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THE RELEASE OF A BRADYKININ-LIKE PULMONARY VASODILATOR SUBSTANCE IN FOETAL AND NEW-BORN LAMBS

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SUMMARY

1. Exposure to glass of whole blood from anaesthetized foetal lambs (at 0·6–0·95 of term) causes the rapid development of a potent pulmonary vasodilator agent.

2. The formation of the vasodilator agent on exposure to glass, its disappearance with time, and the inhibition of its production by soya bean trypsin inhibitor, suggest that it is bradykinin.

3. Small doses of bradykinin (~ 1 ng/kg) cause pulmonary vasodilatation on close arterial injection. Neither this, nor the vasodilatation caused by glass-exposed whole blood, are affected by agents which block the vasodilator actions of acetylcholine, isoprenaline or histamine.

4. Exposure of foetal plasma and/or red cells to glass rarely caused the development of a pulmonary vasodilator agent. Maternal plasma was always active. Injection of the buffy coat from foetal blood caused pulmonary vasodilatation.

5. The capacity of plasma from sheep at different ages to produce kinin(s) was examined by assay on the isolated rat's uterus. In the foetus activity was very low; it increased with age.

6. In adolescent lambs 5–14 weeks after birth injection of ~ 10 ng/kg bradykinin into the pulmonary artery caused only a trifling vasodilata-

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tion; this was greater when pulmonary vasoconstriction was induced by lung collapse, hypoxaemia and hypercapnia.

7. The possible physiological consequences of these findings are discussed.

INTRODUCTION

In previous experiments on the lungs of foetal lambs (Cassin, Dawes, Mott, Ross & Strang, 1964; Colebatch, Dawes, Goodwin & Nadeau, 1965; Campbell, Dawes, Fishman & Hyman, 1967) occasional instances of otherwise unexplained pulmonary vasodilatation were observed. This was attributed to the formation of kinins in the extracorporeal circuit leading from another artery (usually the ipsilateral carotid) to the left pulmonary artery. The present paper describes experiments designed to elucidate this phenomenon, from which it is concluded that kinins are readily produced when foetal blood is exposed to glass, and that their formation depends not so much on glass contact with plasma or red cells but with other formed elements of the blood. The pulmonary (and femoral, Dawes, Lewis, Milligan, Roach & Talner, 1968) circulations of the foetus are found to be very sensitive to brady-kinin, as little as 1 ng causing vasodilatation on close-arterial injection. A brief account of these experiments has been given elsewhere (Campbell, Dawes, Fishman & Hyman, 1966; Dawes, 1966).

METHODS

Foetal lambs. Observations were made on twenty-four foetal lambs of 86–141 days gestation (term is ~ 147 days). The ewes were delivered by Caesarean section under light chloralose anaesthesia (30–40 mg/kg I.V. initially). The lambs were laid alongside the ewe on a warmed table, covered so far as possible with cellulose wadding (B.P.), with the umbilical cord intact.

Blood flow in the left pulmonary artery was measured by a cannulated electromagnetic flowmeter in an extracorporeal loop from the central end of the left carotid (Fig. 1). The details of the preparation and recording instruments are described elsewhere (Cassin *et al.* 1964; Colebatch *et al.* 1965; Campbell, Dawes, Fishman & Hyman, 1967).

New-born lambs. Observations were also made on nine young lambs, 5–14 weeks after birth, weighing 10–21 kg, under light chloralose anaesthesia (40 mg/kg I.V. initially). A tracheal cannula was inserted and positive pressure respiration was given from a Starling Ideal pump. The left chest was opened to give access to the left pulmonary artery. The left hemiazygos vein was tied and divided, and the pericardial insertion around the left pulmonary artery was cleared from the vessel. Heparin was injected (10 mg/kg I.V.). The left pulmonary artery was divided and its ends were reconnected by an external circuit of Perspex cannulae and polyethylene tubing, including an electromagnetic flowmeter gauge head (Wyatt, 1961) and an Elema inductance manometer. Systemic arterial pressure was measured from a femoral artery and left atrial pressure from a catheter in the atrial appendage. Left pulmonary arterial blood flow, the pressures and heart rate (Wyatt, 1957) were recorded on a Schwarzer polygraph. Injections of drugs were given into the external circuit close to the left pulmonary arterial cannula. The left bronchus was occluded, when required,

by inflating a balloon passed down from the trachea. Samples (0·4 ml.) of carotid arteria blood were removed anaerobically and the pH, P_{O_2} and P_{CO_2} were measured at once using Radiometer micro-electrodes. The results were corrected for the difference between electrode and rectal temperature, where necessary.

Generation of kinins from plasma in vitro. Blood was collected, from a polyethylene catheter in a carotid or femoral artery, into ice-cold polypropylene centrifuge tubes (14 ml.) containing 1 mg/ml. solid heparin. The tubes were covered with parafilm, inverted thrice and spun at 2400 *g* for 55 min at 0 °C. The plasma was withdrawn to polypropylene tubes using

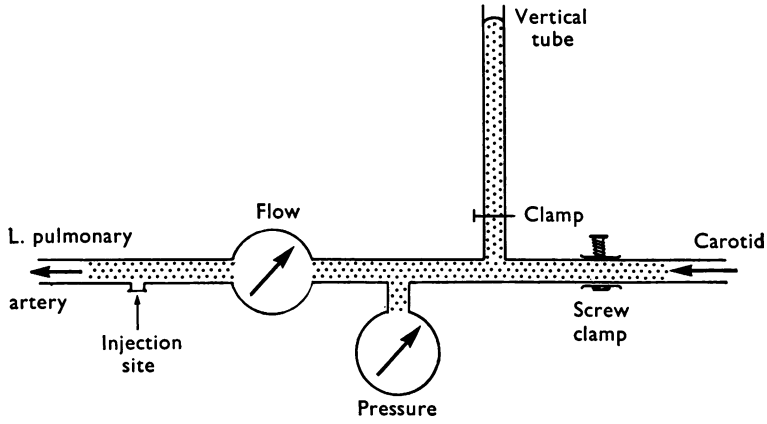

Fig. 1. Schematic diagram of preparation.

plastic syringes, both at 0° C. The cold tubes of plasma were removed to room temperature (23–26·5° C) and a 1 ml. sample was taken at once for assay. After 20 min at room temperature a further control sample of 1 ml. was removed, and one of the following combinations was added to the remaining plasma, in the order stated, and with mixing where necessary:

(*a*) New acid-washed glass ballotini, 0·45–0·5 mm (Jencons Scientific Ltd., No. 8) 0·28 g/ml. plasma, calculated to give about the same surface area (11·6 cm^2/ml.) as that presented by a 5 ml. glass syringe to the plasma in 2 ml. whole blood (no allowance being made for direct occlusion of the surface by erythrocytes).

(*b*) Ethylenediaminetetra-acetic acid (EDTA sodium salt, Analar) 4 mg/ml. plasma; glass ballotini as in (*a*).

(*c*) Soya bean trypsin inhibitor (Koch–Light Laboratories, Colnbrook; batch no. 3896) 1 mg/ml. plasma; EDTA and glass ballotini as in (*b*).

At 5 or 10 min intervals, the incubation tubes were inverted to ensure homogeneity, and 1 ml. samples were withdrawn with plastic syringes and added to 2 ml. 0·9% (w/v) NaCl at 100° C in siliconed glass tubes resting in a boiling water-bath. After 1 min, the tubes were removed, cooled, and centrifuged at 0° C; the supernatant was removed with a plastic syringe and assayed for kinins on the isolated rat uterus (as in Holton, 1948), which had been rendered insensitive to the effects of plasma proteins by previous exposure for 1 min to soya bean trypsin inhibitor (1 mg/ml. de Jalon's Ringer, Margolis, 1958). Synthetic bradykinin (Sandoz, BRS 640, No. 64052, 0·1 mg/ml.) was diluted to 100 ng/ml. 0·9% (w/v) NaCl solution in a 200 ml. siliconed flask for use as standard; in the conditions used, it was shown to retain its potency during assay. Plasma was depleted of kinins by glass contact in the absence of EDTA; recovery of known quantities of added bradykinin after the manipulations outlined above was within the experimental limits of the assay.

RESULTS

Observations in vivo. Previous experiments on mature foetal lambs with unexpanded lungs (Colebatch *et al.* 1965) had shown that manipulations, used to generate a pressure–flow curve *in vivo*, often caused a considerable pulmonary vasodilatation after a latent period of 15–20 sec. In these experiments the lamb was given heparin, and the left carotid artery was connected through a flowmeter to the left pulmonary artery. A vertical polyethylene tube was attached to this connexion (Fig. 1). This vertical tube was filled with blood and inflow from the carotid was arrested; blood then entered the left pulmonary artery from the vertical tube at a progressively decreasing pressure. After a short interval (rarely more than 10 sec) when a sufficient part of the pressure–flow curve had been drawn, inflow from the carotid artery was restored, and pressure and flow rose rapidly. There then ensued a large pulmonary vasodilatation. This could be attributed either to the changes in vascular pressure or to entry of blood into the circulation from the vertical tube.

In the present experiments, in order to distinguish between these two hypotheses, left pulmonary arterial pressure was reduced by tightening a screw clamp between the left carotid artery and the connexion with the vertical tube (Fig. 1). As Fig. 2*a* shows, there was a steady fall in pressure and flow until, after about 20 sec, this was interrupted by an abrupt rise of flow and fall of arterial pressure indicating pulmonary vasodilatation. When the experiment was repeated with the vertical tube clamped off (Fig. 2*b*) there was no such vasodilatation, until at the arrow the clamp was removed, about 2 ml. blood entered the circulation from the vertical tube, and after about 15 sec pulmonary vasodilatation occurred. It was concluded, from this and two qualitatively similar experiments, that entry of blood from the vertical tube must account for the phenomenon, which was not due to the small fall in arterial pressure. Although the vertical tube was made of a plastic, polyethylene, it was connected by a glass T-piece to the flexible plastic tubes which joined the left carotid, flowmeter and left pulmonary artery. It was therefore possible that plasma kinin(s) had been formed by glass contact. Further experiments showed that this was a plausible theory in that a substance having the properties of bradykinin is readily formed in foetal blood, and that bradykinin in very small doses causes intense vasodilatation in the foetal lung. When the glass T-piece was replaced by one of plastic (Perspex) the phenomenon illustrated in Fig. 2 was no longer seen.

Experiments using fresh whole blood. In all subsequent experiments (twenty-one foetal lambs) the left carotid artery was joined to the left pulmonary artery with plastic cannulae and T-pieces (Perspex) and tubes

(polyvinyl and polyethylene) via the flowmeter gauge head, which had silver end-pieces and platinized gold electrodes in a Perspex tube. The external circuit contained neither glass nor a vertical side tube; the lamb was heparinized. When 2–5 ml. blood were withdrawn into a glass syringe from this flow circuit and were re-injected within 5–10 sec, there was a

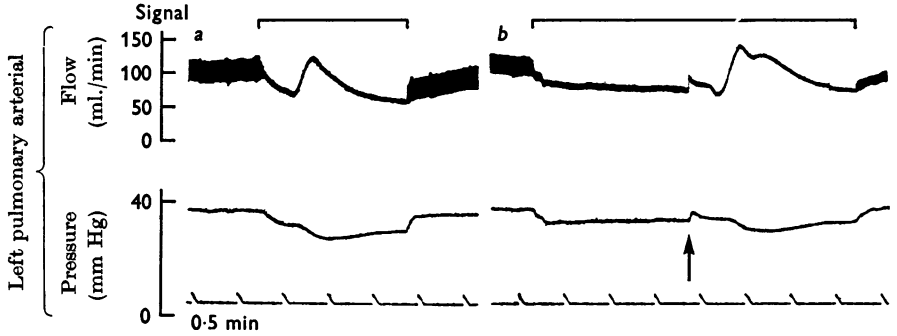

Fig. 2. Foetal lamb, 119 days gestation; preparation as in Fig. 1, with a glass connexion to the vertical tube. The screw clamp was tightened to reduce pulmonary arterial pressure during the upper signal mark; (*a*) with the vertical tube open, or (*b*) with the vertical tube clamped off until the arrow. Pulmonary vasodilatation occurred only when blood entered the lung from the vertical tube.

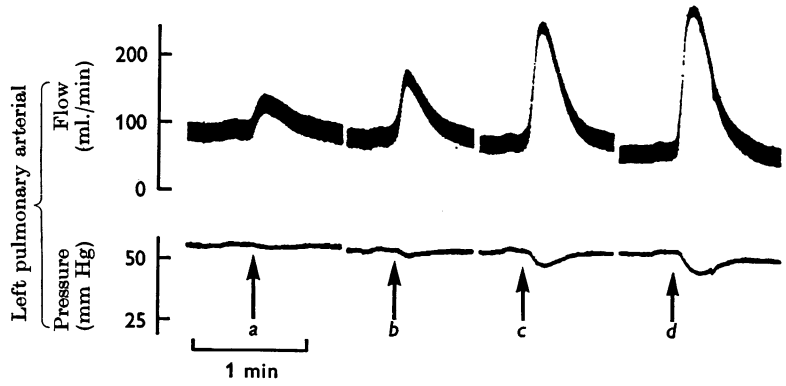

Fig. 3. Foetal lamb, 140 days gestation. Blood was withdrawn from the extracorporeal loop (Fig. 1) into a new 5 ml. glass syringe, held for 1 min at room temperature, and was then re-injected over 5 sec. The procedure was repeated 4 times, using the same syringe at 4 min intervals; the resultant pulmonary vasodilatation increased in size.

transient passive rise in arterial pressure and flow but no alteration in pulmonary resistance (as shown by reference to pulmonary arterial pressure–flow curves). When the blood was allowed to remain in a glass syringe at room temperature for 45 sec or more there was a rise in flow and fall in

arterial pressure on re-injection, due to pulmonary vasodilatation. The magnitude of the effect depended on the nature and previous history of the syringe, the duration of exposure and the temperature.

When a new all-glass syringe was used for the first time the vasodilatation observed on re-injection of blood after 1 min was small (Fig. 3*a*). On the next 3–4 trials there was a progressive increase in the responses (Fig. 3*b*, *c*, *d*), possibly due to the adsorption of Hageman factor on to the glass (Margolis, 1960), after which the responses became steady. This phenomenon was observed with each of ten new glass syringes in five lambs. Repeated washings with saline (0·9 % (w/v) NaCl) or distilled water or boiling the syringe did not change its efficacy in promoting generation of a pulmonary vasodilator agent. But after many exposures to blood, often after several days continuous use, syringes began to lose their efficacy, which could be restored by boiling in N-NaOH. It was therefore important to establish the efficacy of the glass syringes during each experiment.

Given an efficient glass syringe, the magnitude of the vasodilatation observed on re-injection of blood depended on the duration of glass exposure at room temperature, increasing rapidly over the first few min (compare Fig. 4*a* and *b*) to reach a peak between 4 and 16 min, after

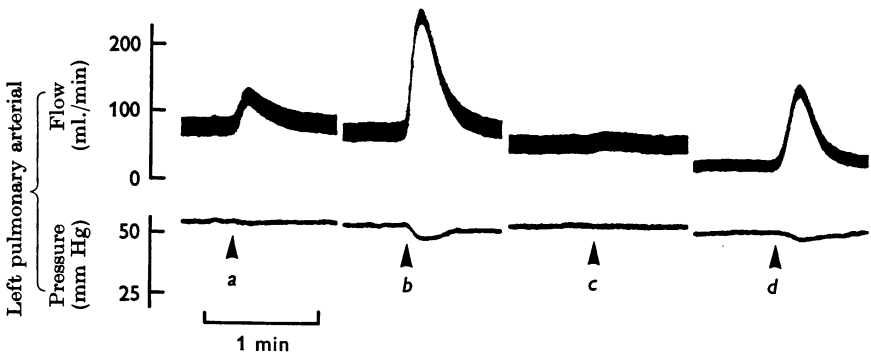

Fig. 4. As Fig. 3. Injection of 2 ml. blood held in a glass syringe at room temperature for 1 min (*a*) or 2 min (*b*) caused pulmonary vasodilatation. Exposure of 2 ml. blood for 2 min in a plastic syringe did not (*c*). Injection of 10 ng bradykinin caused vasodilatation (*d*). Injections were made over 5 sec at 6 min intervals.

which the size of the response slowly declined (Fig. 6). The same results were obtained in four lambs whether the blood was removed into a glass beaker and gently agitated or was held in a glass syringe, both at room temperature. When the blood (in glass) was rapidly cooled in ice water it did not develop vasodilator activity. From each of three lambs blood (2 ml.), mixed in a glass syringe for 2–4 min at room temperature with soya bean trypsin inhibitor (1 mg), did not develop vasodilator properties.

These facts are consistent with enzymic reactions similar to those in plasma kinin formation (e.g. Margolis, 1958, 1960).

When blood was removed into a plastic syringe at room temperature for 0–4 min and was then re-injected it usually caused no change in pulmonary vascular resistance (Fig. 4*c*). However, in two of ten lambs exposure of foetal blood to plastic for 2–10 min at room temperature did cause some activation of a pulmonary vasodilator agent, though it was very much less effective than exposure in glass syringes of similar dimensions. Each of these plastic syringes, of two makes (Gillette; Johnson & Johnson), and the needles, were used once only and then discarded; they came from the same batch, which in other foetal lambs had had a negligible effect.

In four foetal lambs both bradykinin (2–5 ng injected close arterially) and fresh blood exposed in glass syringes for 2 min at room temperature caused undiminished pulmonary vasodilatation after administration of atropine (1 mg/kg), propranolol (0·5–1 mg/kg) and mepyramine (5–10 mg/kg). These abolished the pulmonary vasodilator actions of acetylcholine (1–2 μg/kg) and isoprenaline (1–2 μg/kg) and very greatly reduced that of histamine (2 μg/kg), respectively. In lambs near term arterial injection of small doses of KCl (1 % (w/v) 5–10 mg) caused pulmonary vasodilatation similar to that produced by 5–10 ng bradykinin; in immature foetal lambs it had little effect (Campbell, Dawes, Fishman & Hyman, 1967). It is most unlikely that such large quantities of K^+ could be released from glass exposure of 1–2 ml. blood for 2 min, nor would K^+ disappear from the blood with time.

Bradykinin. Injection of bradykinin into the left pulmonary artery of sixteen mature foetal lambs caused pulmonary vasodilatation (Fig. 4*d*). The size of the response differed according to the circulatory path length. Thus when injected close to the pulmonary arterial cannula the response was large, while distant injection (such as to increase transit time by 10–15 sec) caused a small response (Fig. 5). This might have been partly due to dilution or to absorption into the formed elements of the blood, though destruction in the bloodstream is more likely. Close arterial injection of isoprenaline, which also causes pulmonary vasodilatation, had a slightly greater effect than distant injection, but the difference was not nearly so large as with bradykinin. All subsequent comparisons between the effects of injections of blood or plasma and bradykinin were made after administration at the same point in the circulatory path, usually on close arterial injection.

Changes in the rate of blood flow also altered the size of the response to bradykinin. When flow was low, e.g. 20–30 ml./min. in a mature foetal lamb, the response was apparently less than when flow was 80–100 ml./min.

Also when flow was very high and the vessels were already much dilated, the response was reduced. Injection of 2–10 ng bradykinin during a period of asphyxia (caused by umbilical cord occlusion for 2 min) caused pulmonary vasodilatation of a degree which, taking into account the reduction in pulmonary flow by vasoconstriction, was similar to that in the absence of asphyxia.

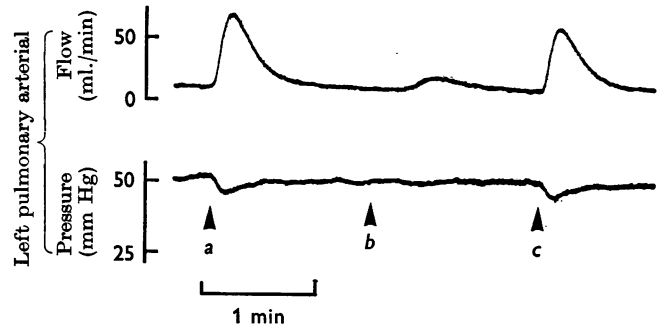

Fig. 5. Foetal lamb, 139 days gestation. Injection of 2 ng bradykinin close arterially (*a*, *c*) caused a larger pulmonary vasodilatation than on distant injection (*b*) into the extracorporeal loop.

The minimal effective dose of bradykinin in causing pulmonary vasodilatation on close arterial injection was < 2 ng in twelve lambs, and often as little as 0·5 ng. There was an increase in response with increasing dose over a range of about tenfold. Injections of bradykinin repeated every 2–4 min produced the same response, provided that there had been no substantial change in resting pulmonary flow. Hence it was possible to make an estimate of the quantity of bradykinin which might have been released on exposure of blood or blood fractions to glass (e.g. Fig. 6). In eight lambs of 104–140 days gestation the pulmonary vasodilator effect of re-injecting 2 ml. whole blood which had been held for 2 min at room temperature in a 12 mm i.d. glass syringe was at least equal to that caused by injection of 1·5 ng bradykinin, and in a further five lambs it was equal to the effect of 5 ng or more bradykinin. In eight lambs of 86–92 days gestation, the effect of 0·5 ml. whole blood held for 1 min at room temperature in an 8 mm i.d. glass syringe was equal to that of 2–5 ng bradykinin. There was no evidence of variation in the capacity of whole foetal blood to produce a bradykinin-like substance with age, over the age range examined.

Experiments on plasma and separated red cells. Foetal whole blood was removed, through a polyethylene catheter inserted via a femoral artery, into a plastic or siliconed glass tube immersed in ice water, and was spun at 2400 *g* for 7·5–30 min. The duration of centrifugation did not affect the

results. In eight of nine lambs 1–2 ml. plasma had no effect on the pulmonary blood vessels on close-arterial injection: in the ninth it caused a very small vasodilatation. The plasma was not activated (to cause the appearance of a pulmonary vasodilator agent) by contact with plastic syringes or tubes. Exposure to glass (e.g. in a glass syringe at 37° C for up to 4 min) caused an activation of doubtful significance in three lambs, and of very small size

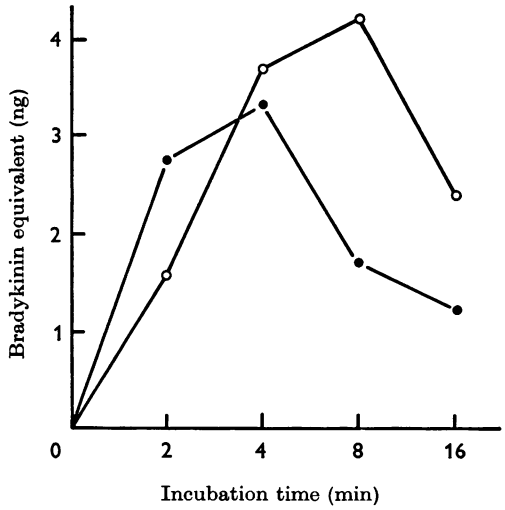

Fig. 6. Observations on the rate of development and disappearance of pulmonary vasodilator activity (expressed in bradykinin equivalence on close-arterial injection) of 2 ml. blood samples withdrawn from foetal lambs of 137 (○) and 141 (●) days gestation into a 5 ml. glass syringe at room temperature.

in another six; the largest response seen is shown in Fig. 7*b*. This activation was always much less than that observed on exposure of maternal plasma (removed at the same time and separated in the same way) to the same glass syringe (Fig. 7*d*). The vasodilator effect of 1 ml. glass-activated plasma was much less than that of 2 ml. glass-activated whole blood, removed from the same foetus (compare Fig. 7*b* and *e*).

In six foetal lambs injection of reconstituted spun red cells and plasma (omitting the buffy coat) after exposure in a glass syringe for 2–4 min failed to produce pulmonary vasodilatation, while exposure of fresh whole blood to glass readily caused formation of a vasodilator agent. Evidently the presence of white cells and/or platelets is normally necessary for the rapid generation of kinins in whole foetal blood. When the buffy coat from 2 to 5 ml. blood was suspended in its original volume of either saline or plasma in a plastic syringe it caused a large vasodilatation, even though it had not been exposed to glass. Presumably centrifugation into a com-

pact sticky layer had led to activation and production of a vasodilator agent.

Kinin formation in vitro from the plasma of sheep at different ages. The rate of kinin formation, on exposure to glass ballotini at room temperature, of plasma from sheep of different ages was measured on the isolated rat's uterus (Table 1). In plasma from five mature foetal lambs no kinin formation was detectable up to 3·5 hr. In the plasma from two of five

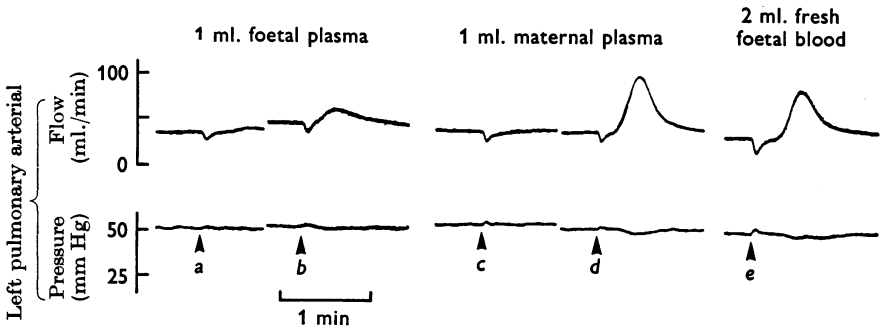

Fig. 7. Foetal lamb, 137 days gestation. Injection of foetal (*a*) or maternal (*c*) plasma without incubation in glass does not cause pulmonary vasodilatation. On incubation in glass for 4 min, both maternal plasma (*d*) and foetal whole blood (*e*) cause a larger vasodilatation than foetal plasma (*b*). The latter was the largest response seen with foetal plasma in nine lambs.

TABLE 1. Rate of kinin production in plasma from sheep of different ages on glass exposure at 24·5° C

	Age	Number	Weight (kg)	Kinin production* (ng/ml. min)
Foetal lambs	130–143 days gestation	5	2·9–4·4	Not detectable
New-born lambs	26–95 days after birth	5	10–21	0–0·72
Adult female (non-pregnant)	5–6 years	2	70	0·13–1·3
Adult female (85–142 days pregnant)	5–6 years	3	49–93	0·56–9·40

* Assayed as bradykinin over first 15 min exposure.

new-born lambs there was some kinin formation, and in all of five adult sheep. Where kinins were detected the peak concentration was observed at ~ 15 min; thereafter it declined rapidly, presumably because of enzymic destruction. So the results shown in Table 1 are expressed as the rate of production over the first 15 min.

The rate of degradation of kinins in plasma is reduced by EDTA. When plasma from foetal lambs was exposed to glass in the presence of EDTA there was evidence of kinin production, but at a rate much less than that

observed in the plasma of new-born lambs or adult sheep (Table 2). The rates were averaged over the first 40 min because this was the minimum time for the production of a measurable quantity in foetal lambs. In adults the rate of production seemed rather greater in pregnancy. Soya bean trypsin inhibitor prevented kinin formation in all of twenty-three tests. In the absence of glass (i.e. in the polypropylene vessels) the rate of kinin production averaged 4 % of that observed in the presence of glass. Once formed the kinin was stable to heat (boiling saline, see Methods) and survived storage at 0° C for 2 weeks.

Adolescent lambs. The reactivity to bradykinin of the pulmonary vascular bed of foetal lambs was so great, compared with that observed in adults of other species (see the Discussion for references) that it was desirable to determine whether this was a species difference. In nine

TABLE 2. Rate of kinin production in plasma from sheep of different ages on glass exposure at 24·5° C in the presence of ethylenediaminetetra-acetic acid

	Age	Number	Weight (kg)	Kinin production* (ng/ml. min)
Foetal lambs	130–138 days gestation	7	3·0–5·0	Mean 0·36 (range 0·11–0·67)
New-born lambs	42–95 days from birth	4	16–21	Mean 2·9 (range 2·3–3·5)
Adult female (non-pregnant)	5–6 years	2	70	1·7–2·9
Adult female (137 days pregnant)	5–6 years	2	79–90	5·2–6·1

* Assayed as bradykinin over the first 40 min exposure.

lambs 5–14 weeks from birth, injections of 0·1–1·0 μg bradykinin into the left pulmonary artery caused only a trifling pulmonary vasodilatation (Fig. 8*a*). It was possible that this was because the lung vessels were already widely dilated. Therefore in six lambs the left lung was collapsed by obstruction of the main bronchus after ventilation with 100 % O_2 for 10 min. The pulmonary vasoconstriction in the left lung was increased still further by ventilating the right lung only with gas mixtures containing 5–7 % CO_2 and 8–12 % O_2. Pulmonary arterial P_{O_2} was reduced to 18–28 mm Hg and P_{CO_2} rose to 47–63 mm Hg. There was a relatively large rise in systemic and pulmonary arterial pressures and a decrease in pulmonary flow. Under these circumstances injection of 0·1 μg bradykinin caused a larger vasodilatation (Fig. 8*b*), though still not as great as that observed in foetal lambs. Injections of 20–100 μg acetylcholine, which in the normally ventilated lamb had either no effect or caused pulmonary vasoconstriction, also now caused vasodilatation.

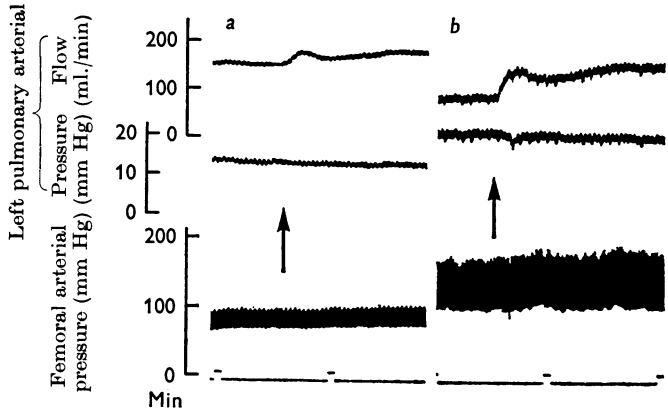

Fig. 8. Adolescent lamb, 10 weeks after birth, 16 kg. Close-arterial injection into the left pulmonary artery of 100 ng bradykinin at each arrow (*a*) when both lungs were ventilated with air, and (*b*) during left pulmonary vasoconstriction caused by collapse of the left lung and underventilation of the right lung with 7 % CO_2 and 21 % O_2 in N_2, to give an arterial P_{O_2} 18, P_{CO_2} 56 mm Hg.

DISCUSSION

The results show that kinin formation can cause artifacts during experiments on foetal lambs in which flow measurement is made by an extracorporeal loop, where this includes glass connexions. When glass is eliminated the effect is usually abolished. In some lambs exposure of blood to plastic material caused the generation of a kinin; this was normally too slow to give trouble *in vivo*. However, subsequent experience with extracorporeal plastic loops between a carotid and pulmonary artery (Campbell, Dawes, Fishman & Hyman, 1967; Campbell, Cockburn, Dawes & Milligan, 1967) or femoral artery (Dawes, Lewis, Milligan, Roach & Talner, 1968) has shown that it is advisable to avoid side-connexions in which small volumes of blood may stagnate and develop vasoactive substances.

Apart from their bearings on this technical problem, the results are of interest because they show that the pulmonary vascular bed of the foetal lamb is exquisitely sensitive to bradykinin, and because in the foetus a bradykinin-like substance is rapidly formed from whole blood, but not so readily from plasma or red cells.

In isolated perfused rabbit lungs large doses of bradykinin caused vasoconstriction (Lecomte & Troquet, 1960). In isolated perfused adult dog lungs the minimal effective vasodilator dose of bradykinin was 0·5–5 μg (Waaler, 1961); the maximum fall in pulmonary vascular resistance was 20 %. There was a tendency for the vasodilatation to be greater when

vascular resistance was high initially. In adult man infusion of 0·3–1·0 μg/kg.min increased cardiac output, but there was evidence of pulmonary vasodilatation only at the higher dosage (Bishop, Harris & Segal, 1965). These results in adults are similar to those observed in adolescent lambs, which suggests that the greater sensitivity to bradykinin in the foetal lamb is not a species difference, but a consequence of the more constricted vascular bed.

Previous work on vasodilator polypeptides has emphasized their formation from plasma, and they are often described as plasma kinins (Gaddum, Hilton, Lewis, Keele & Schachter; quoted by Lewis, 1958). It was therefore surprising to find them produced so readily in the whole blood of foetal lambs, but not from separated plasma or red cells. The presence of some component of the buffy coat appears indispensable to rapid formation of kinin activity in foetal blood. There are several indications that this is the polymorphonuclear leucocyte fraction (Greenbaum, Freer & Kim, 1966; Cline & Melmon, 1966; Zachariae, Malmquist & Oates, 1966; Greenbaum & Kim, 1967).

We also may consider whether these observations suggest a physiological role for bradykinin. For instance, bradykinin might be responsible for the reactive hyperaemia which ensues on the relief of ischaemia in unventilated foetal lungs (Dawes & Mott, 1962). The present experiments show that bradykinin still causes pulmonary vasodilatation on close arterial injection during asphyxia, when the arterial pH is decreased. *In vitro* the activity of kininase from the pseudoglobulin fraction of adult plasma is inhibited at a slightly acid pH, while kinin formation proceeds normally (Edery & Lewis, 1962). Kinin formation from human bradykininogen by polymorphonuclear leucocytes is favoured by an acid pH (Greenbaum & Kim, 1967). So far as they go these *in vitro* experiments suggest that plasma bradykinin concentration might increase during circulatory arrest, to cause vasodilatation on release. Pulmonary vasodilatation on first ventilation of the foetal lungs (or, conversely, vasoconstriction on asphyxia) at present seems unconnected with bradykinin production. Up to 80 % of bradykinin infused intravenously into adult cats disappears during passage through the pulmonary circulation (Ferreira & Vane, 1967), but there is as yet no evidence that it is responsible for the low vascular resistance of ventilated lungs, nor that its production is contingent upon normal adult blood gas tensions and pH. Moreover, Duke & Killick (1952) observed normal pulmonary vasomotor responses to ventilation with CO or N_2 in isolated adult cat lungs perfused either with heparinized blood or with Dextran solution. In the latter instance bradykinin could not be formed from the plasma or formed elements of the blood.

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