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THE TRANSPORT OF ALBUMIN ACROSS THE FERRET *IN VITRO* WHOLE TRACHEA

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SUMMARY

1. The whole trachea of the ferret has been isolated *in vitro* in an organ bath and used to study the transport of bovine serum albumin (BSA) and two dextrans (70000 and 9000 Da) from external buffer solution to air-filled lumen, assessed by fluorescent-labelled tracers.

2. In control conditions, when mucus secretion was not stimulated by drugs, the concentration of albumin in the lumen was over half that in the buffer, and about six times greater than those of the two dextrans.

3. Methacholine and phenylephrine caused large increases in mucus secretion and albumin output and decreases in albumin concentration. The responses were proportional to drug concentration. We concluded that albumin output is increased but diluted with submucosal gland secretion.

4. Salbutamol caused a small increase in mucus secretion and large increases in output and concentration of albumin. The concentration of albumin became greater than that in the external buffer medium. The responses were proportional to concentration of salbutamol.

5. Histamine increased mucus secretion and albumin output and concentration.

6. None of the four drugs increased the output of dextran-70000. Methacholine and phenylephrine increased the output of dextran-9000, but to a far less extent than for albumin.

7. Cooling the trachea and buffer to 4 °C almost abolished the stimulation of mucus and albumin outputs due to methacholine.

8. Increasing the concentration of albumin external to the trachea did not proportionally increase albumin secretion, the logarithmic relationship suggesting saturation of an active transport system.

9. We conclude that albumin is secreted by active transport into the tracheal lumen, and that the rate of transport can be augmented by salbutamol to build up a higher concentration in the lumen than in the external buffer.

INTRODUCTION

Albumin transport into the airways is one of the complications of inflammatory airway diseases such as asthma and the concentrations of albumin in airway secretions from patients with asthma are often high (Persson, 1986; Persson &

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Erjefalt, 1988). For albumin to pass from blood vessels to the lumen of the airways it has to cross two main barriers: the vascular endothelium and the tracheal epithelium. Considerable research has been carried out on the transport of albumin across blood vessel endothelia, and several reports point to an active transport system in some vascular beds (see Discussion).

In contrast to the work with vascular endothelia, there are few direct studies of albumin transport across airway epithelia. It is usually concluded that albumin is an index of transudation and that its transport is passive (Staub, 1984; Rennard, Basset, Lecossier, O'Donnell, Pinkston, Martin & Crystal, 1986). Kwang-Jin, Lebon, Shinbane & Crandall (1985) have shown that albumin may be actively transported across bull-frog alveolar epithelium from lumen to subepithelium, and a similar conclusion was reached by Johnson, Cheng & Boucher (1988) using epithelial cell culture sheets from dog trachea. Jacquot, Benali, Sommerhof, Finkbeiner, Goldstein, Puchelle & Basbaum (1988) have suggested that the serous cells from submucosal glands of the cow can synthesize and secrete albumin.

The ferret *in vitro* whole trachea preparation was developed to assess changes in the volume output of mucus into the tracheal lumen (Webber & Widdicombe, 1987*a*). A preliminary study showed that the preparation could be used to measure albumin transport into the tracheal lumen (Webber & Widdicombe, 1988*a*). In the present study we have used the preparation to examine the pharmacological control of albumin transport across the tracheal wall with no interference from the vasculture. Abstracts of some of the results have been published (Webber & Widdicombe, 1987*c*, 1988*b*).

METHODS

The *in vitro* whole tracheal preparation of the ferret has previously been described in detail (Webber & Widdicombe, 1987*a*). Forty-four ferrets (body weight 0·5–1·4 kg) were anaesthetized with pentobarbitone sodium (30 mg kg^{-1} intraperitoneally). The trachea was exposed and cannulated 5 mm below the larynx with a special Perspex cannula. The ferret was killed with an overdose of pentobarbitone and the chest opened along the mid-line. The trachea was cleared of adjacent tissue, cannulated at the carinal end and was mounted, laryngeal end down, in an organ bath filled with Krebs–Henseleit buffer restricted to the submucosal side (Fig. 1). The composition (mM) of the Krebs–Henseleit buffer was: NaCl, 120·8; KCl, 4·7; KH_2PO_4, 1·2; $MgSO_4.7H_2O$, 1·2; $NaHCO_3$, 24·9; $CaCl_2$, 2·4; glucose 5·6. The buffer was maintained at 37 °C and gassed with 95 % O_2/5 % CO_2. The lumen of the trachea remained air-filled. Secretions were carried by gravity and mucociliary transport to the lower cannula where they collected and could be periodically withdrawn into a polyethylene catheter. The catheters were sealed at both ends and stored frozen until required.

After defrosting, the secretions were washed out of the catheters with 0·5 ml distilled H_2O into 1·5 ml vials. The vials were frozen and stored for use in the albumin assay. Preliminary experiments had shown that frozen storage of up to six months does not affect the concentration of albumin in the mucus samples. Secretion volumes were estimated by the differences in the weights of the catheters with secretions and dried without secretions, and the secretion rates were expressed as $\mu l\ min^{-1}$ (assuming 1 g of secretion is equivalent to 1 ml).

Albumin and dextran transport

To examine the transport of albumin and dextran across the ferret trachea, either bovine serum albumin (BSA) or one of two dextrans was added to the buffer bathing the submucosal surface of the trachea in a concentration of 4 $\mu g\ \mu l^{-1}$. Fluorescent BSA or dextran (20–30 ng μl^{-1}) was also added to the buffer as a marker and enabled an estimate to be made of the total amount of albumin or dextran which appeared in the mucus samples.

The fluorescence of the mucus sample was measured with a fluorimeter, using an excitation wavelength at 550 nm and an emission wavelength at 490 nm. The fluorescent albumin concentration of the mucus samples was estimated from a standard curve relating fluorescence (arbitrary units) to the concentration of fluorescent albumin. The total concentration of albumin in the mucus samples was obtained by multiplying the fluorescent albumin concentration (estimated from the standard curve) by the ratio of non-fluorescent to fluorescent albumin used in

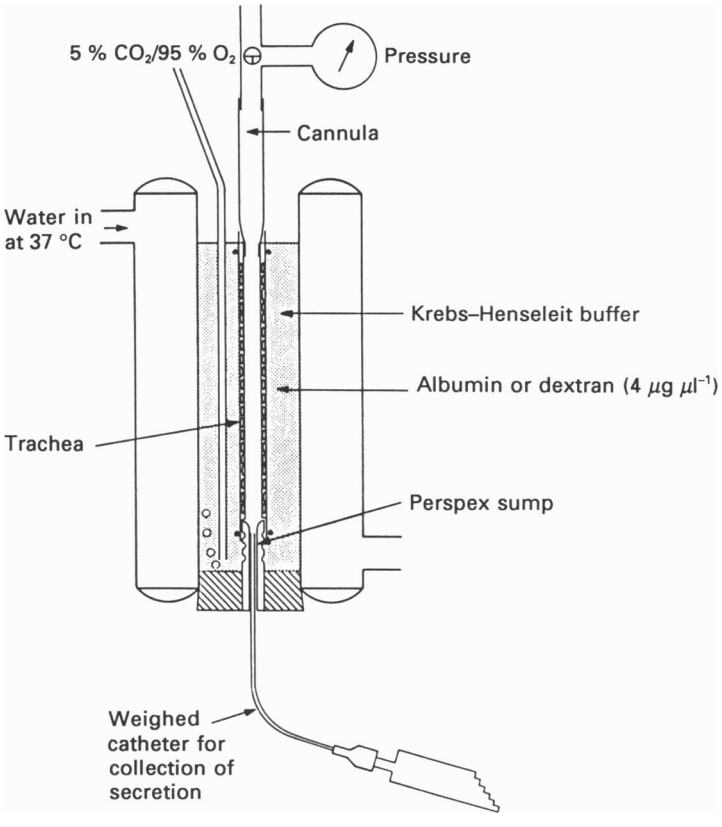

Fig. 1. The ferret *in vitro* whole trachea preparation. Pressure recording from the upper tracheal cannula was not done in the experiments described here.

the experiment. The rate of output of albumin was determined by dividing the total amount of albumin in the mucus sample by the time period over which the sample accumulated. Exactly the same procedure was used to examine the transport of dextrans.

Intraluminal fluorescent albumin concentration might not reflect that of fluorescent albumin in the organ bath since the fluorescent label might be split off or transferred. Therefore, as described elsewhere (Webber & Widdicombe, 1988*a*), nine mucus samples selected randomly were analysed for albumin using affinity chromatography. Linear regression analysis showed a highly significant correlation between the concentration of albumin using this method and the concentration calculated for the same samples by fluorescence measurements (slope = 1·1, $r = 0{\cdot}97$, $P < 0{\cdot}01$). This suggested that the fluorescent label was still attached to the albumin and that the fluorescence of the mucus samples provided an accurate indication of the total albumin concentration.

Addition of drugs

After two 30 min control periods one of the drugs was added to the buffer (containing BSA or dextran) bathing the trachea. Any secretion was withdrawn 30 min after addition of the drug. The

trachea was then washed externally twice and fresh buffer containing no drug was placed in the organ bath. Between two and four control periods of 30 min each were allowed before the next addition of a drug, depending on how long it took the secretion rate to return to the control level. Only the secretion rates in the control periods before and after the drug periods are assessed in the Results section. All four drugs were administered in a random order to each trachea. The concentrations of drugs used in these experiments had previously been shown to produce statistically significant ($P < 0.01$) increases in mucus volume output (Webber & Widdicombe, 1987*b*).

Concentration–response effects of drugs

In each of these experiments, six different concentrations of either methacholine or phenylephrine or three different concentrations of salbutamol were added to the ferret trachea in a random order. Each concentration of secretagogue was left in contact with the trachea for 30 min; any secretion produced was then withdrawn and processed as described above. The trachea was then washed externally twice and fresh buffer containing no secretagogue was placed in the organ bath. Between one and three control periods of 30 min were allowed between each addition of secretagogue depending on how quickly the secretion rate returned to basal levels. All the mucus samples obtained in these experiments were assayed for BSA as described above.

The effect of cooling on BSA transport

After a 30 min control period, methacholine (20 μM) was added to the buffer bathing the trachea. Secretions were withdrawn every 15 min for the first 30 min and at 30 min intervals thereafter until a constant mucus volume output had been obtained. After each 30 min period the buffer surrounding the trachea was replaced with fresh buffer containing methacholine. When a steady mucus volume output had been achieved (2·5–3 h after initial addition of methacholine) the buffer surrounding the trachea was replaced with buffer (containing methacholine) which had been cooled to 4 °C. The water circulating in the water bath was also cooled to 4 °C. After the mucus volume output had been determined for two periods of 30 min, the buffer in the organ bath was replaced with buffer (containing methacholine) which had been warmed to 37 °C, and the circulating water was also warmed to 37 °C. The mucus volume output was then determined for two further 30 min periods. All mucus samples obtained in these experiments were assayed for BSA as described above.

Saturation of BSA transport

Methacholine (20 μM) was used to produce a constant mucus volume output as described above. During this time the submucosal buffer contained 4 $\mu g\ \mu l^{-1}$ BSA and 20–30 ng μl^{-1} fluorescent BSA. When a steady mucus volume output had been achieved, the buffer surrounding the trachea was replaced with buffer containing a different concentration of BSA (1–15 $\mu g\ \mu l^{-1}$) but the same concentration of fluorescent BSA (20–30 ng μl^{-1}). The mucus volume output was then determined for a 30 min period. The buffer was then replaced with buffer containing a further different concentration of BSA and the mucus volume output was again determined for a 30 min period. This process was repeated until seven different concentrations of BSA (range 1–15 $\mu g\ \mu l^{-1}$) had been added to the submucosal buffer.

Statistical analysis of results

All differences between control and drug-induced periods were analysed for statistical significance using a one-way analysis of variance followed by Student's t test for paired observations. A P value of less than 0·05 was considered significant (*) and less than 0·01 highly significant (**).

Materials

The materials used were acetyl-β-methylcholine chloride (methacholine), phenylephrine hydrochloride, salbutamol, histamine dihydrochloride, bovine serum albumin (BSA), FITC-BSA, dextrans (9000 and 70000 Da) and FITC-dextrans (9000 and 70000 Da), all from Sigma.

RESULTS

Outputs and concentrations of BSA and dextrans during control periods

The mucus volume output during control periods (no stimulation of mucus secretion) with BSA in the submucosal buffer was $0{\cdot}08 \pm 0{\cdot}05\ \mu l\ min^{-1}$ (mean ± S.E.M.). Similarly, in control periods with dextran-70000 or 9000 in the submucosal buffer, the mucus volume outputs were $0{\cdot}15 \pm 0{\cdot}08$ and $0{\cdot}14 \pm 0{\cdot}6\ \mu l\ min^{-1}$ respectively. None of these control mucus volume outputs was significantly different from zero.

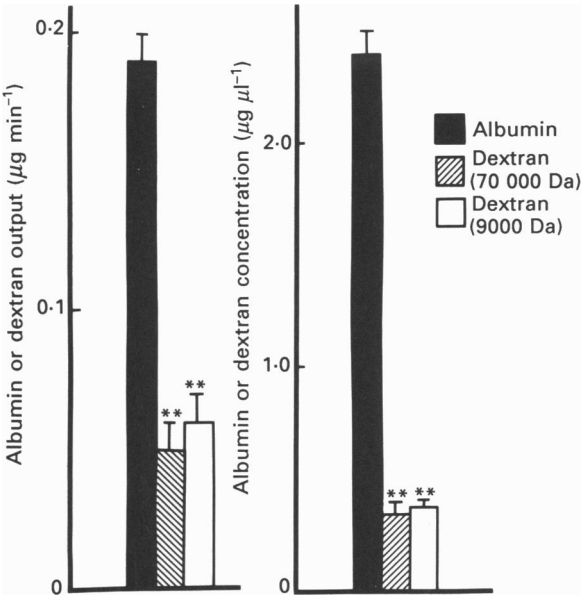

Fig. 2. The output and concentration of albumin (filled bars), dextran-70000 (hatched bars) and dextran-9000 (open bars) in control periods when mucus volume output had not been stimulated by drugs. The results are the means of four to six determinations with S.E.M. shown by vertical lines. $^{**}P < 0{\cdot}01$ for dextrans compared to albumin.

The rate of output of BSA in control periods ($0{\cdot}19\ \mu g\ min^{-1}$) was much greater than the rate of output of dextran-70000 ($0{\cdot}05\ \mu g\ min^{-1}$) or dextran-9000 ($0{\cdot}06\ \mu g\ min^{-1}$; Fig. 2). The concentration of BSA in the mucus collected during control periods ($2{\cdot}4\ \mu g\ \mu l^{-1}$) was also much greater than that of either dextran (0·30 and $0{\cdot}35\ \mu g\ \mu l$ for dextran-70000 and 9000 respectively; Fig. 2). The concentration of BSA in control mucus samples ($2{\cdot}4\ \mu g\ \mu l^{-1}$) compares with a concentration of BSA in the buffer on the submucosal side of the trachea of $4\ \mu g\ \mu l^{-1}$.

The effect of increasing mucus volume output on albumin and dextran transport

The muscarinic agonist methacholine, the α_1-agonist phenylephrine, the β_2-agonist salbutamol and histamine stimulate mucus secretion from the ferret *in vitro* trachea (Webber & Widdicombe, 1987*b*). They were used to see what effect increasing the mucus volume output had on albumin and dextran movement across the tracheal wall. Each concentration of drug produced approximately 70–80% of the maximum increase in mucus volume output possible with the particular drug.

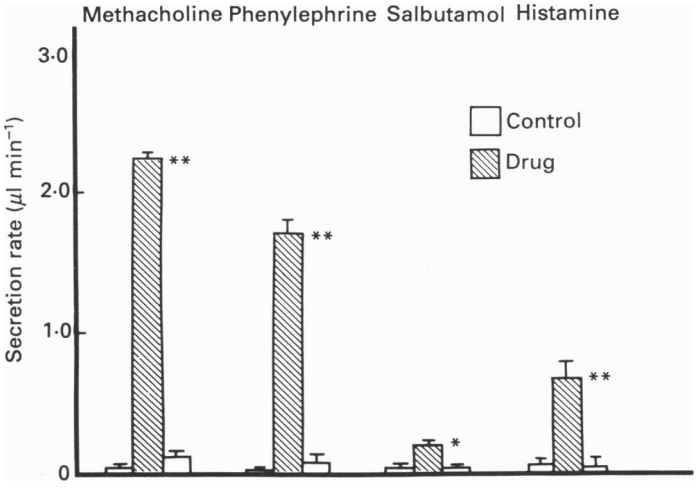

Fig. 3. The effect of drugs on mucus volume output from the ferret trachea. Empty bars denote control periods and hatched bars drug-induced periods. The results are the means of four to six determinations with S.E.M. shown by vertical lines. ** $P < 0{\cdot}01$, * $P < 0{\cdot}05$ for response to drug compared to control.

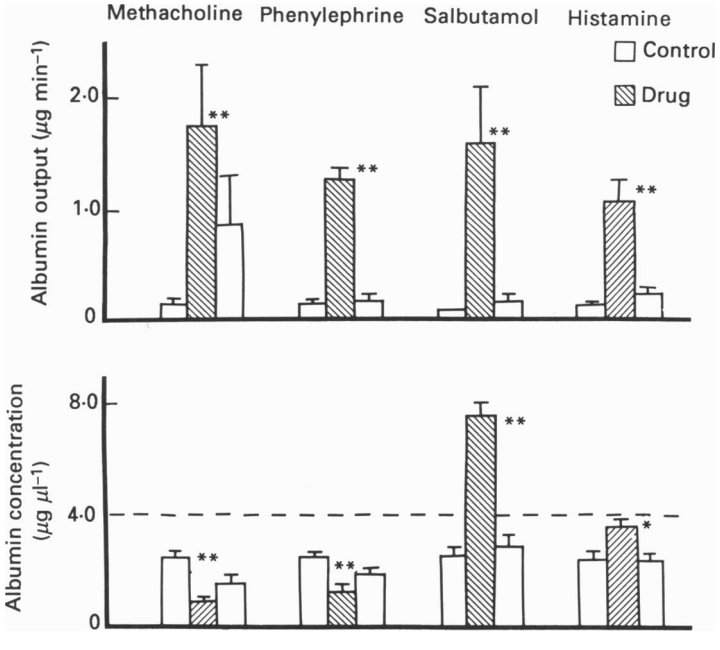

Fig. 4. The effect of drugs on the concentration and output of BSA from the ferret trachea. Empty bars denote control periods and hatched bars drug-induced periods. The results are the means of four to six determinations with S.E.M. shown by vertical lines. The interrupted line on the lower histogram represents the albumin concentration in the organ-bath buffer. ** $P < 0{\cdot}01$, * $P < 0{\cdot}05$ for response to drug compared to control.

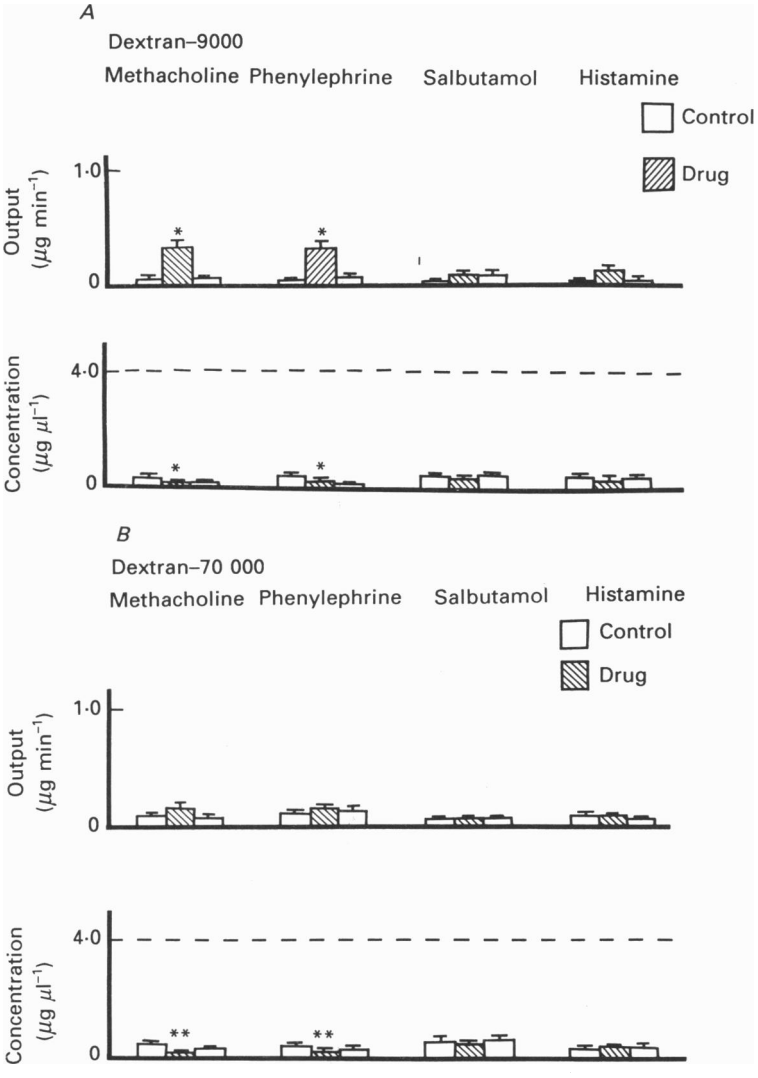

Fig. 5. The effect of drugs on the concentration and output of dextran-70000 (*A*) and dextran-9000 (*B*) from the ferret trachea. Empty bars denote control periods and hatched bars drug-induced periods. The results are the means of four to six determinations with S.E.M. shown by vertical lines. The interrupted lines represent the dextran concentrations in the organ-bath buffer. ** $P < 0{\cdot}01$, * $P < 0{\cdot}05$ for response to drug compared to control.

Methacholine (0·02 mM), phenylephrine (0·1 mM), histamine (0·1 mM) and salbutamol (0·1 mM) increased mucus volume output (Fig. 3). All four drugs produced highly significant increases in the rate of output of BSA (Fig. 4). However, the concentrations of BSA in methacholine- and phenylephrine-induced mucus (0·8 and 1·2 $\mu g\ \mu l^{-1}$ respectively) were significantly lower than preceding control values (2·3 and 2·4 $\mu g\ \mu l^{-1}$), whereas salbutamol and histamine significantly increased the albumin concentration (7·4 and 3·3 $\mu g\ \mu l^{-1}$ respectively) from control levels (2·5 and

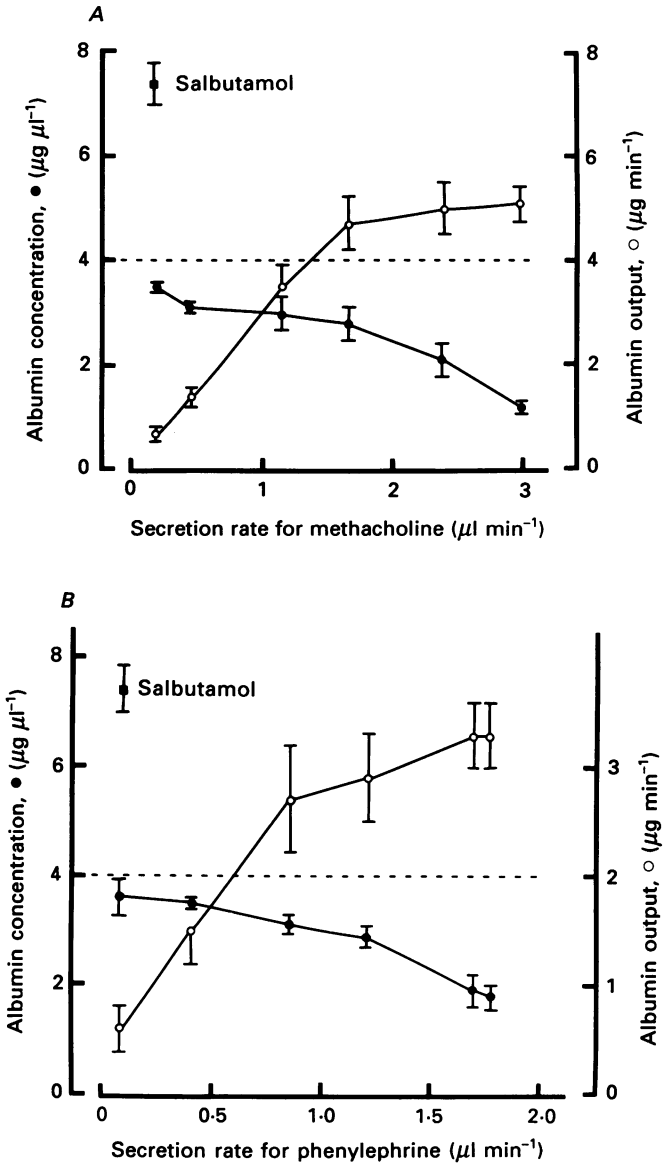

Fig. 6. For legend see facing page.

2·2 $\mu g\ \mu l^{-1}$; Fig. 4). The concentration of albumin in salbutamol-induced secretion was nearly twice as high (7·4 $\mu g\ \mu l$) as that in the submucosal buffer (4 $\mu g\ \mu l$).

None of the drugs had a significant effect on the output of dextran-70000 compared to previous controls (Fig. 5*A*). The concentrations of dextran-70000 were reduced in mucus produced by methacholine and phenylephrine (0·06 and 0·11 $\mu g\ \mu l^{-1}$ respectively; controls 0·40 and 0·32 $\mu g\ \mu l^{-1}$), but were not significantly changed by histamine or salbutamol (Fig. 5*A*).

Methacholine and phenylephrine increased the output of dextran-9000 (0·32 and

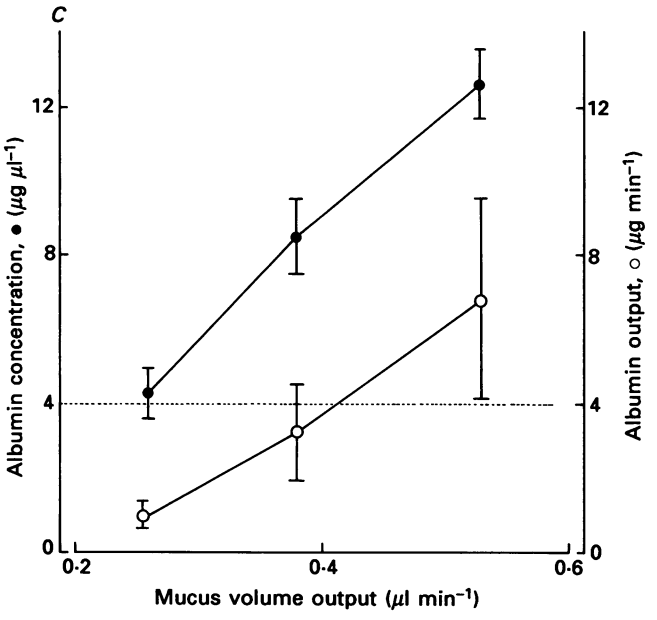

Fig. 6. The effect of increasing mucus volume output by methacholine (*A*), phenylephrine (*B*) and salbutamol (*C*) on the concentration (●) and output (○) of BSA from the ferret trachea. Points are means of four to six determinations and vertical bars represent S.E.M. The interrupted lines show the concentration of BSA in the external buffer. For salbutamol the three mucus volume outputs correspond to concentrations of 30, 100 and 300 μM.

0·32 μg min^{-1} respectively; controls 0·05 and 0·05 μg min^{-1}; Fig. 5*B*). However, the concentrations of dextran-9000 were significantly reduced (0·15 and 0·22 μg μl^{-1} respectively; controls 0·33 and 0·29 μg μl^{-1} respectively). Histamine and salbutamol had no significant effect on either the output or concentration of dextran-9000 (Fig. 5*B*).

Concentration-related effects of methacholine, phenylephrine and salbutamol on albumin output and concentration

Methacholine (0·03–50 μM), phenylephrine (0·3–300 μM) and salbutamol (30–300 μM) produced concentration-dependent increases in mucus volume output from the trachea (0·19–2·97, 0·16–1·82 and 0·26–0·53 μl min^{-1} respectively). As the mucus volume output produced by methacholine or phenylephrine was increased, there was a concentration-dependent increase in the rate of output of BSA and a concentration-dependent decrease in the BSA concentration (Fig. 6*A* and *B*). In contrast, as the mucus volume output produced by salbutamol was increased, there were concentration-dependent increases in both the BSA output and concentration (Fig. 6*C*). At low concentrations of methacholine and phenylephrine, the concentrations of BSA in the mucus (3·4 and 3·6 μg μl^{-1} respectively) approached those of the submucosal buffer (4 μg μl^{-1}) but were still lower than the BSA concentration produced by any concentration of salbutamol (Fig. 6*C*).

The effect of cooling on BSA transport

Addition of methacholine (20 μM) to the external buffer increased mucus volume output ($2{\cdot}58 \pm 0{\cdot}39$ μl min^{-1}; control, $0{\cdot}07 \pm 0{\cdot}05$ μl min^{-1}). There was a simultaneous increase in albumin output ($4{\cdot}76 \pm 0{\cdot}59$ μg min^{-1}; control, $0{\cdot}26 \pm 0{\cdot}08$ μg min). On continued application of methacholine both the mucus volume output and the albumin output decreased but reached steady 'maintained' levels approximately 2·5–3 h after the drug had first been added to the buffer (0·56 μl min^{-1} and 2·4 μg min^{-1} respectively). The albumin concentrations were close to that of the external buffer ($3{\cdot}9 \pm 5$ μg μl^{-1}).

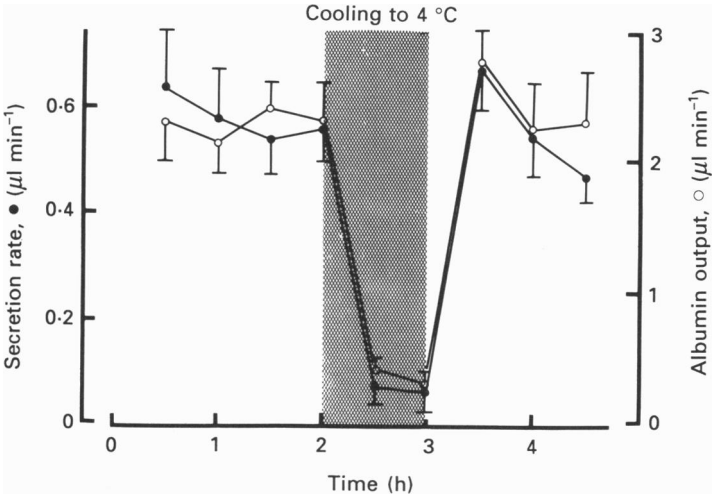

Fig. 7. The effect of cooling the tracheal external buffer and the water bath to 4 °C on the mucus volume output (●) and the BSA output (○) during maintained methacholine-induced mucus secretions. Points are the means of six determinations and the vertical bars represent S.E.M.

During cooling the trachea to 4 °C there were highly significant ($P < 0{\cdot}01$) decreases in mucus volume output (0·07 μl min^{-1}) and albumin output (0·4 μg min^{-1}; Fig. 7). When the trachea was rewarmed to 37 °C both the mucus volume output and the albumin output increased to values not significantly different from those immediately preceding the cooling period (0·68 μl min^{-1} and 2·7 μg min^{-1} respectively). Cooling had no significant effect on albumin concentrations (control before, $3{\cdot}9 \pm 0{\cdot}5$; during cooling, $4{\cdot}1 \pm 0{\cdot}5$; control after, $3{\cdot}8 \pm 0{\cdot}6$ μg μl^{-1}).

Saturation of BSA transport

Continuous application of methacholine (20 μM) to the buffer (containing 4 μg μl^{-1} BSA and 20–30 ng μl^{-1} fluorescent BSA) bathing the trachea produced a maintained mucus volume output ($0{\cdot}63 \pm 0{\cdot}12$ μl min^{-1}). The fluorescent BSA and the total BSA concentrations of the secreted mucus were also constant ($24{\cdot}0 \pm 1{\cdot}1$ ng μl^{-1} and $2{\cdot}40 \pm 0{\cdot}11$ μg μl^{-1} respectively). When the non-fluorescent BSA concentration of the

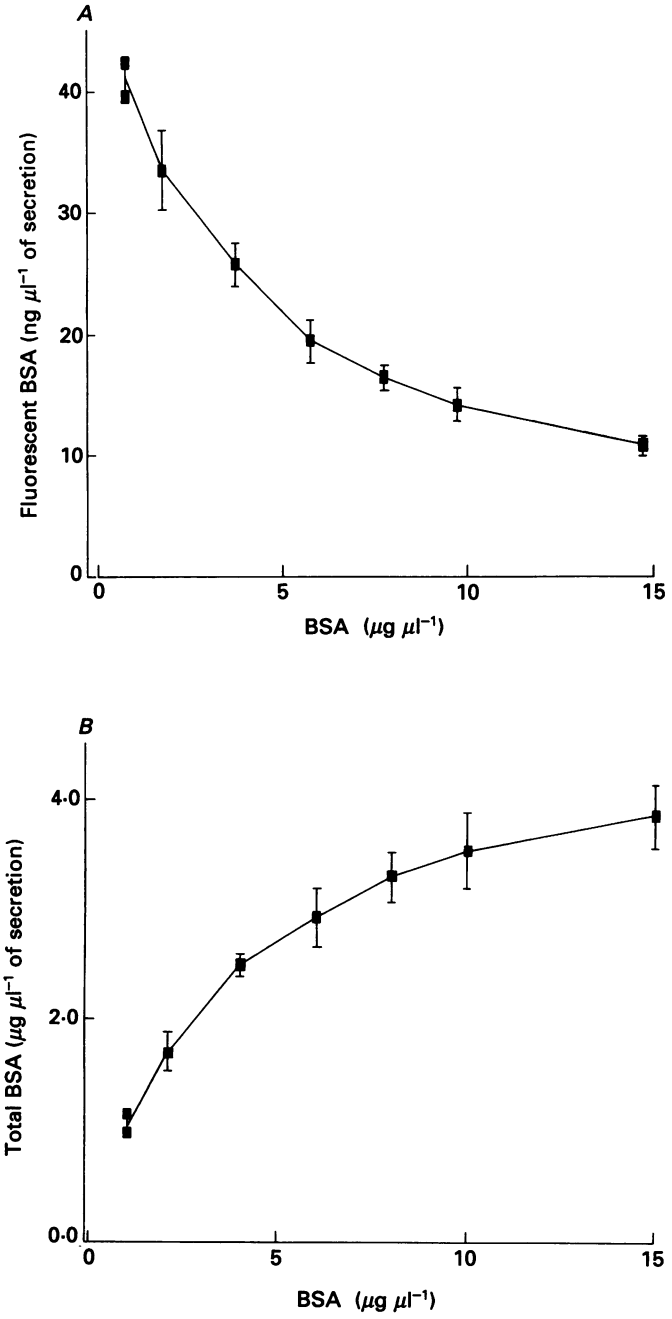

Fig. 8. The effect of increasing the concentration of BSA in the submucosal buffer on the concentration of fluorescent BSA (*A*) and the total concentration of BSA (*B*) in the maintained mucus volume output induced by methacholine. Except where indicated, points are means of four determinations and the vertical bars represent S.E.M.S.

submucosal buffer was increased, the measured fluorescent BSA concentration in the secreted mucus decreased (Fig. 8*A*) and the calculated total BSA concentration increased (Fig. 8*B*). The decrease in the fluorescent BSA concentration was exponential (as shown by a log plot) and approached a minimum when the submucosal BSA concentration was about 15 $\mu g\ \mu l^{-1}$ (Fig. 8*A*); similarly, the total BSA concentration increased exponentially and approached a maximum when the submucosal BSA concentration was about 15 $\mu g\ \mu l^{-1}$ (Fig. 8*B*).

DISCUSSION

Our results strongly suggest that albumin is transported actively across the airway mucosa into the lumen. This conclusion is based on five observations.

(1) Under resting conditions the albumin concentration in the lumen was considerably higher than that of dextrans of the same or of considerably smaller molecular mass. Even if equilibrium had not been reached, the differences in albumin and dextran concentrations are unlikely to be due to passive diffusion.

(2) The application of secretagogues greatly increased the output of albumin but had no effect on the output of dextran-70000. Although the output of dextran-9000 was increased with methacholine and phenylephrine, the changes were far smaller than those of albumin. Therefore it is unlikely that the increased albumin output with secretagogues is due to increased permeability of the mucosa and diffusion of albumin.

(3) In the case of salbutamol the luminal concentration of albumin exceeded that of the surrounding buffer. This could only have been produced by active secretion of albumin or by active reabsorption of ions and water. However the increase in total albumin output due to salbutamol and the lack of effect of salbutamol on the concentrations of either dextran are inconsistent with the reabsorption of ions and water. The output and concentration of albumin depended on the concentration of salbutamol, a result consistent with the view that salbutamol is augmenting an active secretory process.

(4) Cooling the preparation to 4 °C reduced the albumin output (and also the mucus volume output). An inhibition of comparable size would not be expected from cooling a passive diffusional mechanism.

(5) Increasing the submucosal concentration of albumin did not increase albumin output linearly, but instead there was a logarithmic response with 'saturation' when the external albumin concentration was about 15 $\mu g\ \mu l^{-1}$. This relationship is consistent only with an active transport process. The concentration of albumin used in most of the experiments was 4 $\mu g\ \mu l^{-1}$, on the steep part of the graphical relationship. It may be significant that many interstitial fluids are thought to have an albumin concentration in the range 5–10 $\mu g\ \mu l^{-1}$ (Taylor & Granger, 1984); thus the active albumin transport would be able effectively to clear albumin from the interstitial fluid, although the final balance would also depend upon the input of albumin from the blood vessels into the interstitium. We have not shown whether the dextran transport system can be saturated since, whatever the result of such experiments might be, they would not invalidate the conclusion that the

physiologically natural substance albumin is actively transported across the epithelium.

We have expressed our results in terms of mass of molecules rather than moles. Since albumin and dextran-70000 have very similar molecular masses, re-expression as moles would make no significant difference. However dextran-9000 is eight times smaller in molecular mass than albumin and dextran-70000. Thus Figs 2 and 5 could be redrawn to show that dextran-9000 is passing across the epithelium about eight times faster than albumin or dextran-70000, which would be expected on a molar basis.

Although we cannot be sure that fluorescent-labelled BSA would be actively transported at the same rate as ferret serum albumin, we eliminated the possibility that the fluorescent label was important. Thus analysis by fluorescence gave nearly identical results to analysis by affinity chromatography (Webber & Widdicombe, 1988*a*). The question remains whether the ferret airway mucosa might handle BSA in a quantitatively different way from ferret serum albumin.

The secreted albumin might come from either submucosal glands or the epithelium. The cooling experiment does not clarify this point, since both total mucus output and albumin output were decreased proportionately. However pharmacological studies on airway secretions are more informative.

Salbutamol is a weak stimulant of total secretory flow but was a potent stimulant of albumin secretion showing a concentration–response relationship. In the ferret, salbutamol produces glandular secretion mainly from mucous cells (Gashi, Nadel & Basbaum, 1984), which could therefore be a source of albumin. However salbutamol does not increase lysozyme secretion from the ferret trachea (Webber & Widdicombe, 1987*b*); since lysozyme is usually considered a marker for serous cell secretion (Tom-Moy, Basbaum & Nadel, 1983), it seems unlikely that these cells are the main source of albumin. Jacquot *et al.* (1988) have shown that salbutamol causes cell cultures of bovine submucosal gland serous cells to synthesize and secrete albumin. Either there is a species difference, or in our experiments any contribution to albumin secretion by serous cells must be completely outweighed by that from the epithelium.

The concentrations of albumin decreased during the stimulations by methacholine and phenylephrine, which suggests that glandular mucus is diluting the main fluid source of albumin, and that a substantial proportion of the albumin is being secreted by the epithelium. Methacholine promotes secretion from both serous and mucous cells in submucosal glands in the ferret (Tom-Moy *et al.* 1983), whereas phenylephrine acts predominantly on serous cells via α_1-adrenoceptors (Basbaum, Ueki, Brezina & Nadel, 1981; Gashi *et al.* 1984). Any possible increase in albumin output from mucous cells must be diluted by serous cell secretions lacking albumin.

With regard to epithelial transport, salbutamol promotes the secretion of chloride and sodium into the airway lumen by a β_2-adrenoceptor mechanism (Al-Bazzaz, 1986). It also augments the output of ^{35}S-labelled macromolecules, probably muco-glycoproteins, from the ferret trachea, the source probably being the glycocalyx of the epithelium (Kyle, Widdicombe & Wilffert, 1988). Methacholine may have similar actions, but α_1-agonists such as phenylephrine are said not to affect epithelial ion transport (Al-Bazzaz, 1986).

Our tentative conclusion is that the albumin is coming mainly from epithelial

secretion with a poor correlation between drug-induced ion and albumin transports. However there could be a contribution from gland mucous but not serous acini. The role of the latter is mainly to dilute secretions containing albumin, thereby lowering its concentration.

Active transport of albumin across the intact airway mucosa does not seem to have been described before. Albumin may be actively transported across bull-frog alveolar epithelium out of the lumen (Kwang-Jin *et al.* 1985). Sheets of cultured epithelial cells of the dog trachea can transport albumin from luminal to submucosal side (Johnson *et al.* 1988). The tracheal epithelium has different ion transport mechanisms in dog and ferret (Boucher, Bromberg & Gatzy, 1980; Al-Bazzaz, 1986), so albumin transport could also be different. Significant secretion of albumin into the lumen of the bronchi seems unlikely because it would lead to progressive increases in albumin concentrations centrally; according to Boucher *et al.* (1980) the bronchial epithelium is primarily absorptive while that of the trachea is secretory, at least in terms of ions and water. Another possibility is that transport of albumin may be bi-directional, the dominant direction depending on experimental conditions. We have not studied the possibility of transport, active or passive, of albumin from the lumen into the submucosal interstitial space. Nor have we eliminated the possibility that albumin may diffuse passively into the lumen and then, by some transformation, be prevented from back-diffusion into the submucosa: however such a hypothesis would require that the albumin transformation and/or back-diffusion are affected by cooling, drugs such as salbutamol, and saturation mechanisms in such a way as to mimic active transport. This seems unlikely.

The possibility of bidirectional transport is consistent with the fact that during prolonged weak secretion due to methacholine in the cooling experiments, the albumin concentrations came close to that of the external medium. This result is unlikely to be due to free diffusion of the albumin, since dextran-9000 concentrations were always far lower than those of albumin. Histamine did not increase the diffusibility of macromolecules through the epithelium; although it increased the concentration of albumin it did not increase that of dextran-9000. Histamine is a rather weak stimulant of submucosal gland secretion in the ferret trachea (Webber & Widdicombe, 1987*b*) and increases sodium and chloride transport across the dog tracheal epithelium *in vitro* (Marin, Davis & Nadel, 1977).

Active transport mechanisms for albumin are established in some capillary endothelia, including those in the pulmonary circulation (Shasby & Shasby, 1985; Del Vecchio, Siflinger-Birnboim, Shepard, Bizios, Cooper & Malik, 1987; Cooper, Del Vecchio, Minnear, Burhop, Selig, Garcia & Malik, 1987). Transport of albumin across monolayers of cultured endothelial cells shows the greater flux from interstitium to lumen, although there is one report to the contrary (Seflinger-Birnboim, Del Vecchio, Cooper & Malik, 1986). The transport depends on the concentration of albumin (Shasby & Petersen, 1987) and is blocked by cyanide, supporting the view that it is active (Shasby & Roberts, 1987).

The mechanism of the active transport of albumin is not known but it may involve specific receptors. ^{125}I-Albumin binds specifically, saturably and reversibly to isolated hepatocytes, adipocytes and erythrocytes (Ockner, Weisiger & Gollan, 1983). Furthermore, a novel albumin-binding glucoprotein secreted by endothelial cells in culture has been reported (Sage, Johnson & Bornstein, 1984). Recent studies have

shown that albumin may be transported across the endothelia of capillaries and post-capillary venules by a receptor-mediated transcytosis (Ghitescu, Fixman, Simionescu & Simionescu, 1986; Simionescu, Ghitescu, Fixman & Simionescu, 1987).

We can only speculate about the role of albumin in airway secretions. It could change the rheological properties of mucus (List, Findley, Forstner & Forstner, 1975), the adhesiveness of mucus to epithelial surfaces, or the rheology of the periciliary fluid in which the cilia beat. Another possibility is that the albumin may carry compounds of importance with it, such as lipids. Albumin can bind various mediators such as leukotrienes (Lamm, Selfe & Albert, 1988) and might therefore render potentially active luminal agents less effective. Albumin may also act as a luminal antioxidant (Halliwell, 1988), preventing the formation of oxygen free radicals by binding to copper ions which are necessary for the formation of free hydroxyl radicals (Gutteridge, 1986). In this respect a bi-directional albumin transport system might have physiological advantages.

Our results show that albumin can be actively transported into the tracheal lumen, and that the rate of transport can be pharmacologically controlled. The patho-physiological importance of this system, and whether it is involved in therapeutic use of inhaled drugs, remain to be studied.

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