

# Action of serum on the output of secretory glycoproteins from human bronchi in vitro

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**ABSTRACT** The effect on mucin output of placing dilute serum from healthy donors in contact with the luminal surface of human airway has been studied. Bronchi were dissected from lungs removed at operation. Mucins, radiolabelled biosynthetically, were collected from the luminal aspect of the bronchi, which were mounted in Ussing chambers. Serum added to the luminal aspect of the tissue, at dilutions ranging from 1:100 to 1:10 of Krebs-Henseleit solution, consistently increased the output of radiolabelled mucins. The concentrations of serum tested in these experiments lie within the range commonly found in sputum coughed from the lungs of those with inflamed airways. Serum diluted to 1:2500, which is roughly the concentration found in the normal human airway, had little or no effect on bronchial secretion. Increased leakage of serum into the inflamed airways is suggested as one of the stimuli that increase bronchial secretion.

Alteration in the balance between the production of mucus and its clearance is important in the pathogenesis of respiratory disease. Where secretion outstrips clearance mucus will accumulate in the airway, narrow the lumen, and predispose to infection. In diseases where the airway mucosa becomes inflamed secretions often block air passages and this occurs in acute or chronic bronchitis,<sup>1</sup> asthma,<sup>2</sup> and cystic fibrosis.<sup>3</sup> In such diseases serum proteins, normally present in the airways only at low concentrations,<sup>4</sup> leak into the lumen.<sup>5</sup> Blood in the airway lumen causes mucus secretion in animals,<sup>6</sup> and recent reports<sup>7</sup> have suggested that dilute serum proteins cause secretion into the cat trachea. If the human respiratory tract behaves in the same way, serum proteins may be one of the stimulants that augment mucus output into the diseased airway. We wished to determine whether human serum in contact with the luminal surface of the bronchus evokes secretion.

## Methods

The experiments were performed by the method of Phipps *et al.*<sup>8</sup> Bronchi free of tumour were dissected

from lungs freshly removed at operation and immersed in oxygenated Krebs-Henseleit solution at 0°C for transport to the laboratory. There they were opened, pinned flat on a wax surface with luminal side uppermost, and then mounted between the two halves of an Ussing chamber. Krebs-Henseleit solution, gassed with 5% carbon dioxide in oxygen and warmed to 37°C, was circulated through both half chambers. Four mCi of sodium <sup>35</sup>S-sulphate was added to the submucosal aspect of the Ussing chamber at the beginning of the experiment to radiolabel the secretory glycoproteins synthesised in the bronchial wall. For the experiments on the effect of serum, 18 pieces of mainstem, lobar, or segmental bronchi from 11 patients were used. Serum, diluted as described below, was added to the luminal half chamber at intervals. In addition, 13 control studies were performed on bronchial tissues from nine patients, to define the pattern of output of radiolabelled macromolecules in the absence of any stimuli.

At 15 minute intervals liquid was drained from the luminal side of the chamber, which was then refilled with 15 ml Krebs-Henseleit solution. In experiments in which serum had been given, the fluid collected was dialysed exhaustively against 0.15 mol/l sodium chloride (to prevent the precipitation of serum proteins) containing 0.01% (w/v) sodium azide and 0.01% sodium sulphate. In the control experiments the fluid collected was dialysed against distilled water, with the same additions. The

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final change of dialysis water contained 6 mol/l urea to dissolve insoluble mucus.

To ensure that serum did not bind with sulphur-35 in vitro and so enrich the radioisotope content of the samples containing serum, we mixed  $^{35}\text{S}$ -sulphate with serum diluted in 10 volumes of Krebs-Henseleit solution and dialysed this sample in the same way as washings from the Ussing chambers. Dialysis removed all the sulphur-35.

The amount of radiolabelled macromolecule in each sample was measured by counting in a liquid scintillation counter (Intertechnique-SL30). A correction was made for quenching by the external standard channels ratio.<sup>9</sup> The rate of output was expressed in becquerels per minute of collection period.

The serum was obtained from blood taken from healthy subjects and allowed to clot at room temperature (about 20°C) before being centrifuged. Serum was diluted with Krebs-Henseleit solution to give dilutions varying from 1 part of serum in 10 volumes to 1 part in 100 volumes.

In eight experiments the most concentrated serum (1:10) was put in the luminal half chamber 2.5 hours after the start of the experiment. In six further experiments the effect of increasing concentrations of serum were investigated: 1:100 at 2.5 hours, 1:30 at 3.5 hours, and 1:10 at 4.5 hours. In a series of four experiments serum diluted 1:2500, about the concentration found in sputum from healthy lungs, was administered at 2.5 hours and then the 1:10 serum given at 3.5 hours.

The output of radioactivity after the addition of serum was calculated as the percentage in output over that in the immediately preceding collection period. Changes in mucus output are expressed as the median percentage change and range. The significance of a change in output which occurred in the presence of serum was tested against the changes that occurred at the same stage of control experiments by the Mann-Whitney U test.<sup>10</sup> This non-parametric test was used as the data did not appear to be normally distributed.

In a single experiment the material collected from the luminal chamber throughout the experiment was pooled and concentrated to 2 ml above an Amicon XM 50 membrane. This membrane retains molecules of molecular weights above 50 000. The 2 ml was fractionated by gel exclusion chromatography on a Sepharose CL 2B column (dimensions 2.6 × 70 cm) and eluted with 10 mmol/l sodium phosphate buffer (pH 7.2) containing 6 mmol/l urea and 0.01% w/v sodium azide.<sup>11</sup> The eluate material was collected as 45 fractions; the radioactivity of each fraction was counted on the scintillation counter. The void volume of the column was defined with dextran blue.

## Results

### OUTPUT OF RADIOLABELLED MACROMOLECULES IN CONTROL EXPERIMENTS

In the 13 control experiments, in which no stimulus was given, the rate of output of bound radioactivity tended to increase during the experiments; but the percentage changes in mucus output in the collections made where stimuli were normally given (2.5, 3.5, and 4.5 hours) over those in the immediately preceding collection periods were generally not large (table): the median change at 2.5 hours was +18.5% (range -28% to +39%); at 3.5 hours +1% (-44% to +40%); at 4.5 hours +5% (range -70% to +53%).

### EFFECT OF SERUM ON THE OUTPUT OF RADIO-LABELLED MACROMOLECULES

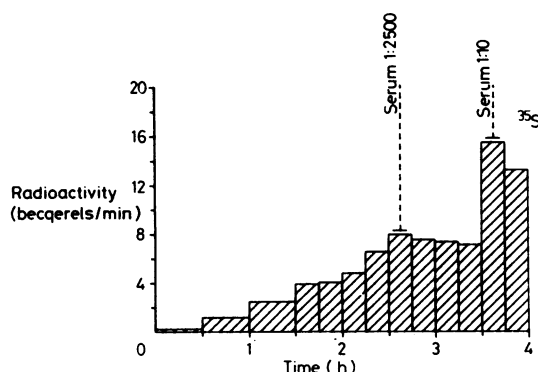
In one series of eight experiments serum diluted 1:10 in Krebs-Henseleit solution was placed in the luminal half of the Ussing chamber for the sampling period beginning at 2.5 hours. The median change over the output in the preceding collection period was +104% (range +14 to +392). The change in output was significantly greater than that which occurred at the corresponding time in control experiments ( $p < 0.05$ ).

In another series of six experiments increasing

*Changes in output of  $^{35}\text{S}$ -radiolabelled mucins at 2.5, 3.5, and 4.5 hours from the start of the experiments in control tissues (upper line) and in tissues treated with three concentrations of serum (lower line)*

	At 2.5 h	At 3.5 h	At 4.5 h
<i>Control tissues</i>			
Median change in output (%)	+18.5	+1	+5
Range	-28 to +39	-44 to +40	-70 to +53
n	12	13	13
<i>Tissues treated with serum</i>			
Dilution 1:100		Dilution 1:30	Dilution 1:10
Median change in output (%)	+140†	+85†	+76*
Range	+9 to +306	0 to +287	-44 to +225
n	6	6	6

\* $p < 0.05$ ; † $p < 0.01$  for significance of difference over controls.



Output of macromolecularly bound radioactivity during an experiment. Very dilute serum (1:2500) was added to the luminal half of the Ussing Chamber at 2.5 hours and was followed by little change in the output of bound radioactivity. The output of bound radioactivity increased substantially after the addition of serum at a dilution of 1:10.

concentrations of serum (1:100, 1:30, and 1:10) were placed in the luminal half of the Ussing chambers at 2.5, 3.5, and 4.5 hours respectively. All concentrations increased the output of macromolecularly bound radiolabel, through the effects were not obviously dose related. The results are summarised in the table. The increases in output after exposure to serum are all significantly greater than those occurring at the same time in control experiments.

In a series of four experiments very dilute serum (1:2500) was added to the luminal half of the Ussing chamber after 2.5 hours and then serum diluted 1:10 was given at 3.5 hours (fig). In these experiments the very dilute serum had little effect (median change +24.5%, range +1% to +46%: not significantly different from controls at 2.5 hours). Serum (1:10) at 3.5 hours again stimulated secretion (median change +155%, range +63% to +200%:  $p < 0.001$  when this was compared with controls at 3.5 hours). In these four experiments the effect of the 1:10 serum at 3.5 hours was greater than that of the 1:2500 serum at 2.5 hours ( $p < 0.05$ ).

Fractionation of material from the luminal chamber resulted in a single radiolabelled peak in the excluded volume of the Sepharose CL 2B column. No peak was found in the included volume.

## Discussion

The presence of serum in the luminal half of the Ussing chamber increased the rate of release of radiolabelled macromolecules from the bronchi.

The increased concentration of bound radioactivity in the washings from the Ussing chamber cannot be explained by binding between  $^{35}\text{S}$ -sulphate ion and serum protein for two reasons: firstly, dialysis completely dissociated  $^{35}\text{S}$ -sulphate from serum with which it had been mixed; secondly, gel filtration of radiolabelled washings and serum after dialysis resulted in a peak of radioactivity in the excluded volume, but there was no peak in the included volume, in which serum proteins are known to elute.<sup>11</sup>

The radiolabelled macromolecules are likely to be mucins for the reasons given by Phipps *et al.*<sup>8</sup> The effects of the very dilute serum (1:2500) were small or absent, suggesting that the traces of serum proteins thought to be present in healthy bronchi<sup>4</sup> play no important part in regulating normal secretion. When serum was given at a range of concentrations (from 1:100 to 1:10) commonly seen in sputum from inflamed airways<sup>12,13</sup> it stimulated secretion of radiolabelled macromolecules. Over this range the effects were not obviously dose related.

The effect reported here resembles that seen in the cat trachea *in vivo*,<sup>7</sup> where diluted serum strongly enhanced mucin secretion. A similar finding has been described in the rabbit trachea *in vitro*, where contact with serum from healthy human donors induced secretion.<sup>14</sup> In the cat it was shown that several high molecular mass fractions of serum, probably proteins, stimulated secretion. Neither the mechanism of action nor the cells which discharge the mucus was elucidated in our human studies.

Is the additional secretion in response to serum an adaptive response? Provided that the cilia are working effectively, extra secretion would dilute the serum and assist its removal from the airway together with the irritants, allergens, bacteria, or leucocytes causing the inflammatory response. Where ciliated cells are sparse or ineffective, such as during attacks of asthma,<sup>2,15</sup> the additional secretions would accumulate unless coughing removed them, leading to narrowing of the airways, increasing resistance to airflow, and predisposing to infection.

These results show that serum, at dilutions which are commonly found in the diseased airway, can evoke secretion of macromolecules of the type found in mucus.

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