AN ORGAN CULTURE STUDY OF THE EFFECT OF DRUGS ON THE SECRETORY ACTIVITY OF THE HUMAN BRONCHIAL SUBMUCOSAL GLAND

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SUMMARY

1. The incorporation of tritiated glucose into bronchial gland cells enables their glycoprotein secretion to be followed by radioautography. The number of cells from which secretion is observed after 4 h of cell culture is the secretory index.

2. Large variations in secretory index were observed between the bronchi of different subjects, but the secretory index was proportional to gland size.

3. The secretory index was increased by parasympathomimetic drugs and diminished by parasympatholytic drugs, the magnitude of the effect being proportional to gland size in the former and inversely proportional in the latter case.

4. Sympathomimetic drugs, bradykinin and mucolytic drugs had no effect on the secretory index.

5. In cystic fibrosis, bronchitis and bronchiectasis gland size and not the disease process was the main determinant of the secretory index.

Key words: bronchial submucosal secretion, parasympathomimetic drugs, parasympatholytic drugs, sympathomimetic drugs, mucolytic drugs, cystic fibrosis, bronchitis.

Our understanding of the control of mucus secretion in the bronchial tree derives mainly from animal studies although the complexity of the secretion in both animal and man makes investigation difficult. Many studies (C. Rossbach, 1882, cited by Calvert, 1896; Calvert, 1896; Perry & Boyd, 1941) used assessment of volume of collected bronchial fluid to trace the effect of drugs, a method that did not distinguish different types of gland secretion from transudate or exudate. Calvert (1896) suggested that vasoconstriction and vasodilatation have a profound effect on the volume of bronchial fluid.

Florey, Carleton & Wells (1932), by collecting secretion from the opened trachea in the cat and dog and studying microscopic sections of the bronchial wall, were able to show that the
glands secreted mucus in response to vagal stimulation. They tried to collect separately the submucosal gland secretion and that of the surface goblet cells by blotting the surface and stimulating the vagus nerve. They failed, because tracheal fluid formed so quickly, evidently by transudation since the goblet cells were still replete with secretion.

Trowell's (1959) method of organ culture applied to the human bronchus has facilitated the study of mucus secretion from individual cells. In man mucus is secreted by the goblet cells of the surface epithelium as well as by the serous and mucous cells in the submucosal glands, each cell type secreting a range of acid glycoproteins. With Trowell's (1959) method strips of adult tissue in an artificial medium retain their normal histological appearance for about a week. After the addition of radioactive precursors to the culture medium the radioactive label can be followed in its progress through the cell by radioautography. Drugs also can be added and their effect on the passage of the radioactive label through the gland cells observed. Since, in the case of human bronchi the radioactive label is discharged from the cell after about 4 h of incubation, the tissue survival-time necessary for the experiment is well within the acceptable period. In our previous studies glucose and threonine were used to follow metabolism of the mucous and serous cells (Sturgess & Reid, 1972); in the present study of drug effects, tritium-labelled glucose only has been used.

Our purpose was to explore the possible effect of the autonomic nervous system on mucus secretion by the human bronchial submucosal glands, by using a range of drugs and their antagonists, as well as other humoral and therapeutic agents. The variation in drug response of glands from different individuals is here related to gland size.

**MATERIALS AND METHODS**

Specimens of the type described by Sturgess & Reid (1972) were used. For the drug studies described below these were forty-five bronchial rings from forty-three surgical resection specimens and seventeen from seven autopsy specimens. The resections were carried out mainly for carcinoma of the bronchus; the bronchi, otherwise normal, included glands of normal size or of various degrees of hypertrophy: the autopsy bronchi were normal. Resection specimens usually provided only one ring of bronchus but at autopsy as many as three were obtained from different levels in the bronchial tree. Resection specimens were also studied from four children with cystic fibrosis.

**Organ culture**

The method of organ culture used in this study has been described in detail (Trowell, 1959; Sturgess, 1970; Sturgess & Reid, 1972). From each specimen of bronchus, one ring 0.5 cm deep was taken for histological examination and assessment of gland size, while the remainder was used for organ culture. Strips of 'explants' of submucosal tissue, including the glands and 1 mm wide and deep and 1 cm long, were dissected from the bronchial wall and placed on lens tissue on stainless-steel grids and moistened with tissue-culture medium (TC 199). Three strips or explants of bronchus were placed on one lens tissue and were treated in a similar way so that each result is the mean of findings on two or three of the explants depending on whether enough satisfactory gland acini were found in only two of the explants. Sections were examined from two levels in each explant.

Explants were incubated at 37°C in O\textsubscript{2} + CO\textsubscript{2} (95:5) for 1 h to allow the tissue to equilibrate.
Then the explants, still on the lens tissue, were transferred to clean grids with fresh culture medium to which \[^3\text{H}\]glucose (TRA 85, The Radiochemical Centre, Amersham, Bucks., U.K.) was added as a marker for glycoprotein secretion to give a concentration of 10 $\mu$Ci/ml. Drugs under test were added at this stage, made up in sterile solution without preservative and diluted in tissue-culture medium with the final dilution prepared in medium containing radioactive glucose. For each drug concentrations were used in progressive 10-fold dilutions. In Table 1 the values represent the range of concentration tested: within the range, all dilutions in 10-fold steps have been studied, e.g. acetyl choline was studied in concentrations of 0·1, 1·0, 10 and 100 $\mu$g/ml.
After incubation explants were fixed in neutral buffered formalin, dehydrated, cleared and embedded in paraffin wax. Serial sections, 4 μm thick, were taken from at least two levels of each explant; at each level one section was stained with Haematoxylin and Eosin (H & E) and one with Alcian Blue and periodic acid–Schiff (AB/PAS) for histological studies. From further sections, pre-stained with PAS, radioautographs were prepared by using the stripping film technique (Pelc, 1947).

**Assessment of drug effect from the radioautograph**

After incubation with $[^3]$H$glucose$ the radioactive label was taken into the gland cells at their base, progressed to the apex and was discharged. The progress of radioactive label can be traced by assessing its position in the cell. Because of the shape and size of the lumen of the mucous acinus the discharge of radioactive label on to the cell surface can be followed, and the number of mucous cells secreting at any given time expressed as a percentage of all mucous cells. In the case of the serous acinus this quantitative method was not practicable because the lumen was rarely cut in cross-section. For the serous cells the presence of granules within the cell was recorded and it was found that lack of granules corresponded with increased secretory activity from the mucous cells. The number of mucous cells discharging radioactive medium after 4 h of incubation was taken as the secretory index (SI). To assess the effect of drug treatment, the difference between the mean SI of six drug-treated explants and six controls was expressed as a percentage of the control value.

Variation in secretory activity within a series of three explants was studied in material from five bronchi. The maximum variation in the secretory index for any one of the bronchi was within $\pm 15\%$ of the mean for the two levels in each explant. A greater variation than 15% was thus taken to indicate a significant effect of a drug on secretory activity. It has been shown that the SI varies between different subjects but is similar at any level in the bronchial tree from one subject (Sturgess & Reid, 1972).

**RESULTS**

A number of additional Tables (A–E) have been lodged as *Clinical Science* Tables 42/45–49 with the librarian at the Royal Society of Medicine, from whom copies may be obtained. These provide detailed information about (a) variation in secretory pattern in tissue from one bronchus, (b) the effect of sympathomimetic and sympatholytic drugs, corticosteroids, humoral agents and their antagonists on the SI, and (c) the effect of drugs on the SI in cystic fibrosis and bronchitis.

**Effect of drugs on human submucosal gland secretion**

*Parasympathomimetic drugs.* Parasympathomimetic drugs increased the SI of the mucous cells in all glands studied (Table 2) and where investigated a dose-related response was seen. Secretion also occurred from the serous cells. At doses above 100 μg/ml both acetylcholine and pilocarpine were toxic as judged by the altered histological appearance of the tissue. After 6 h of incubation no further effect was produced by acetylcholine, probably because it is rapidly destroyed. Pilocarpine, perhaps because it is more stable, caused an increased concentration of radioactive label in the gland lumen until at least 18 h of incubation, the longest time studied. The same concentration of acetylcholine produced a widely different response in the SI in different subjects, thus 1 μg/ml caused increases between 18 and 140% in different bronchi.
Drug control of bronchial mucous gland

Explants from one bronchus were incubated with [3H]glucose for 4 h and then washed and transferred to an organ culture to which acetylcholine had already been added. After only 1 h a significant increase in the secretory rate (45%) had occurred, perhaps by a direct effect on cell discharge, perhaps indirectly through an increase in cell synthesis. Other explants were

<table>
<thead>
<tr>
<th>Table 2. Effect of parasympathomimetic and parasympatholytic drugs on the secretory index of mucous cells. The values represent % increase (+) or decrease (−) in the secretory index as compared with the corresponding control tissue. Each row of values represents different specimens from the same bronchus.</th>
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<tbody>
<tr>
<td><strong>Parasympathomimetic drugs</strong></td>
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<td>Acetylcholine</td>
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<td>Carbachol</td>
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<tr>
<th><strong>Parasympatholytic drugs</strong></th>
<th><strong>Concentration (µg/ml)</strong></th>
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<td>0.5</td>
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<tr>
<td>Atropine</td>
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<td>−60</td>
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<td>−71</td>
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<td>Hyoscine</td>
<td>−36</td>
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incubated with acetylcholine for 1 h and then washed and incubated in the medium containing radioactive precursor. This preincubation with acetylcholine caused a 30% increase in the SI. Whether this effect detected after 4 h was produced by discharge within the first hour or whether it represents a continuing effect on synthesis is not certain and requires further study.

Parasympatholytic drugs. The effect of atropine and hyoscine was to reduce the SI in the mucous cell population (Table 2) but in no case was the secretion inhibited completely. The secretory granules in the serous cells were relatively little affected by the parasympatholytic drugs. A dose-related response to atropine was demonstrated up to a concentration of 50 μg/ml; at concentrations above this no additional inhibition was seen. The degree of inhibition of secretion varied widely in bronchi from different subjects, 0·5 μg/ml of atropine producing between 18 and 73% reduction in SI.

A comparison of the effect of acetylcholine with that of atropine for explants from the same bronchus is given in Fig. 1. In control bronchi after 4 h of incubation radioactive label was being discharged from 40% of the cells and was at the apex in 40%. Acetylcholine had little effect on the percentage of cells in which label had reached half way up the cell or less, but it increased the number of cells discharging, suggesting that it influenced those cells that were near secretion. This suggests that the effect of the stimulus is on the discharge of secretion rather than on its synthesis. Atropine reduced the passage of radioactive label through the cell, so

![Graph](image-url)
that the number of cells discharging were fewer and in more cells the tracer had reached only half way up. This suggests reduction in uptake of metabolite or synthesis, perhaps because of reduced discharge.

*Sympathomimetic and sympatholytic drugs.* No sympathomimetic or sympatholytic drug had any effect on secretion either from mucous or serous cells; the drugs investigated included α- and β-blocking agents.

*Sodium salicylate.* Sodium salicylate showed a dose-dependent reduction in the SI of the mucous cells and a similar effect on the serous cells, accompanied by reduction in the amount of glucose incorporated into the cells. Doses above 100 μg/ml were toxic to both cell types, as judged by the presence of vacuolization.

*Corticosteroid hormone.* In only some of the specimens of bronchus treated with hydrocortisone was the SI significantly reduced but for these bronchi all explants responded and the response was dose-related. The cortisone derivatives betamethasone, dexamethasone and triamcinolone, although more potent than hydrocortisone, gave neither a more consistent nor a greater effect than hydrocortisone.

This group of drugs is different from those described above in that not all subjects were affected, which would seem rather to imply a tissue sensitivity than to reflect drug potency or concentration. In all specimens studied the corticosteroid-treated explants stained more intensely with PAS and their histological appearance after 24–48 h was better defined.

*Nicotine.* On only one of the four bronchial specimens used did nicotine have any effect. At 10 μg/ml of nicotine both mucous and serous cells appeared vacuolated but at higher concentrations the nicotine produced marked necrosis. The significance of the one case of increased secretion is not clear but, as with steroids, suggests a tissue susceptibility.

*Other drugs.* A series of other drugs and humoral agents was tested, chosen because they have been claimed to act either therapeutically or physiologically on the bronchial tree. None of these produced any effect. Two mucolytic agents, N-acetyl cysteine and a vasicine derivative tested in organ culture for periods up to 15 h showed no effect on mucous or serous cell secretion. Mucus accumulates on the surface of explants during organ culture but at physiological concentration neither mucolytic agent had any apparent effect. With N-acetylcysteine solubilization was minimal up to 100 μg/ml, and higher concentrations produced necrosis of tissue and hence no mucus secretion. Vasicine was toxic to tissues at concentrations higher than 1 mg/ml.

Bradykinin, histamine and their antagonists had no effect on bronchial secretion, nor did potassium iodide or sodium cromoglycate.

*Variation in individual response to drugs*

The response by glands at three different levels in the same bronchial tree, trachea, main bronchus and lobar bronchus was investigated (Table 3). When the drug affected the SI the effect was similar at each of the levels of the bronchial tree studied. However, there was a wide variation in the response of glands in different bronchi to acetylcholine and atropine. The individual response was therefore related to gland size estimated as the gland/wall ratio (GWR) (Reid, 1960). The percentage increase in SI caused by incubation with acetylcholine was compared with the degree of gland hypertrophy in bronchi from eleven subjects. A significant relationship between gland size and sensitivity to acetylcholine is shown in Fig. 2 ($P<0.001$), the hypertrophied gland showing an enhanced response to acetylcholine. For example, when the
GWR was higher than 0.6, the SI was increased by 100–120% while in the more normal gland with a GWR less than 0.4 the increase was only 20–40%.

The variation in response of bronchi from twelve subjects to incubation with 5 μg/ml of

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Trachea</th>
<th>Main bronchus</th>
<th>Lobar bronchus</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td>24.3</td>
<td>25.1</td>
<td>22.8</td>
</tr>
<tr>
<td>Acetylcholine</td>
<td>58.9</td>
<td>58.0</td>
<td>56.4</td>
</tr>
<tr>
<td>Atropine</td>
<td>10.0</td>
<td>8.8</td>
<td>12.0</td>
</tr>
<tr>
<td>Isoprenaline</td>
<td>25.2</td>
<td>24.0</td>
<td>23.6</td>
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<tr>
<td>Guanethidine</td>
<td>28.0</td>
<td>26.2</td>
<td>27.1</td>
</tr>
</tbody>
</table>

Fig. 2. Relationship between the degree of gland hypertrophy (gland/wall ratio) and the percentage stimulation (increase in the SI) caused by acetylcholine (1 μg/ml). Each point represents specimens from a different bronchus. The effect of acetylcholine is greater on the hypertrophied gland ($P<0.001$).
atropine (Fig. 3) showed that inhibition by atropine was less in the hypertrophied gland 
\((P<0.01)\); with the gland/wall ratio more than 0.5, less than 30% inhibition occurred, while 
40–70% was found in a gland with a GWR below 0.5.

\[\text{Fig. 3. Relationship between the degree of gland hypertrophy (gland/wall ratio) and the percentage} \]
\[\text{inhibition (decrease in SI) caused by atropine (5 \mu g/ml). Each point represents specimens from a} \]
\[\text{different bronchus. The effect of atropine is less in the hypertrophied gland (}\ P<0.001\).} \]

**Drug response in cystic fibrosis**

Bronchi from four patients with cystic fibrosis were included in this study since it has been 
suggested that this disease causes dysfunction of the autonomic nervous system.

Acetylcholine increased the SI in all cases and, as in chronic bronchitis, the increase was 
related to gland size. The response to atropine, however, was peculiar in that in only two cases 
was an inhibitory effect demonstrated. The two unaffected cases had the highest SI in the series 
and, while these would be expected from their large gland to show a lesser response to atropine, 
they are the only two cases in the whole series in which atropine produced no effect at all.

Isoprenaline and guanethidine showed no significant effect on SI nor did hydrocortisone in the 
one case in which it was tested.

**DISCUSSION**

For the first time the effect of drugs has been shown directly on the human bronchial glands. 
The stimulatory effect of parasympathomimetic drugs on both mucous and the serous cells has
been demonstrated and partial inhibition produced by their antagonists. While there was uniformity in the response throughout one bronchial tree, wide variation was found between different individuals. This has been shown to be related to gland size; the larger the gland the greater the effect of stimulation and the less effective is inhibition, thus explaining why with hypertrophied glands in disease, drug control of hypersecretion has proved so disappointing. Since in bronchial mucous gland hypertrophy the individual gland-cells are increased in size and discharge through them is raised even under resting conditions (Sturgess & Reid, 1972), it is apparent that in disease there is greatly increased activity per unit volume of cytoplasm.

The altered sensitivity may be analogous to that of the submaxillary gland following recurrent parasympathetic stimulation. This leads to ‘augmented secretion’ in response to a given parasympathomimetic drug dose and ‘tolerance’ develops to atropine (Burgen & Emmelin, 1961).

Biochemical studies of sputum suggest that drugs have a similar effect on gland secretion in vivo to that demonstrated in organ culture. The relative contribution of gland secretion and tissue fluid can be traced using the concentration and ratios of selected substances such as neuraminic acid and fucose (Reid, 1968; Keal, 1971a, b). In one patient atropine caused a decrease in total secretion as well as a decrease in the mucus contribution, suggesting some decrease in gland secretion. In another patient producing a large amount of sputum, it was the antihistamine, chlorpheniramine, that caused a decrease in volume with a relative increase in the mucus component, suggesting an effect on transudate. In bronchorrhoea the response of the large sputum volumes to corticosteroids has been studied in some detail (Reid, 1968; Keal, 1971a). In certain patients corticosteroids decreased the volume of bronchial secretion and increased the neuraminic acid content without changing the dry-weight yield, suggesting that the nature of the gland secretion is altered. Some patients did not respond to corticosteroids; these were usually those with associated chronic bronchitis.

The main action of the parasympathomimetic drugs would seem to be on discharge, the lesser effect on synthesis may be direct or secondary to the change in discharge rates. The interaction between these two mechanisms calls for further study.

A number of the drugs that we have shown to be ineffective, such as the sympathomimetic drugs, have been reported to affect total bronchial secretion; for example Kountz & Koenig (1930) reported an increase in secretion in the dog after sympathomimetic drugs and Ratner (1959) reported a reduction in the amount of bronchial secretion produced by antihistamines. Any such overall effect may reflect change in transudate or exudate rather than in mucus secretion.

For the periods of drug treatment studied here we have failed to show any effect by the sympathetic system. Wells (1963) has shown in animal experiments that in the salivary gland this system is concerned with basal growth and tissue maintenance, but our experiments being limited to a day, and usually less that 6 h duration, may not have sufficed to detect this effect. Certainly sympathomimetic drugs seem to have no short-term effect on secretion.

Corticosteroid hormones showed a peculiar effect in that not all patients responded although in those that did the decrease in secretion was dose-related. This seems to reflect a difference in tissue sensitivity, the ‘refractory’ material not being affected by higher doses or by the more potent derivatives.

For the first time salicylate has been shown to decrease the amount of glucose incorporated into the secretory cells of the bronchial glands. An inhibitory effect on connective tissue
Drug control of bronchial mucous gland

mucopolysaccharide synthesis was reported by Whitehouse & Böström (1961); Allen & Kent (1968) reported that in the sheep colon salicylate blocked a stage in protein synthesis. A high dose of sodium salicylate was necessary in our studies but this has a low diffusing capacity and a more soluble derivative may penetrate the cell membrane more easily.

In several respects the bronchial submucosal glands are similar throughout the bronchial tree. The degree of drug effect for glands at different levels in the airways is similar and similarity in gland size (Lamb & Reid, 1972) and in secretory activity (Sturgess & Reid, 1972) has been reported. This is not achieved by uniformity of cells since the mucous and the serous cells each represent a mixed population. The basis for this control must be a matter for conjecture but, in some way, at different airway levels a mixed cell population behaves in a similar way.

ACKNOWLEDGMENTS

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REFERENCES


