J. Physiol. (1961), **157**, *pp.* 475–483
With 4 text-figures
Printed in Great Britain

THE EFFECT OF BRADYKININ IN AN ISOLATED PERFUSED DOG LUNG PREPARATION

BY B. A. WAALER*
From the University Laboratory of Physiology, Oxford

(*Received* 23 *January* 1961)

The polypeptide bradykinin has attracted much attention since the first description of its actions and properties by Rocha e Silva, Beraldo & Rosenfeld in 1949. Following the recent isolation of bradykinin (Elliott, Lewis & Horton, 1960*a*) and the determination of its structure (Elliott, Lewis & Horton, 1960*b*), it has been shown that the pure polypeptide possesses all the biological properties demonstrated for crude preparations (Elliott, Horton & Lewis, 1960).

In the systemic circulation bradykinin causes pronounced vasodilatation and increased capillary permeability in several species. However, the effect of bradykinin on the pulmonary vascular bed has not been examined. The only reported effect of bradykinin in the lungs is that of Collier, Holgate, Schachter & Shorley (1959), who showed that bradykinin causes bronchoconstriction when injected intravenously into guinea-pigs. Bronchoconstriction was also observed in isolated perfused guinea-pig lungs, although here much larger doses of bradykinin were necessary.

In the present experiments on an isolated perfused dog lung preparation, bradykinin caused vasodilatation of the pulmonary vascular bed, which, however, seemed to be less sensitive than other vascular fields. The airways of the isolated perfused dog lung preparation were never constricted and in some experiments were slightly dilated by bradykinin.

A preliminary communication on the findings has already been published (Waaler, 1960).

METHODS

Mongrel dogs (17–25 kg) were premedicated with morphine (2 mg/kg) and injected intravenously with heparin (Boots powdered heparin, 3 mg/kg). In some of the experiments atropine sulphate (1 mg) was also injected intravenously. After inserting a cannula into a femoral artery under local anaesthesia exsanguination was carried out. During collection of the blood more heparin (6 mg/100 ml. blood) was added to the blood, which was then filtered through one layer of linen (mesh 50 μ by 250 μ) and stored at 36° C until used undiluted for the subsequent perfusion.

Perfusion. Either the left apical together with the left cardiac lobe, or the left diaphragmatic lobe alone were used, perfusion of both the pulmonary and the bronchial

* Research Fellow, the Norwegian Research Council for Science and the Humanities.

circulation being carried out as previously described (Daly, 1956; Daly & Waaler, 1960). The pulmonary circulation was perfused either at constant volume inflow or at a constant head of perfusion pressure at 14–22 mm Hg. The reduced systemic circulation, including the bronchial vascular system, was perfused at constant volume inflow. This perfusion was carried out through a cannula inserted into the lower end of the thoracic part of aorta, the ascending aorta being clamped close to the heart. The main bronchial artery or arteries, which in the dog come off from the 5th–8th intercostal arteries on the right side, were segregated and preserved. The other arterial branches from the aorta were ligated. Some of the blood passing through the aorta, however, was short-circuited back to the blood reservoir through small arterial leaks or by means of a cannula inserted into an aortic branch. This arrangement prevented stagnation of blood in the aorta.

Cleaning of equipment and measurements of pressures and flow were carried out as described by Allison, Daly & Waaler (1961).

Pulmonary vascular resistance. The pulmonary vascular resistance (PVR) was calculated according to the formula:

$$\text{PVR} = \frac{\text{Pulmonary arterial pressure (mm Hg)} - \text{Left atrial pressure (mm Hg)}}{\text{Left atrial flow (l./min)} \times x/\text{m}^2 \text{ body surface}},$$

where x equals the ratio of total lung weight to the weight of the perfused lobes, as given by Rahn & Ross (1957).

Ventilation. The lung lobes were ventilated with 6 % CO_2 in air by a Starling 'Ideal' pump at a peak inspiratory pressure of 80–90 mm of water. The ventilation overflow was measured by the method of Konzett & Rössler (1940).

Bradykinin. G. P. Lewis kindly supplied pure bradykinin (Elliott *et al.* 1960*a*) and also a partly purified sample (P_3). Both preparations were used diluted in NaCl solution 0·9 g/100 ml. The dilutions were made up freshly before each experiment. The bradykinin-containing solutions were injected into the pulmonary arterial inflow tubing or into the aortic inflow tubing, from which the bronchial arteries were being perfused. The injections were carried out with fine needles, the volumes (0·1–1·0 ml.) being kept constant for each experiment.

Saliva was collected from human subjects by their spitting into a container, and from the dogs by collecting the spontaneous flow after morphia medication. Both types of saliva were centrifuged at 1200 *g* for 30 min, the clear supernatant liquid being used.

RESULTS

Effect on pulmonary circulation. The effect of injections of bradykinin into the pulmonary arterial inflow tubing was examined in eight atropinized and three non-atropinized preparations. The usual response to such an injection was a fall in PVR, which was obtained in nine experiments; no response was obtained with one preparation and an increase in PVR in another. The minimal effective dose producing a vasodilator response, as tested in three preparations, varied from 0·5 to 5μg of pure bradykinin. From 2 to 10μg produced the maximal dilator effect, giving a fall in PVR of from $\frac{1}{2}$ to 20 %. The dilator effect was evident in 15–30 sec, reached its maximum in 1–2 min and lasted for 2–20 min after the injection. When the injection of bradykinin was repeated several times, the responses diminished, even when the intervals between injections were as long as 15–20 min. This tachaphylaxis is illustrated in Fig. 1, which shows

the responses to three successive injections of pure bradykinin. With each subsequent injection the size and duration of the dilator response diminish. In the experiment of Fig. 1 the PVR was spontaneously and gradually falling during the period of the tests, as shown by the decline in perfusion

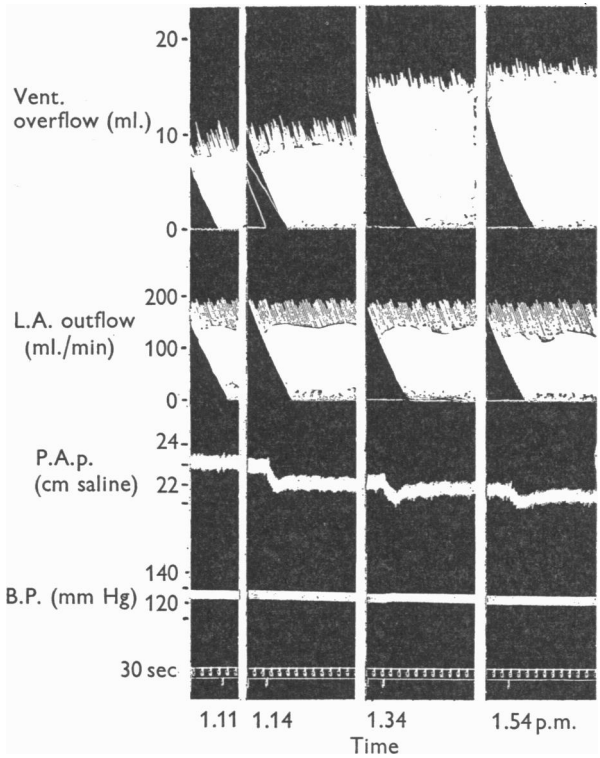

Fig. 1. Effect of successive injections of bradykinin into pulmonary artery of isolated perfused lung lobe preparation. Dog, *m*, 17 kg. Left apical and cardiac lobes perfused. Left atrial pressure = 6·5 cm saline. Injections into pulmonary arterial inflow tubing of 0·2 ml. 0·9 % NaCl solution at 1.11 p.m., of 10 μg pure bradykinin (in 0·2 ml. 0·9 % NaCl) at 1.14 p.m., 1.34 p.m. and 1.54 p.m. Vent = ventilation; L.A. = left atrial; P.A.p. = pulmonary arterial perfusion pressure; B.P. = perfusion pressure in reduced systemic circulation (bronchial circulation). Ventilation pump stroke = 77 ml. Pulmonary vascular resistance (PVR) range during these observations = 7·4–6·5.

pressure. A gradual fall in PVR during the initial 1–2 hr of perfusion is a usual feature of most experiments of this type.

Injections of the crude bradykinin P_2 caused vasomotor effects indistinguishable from those given by pure bradykinin, the relative potency of the crude material in this preparation being about 1/200 of that of the

pure polypeptide. This relationship agrees well with what Elliott *et al.* (1960) found when comparing pure bradykinin with P_2 on other biological preparations.

There was a tendency for bradykinin to cause more marked vasodilatation when the PVR was high than when it was low. This is illustrated in Fig. 2 which includes the maximal responses to bradykinin in ten experiments in which tests were carried out at different PVR levels. The high

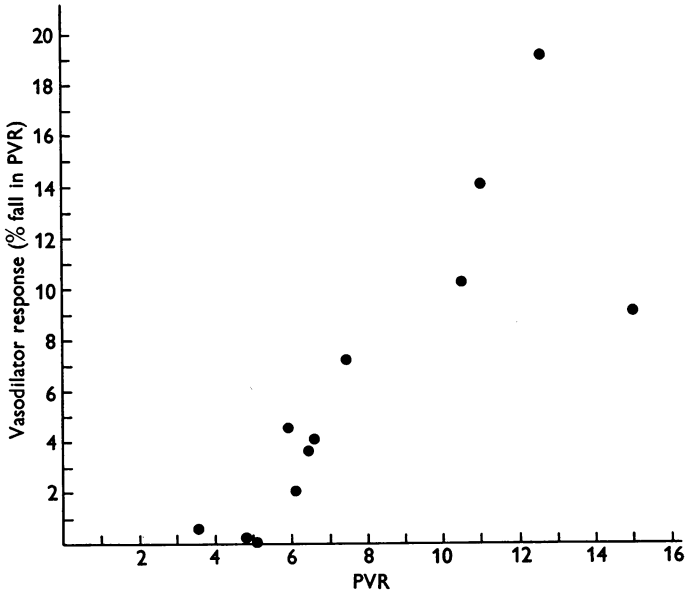

Fig. 2. Vasodilator responses to bradykinin in isolated perfused dog lung lobe preparations at various levels of pulmonary vascular resistance (PVR). Pure bradykinin or P_2 (partly purified substance) injected into pulmonary artery of preparations. The responses are the maximal ones obtained in 10 preparations, two of which are represented by two observations each.

PVR levels at which some of the tests were carried out occurred either during the pulmonary vasoconstriction which follows the start of bronchial circulation perfusion in a handled lung (Daly & Waaler, 1960) or at a late stage in an experiment, when the PVR tends to rise. Because of the tendency for tachaphylaxis, only one or two responses from each preparation are included in the figure. Where one preparation is represented by two observations, the last of them was separated from any previous injection of bradykinin by an interval of 1 hr or more. The vasomotor response to bradykinin was unaffected by atropine, the atropinized preparations giving vasodilator responses as great as those given by the non-atropinized ones.

Effect on bronchial circulation. Bradykinin caused a fall in the perfusion pressure of the reduced systemic circulation when injected into the aortic inflow tubing, or when added to the blood perfusate in the reservoir. Since the reduced circuit consisted of the aorta and the bronchial vascular bed only, this fall in pressure is interpreted as being due to a vasodilatation in the bronchial circulation. Because of the shunting of a considerable amount of blood from the aorta back to the reservoir (see Methods), this perfusion circuit did not allow more than a qualitative evaluation of its vasomotor responses.

Effect on the airways. Injections of pure bradykinin or of P_2 into the pulmonary artery either caused a small bronchodilatation (Fig. 3) or had no effect on the tidal air (Fig. 1). Small bronchodilator responses were seen in one out of three non-atropinized preparations and in four out of eight atropinized ones. The bronchodilator effect never caused more than a 5% increase in tidal air. Like the vascular effect of bradykinin the bronchodilator effect also diminished on repeated injections, as is illustrated in Fig. 3.

Injections of bradykinin through the aortic inflow tubing into the bronchial circulation also caused either a small bronchodilatation (one test) or no change in tidal air (two tests). Bradykinin did not cause bronchoconstriction in any of these experiments whether injected into the pulmonary or into the bronchial circulation.

Effect of saliva on the isolated dog lung. Dog saliva, known to contain a bradykinin-forming enzyme, caused a fall in PVR in five out of six tests when added to the blood perfusate. The response to the addition of 1–2 ml. of saliva was of the same magnitude as that obtained with injections of 10μg pure bradykinin into the pulmonary artery. This vasodilator effect occurred about 30 sec after the addition of saliva to the blood reservoir, and it lasted for 3–10 min. The preparation which responded to injections of pure bradykinin with pulmonary vasoconstriction gave a constrictor response also to the addition of dog saliva. In one of the tests there was a moderate bronchodilatation, in the remaining five no effect on the tidal air.

In contrast to dog saliva human saliva always caused a marked increase in PVR and a reduction in tidal air. The effects of dog and human saliva on the pulmonary and bronchial circulations and on the airways are shown in Fig. 4. Addition of 0·5–2 ml. of human saliva to the perfusion blood in nine other experiments gave results similar to those illustrated by the last observation in this figure. The vasoconstrictor response to human saliva started 0·5–1·25 min after the addition of the saliva to the blood reservoir, and reached its maximum 1·25–13 min after the addition. However, if the saliva was left in contact with cell-free plasma for 10–20 min, the

addition of this mixture to the reservoir caused a more rapidly developing vasoconstriction which reached its maximum within 1·5 min. In two of the ten tests with addition of human saliva the vasoconstrictor response was preceded by a short-lasting fall in PVR.

In preparations perfused at a constant head of pressure (as in Fig. 4) the flow did not recover after being reduced by the effect of added human saliva. In preparations perfused at constant volume inflow (pulsatile flow) the PVR usually returned to the previous value within 15–30 min.

When human saliva was added to the blood reservoir the broncho-constrictor response started 0·5–4 min later, thus sometimes (as in the experiment of Fig. 4) being delayed in relation to the vasomotor response.

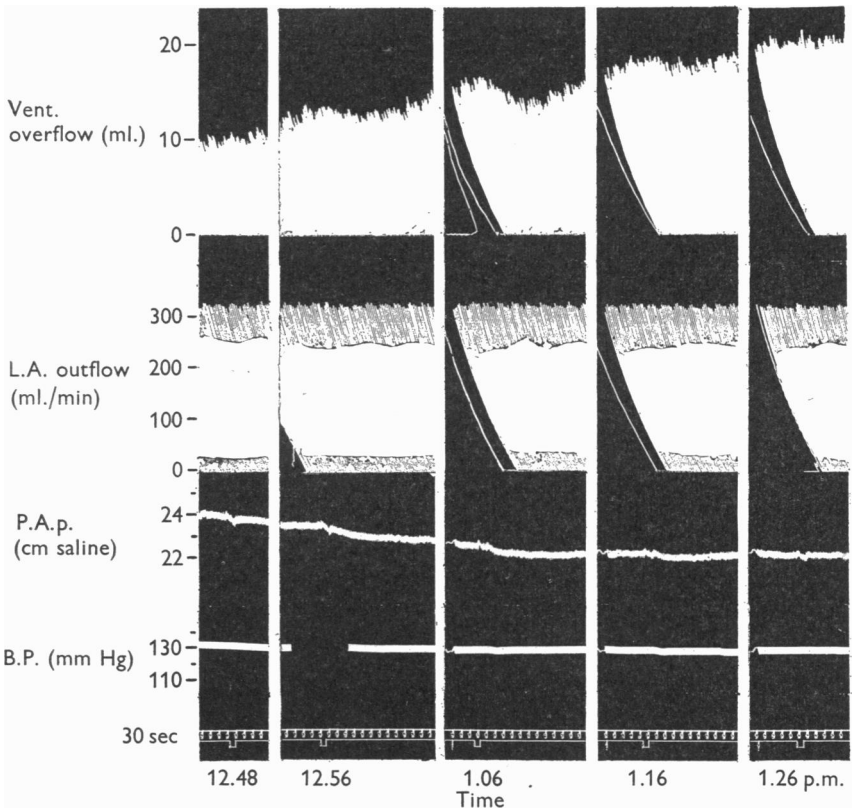

Fig. 3. Effect of successive injections of bradykinin (P_2) into pulmonary artery of isolated perfused lung lobe preparation. Dog, *m*, 23 kg. Left apical and cardiac lobes perfused. Left atrial pressure = 3·5 cm saline. Ventilation pump stroke = 85·5 ml. Injections into pulmonary arterial inflow tubing of 100 μg of P_2 at 12.48 p.m., 1 mg P_2 at 12.56 p.m., 5 mg P_2 at 1.06 p.m. and 1.16 p.m. and of 1 ml. 0·9% NaCl solution at 1·26 p.m. Each dose of P_2 dissolved in 1 ml. of 0·9% NaCl solution. Abbreviations as in Fig. 1. PVR range during these observations = 6·6–6·0.

Addition of human saliva to the blood perfusate usually had little effect on the bronchial circulation perfusion pressure (see Fig. 4), this pressure showing a small to moderate rise in three preparations, a fall in three and no change in another two. In the remaining two preparations tested the bronchial circulation was not perfused.

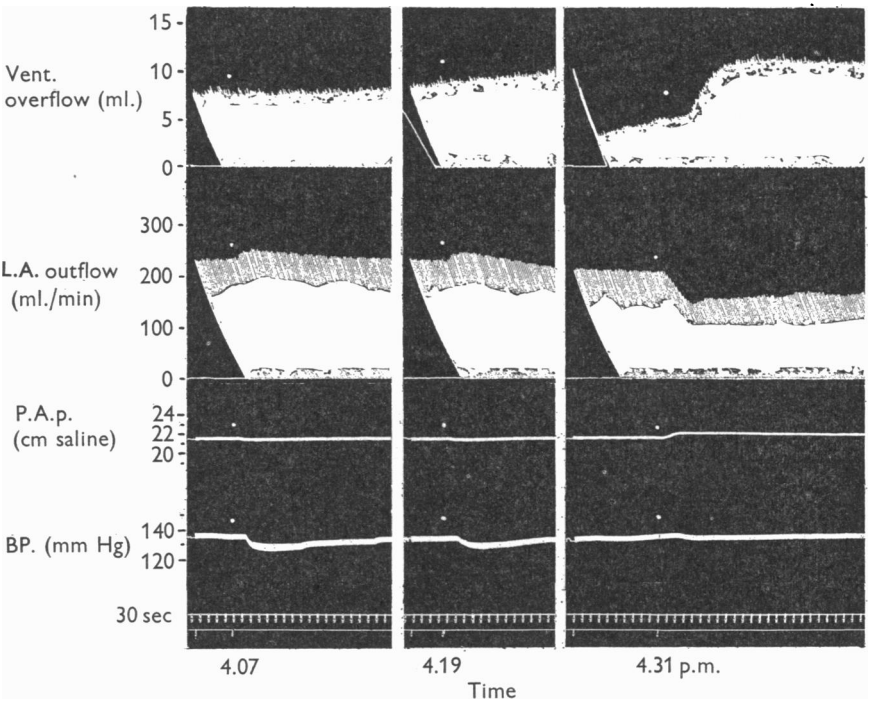

Fig. 4. Effect of addition of dog and human saliva to blood perfusate of isolated lung lobe preparation. Dog, *f*, 17 kg; 1 mg atropine sulphate intravenously before exsanguination. Left apical and cardiac lobes perfused. Left atrial pressure = 4 cm saline. Ventilation pump stroke = 44 ml. to 4.26 p.m., then reduced to 36 ml. Addition to blood reservoir of 1 ml. dog saliva at 4·07 p.m., 2 ml. dog saliva at 4.19 p.m. and 1 ml. human saliva at 4.31 p.m. Abbreviations as in Fig. 1. PVR range during these observations = 6·5–10·7.

DISCUSSION

In isolated lung lobes of the dog, perfused with autologous blood through both the pulmonary and the bronchial circulations, vasodilatation was the predominant response of the pulmonary vascular bed to bradykinin injections. The reduction in pulmonary vascular resistance (PVR) caused by bradykinin was, however, never very marked. Bradykinin was also found to dilate the bronchial vascular system, but no comparison between this response and that of the pulmonary vascular bed was feasible, because

the systemic perfusion circuit was unsuitable for quantitative evaluations of vasomotor responses.

There are two explanations of the fact that the vasodilator effect of bradykinin in the pulmonary circulation was stronger at high levels of PVR than at low levels. One interpretation is that bradykinin may act more powerfully on vessels which have a greater vasoconstrictor tone than on more relaxed vessels, another that bradykinin may be acting selectively on types of vessels which remain open at the high PVR levels, whilst non-responsive vessels are closed down.

The effect of bradykinin on the airways in the isolated lung differs from that obtained in the guinea-pig, in which Collier *et al.* (1959) have found that bradykinin causes bronchoconstriction not only *in vivo* but with large doses in isolated perfused lungs as well. However, the recent finding of Collier & Shorley (1960), that a bronchodilator response to bradykinin in the guinea-pig may be unmasked when the bronchoconstrictor response is prevented by acetylsalicylic acid, suggests that in the guinea-pig too there is sometimes a bronchodilator component in the response to bradykinin.

The effect of dog saliva may be fully explained in terms of bradykinin formed when the bradykinin-forming enzyme in saliva comes into contact with plasma globulins. However, bradykinin formation cannot explain the completely different response to human saliva. Walaszek & Huggins (1959) have recently shown that when amylase, a constituent of human but not of dog saliva, is incubated with plasma proteins, a polypeptide is formed which has a vasoconstrictor effect. It is conceivable therefore, that the pulmonary vasoconstrictor and bronchoconstrictor responses to human saliva in our preparation are due to such a polypeptide.

SUMMARY

1. Injections of pure bradykinin into the pulmonary artery of a perfused isolated dog lung preparation caused a small or moderate pulmonary vasodilatation. The vasodilatation was more marked at high than at low levels of pulmonary vascular resistance.

2. Whether the preparation was atropinized or not, bradykinin caused either a small bronchodilatation or had no effect on the tidal air.

3. Both the vasodilator and the bronchodilator responses to bradykinin were reduced on repeated injections.

4. Additions of dog saliva to the blood perfusate caused effects similar to those given by injections of large doses of pure bradykinin. Human saliva, however, caused marked pulmonary vasoconstriction and bronchoconstriction when added to the blood perfusate.

I wish to express my thanks to Professor I. de Burgh Daly, F.R.S. and to Dr G. P. Lewis for valuable advice and encouragement, to Professor E. G. T. Liddell, F.R.S. and to Professor Sir Lindor Brown, F.R.S. for their kindness in granting laboratory facilities, and to Mr Nolan Howland and Miss Diana Jones for technical assistance. This work was done during a period in which I received grants from the Norwegian Research Council for Science and the Humanities, from the University of Oslo, and from Dr A. Malthes Legacy.

REFERENCES

ALLISON, P. R., DALY, I. DE BURGH & WAALER, B. A. (1961). Bronchial circulation and pulmonary vasomotor nerve responses in isolated perfused lungs. *J. Physiol.* **157**, 462–474.

COLLIER, H. O. J., HOLGATE, J. A., SCHACHTER, M. & SHORLEY, P. G. (1959). The bronchoconstrictor action of bradykinin in the guinea-pig. *Brit. J. Pharmacol.* **15**, 290–297.

COLLIER, H. O. J. & SHORLEY, P. G. (1960). Analgesic antipyretic drugs as antagonists of bradykinin. *Brit. J. Pharmacol.* **15**, 601–610.

DALY, I. DE BURGH (1956). Pulmonary vascular responses in an innervated isolated perfused left lung preparation. *J. Physiol.* **132**, 42–43*P*.

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

ELLIOTT, D. F., HORTON, E. W. & LEWIS, G. P. (1960). Actions of pure bradykinin. *J. Physiol.* **153**, 473–480.

ELLIOTT, D. F., LEWIS, G. P. & HORTON, E. W. (1960*a*). The isolation of bradykinin: A plasma kinin from ox blood. *Biochem. J.* **74**, 15–16*P*.

ELLIOTT, D. F., LEWIS, G. P. & HORTON, E. W. (1960*b*). The structure of bradykinin. *Biochem. J.* **76**, 16*P*.

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

RAHN, H. & ROSS, B. B. (1957). Bronchial tree casts, lobe weights and anatomical dead space measurements in the dog's lung. *J. appl. Physiol.* **10**, 154–157.

ROCHA E SILVA, M., BERALDO, W. T. & ROSENFELD, G. (1949). Bradykinin, a hypotensive and smooth muscle stimulating factor released from plasma globulin by snake venoms and by trypsin. *Amer. J. Physiol.* **156**, 261–273.

WAALER, B. A. (1960). The effect of bradykinin in an isolated perfused dog lung preparation. *J. Physiol.* **154**, 57–58*P*.

WALASZEK, E. J. & HUGGINS, C. G. (1959). Substance A: a hypertensive polypeptide from fraction IV-4 of plasma protein. *J. Pharmacol.* **126**, 258–263.