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EFFECT OF DOPAMINE ON THE ISOLATED PERFUSED LUNG LOBES OF THE DOG

BY

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Dopamine (β-3:4-dihydroxyphenylethylamine) has a pressor action on the pulmonary vascular bed in isolated perfused lung lobes of the dog. Its pulmonary vasoconstrictor effect in this preparation is about 1/20–1/30 that of adrenaline or noradrenaline. When compared with adrenaline, dopamine is less effective as a vasoconstrictor in the bronchial vascular system than in the pulmonary circulation. Even very large doses of dopamine had no bronchomotor effect in the isolated perfused lung lobes. Addition of dopamine alone, or of dopamine together with some " new " blood, sometimes caused a potentiation of the pulmonary vasoconstrictor responses to sympathetic nerve stimulation.

The demonstration of a relatively large dopamine (β-3:4-dihydroxyphenylethylamine) content in lung tissue by Euler & Lishajko (1957) and by Schümann (1958) has some interesting aspects. According to these investigators, more than 90% of the total amount of catecholamines in lung tissue is dopamine, and it is possible that it may have functions other than serving as precursor in local noradrenaline formation. Schümann (1959) has speculated that dopamine may have its own functions and act as a local hormone. It was therefore thought of interest to test the effect of dopamine when introduced into the circulation of isolated perfused lungs. The preparation used was one in which both the pulmonary and the bronchial circulations were perfused (Daly & Waaler, 1960; Allison, Daly & Waaler, 1961). Since in the dog the bronchial vascular system supplies several important intrapulmonary structures such as the nerves and the airways down to the respiratory bronchioles, an adequate perfusion of this system may be of great importance for the reactivity of the preparation to drugs. It is well known that vasomotor responses to sympathetic nerve stimulation cannot be obtained in such a preparation unless the bronchial vascular system is being perfused (Daly & Euler, 1932; Allison, Daly & Waaler, 1961). It should also be emphasized that a preparation of this type can be perfused for several hr at a pulmonary vascular resistance which is well within the range for the normal resting dog (Daly & Waaler, 1960). This allows testing of vasomotor drugs to be carried out during periods in which the vascular tone in the pulmonary circulation is probably not much different from that occurring *in vivo*.

METHODS

Mongrel dogs (11 to 29 kg) were premedicated with morphine sulphate (2 mg/kg) and injected intravenously with heparin (Boots' powdered heparin, 3 mg/kg) and in most experiments also

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with atropine sulphate (1 mg). They were then exsanguinated through a cannula which under local anaesthesia had been inserted into a femoral artery. The blood was collected in a glass cylinder containing heparin 6 mg/kg body weight, the total amount of heparin used thus being about 9 mg/kg body weight. Penicillin ("Crystapen" crystalline penicillin G, Glaxo, 5 i.u./ml. blood) was added, the blood was filtered through a piece of linen and stored at 36° C in stoppered bottles until needed for perfusion.

The left apical and the left cardiac lobes, together with the heart and its attached vessels, including the descending aorta with the segregated bronchial artery or arteries (which in the dog come off from the 5th to 8th intercostal arteries on the right side), were removed from the body and arranged for perfusion in a warmed perspex tray. The left thoracic vagosympathetic nerve was also preserved and included in the preparation.

Perfusion. The pulmonary circulation was perfused either at a constant head of pressure or at a constant volume inflow through a cannula tied into the pulmonary artery. The outflowing blood was collected through a cannula inserted into the left auricle and the flow measured by leading this blood through a modified Gaddum flow recorder. The pulmonary arterial pressure was measured by a saline manometer and a Marey tambour. The bronchial circulation was perfused at a constant volume inflow through a cannula inserted into the abdominal end of the thoracic part of the aorta. All branches from the aorta, other than the bronchial artery or arteries and some small arterial twigs to the left thoracic vagosympathetic nerve, were ligated. There was one exception to this procedure: the blood flow in the reduced systemic circulation was small, and in order to prevent stagnation of the blood in the aorta an arterial branch was cannulated and a controlled amount of blood from it allowed to spill back into the reservoir. The perfusion pressure in the systemic circuit was measured by a mercury manometer. Perfusion of the pulmonary circulation and of the reduced systemic circulation were carried out by two Dale-Schuster pumps, which drew blood from a common blood reservoir with a volume of about 250 ml. The total volume of the external perfusion circuits was about 450 ml.

Ventilation. Positive pressure ventilation with 6% carbon dioxide in air or 6% carbon dioxide in oxygen was carried out with a Starling "Ideal" respiration pump at peak inspiratory pressures of 80 to 95 mm of water and an expiratory pressure of 30 mm of water. The tidal air was controlled by measuring the ventilation overflow according to the method of Konzett & Rössler (1940).

Dopamine hydrochloride (California Corporation for Biochem. Research), (−)-noradrenaline bitartrate ("Levophed," Bayer, London) and adrenaline chloride (Parke, Davis, Hounslow) were used. All doses are referred to in terms of the bases. The substances were dissolved in small volumes of 0.9% sodium chloride solution and either added to the blood reservoir or injected into the pulmonary arterial inflow tubing. The amount of saline in which injected doses of the substances were dissolved was kept constant in each experiment, usually being 0.1 to 0.3 ml.

Pulmonary vascular resistance. The pulmonary vascular resistance, corresponding to the whole of both lungs, was calculated as previously described (Daly & Waaler, 1960).

RESULTS

Circulatory effects. When added to the blood reservoir or injected into the pulmonary arterial inflow tubing, dopamine caused pulmonary vasoconstriction. Its vasoconstrictor effect was less marked, however, than that of noradrenaline or adrenaline. The ratio of equipotent doses of noradrenaline, adrenaline and dopamine was about 1:1:25. Various preparations differed in the sensitivity of their pulmonary vascular bed to all the three amines, but the relative sensitivity to each of them remained the same. The responses in one preparation to small doses of adrenaline, noradrenaline and dopamine injected into the pulmonary arterial inflow tubing are shown in Fig. 1. Fig. 2 shows the responses in another preparation to

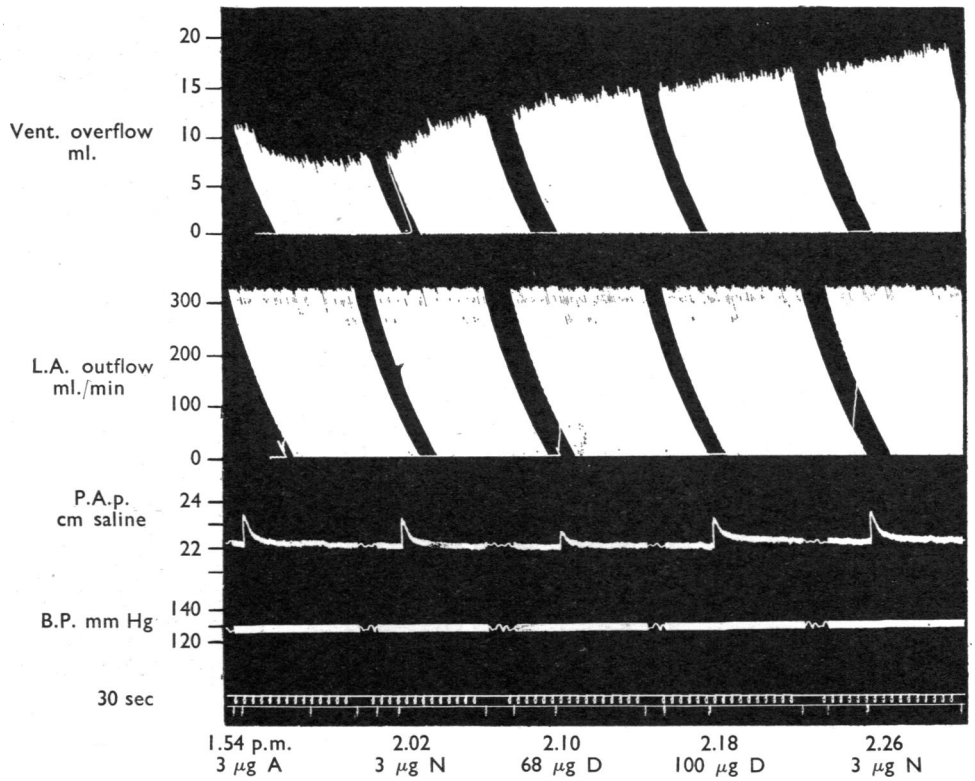

Fig. 1. Effect of injections of adrenaline (A), noradrenaline (N) and dopamine (D) into pulmonary artery of isolated dog lung perfused at constant volume inflow. Dog: ♂, 23 kg, left apical and left cardiac lobes perfused. Tracings from above: ventilation (Vent.) overflow (respiration pump stroke=70 ml.), left atrial (L.A.) blood outflow, pulmonary arterial perfusion pressure (P.A.p.), perfusion pressure in reduced systemic circulation (i.e. aorta and bronchial circulation) (B.P.) and time marker and signals. Left atrial pressure=3.5 cm of saline. All doses of catecholamine were dissolved in 0.15 ml. of 0.9% sodium chloride solution and injected in the course of 5 sec.

additions of the three amines to the blood reservoir. Vasoconstrictor responses of similar duration and magnitude were obtained in preparations perfused at constant pressure.

The additions to the perfusate in the reservoir of dopamine caused rather prolonged vasoconstrictor responses in this isolated lung preparation, similar to responses produced by additions of adrenaline or noradrenaline. The rate at which the responses declined is seen in Fig. 2. A dose of 0.5 to 1 mg of dopamine gave a response which needed more than 0.5 hr to disappear.

It will be seen from Fig. 2 that doses of dopamine which caused a definite constriction in the pulmonary vascular bed did not have any appreciable vasoconstrictor effect in the reduced systemic (bronchial) circulation. Since the two circulations were perfused with blood from a common reservoir, to which the test substances were added, the concentration of the amines in the blood entering the two circuits was

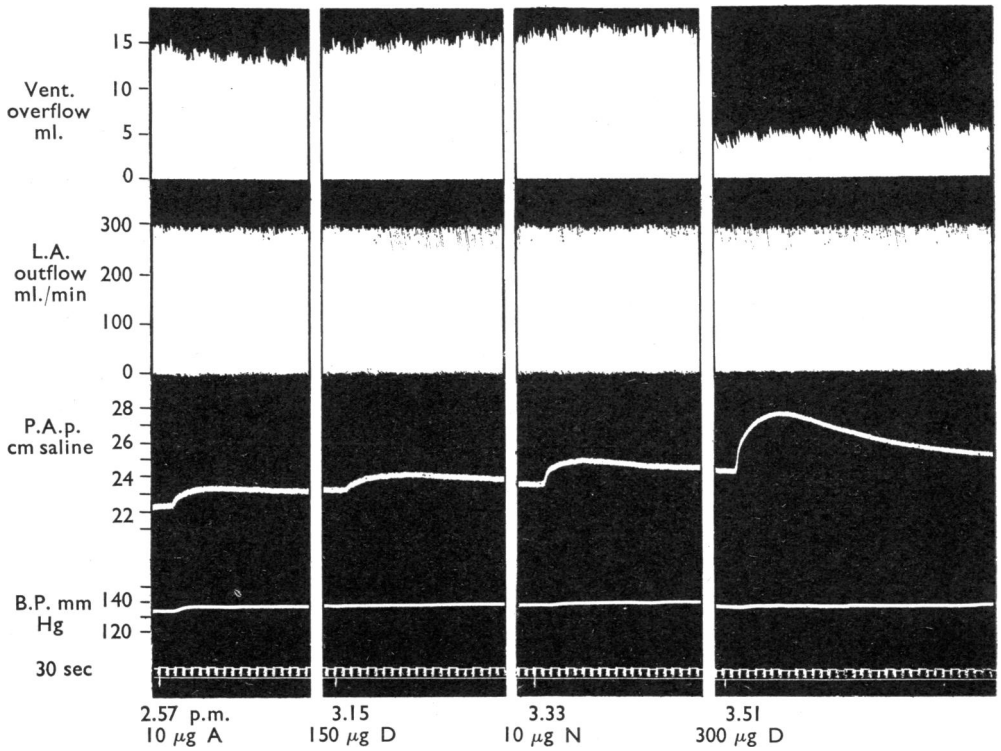

Fig. 2. Effect of adding adrenaline (A), dopamine (D) and noradrenaline (N) to the blood reservoir of an isolated dog lung perfused at constant volume inflow. Dog: ♂, 19 kg, left apical and left cardiac lobes perfused. Tracings as in Fig. 1. Respiration pump stroke=66.5 ml. up to 3.48 p.m., then reduced to 52 ml. Left atrial pressure=4 cm saline. All doses of catecholamine dissolved in 0.2 to 0.3 ml. of 0.9% sodium chloride solution and added to blood reservoir (volume 250 ml.) during stirring.

the same. The preparation was not suitable for quantitative evaluations of vasomotor responses in the bronchial circulation, because of the shunting of blood back from the aorta to the reservoir. This constant leak of arterial blood reduced the amplitude of recorded pressure changes caused by vasomotor events in the bronchial vascular system. In spite of this disadvantage, however, some comparison between the effect of various substances on the bronchial vascular bed was possible. It is seen from Fig. 2 that 10 μg of adrenaline had about the same vasoconstrictor effect in the pulmonary circulation as had 150 μg of dopamine. This dose of adrenaline, however, had an appreciable constrictor effect in the bronchial circulation whereas 300 μg of dopamine had hardly any effect on the perfusion pressure in this circulation. This difference between adrenaline and dopamine was observed in several preparations. Noradrenaline seemed to be intermediate to adrenaline and dopamine in its relative vasoconstrictor effect on the bronchial vascular bed.

Effect on tidal air. Dopamine never caused any changes in the tidal air of these preparations, not even when added in doses large enough to increase the pulmonary

vascular resistance by 100%. Adrenaline always gave bronchodilatation, even after many hr of perfusion (Figs. 1 and 2). Daly, Hebb & Petrovskaia (1941) reported that in a perfused lung preparation adrenaline caused bronchodilatation in the initial part of an experiment, and bronchoconstriction after approximately 2 hr of perfusion. The reason for the constant finding of a bronchodilator response to adrenaline in the experiments here described may be in some way connected with the perfusion of the bronchial vascular system. In the preparation used by Daly, Hebb & Petrovskaia (1941) the bronchial circulation was not perfused. Noradrenaline in doses large enough to cause a marked increase in pulmonary vascular resistance had no effect on the tidal air in the present preparation.

Effect on vasoconstrictor responses to sympathetic nerve stimulation. In some of the preparations, stimulation of the postganglionic sympathetic nerve fibres to the lung was carried out at 10 min intervals. The stimuli were applied to the left thoracic vagosympathetic nerve. Such stimulation regularly gives pulmonary vasoconstrictor responses in this type of preparation (Daly, 1958 ; Allison, Daly & Waaler, 1961). Because the preparation was atropinized, no bronchoconstrictor response to stimulation of the parasympathetic fibres in the vagosympathetic nerve occurred. The pulmonary vasoconstrictor effect of nerve stimulation could be shown to persist during short periods of bronchial circulation interruption. It was therefore due to

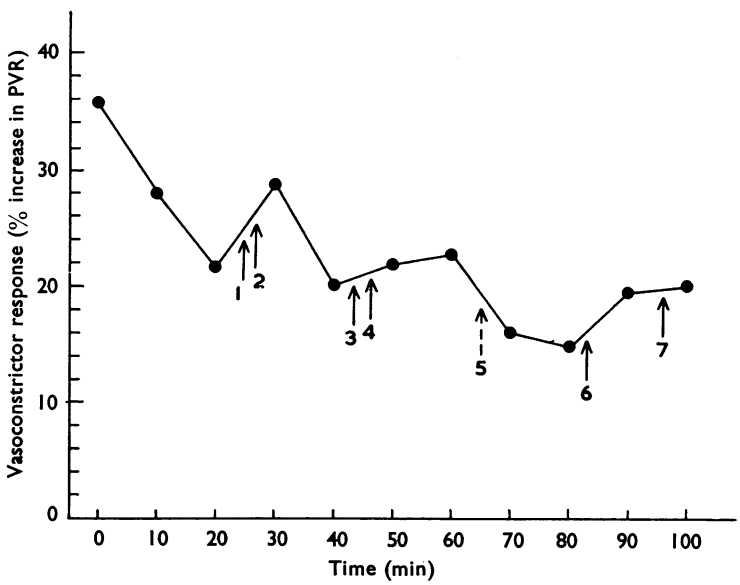

Fig. 3. Effect on declining pulmonary vasoconstrictor responses to sympathetic nerve stimulation of additions of dopamine. Isolated dog lung perfused at constant pressure. Each point represents a vasoconstrictor response to a stimulus applied to the left thoracic vagosympathetic nerve. Responses expressed as % increase in calculated pulmonary vascular resistance (PVR). Dopamine, dissolved in small amounts of 0.9% sodium chloride solution (0.1 to 0.3 ml.), added to blood reservoir during stirring. At 1 and 2, 50 μg of dopamine; at 3 and 4, 150 μg of dopamine; at 5, 110 ml. of "new" blood; and at 6 and 7 100 μg of dopamine was added. " New " blood was without effect in this preparation.

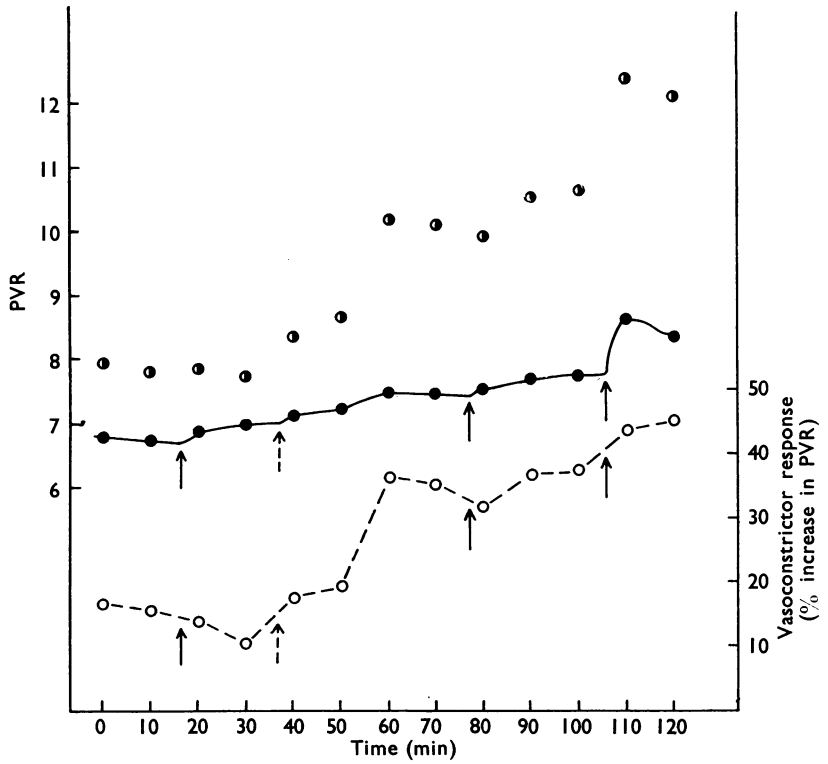

Fig. 4. Effect on declining pulmonary vasoconstrictor responses to sympathetic nerve stimulation of additions of dopamine and " new " blood. Isolated dog lung perfused at constant volume inflow. Stimuli applied to left thoracic vagosympathetic nerve. ● represents the calculated pulmonary vascular resistance (PVR) at beginning of each stimulation, ◑ the pulmonary vascular resistance at peak of vasoconstrictor response to each stimulation. In the lower curve ○ shows the vasoconstrictor responses expressed as % increase in pulmonary vascular resistance. Solid arrows=dopamine, 50 μg, 50 μg and 100 μg (in 0.1 to 0.2 ml. 0.9% sodium chloride solution), added to blood reservoir (volume 250 ml.) during stirring. Broken arrow=addition to blood reservoir of 60 ml. of " new " blood.

constriction of the pulmonary vascular bed proper, and not due to passive effects on the pulmonary circulation caused by events in the bronchial circulation (Daly, 1958). When such stimulations are carried out at 10 min intervals for some time, the marked vasoconstrictor responses which are usually obtained sometimes start to decline (Fig. 3). It was then observed that addition to the blood perfusate of dopamine 50 to 300 μg sometimes caused a moderate potentiation of the vasoconstrictor response to one, two or three of the subsequent sympathetic nerve stimulations (Fig. 3). Sometimes the very first vasoconstrictor response after dopamine addition was reduced, the following one or two showing some potentiation. Potentiation of the vasoconstrictor responses to nerve stimulation occurred after 10 out of 17 additions of dopamine in 8 experiments. The potentiating effect of dopamine was particularly marked on some occasions where dopamine and a small amount of " new " blood were added to the preparation together or one after the other. " New "

blood denotes blood which had been stored at 36° C since death of the animal. This " new " blood sometimes caused potentiation of the vasoconstrictor responses to sympathetic nerve stimulation when added alone, but even when it failed to do so a subsequent addition of dopamine would usually initiate a potentiation of the responses. On two occasions the opposite sequence of events was observed; addition of dopamine failed to potentiate the responses, but a subsequent addition of " new " blood initiated such potentiation (Fig. 4). Further addition of dopamine then caused further potentiation of the responses.

Continuous infusion of dopamine 5 to 25 μg/min caused no potentiation of the vasoconstrictor responses to sympathetic nerve stimulation. On the contrary, the responses tended to become less marked during the period of infusion. On stopping the infusion the responses regained their pre-infusion size in one, showed a small potentiation in another, and continued to decline in the third experiment.

DISCUSSION

Dopamine has a definite vasoconstrictor effect on the pulmonary vascular bed in isolated perfused dog lungs. It is considerably less effective than noradrenaline and adrenaline. When compared with adrenaline, however, dopamine seems to be a less potent vasoconstrictor in the bronchial vascular system than in the pulmonary vascular bed. This difference might have a bearing on the possible role of dopamine in the lungs.

The vasoconstrictor effect of dopamine in isolated perfused lungs wears off slowly and at about the same rate as that of noradrenaline and adrenaline. The rate of destruction of catecholamines in this isolated perfused lung preparation must be fairly slow since infusion of as little as 5 μg/min of dopamine caused a gradual rise in pulmonary vascular resistance.

The effects of dopamine on the pulmonary vasoconstrictor response to sympathetic nerve stimulation are interesting. During continuous infusion of dopamine, and sometimes also just after the addition of one single dose, the responses were diminished. This is compatible with the view that some dopamine might then be fixed to receptor sites and prevent the vasoconstrictor transmitter from acting when released by nerve stimulation (Burn & Rand, 1958). One explanation of the potentiating effect on these responses produced by dopamine, alone or in combination with " new " blood, which was observed in some of the preparations, is that dopamine was taken up at the transmitter site and transformed to noradrenaline, which may be the sympathetic transmitter in the organ. Since " new " blood and dopamine when added together sometimes caused a very marked potentiating effect on the vasoconstrictor response to sympathetic nerve stimulation, this blood might have provided an enzyme or other material necessary for such uptake and/or transformation. Speculating on these lines it would be expected that if perfused lungs became deficient in sympathetic transmitter they would give only weak vasoconstrictor responses to nerve stimulation. If, however, this deficiency were remedied by addition of dopamine and of some additional components in " new " blood, then the responses might be potentiated, as was indeed found in some of the experiments.

The reason why dopamine and "new" blood, either separately or together, sometimes failed to potentiate the vasomotor responses to nerve stimulation is obscure. It may be that the transmitter mechanism was in these experiments already working under optimal conditions.

Whatever the underlying mechanisms responsible for the potential ability of dopamine to restore the declining vasoconstrictor response to sympathetic nerve stimulation, these observations suggest a close relationship between dopamine and the transmitter apparatus concerned.

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REFERENCES

ALLISON, P. R., DALY, I. DE BURGH & WAALER, B. A. (1961). Bronchial circulation and pulmonary vasomotor nerve activity. *J. Physiol. (Lond.)*. In the press.

BURN, J. H. & RAND, M. J. (1958). Vasomotor reversal and reserpine. *J. Physiol. (Lond.)*, **143**, 73–74*P*.

DALY, I. DE BURGH (1958). Intrinsic mechanisms of the lung. *Quart. J. exp. Physiol.*, **43**, 2–26.

DALY, I. DE BURGH & EULER, U. S. VON (1932). The functional activity of the vasomotor nerves to the lungs in the dog. *Proc. Roy. Soc. B.*, **110**, 92–111.

DALY, I. DE BURGH, HEBB, C. O. & PETROVSKAIA, B. (1941). Adrenaline bronchoconstriction in isolated blood perfused lungs. *Quart. J. exp. Physiol.*, **31**, 129–159.

DALY, I. DE BURGH & WAALER, B. A. (1960). Bronchial circulation and pulmonary vascular resistance (PVR) in isolated perfused lung lobes. *J. Physiol. (Lond.)*, **153**, 14*P*.

EULER, U. S. VON & LISHAJKO, F. (1957). Dopamine in mammalian lung and spleen. *Acta physiol. pharmacol. neerl.*, **6**, 295–303.

KONZETT, H. & RÖSSLER, R. (1940). Versuchsanordnung zu Untersuchungen an der Bronchialmuskulatur. *Arch. exp. Path. Pharmak.*, **195**, 71–74.

SCHÜMANN, H. J. (1958). Über den Hydroxytyramin- und Noradrenalingehalt der Lunge. *Arch. exp. Path. Pharmak.*, **234**, 282–290.

SCHÜMANN, H. J. (1959). Über den Hydroxytyramingehalt sympathischer Nerven und sympatisch innervierter Organe. *Arch. exp. Path. Pharmak.*, **236**, 44–46.