The release and detection of bronchoactive substances by serially perfused isolated lungs

A residual increase in air overflow volume during anaphylaxis of the Konzett Rössler preparation of guinea-pig lungs *in vivo* occurred after pretreatment of the animal with antagonists of histamine, 5-hydroxytryptamine (5-HT), the Hounslow preparation of slow-reacting substances in anaphylaxis (SRS-A–H), and bradykinin (Collier & James, 1967). This increase was intensified by blockade of β-receptors for adrenaline and was not prevented by destruction of the central nervous system. That papaverine lessened the residual effect suggested that it was wholly or partly due to bronchoconstriction. Suggested mechanisms, by which this residual bronchoconstriction might occur, included the failure of the antagonists used to suppress a known humoral factor and the release of an unidentified factor not susceptible to these antagonists (Collier & James, 1967). A preparation of serially perfused isolated lungs described below has been developed to explore these possibilities.

Guinea-pig lungs were isolated by the technique of Bhattacharya & Delaunois (1955). The guinea-pig was lightly anaesthetized with phenobarbitone (30 mg/kg, i.p.), the trachea was cannulated and artificial ventilation was established with a pump of 5–8 ml stroke volume at a rate of 40 strokes/min. The pulmonary artery and vein were cannulated and the lungs perfused by a peristaltic pump at 8–12 ml/min with Tyrode solution containing 2·5% w/v polyvinyl pyrrolidone at 37°. The lungs were then excised and suspended in a heating jacket containing water at 37° (Fig. 1). The pressure in the trachea was measured by a pressure transducer in the side-arm of the tracheal cannula. The perfusion pressure was measured by a Statham pressure transducer from a needle inserted above the pulmonary artery. The lungs were suspended from a strain gauge to detect increase in weight and hence oedema formation.

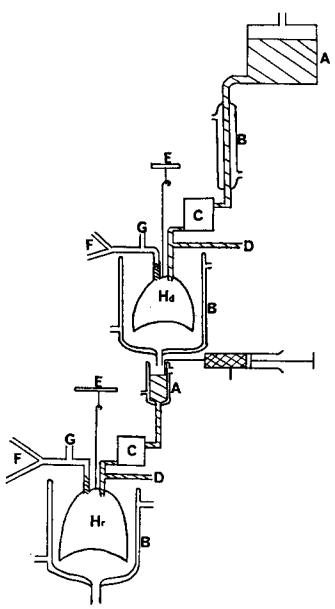

FIG. 1. Diagram to show the arrangement of the apparatus. A, reservoir; B, heating jackets; C, peristaltic pump; D, to Statham pressure transducer; E, strain gauge; F, to respiratory pump; G, to air pressure transducer; Hd, donor lung; Hr, recipient lung; I, slow injection apparatus.

A second pair of lungs (the recipient), taken in the same way from another animal, was suspended in series with the first (the donor), so that the effluent from the donor was perfused through the pulmonary artery of the recipient (Fig. 1). In most experiments the donor lungs were from guinea-pigs that had been sensitized with ovalbumen as previously described (Collier & James, 1967), whereas the recipient preparations were always from non-sensitized animals. Antigen was administered to the donor lungs by injection into the pulmonary artery. Drugs or antigen were administered to the recipient lungs via the reservoir collecting perfusate from the donor preparation (Fig. 1).

In the first experiment, sensitized donors were used. Whereas antigen administered only to the recipient elicited no response, administration of antigen to the donor elicited from it a sharp increase in tracheal pressure, usually accompanied by an increase in perfusion pressure, followed by an increase in weight (Fig. 2, left). Some minutes later, there was an intense increase in the tracheal pressure of the recipient, usually without obvious change in the perfusion pressure or lung weight. This experi-

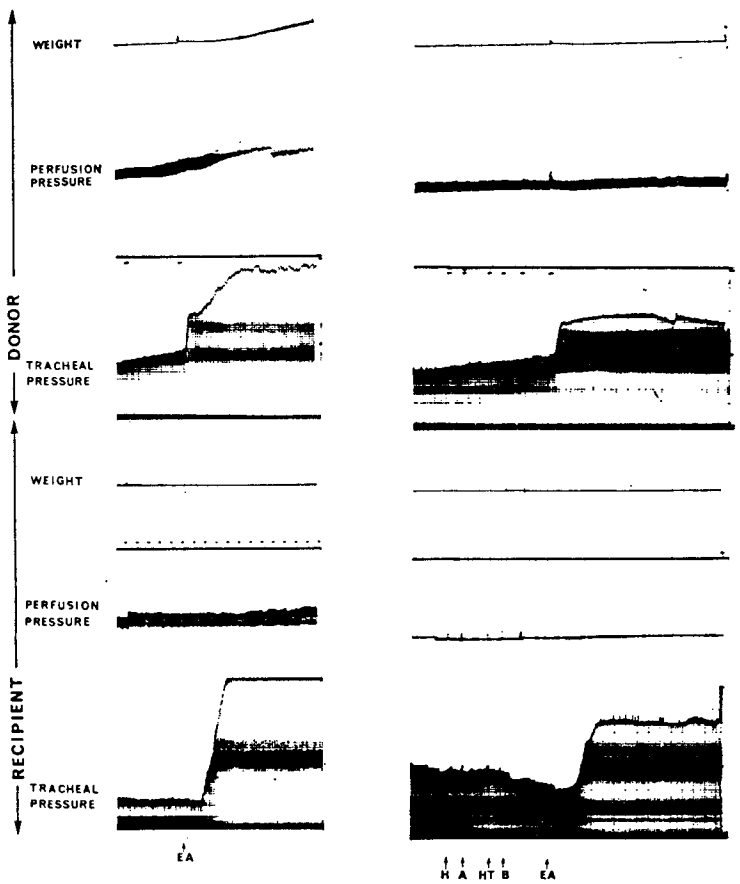

FIG. 2. Transfer of humoral factors from a sensitized to a non-sensitized isolated lung. Donor lungs were sensitized to ovalbumen. Recipient lung was not sensitized. Time 1 min. *Left-hand panel*: EA, 5 mg ovalbumen added to donor perfusate. *Right-hand panel*: Recipient lung perfused with Tyrode containing 10 μg/ml of mepyramine, 1 μg/ml of atropine, 1 μg/ml of methysergide and 100 μg/ml of meclofenamate sodium. A 0·1% solution of meclofenamate was given in the form of an aerosol to the recipient lung. H, 1 μg histamine; A, 5 μg acetylcholine; HT, 1 μg 5-HT; B, 1 μg bradykinin, all added to perfusate to recipient lung only. EA, 5 mg ovalbumen added to perfusate to donor lung.

ment was made seventeen times, and on sixteen occasions the tracheal pressure response of the recipient was observed. In one experiment, neither donor nor recipient responded to antigen.

In a second experiment, also in seventeen serially perfused preparations, sensitized donors were again used, but the recipient was perfused with mepyramine (10 μg/ml), atropine (1 μg/ml), methysergide (1 μg/ml) and meclofenamate sodium (100 μg/ml). A solution of 0·1% meclofenamate sodium in 0·9% w/v sodium chloride was also administered in an aerosol to the recipient by means of its respiration pump. After this treatment, the recipient was unresponsive to the following agonists: histamine (1 μg), acetylcholine (5 μg); 5-HT (1 μg), bradykinin (1 μg) or of SRS-A-H (0·25–1·0 mg). When antigen was administered to the donor an increase of tracheal pressure occurred in all donors and in fourteen of seventeen recipients, an example is illustrated in Fig. 2, right.

In a control experiment, made at the same time as the second experiment, twelve serially perfused lung preparations were used. The donors lungs were excised from non-sensitized guinea-pigs. No donors responded to antigen and nine of the recipients were also unresponsive; but there was a marginal increase in tracheal pressure in three of the recipients. The difference in the proportion of recipients responding between the control and test preparations was statistically significant ($P < 0{\cdot}001$).

The bronchoconstriction elicited in non-sensitized lungs by fluid received from sensitized lungs after challenge with antigen can safely be attributed to one or more humoral factors. That, after antagonism of histamine, acetylcholine, 5-HT, kinins and SRS-A-H, the recipient still responded vigorously to fluid from the donor indicates either an overwhelming amount of one or more of these factors was liberated, or that one (or more) other bronchoconstrictor factor(s) was released. Because, in the above experiments, challenge doses of known humoral factors failed to elicit a response from non-sensitized lungs protected by antagonists, the possibility seems the more likely that other unidentified bronchoconstrictor factors are involved. Among candidates for an unidentified factor is prostaglandin $F_{2\alpha}$, which is released in anaphylaxis of isolated guinea-pig lungs (Piper & Vane, 1969) and which, in the guinea-pig *in vivo*, is a bronchoconstrictor that is not antagonized by meclofenamate (James, 1969). Another possibility is that the substance transmitted to the recipient from the donor is rabbit aorta contracting substance (RCS), which Piper & Vane (1969) have shown to be released from guinea-pig isolated lungs in anaphylaxis. RCS contracts the isolated trachea of the guinea-pig and fenamates do not antagonize this effect, although they block the release of RCS (Piper & Vane, 1969). We have not enough evidence to decide between these or other possible humoral factors.

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