the dilution differences of the acetylcholine, equal vasoconstrictor effects at high and at low perfusion pressures were obtained.

These results indicate that the discrepancies in the response of the pulmonary vascular bed to acetylcholine at different perfusion pressures depend very largely if not solely on hæmodynamical factors which determine the dilution of acetylcholine in the blood entering the lungs. In some preparations the response to acetylcholine is weak and evanescent, and it is not possible to state categorically that the dilution effect described is the only one operative; however, with the doses employed we have not as yet obtained any satisfactory evidence that the state of the blood-vessels at different pressures is in any way responsible for the phenomena observed.

Effect on Blood Outflow.—In preparations perfused at constant pressure, acetylcholine caused either a slight increase in outflow or more rarely a slight decrease (figs. 2, 3, 4 and 7): in those perfused at constant inflow, the outflow increased in eleven experiments, diminished in three,

showed a diphasic outflow curve in five, and had no effect in three. These changes appeared to be irrespective of the action of acetylcholine on the inflow (figs. 2 and 3).

In three experiments in which small doses of acetylcholine have initially caused a diminution in outflow, raising their value brought about an increased outflow. One experiment may be quoted to illustrate this effect. A series of injections of acetylcholine were made into the pulmonary arterial tubing of an uneserinised preparation perfused at

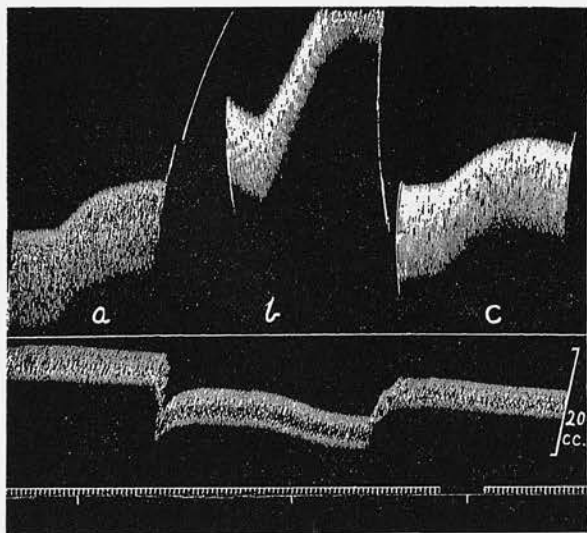


FIG. 6.—Dog. 8 kg. I.P.L. Eserinised preparation. Three injections of A.C. 300 γ into venous reservoir. Perfusion pressure of (a) 10.5 cm. blood, (b) 15 cm. blood, (c) 10.5 cm. blood. Top tracing = P.A.p.; 2nd = T.A. showing complete bronchoconstriction; 3rd = volume of venous reservoir; 4th = 10 secs.; 5th = signal.

constant blood inflow. The doses injected were 10, 25, 50, 200, 400, 600, and 1000 γ , the corresponding changes in the blood-volume of the venous reservoir were -0.1, -0.2, +0.3, +0.3, +0.6, +0.9, and +1.5 c.c. The pulmonary arterial pressure only showed a slight fall of from 1 to 2 mm. blood after each injection.

Action of Acetylcholine in Nicotinised Preparations. Dale [1914] has shown that acetylcholine exhibits both a muscarine-like and nicotine-like action, the former being abolished by atropine but unaffected by nicotine. Figs. 2 and 3 demonstrate that the rise or fall in pulmonary arterial pressure which acetylcholine may produce are unaffected by doses of nicotine sufficiently large to paralyse the ganglia. Although the fall in pulmonary arterial pressure due to acetylcholine in fig. 3 occurs after ergotoxine, we have obtained a pressure fall in nicotinised preparations in the absence of ergotoxine. The additional fact that

atropine abolishes both types of pulmonary vasomotor response of acetylcholine [Daly and Euler, 1932] makes it clear that both constrictor and dilator effects are due to the muscarine-like action of the ester.

Action of Eserine.—Eserine itself causes a rise in pulmonary arterial pressure with or without an increase in outflow from the lungs. We have only twice observed a fall in pressure, and this change was associated with a large increase in outflow.

One peculiar effect of eserine is worthy of mention, namely, its delayed

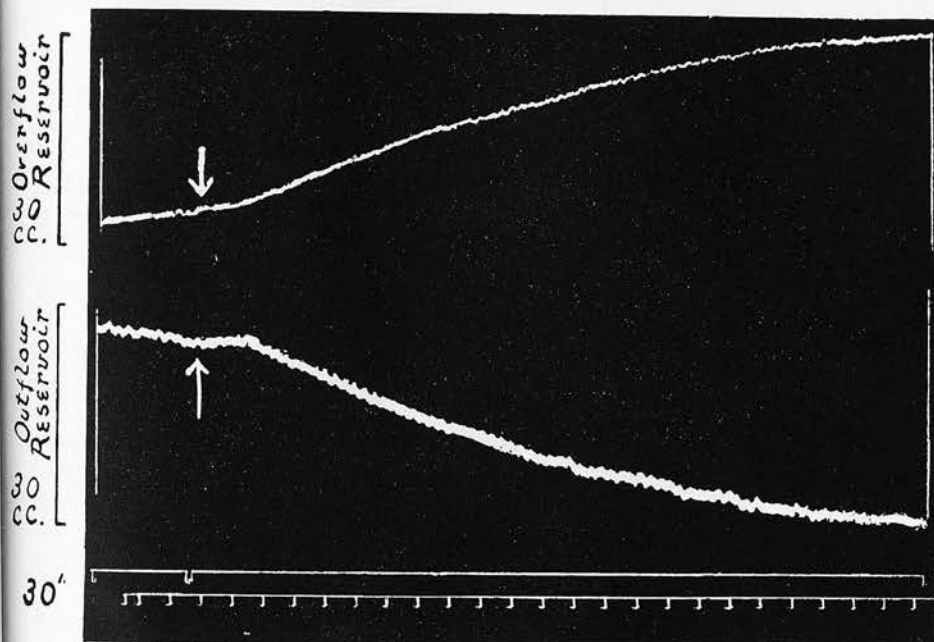


FIG. 7.—Dog. I.P.L. Eserinised preparation. Constant pressure perfusion at 16.5 cm. blood. Upper tracing = volume of overflow reservoir; lower = volume of venous reservoir. Time = 30 sec. Effect of 50 γ A.C.

action both on the blood-vessels and bronchioles. The usual response of the isolated lungs is an inflow constriction within a few seconds of injection followed, after a delay of a few minutes, by a bronchoconstriction more or less complete (fig. 3), but sometimes the vascular response may be delayed for as long as five minutes (fig. 8). Unless this phenomenon is recognised, errors in interpretation may arise by the delayed response of eserine being superimposed upon that due to a subsequent injection of some other substance.

Eserine enhances the vasodilator and the vasoconstrictor inflow effects of acetylcholine as well as the bronchomotor. On a number of occasions we have been able to confirm the observation of Gaddum and

Holtz [1933] that eserine may convert an acetylcholine inflow vasodilatation to constriction: we agree with their suggestion that it may be due to an increase in the effective concentration of the ester consequent upon the inhibition of the esterase action. We have, however, observed in two experiments eserine to convert an inflow vasoconstriction response to acetylcholine to one of vasodilatation, which suggests that the ester may be acting on two different parts of the vascular bed, the increase in its effective concentration in these experiments having a larger effect on those vessels which react by dilating than

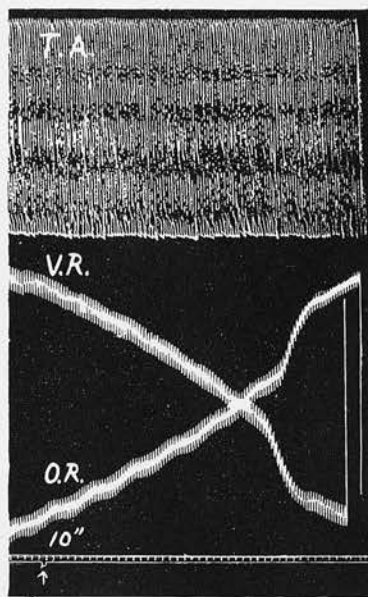


FIG. 8.—Dog. 16 kg. I.P.L. Constant perfusion pressure. Eserine 2.0 mg. at signal. Vascular response delayed 5' 20".

on those which constrict. Eserine appears to have some other action than that of rendering small quantities of acetylcholine in the tissues effective, because when injected subsequently to acetylcholine which has produced a rise in pulmonary arterial pressure it has caused a well-marked fall in pressure, although a further dose of acetylcholine has produced a greater rise in pressure than the first ester injection.

Bronchomotor Effects and Vasomotor Response.—It has been suggested that the pulmonary vascular response to drugs is in part, if not in some cases wholly, dependent upon the bronchial effects, the changes in blood pressure or flow being due to compression of the blood-vessels. In the isolated lungs of the guinea-pig under positive pressure intratracheal ventilation, Dale and Narayana [1935] working in these laboratories have shown that adrenaline produces a decrease in outflow in the

absence of any action on the bronchi, but if the tone of the bronchial muscles is already high and bronchodilatation takes place, then an increase in outflow occurs. Apparently the relaxation of the bronchial musculature by adrenaline releases the existing compression of the blood-vessels. On the other hand, acetylcholine in their experiments with one exception produced a diminution in outflow whether bronchoconstriction was present or absent and it therefore had a true vascular effect.

Since perfused lungs under negative pressure ventilation are not strictly comparable with those respired under positive pressure ventilation, it is desirable to examine how far the vasoconstriction and vasodilatation effects due to acetylcholine in our own experiments are due to concomitant bronchomotor effects. A scrutiny of the tracings obtained from over fifty preparations showed that acetylcholine is able to produce a rise or a fall in pulmonary arterial pressure (1) during concomitant bronchoconstriction as evidenced by a diminishing tidal air, (2) when bronchoconstriction is complete due to a previous dose of eserine or acetylcholine—the tidal air being zero, and (3) when the bronchi are partially constricted from a previous dose of acetylcholine, the subsequent injections of the ester producing a vascular response but no further change in the tidal air. Some of these effects are shown in the figures reproduced. Evidence that acetylcholine is able to produce a fall in pulmonary arterial pressure associated with a powerful bronchoconstriction has already been presented by Daly and Euler [1932].

In interpreting these results it should be remembered that the tidal air changes are taken as indicative of the degree of bronchoconstriction, a criterion which, from the quantitative aspect, is open to criticism because in the presence of complete constriction of a part of the bronchial tree the tidal air will not undergo any further alterations in response to changes in calibre of those bronchioles which are situated peripheral to the site of complete constriction. Even so, it is difficult to conceive of bronchomotor effects solely determining the changes in vascular pressure under such a variety of conditions as we have described, although it must be admitted that by measuring the tidal air we are in no way obtaining information of events taking place in the finer air tubes peripheral to a constricted bronchus. An additional incentive to caution in our interpretation arises from the lack of knowledge of the function and reactions of the large quantity of smooth muscle in the lungs which cannot be considered to belong to the bronchial musculature proper [Baltisberger, 1921; Macklin, 1929]. When contracting this muscle system may reduce air entry by pressure on the alveolar surfaces, and indeed may also be responsible for pressing blood out of the lung capillaries. That isolated pulmonary blood-vessels are responsive to acetylcholine is shown by the work of Franklin [1932], and until clearer light has been thrown upon these mechanisms we will have to content

ourselves with the statement that the vascular response to acetylcholine is at any rate in part independent of the bronchomotor effects.

ADRENALINE.

The action of adrenaline in various doses has been tested on twenty preparations perfused at constant blood inflow and on twenty-four perfused at constant blood-pressure. The main results are incorporated in the table, but no indication is given therein of the effects of altering the dose of adrenaline in any given preparation. A minimal effective

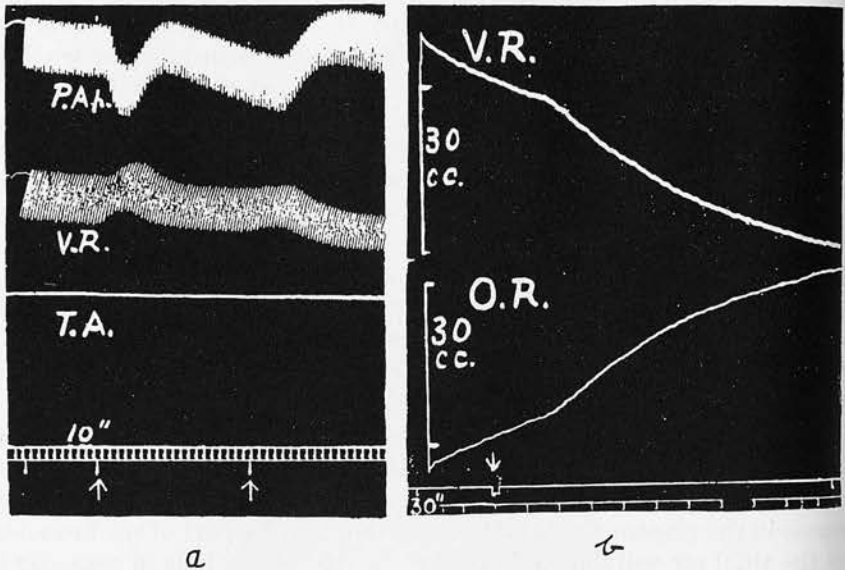


FIG. 9. *a*.—Dog. 8.0 kg. I.P.L. Constant inflow perfusion. Two successive doses of adrenaline (cryst.) 50 γ .
b.—Dog. 21.0 kg. I.P.L. Constant perfusion pressure at 7.5 cm. blood. Histamine 2.5 γ injected into P.A. at signal. Inflow constriction. No effect on outflow.

dose of adrenaline generally causes an increase in venous outflow with only a small change or none at all of the pulmonary arterial pressure (fig. 11). Larger doses then tend to raise the pressure considerably without causing a comparable increase in outflow. Definite indications of a pulmonary arterial diminution (or increase in inflow) were obtained in only five experiments, but the responses, except where otherwise indicated in the table, were definite. Reports in the literature of a vasodilator response to adrenaline in the dog are rare, and have in some instances been attributed to the presence of chloretone in the preparation used, a complication which does not arise in our experiments. In one experiment only did we observe a pressure fall in response to adrenaline, and it was remarkable that a subsequent injection caused a rise

pressure after a long latent period (fig. 9, *a*) associated with a reduction in venous outflow.

The effect of adrenaline on the blood *outflow* requires some discussion in view of the observations made by Gaddum and Holtz [1933]. In two out of ten of their experiments, the lungs being without respiration and under constant pressure perfusion, adrenaline caused an increase in lung volume and a diminution in outflow, associated with the usual rise in pulmonary arterial pressure. Hence the drug appeared to have a constrictor action on the outflow. In our experience such an effect was observed only on three occasions and then was slight, the usual outflow response to adrenaline being an increase (table and fig. 10, *a*). Gaddum and Holtz also observed this augmentor effect on the outflow in five out of ten experiments, and concluded that the effect of adrenaline on the outflow was variable. We are not in entire agreement with the sense of this statement and would prefer to stress the importance of an outflow augmentation and the unusual occurrence of outflow constriction in lungs under negative pressure ventilation. We incline to this view chiefly because the augmentations in outflow were, in general, much larger than the diminutions, a fact not evident from the table.

TABLE.—ACTION OF ADRENALINE AND HISTAMINE ON ISOLATED PERFUSED LUNGS.

	No. of expts.	No. of tests.	Dose γ	P.A.p.	Inflow.	Outflow.
Adrenaline	1	1	50	+		-
	2	2	20		-	-sl.
	10	19	2-200	+		+
	11	19	10-250		-	+
	5	7	2-50	+sl.		0
	4	5	5-40		-sl.	0
	3	6	2-40	0		+
	3	4	10-100		0	+
	1	1	50	-		+
	1	2	100		+sl.	+
3	4	4-200		+sl.	0	
Histamine	6	8	0.5-300	+		-
	1	1	800		-	-
	1	1	100	+		0
	6	6	2-250		-	0

In confirmation of Daly and Euler [1932] and of Gaddum and Holtz [1933] we find that ergotoxine converts the adrenaline inflow diminution to an augmentation (fig. 10, *b*). This reversal may occur in preparations perfused at constant pressure, and therefore is not dependent upon the

augmented pressure produced by ergotoxine. In one experiment only did we obtain satisfactory evidence that ergotoxine enhanced the effect of adrenaline in increasing the outflow.

The blood volume changes of the lungs in response to adrenaline

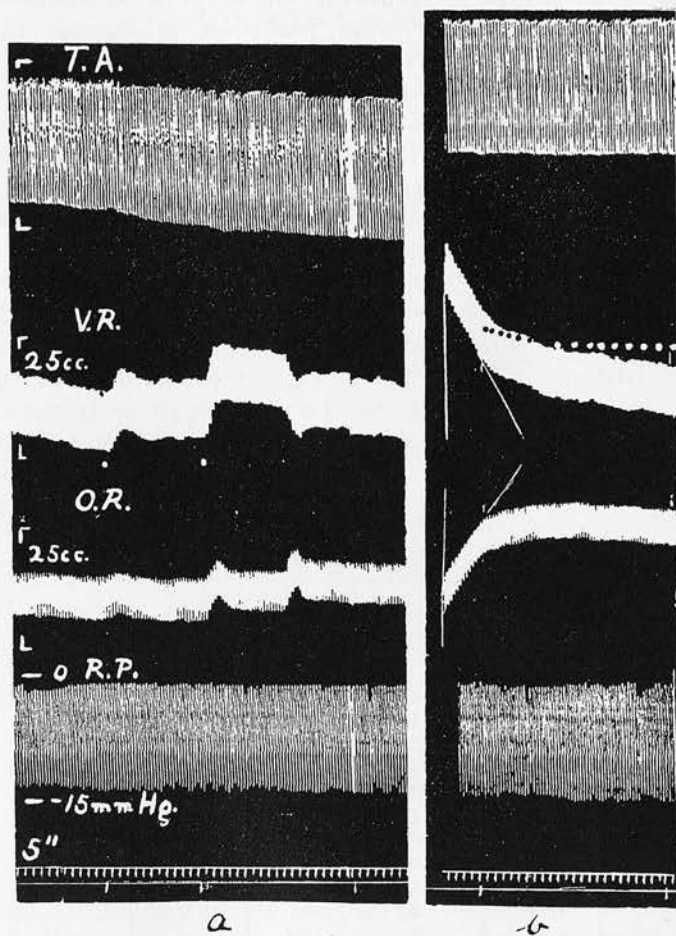


FIG. 10. *a*.—Dog. 17.5 kg. I.P.L. Constant perfusion pressure. 1st signal =adrenaline 20 γ ; 2nd =adrenaline 80 γ ; 3rd =mechanical constriction of inflow. The overflow reservoir tracing is horizontal because blood just fails to spill over.
b.—Same animal after ergotoxine. At signal, adrenaline 80 γ .

varied from 0.7 to 15 c.c. (with adrenaline doses from 2 to 250 γ). Gaddum and Holtz reported volume alterations of less than 1.0 c.c. in unventilated lungs. The larger values obtained in our preparations were not entirely unexpected since the lungs contain much more blood when under negative pressure ventilation than when in the collapsed condition. We have, however, compared the blood volume changes due to adrenaline injections into lungs under negative pressure ventilation

and in the same preparations after the ventilation had been stopped. We obtained almost identical results. Since the blood volume changes of the lungs in Gaddum and Holtz's experiments were very much lower than in our unventilated preparations we are not at all clear as to the

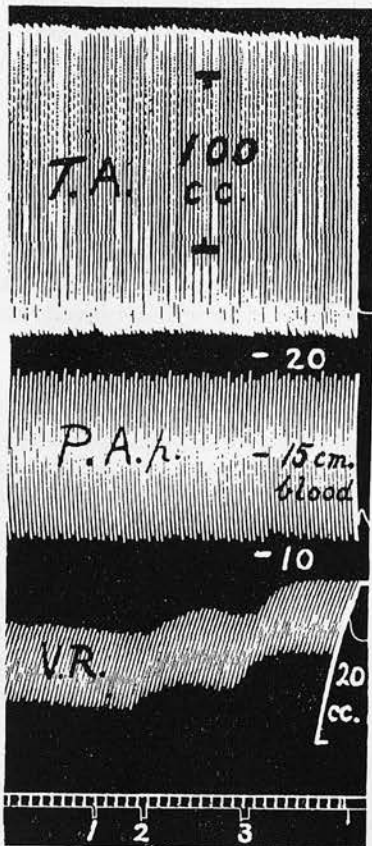


FIG. 11.—Dog. 18 kg. I.P.L. Constant inflow perfusion. 1st signal = 0.5 γ adrenaline injected into P.A.; 2nd = 2.0 γ adrenaline; 3rd = 5.0 γ adrenaline. Time = 10 sec.

reason for this discrepancy. It might be that the blood-vessels directly or indirectly responsible for the adrenaline alterations in lung blood volume remain dilated after the cessation of ventilation in those preparations which have been initially under negative pressure respiration. We have not carried this aspect of the investigation further.

HISTAMINE.

Fourteen isolated lung preparations were tested. With doses varying from 0.5 to 300 γ we have always obtained a diminution in inflow or a rise in pulmonary arterial pressure, the outflow showing a

decrease or no change (see table). The tidal air when affected always showed a diminution. These results are in agreement with other workers. At one period of the research we were suspicious that small doses of histamine caused a slight inflow augmentation, but careful analysis of the tracings and repetition of the experiments failed to confirm this view. Fig. 9, *b* shows that 2.5 γ histamine produces a significant diminution in inflow; since the venous reservoir tracing is almost an exact mirror image of the overflow reservoir tracing, no direct action on the outflow is detectable.

DISCUSSION AND CONCLUSIONS.

Our main objective—the determination of the action of the smallest effective doses of acetylcholine, histamine, and adrenaline on the pulmonary vascular bed of the dog—although achieved, only reveals the action of these substances on the pulmonary blood inflow and outflow and throws little light on their point of action. We are aware that the results described are only strictly applicable to the lungs of the dog under experimental conditions which probably lead to a rapid loss of tone of the blood-vessels. We would expect from the researches of Dale and Richards [1918], Burn and Dale [1926], Krogh [1929], and Burn [1932], on the part played by tone of the systemic blood-vessels in determining their responses, that the pulmonary vascular system would be governed by somewhat similar considerations. Indeed, recently we have collected evidence, as yet incomplete, that such at any rate in part is the case, and in consequence we hesitate in a discussion to accept our observations as illustrating in every respect the effects which would occur in the whole animal.

We may state our conclusions briefly. In isolated lungs perfused with defibrinated blood and placed under negative pressure ventilation, small doses of *acetylcholine* (1) increase the pulmonary inflow (or diminish the pulmonary arterial pressure), and (2) slightly diminish or increase the outflow. If in any given experiment the minimal effective dose causes a decrease in outflow, it is converted to an increase by raising the dose. Large doses of acetylcholine (1) decrease the pulmonary inflow (or raise the pulmonary arterial pressure), and (2) slightly increase the outflow.

In preparations perfused at constant inflow minimal effective doses of *adrenaline* have little or no action on the pulmonary arterial pressure, but in general cause an increase in outflow which leads to a diminution in the blood content of the lungs. Larger doses cause an increase in the pulmonary arterial pressure with an increase in venous outflow. Exceptions to these actions of adrenaline have been observed but they are relatively uncommon.

Histamine in all concentrations always increased the pulmonary

arterial pressure or decreased the inflow, and also diminished the outflow.

These results, carried out on preparations under negative pressure ventilation, differ in some respects from those of Gaddum and Holtz [1933] performed on non-ventilated preparations.

Franklin [1932], working on isolated vessels, found that the smallest effective dose of *acetylcholine* relaxed the extrapulmonary arteries and contracted the smaller and larger extrapulmonary veins: there was no action on the intrapulmonary arteries, and the responses of the intrapulmonary veins were slight and variable. These results would correspond to an inflow increase and outflow decrease in the perfused preparations, a phenomenon which we observed in a few experiments with small doses. Gaddum and Holtz's results differ in that they never observed an outflow diminution. It is true that the more usual effect of *acetylcholine* on the outflow in our experience is augmentation, but when diminution occurred it was definite (fig. 2, c). The failure of Gaddum and Holtz to obtain evidence of a diminution in outflow with small doses we are unable to explain except on the score of its relatively rare occurrence. Again, the failure of Franklin to obtain definite evidence of relaxation of the pulmonary veins with the larger doses of *acetylcholine* may have been due to the isolated veins being already fully relaxed. These discrepancies no doubt will disappear when more is known of the action of *acetylcholine* under conditions of varying tone of the blood-vessels.

In the presence of nicotine in sufficient doses to paralyse the ganglia, *acetylcholine* is still able to augment or to reduce the pulmonary arterial pressure. These effects being enhanced by *eserine* and suppressed by *atropine* support the evidence already brought forward by Daly and Euler [1932] of the presence of pulmonary cholinergic vasodilator fibres. These workers found that excitation of the thoracic vagosympathetic caused vasodilatation, the effect being enhanced by *eserine* and suppressed by *atropine*. Although *acetylcholine* in large doses causes pulmonary arterial pressure augmentation which is also enhanced by *eserine* and suppressed by *atropine*, the evidence for the presence of functionally active cholinergic pulmonary vasoconstrictor fibres is not secure for we have not as yet succeeded in demonstrating that pulmonary nerve-excitation may produce a vasoconstriction which responds in a similar manner to the exhibition of *eserine* and *atropine*. Until this has been done we shall be uncertain whether the constrictor action of such large doses is not due to their toxic action or to a type of action on the peripheral neuromuscular apparatus which is not brought into activity by nerve impulses.

Several questions arise as to the nature of *adrenaline* action in the normal animal in view of the result that minimal effective doses may exert their chief effect by augmenting the venous outflow. If this were

the only action of the increased circulating adrenaline during exercise, the onward flow of blood through the lungs would be facilitated. However attractive this hypothesis may appear on teleological grounds, we hesitate to accept it without further investigation. It fails to explain why electrical excitation of the sympathetic nerve-supply to the lungs leads to a large increase in pulmonary arterial pressure; moreover, the augmentation of venous outflow by adrenaline may be due either to a capillary constriction without significant changes in the vascular resistance or to a venous dilation. Our investigations do not enable us to decide between these two alternatives.

Gaddum and Holtz [1933] found in non-ventilated lungs that where definite evidence was obtained that a drug was acting on the pulmonary veins, this action was such as to neutralise the change in lung volume which would have occurred if the drug had acted solely on the inflow. In this respect the lungs under negative pressure ventilation behave in a different manner. An examination of our protocols reveals that no categorical statement can be made as to the precise action of adrenaline and of acetylcholine on the blood volume of the lungs. In the majority of experiments, however, the effect of adrenaline has been to deplete the lungs of blood by diminishing the inflow and increasing the outflow; this also has been the action of large doses of acetylcholine, although the outflow effect has been relatively smaller. Minimal effective doses of acetylcholine when producing a diminution of outflow also increase the inflow, thus leading to an augmentation of lung blood volume. At the moment we wish to stress these particular results as apparently indicating that negative pressure ventilation may play an important rôle in determining the response of the pulmonary vascular system to drugs: their significance is as yet obscure.

SUMMARY.

In isolated lung preparations of the dog under negative pressure ventilation and perfused with defibrinated blood

1. Acetylcholine in the smallest effective doses causes a slight fall in pulmonary arterial pressure (or an increase in inflow), and in large doses a rise in pulmonary arterial pressure (or decrease in inflow). With small doses the venous outflow may show a slight decrease which is converted to an increase on raising the dose of acetylcholine.

2. The effects described above are in part, if not solely, independent of bronchomotor mechanisms.

3. The pulmonary arterial pressure rise or fall due to acetylcholine injections occur in nicotised preparations, are enhanced by eserine and suppressed by atropine.

4. The apparent relationship between the type of vascular response to acetylcholine and the height of the perfusion pressure which is

described, is determined by factors which are peculiar to the perfusion apparatus and which govern the concentration of the ester in the blood reaching the lungs. No evidence has been obtained that the degree of distension of the pulmonary blood-vessels determines their response to acetylcholine.

5. Adrenaline in small doses may have no effect upon the pulmonary arterial pressure yet cause an increase in venous outflow.

6. A method is described for measuring the blood inflow and outflow from lungs under negative pressure ventilation.

The expenses of the research have been defrayed by a grant to one of us (I. de B. D.) from the Government Grant Committee of the Royal Society, to whom we express our thanks.

REFERENCES.

- BALTISBERGER, W. (1921). *Z. Anat. Entwickl.* **61**, 249.
- BARCROFT, J. (1934). *Features in the Architecture of Physiological Function*, p. 342. Cambridge.
- BARTOSCH, R., FELDBERG, W., and NAGEL, E. (1932). *Pflügers Arch.* **230**, 129.
- BARTOSCH, R., FELDBERG, W., and NAGEL, E. (1932 a). *Ibid.* **230**, 674.
- BARTOSCH, R., FELDBERG, W., and NAGEL, E. (1933). *Ibid.* **231**, 616.
- BERRY, J. L., and DALY, I. DE BURGH (1931). *Proc. Roy. Soc., B*, **109**, 319.
- BURN, J. H. (1932). *J. Physiol.* **75**, 144.
- BURN, J. H., and DALE, H. H. (1926). *Ibid.* **61**, 185.
- DALE, A. S., and NARAYANA, B. (1935). *Quart. J. Exp. Physiol.* **25**, 85.
- DALE, H. H. (1914). *J. Pharmacol.* **6**, 147.
- DALE, H. H., (1929). *Lancet*, i, 1285.
- DALE, H. H. (1933). *J. Physiol.* **80**, 10 P.
- DALE, H. H., and RICHARDS, A. N. (1918). *Ibid.* **52**, 110.
- DALE, H. H., and SCHUSTER, E. H. J. (1928). *Ibid.* **64**, 356.
- DALY, I. DE BURGH (1928). *Ibid.* **65**, 422.
- DALY, I. DE BURGH (1933). *Physiol. Rev.* **13**, 149.
- DALY, I. DE BURGH, and EULER, U. S. v. (1932). *Proc. Roy. Soc., B*, **110**, 92.
- DALY, I. DE BURGH, PEAT, S., and SCHILD, H. (1935). *Quart. J. Exp. Physiol.* **25**, 33.
- DUNN, J. SHAW (1919). *J. Physiol.* **53**, 3 P.
- EULER, U. S. v. (1932). *Ibid.* **74**, 271.
- FRANKLIN, K. J. (1932). *Ibid.* **75**, 471.
- GADDUM, J. H., and HOLTZ, P. (1933). *Ibid.* **77**, 139.
- HIROSE, Y. (1932). *Arch. exp. Path. Pharmak.* **165**, 401.
- HUNT, REID (1918). *Amer. J. Physiol.* **45**, 197.
- KROGH, A. (1929). *The Anatomy and Physiology of the Capillaries*. New Haven and London.

MACGREGOR, R. G. (1933). *J. Physiol.* **80**, 65.

MACKLIN, C. C. (1929). *Physiol. Rev.* **9**, 1.

SPINELLI, A. (1932). *Boll. Sez. Ital. Soc. Int. Microbiol.* **4**, 257.

THORNTON, J. W. (1934). *J. Physiol.* **82**, 14 P.

TRIBE, E. M. (1914). *Ibid.* **48**, 154.

TRONCI, L. (1934). *Sperimentale*, **88**, 719.

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