# The Bronchial Epithelium in Disease

## The Scientific Basis of Medicine

**With Professor Lynne Reid, Institute of Diseases of the Chest, University of London.**

**University of London Audio-Visual Centre, 1971.**

**Introduced by Dr Ian Gilliland.**

**Produced by Peter Bowen.**

**Made for British Postgraduate Medical Federation.**

**Black and White**
**Duration: 00:33:38:21**

**00:00:00:00**

<**Dr Ian Gilliland to camera**>

Professor Lynne Reid is Professor of Experimental Pathology in the Institute of Diseases of the Chest at the Brompton Hospital. She is the author of much experimental research on the pathology of the lungs and, indeed, her work has largely been responsible for the renewed scientific interest in chronic bronchitis. Her contributions are internationally known. Professor Lynne Reid.

<**Professor Lynne Reid to camera, then leans down to show model of bronchial tree, then back to camera**>

The bronchial epithelium is part of the surface of the body, in contact with the outside air. This air is warmed and humidified in the nose before it reaches the epithelium

that lines every one of the airways shown here. You see that there are airways large and small, carrying air into every part of the lung. But this air also takes with it the pollutants from the atmosphere, some of them personally administered, such as tobacco smoke, others less under personal control – the pollutants that society thrusts upon us.

The disease we'll be discussing this afternoon, chronic bronchitis, is essentially a disease of pollution; it is the result of irritation of the surface epithelium.

<**Reid over slide showing different epithelial cells, then to camera**>

The epithelium lining the airways is a pseudostratified ciliated columnar. The cell with which we're mostly going to be concerned is the goblet cell. Here you see goblet cells seen as individual black dots. The other cell whose evidence you can just detect in this picture is the ciliated cell; you see here the fringe of cilia that waft the mucous layer toward the larynx and take with it entrapped particles. Mucous is also secreted by cells of the sub-mucosal gland; these glands also include two cell types – you see here the black staining ones are the mucous cells, the greyer staining ones are acinar or tubules, consisting just of serous cells.

Recently, new anatomical details have come to light about these glands. For the first time a 3-dimensional reconstruction has been made of the human bronchial sub-mucosal gland.

<**Reid over diagram of 3-dimensional model of bronchial sub-mucosal gland**>

This is a diagrammatic of the representation of the reconstruction. Here would be the lumen of the airway, this the surface of the epithelium, here the duct. The first part of the duct, to a distance of about 200 mu, is lined by the same pseudostratified ciliated columnar epithelium. Beyond this the duct is lined by a special collecting duct cell that you'll see shown in a moment. From this arise, in this picture, 11 tubules. Each of these tubules is lined by mucous cells at its point of origin and the serous cells are found only at the periphery. Tubules A and B are enlarged in this picture and here

you see, shown in the heavy black line, the mucous cell part, with the serous cells by the interrupted line that open either at the end of the main pathways or as the pouches here at the side. This means that in each case the secretion of the serous cell passes over that of the mucous, out into the duct and over the connecting duct.

<**Reid over slide and electron micrograph showing section of part of the sub-mucosal gland, then to camera**>

This is a section of part of the gland, in this case stained with alcian blue, and you see here are tubules of mucous cells distended with secretion that stains black. Here are the serous cells showing just granules. Here, the duct, with secretion in the lumen, but you see that this duct cell, the collecting duct cell, is high, maybe up to 70 mu in height but it has no secretory granule stained. And the electron microscope reveals that this cytoplasm is stuffed with mitochondria. Here is one mitochondrion and you see the large numbers that pack this cell.

This structure is reminiscent of what we see in the salivary gland, save that this particular duct cell does not have a striated base. Almost certainly it's concerned with control of the fluid and electrolyte content of the secretion.

**00:05:19:00**

In chronic bronchitis, these glands are enlarged. We accept now that the definition of chronic bronchitis be a clinical one and in 1965 the MRC gave us the definition for chronic bronchitis that it be the condition characterised by excessive production of mucous, enough to produce sputum. For epidemiological purposes, it was necessary to set arbitrary limits and the diagnosis of chronic bronchitis is now made if a patient has produced sputum most days for at least one season of the year and for at least two years.

The enlargement of the glands can be used to make the diagnosis pathologically, and we found the best way to assess gland size is to measure it and to assess it as a gland/wall ratio.

<**Reid over diagrams showing gland to wall ratio in normal and bronchitic patients**>

If we choose one of the large glands, we can here find a point, or level, where the epithelium is roughly parallel to the cartilage and, at the same point, measure the gland thickness or the inner wall thickness. This can then be assessed as a gland to wall ratio and you see here that we have the ratio being in the normal, 1 is to 3. The advantage of the ratio is that it is much the same in children as in adults, in males as in females. Above the age of 4, we find that below 1 is to 3 is the normal value, in the child below 4 years of age it's rather higher.

In the bronchitic, which you will see in this next slide, you see that that gland has increased in absolute depth and now represents one half of wall thickness. You'll also notice that there is an increase in the number of goblet cells on the surface and the next slide shows an even more severe degree of hypertrophy where the gland is now 2 is to 3. If you choose a large airway it's possible to find perhaps 3 or 4 sites where these measurements can be made and any patient can be then assessed by taking the mean of these 4 measurements.

<**Reid over graphs showing gland/wall ratios in normal people and people with chronic bronchitis**>

This graph summarises our findings in a group of normal subjects, first of all, and by this we meant people who had never smoked. You see, shown here on the vertical axis is thickness – in the normal subject, the gland/wall ratio is about 0.26. You see the gland thickness shown by the coarse stippling, the wall thickness by the fine, this giving a gland/wall thickness of a quarter, that is the mean for the group, and the normals, as I said before, show values under one third.

This group of patients with a chronic bronchitis had produced coughs and sputum for at least 5 years and you see here the increase in gland is such that there is now a

gland/wall ratio of 0.59. What was important was that there was no overlap between these two groups, so here we had a test that gives us a significant result.

When we look at a group of patients with surgical bronchiectasis, although the mean values are within the range of the bronchitic group, there was overlap with the normals because some of these had, in fact, had resection because of haemoptysis, not because of persistent infection. The last column shows a group of patients, a smaller group, 8 patients with emphysema, and thought to be without sputum – there's one bronchitic hidden here and if that patient were removed, in fact, the value for this group is absolutely the same as the normal.

<**Reid over graph comparing sputum history in patients with small and large glands**>

This raised the question as to whether in the community there are two sorts of people, those with small glands and those with large. To investigate this we studied a series of resection specimens. In this the patients were assessed by their long-term sputum history, we disregarded the sputum pattern for the few months before operation and divided the patients into half ounce daily sputum groups, save that we divided this group here more finely, distinguishing no sputum at all, a trace and a half an ounce. And you see that over this group of increasing sputum production, the thickness shown here as before, reveals that the gland increases steadily with sputum production, the gland/wall ratio also increases, but most important – even this group of subjects who produced only a trace of sputum in the morning, already showed a degree of gland hypertrophy significantly different from the normal.

<**Reid to camera, then over slides showing different levels of goblet cells in the bronchioli, then to camera**>

So much for the glands. If we move down the smaller airways of the lung to the bronchioli, we find that there is an increase in goblet cells. Here on this slide you see a normal bronchiolus, thin epithelium, with virtually no goblet cells at all. But in the next slide you will see what happens in patients with a chronic bronchitis. You see

here that the cells are increased in height and each of these cells is distended with secretion. You can't detect that it's also staining brightly red because of the mucous included in the cell.

These then are the fundamental changes in patients with chronic bronchitis: increase in gland size, increase in goblet cell number in large airways and their appearance in large numbers in peripheral small airways where they are normally sparse.

**00:10:41:00**

These patients are commonly short of breath and in the past we would have felt that the most important reason for the shortness of breath was the superadded infection. In fact, as one studies the lung of patients who die from this condition, it is easy to see the ravages that infection has produced. And if one studies the bronchograms of patients who have had chronic bronchitis for some years, it's possible to show that there is interference with conduction along airways, the reason for airways obstruction.

<**Reid over bronchograms showing chest of normal and bronchitic patients, then to camera**>

In this bronchogram you see on the left, the filling is normal, the whole pattern is normal. The left lung is filled well out to the periphery; we can regard a bronchogram as a respiratory test, and you can see here that it has, in fact, conducted air well out into small airways. In the bronchogram on the right, from a patient who had been a bronchitic for many years, had been short of breath for many years, the bronchogram was technically adequate in that the patient had been postured well before it was done. There is plenty of radio opaque medium in the airways and the patient sat in the upright position for 20 minutes before this picture was taken. But you'll see that, in fact, the main airways have not been able to conduct beyond this level, and these are still quite large airways; there is complete absence of peripheral filling in this picture. Furthermore, there are also points of bronchiectasis and bronchiolitis obliterans.

It's possible to see such changes of non-filling, even where the airways that have filled may still be normal in shape and have parallel walls. And this is probably, in part, because there is sputum present and clearly airways obstruction can develop just from accumulation of secretion in the lumen.

Increasingly in the last few years, the early stages of this condition have been studied, the pre-clinical stages if you like. And I want to mention to you some work of Dr Ian Gregg, carried out in his practice at Roehampton – he and his colleagues deal with a pleasant part of London where atmospheric pollution isn't too high. One of the first studies he carried out was to look into the respiratory function of normal men in the practice, that is, normal from the point of view of their lungs, and normal meant that also they had never smoked.

<**Reid over diagrams detailing research by Dr Ian Gregg into respiratory function in different groups of normal and unwell patients, then to camera**>

Here you see, in the top left-hand corner, shown the results for the normal group. Here on the horizontal axis age is plotted, 20 to 60; on the vertical their peak expiratory flow is indicated as a percent of the predicted value. And you see that between 20 and 60 there is virtually no peeling off from the predicted value of this group. At the bottom right-hand corner, you see a group of patients with a chronic bronchitis, this time with inverted commas because these were patients who had presented to Dr Gregg or his colleagues because of respiratory symptoms. These were patients who had complained of something wrong with their chest. And you will see that all of these fall well below the normal distribution – these are patients, by the way, come up to 80, and even these two probably represent a fall from a higher level. So, when the patient presents to the doctor, he already has severe limitation of respiratory function. In this group Dr Gregg has studied smokers with expectoration, so this group would come within the MRC definition of a chronic bronchitis. And you see that already a large number of these subjects show impairment of respiratory function. Dr Gregg also recognised an earlier group of bronchitis that wouldn't come within the MRC definition, a group that had just throat clearing. They would reply to

the question: “do you clear your throat in the morning?”, “yes”, although they would reply to the question: “do you produce sputum?”, “no.” So this group with throat clearing already shows that there is, in fact, impairment in many of them, of respiratory function. This is the group of symptomless smokers. Here is a group of ex-smokers who’ve never had mucous hypersecretion, and that, of course, is the group that is the most normal of all.

So we come back to this, then, that even the stage of mucous hypersecretion seems to be associated with severe restriction of respiratory function. What is the cause of this bronchial mucous gland hypertrophy? If we turn to the epidemiologists, their studies tell us that one of the most important causes of mucous hypersecretion and, of course, hypertrophy, is the habit of tobacco smoking. It’s so important that you can’t really investigate any other possible cause unless you’ve made allowance for the tobacco smoking of the population under investigation. But if this is so, there is evidence that certain other conditions, such as dusty occupation, even environmental atmospheric conditions, may contribute to the prevalence of a chronic bronchitis.

**00:15:53:00**

To investigate this condition experimentally, we turned to the experimental animal, to possible irritants, to see if we could produce the same sort of changes in the laboratory animal. To do this we chose the rat, which is one of the few small animals that has sub-mucosal or bronchial glands, and is reasonably well-endowed with goblet cells, even when there is no infection in the lung. The mouse, the hamster, the guinea pig and the rabbit, these have virtually no glands.

<**Reid over slides showing rat lungs; one normal, the other after having been exposed experimentally to sulphur dioxide in large quantities, then to camera**>

And you see, here, on this slide, the two lungs of a rat are mounted side by side. The right lung is rather inconveniently divided into 5 lobes, but the left lung is conveniently divided into 1 lobe, and here you see the pulmonary artery in this structure. Here are also small airways, but again, the lung, quite even, quite normal. We exposed these

animals to sulphur dioxide, in large doses, 400 parts per million, although the worst London smog is only included 2 parts per million, but we were not interested in sulphur dioxide as a pollutant, rather as a tool to produce goblet cells. And you'll see in this next slide that after 3 ½ weeks, we had produced increase in mucous production so that there is impaction of mucous out here in the alveoli and here you see impaction even in small airways.

We wondered whether this particular secretion had been aspirated from higher up in the bronchial tree. We inserted India ink solution into the noses of these animals and did not find India ink in the lung, so it seemed that this excessive production had come from the local, small airways, where we were able to show that there was an increase in goblet cells. And you see here, shown diagrammatically, the way we made these quantitative studies.

<**Reid over series of diagrams and tables showing results of comparison between normal rat epithelium and that of rat exposed experimentally to sulphur dioxide**>

Here you see the left lung of the rat, the trachea and main bronchus as cartilage and glands in the wall, and it's possible to cut this along in such a way that you finish up with an ideal section along which you can see the epithelium of this axial pathway and identify side branches of varying degrees of smallness.

First of all we measured the gland size. And here you see shown the sort of gland size we found in the control animals. It's not possible to measure gland size in the rat as it is in the human, but it is possible to assess size by making measurements of the length of the gland, and also the depth. You see here shown that after sulphur dioxide exposure, there is an increase in length and an increase in depth.

And shown here are the results for the mean and the maximum. First of all are the control animals – here you see the two figures, and if you look first at the mean values, you'll see that as exposure increases, up to 6 weeks, there's a steady rise in

the mean value and also a steady rise in the maximum value. So we have, in these animals, produced an increase in the size of the glands.

When it came to studying the goblet cell number along the airways, we first of all, counted in the non-exposed or control animals – you see here, shown on the vertical, the number of goblet cells for 5 high power fields. Along here the time of exposure, this was a 6 weeks experiment. Four levels of the airways were studied, and you see that in the distal small airways of the control animals there are no goblet cells; in the larger ones there are goblet cells. During the first week of exposure, we found there was a fall in the number of goblet cells. This was associated with severe ulceration of the epithelium and, I would point out, that although these animals were still exposed to large doses of sulphur dioxide, this epithelium healed, the epithelium differentiated and you see, differentiated to produce further goblet cells until, at 6 weeks, the number is significantly higher than in the control animals. But what was more important was that in the distal small airways, where there are normally no goblet cells, we now had quite a large number present.

**00:20:16:00**

<**Reid to camera, then over chart showing similar experiment to above in which mitosis is used as a way of assessing ulceration of the glands, then back to camera**>

We wondered whether in the distal small airways we'd missed a minor degree of ulceration. And so we counted the cells in mitosis, hoping that mitosis would be a more sensitive way of assessing whether there had been ulceration of the glands.

And you see shown in this chart a similar experiment in which the exposure times being for 6 weeks, but here on the vertical axis is shown the percent of the total cells that were dividing. You see that at all levels of the bronchial tree in the control animals, the number of cells in division is extremely low. These experiments, by the way, were done with colchicine which arrests the cells in mitosis; we've repeated these experiments using tritiated thymidine which show the number of cells that go

into mitosis in a given time and as these have given to date the same results, it implies that this represents a true increase in mitosis, not just an increase in the duration.

And you see that during this first week when we know there was ulceration in the large airways, we have a peak in the cells dividing – this presumably representing the healing phase. This peak drops but the increase does level off to be above normal values and this is, again, a representation of the continuing mild damage that occurs. But if you look here, in the distal airways, there is no significant increase in the number of cells in mitosis, although you will recall the goblet cells increased steadily.

From this we can presume that goblet cell increase does not necessarily follow ulceration, it evidently can occur by changes that occur within persisting epithelium and, at the moment, we are pursuing these changes with the electron microscope.

Another way we've studied the changes in the goblet cells was by looking at the type of acid glycoprotein, or mucopolysaccharide they include.

<**Reid over illustrations showing distribution of goblet cells following exposure to different enzymes. Then to camera**>

And you see here, shown in a slightly different way, the number of goblet cells here in the normal. And this particular enzyme, RDE, made it possible for us to analyse one type of acid glycoprotein – you see here that after treatment with this enzyme we have been able to affect a large number of the peripheral ones. After sulphur dioxide exposure there is a large increase in the total number of goblet cells, but you see great increase in the effect of this enzyme, particularly in the periphery. And from this and other tests it's possible to say that this represents a great increase in the sulphur-producing cells and this change is seen not only after sulphur dioxide but after other types of irritant.

We have, then, got two ways of assessing – if you like, of titrating – damage to the pulmonary epithelium. One is by counting the number of goblet cells, the other is by counting the cells in division. And if one can over-simplify this a little, it's possible to suggest that the goblet cell increase is related to the problem of bronchitis, the increase in cells in division is more closely related to the problem of carcinoma.

We carried out a further series of experiments in association with Professor Passey, in which we investigated the effect of tobacco smoke; tobacco smoke arising from two types of tobacco. You're probably aware that there are two main types of tobacco in current social use: the cigarette tobacco, Gold Leaf Virginia tobacco, is prepared by flue curing in which the tobacco is dried at high temperatures. Burley, or cigar tobacco, is prepared by air curing at which the tobacco is dried at lower temperatures.

Cigarettes were prepared with both these types of tobacco but each cigarette included a cigarette paper and the same weight of tobacco.

<**Reid over graphs showing goblet cell frequency after exposure to tobacco smoke, then to camera**>

And we were able, as shown here, to show, first of all, that even with 10 cigarettes a day, for a month, it was possible to show, in these rats, a significant increase in the goblet cell numbers shown by the black and shaded columns, over the control, in the trachea and at each of these four levels along the bronchial tree. The fact that we hadn't shown that the cigars were more benign than the cigarettes was a little disappointing to our colleagues who'd switched from cigarettes to cigars.

You see in the next graph that when we compared the 5, 10 and 20 cigarettes a day, we have got a good dose relationship. And we have, in fact, in this method got a sensitive way of detecting the effect on the bronchial tree of a variety of irritants. The 5 and 20 animals were exposed on the same occasion, the 10 was a separate experiment, hence there is a slight difference in the controls. But in none of these

groups was there a significant difference in the goblet cell increase produced by the two types of tobacco.

When we turn to look at the cells in mitosis, the results were much more encouraging for the cigar smokers in that we found the cigar tobacco was much more benign as judged by this change. We couldn't carry out these experiments at exposures above 20 cigarettes a day because there were so many cells in mitosis we had hardly any differentiation into [*tape jumps*] goblet cells at all. But you've seen from what I've said that here, with these two ways of titrating, a given irritant may not rate as badly by both methods of testing and, for example, we've seen a dissociation between the two in comparing the cigar and the cigarette. So these methods of comparing do offer us a satisfactory laboratory tool.

**00:26:16:00**

I now want to refer to another model of chronic bronchitis in which the changes are being produced by a drug. We've been able to produce them both by isoprenaline and by pilocarpine.

It's long been known to the physiologists that isoprenaline, a sympathomimetic drug, (or isoprotenerol, to give it its other name), that isoprenaline produces an increase in size of the salivary glands. Dr Sturges investigated the effect of these drugs, or first of all, of isoprenaline on the bronchial glands. She used pilocarpine as a control drug, pilocarpine being a parasympathomimetic drug. And much to our surprise, we found not only an increase in the size of the bronchial sub-mucosal glands, but an increase in the number of goblet cells in the surface epithelium.

<**Reid over graphs showing the effects of isoprenaline and pilocarpine on the bronchial glands, then to camera**>

Analysing this further, we found that the two drugs did not act in the same way. Here in the left, you see analysed, the total number of four types of goblet cell: they are types that are distinguished on their size and on their staining properties. The

number, per 6mm of trachea, and you see here the increase that was achieved after these animals had had 12 injections of the drug. This particular one being pilocarpine. So you'll see that here, on the right, there is an increase in total but the increase in total has been achieved by an effect on each of the four types of goblet cell. When we look at the effect of isoprenaline, again, the control animals would give you the sum total of those four per 6mm of trachea; the animals treated with isoprenaline, an increase in total, but this increase has been achieved by affecting only two types of goblet cell, both these types being cells that produce acid glycoprotein.

So we have here a difference in the effect of these two drugs. When Dr Baldock took the analysis of the dividing cells further, by applying tritiated thymidine to animals who had been treated in these ways, he has been able to show, and these are preliminary results, that it looks as though isoprenaline does not produce an increase in the number of cells dividing, but pilocarpine does. One working hypothesis is that the pilocarpine is simply producing a work hypertrophy in that it's producing greater activity in all cells, but it looks as though isoprenaline is, in some way, interfering with intracellular pathways and this is perhaps the basis for its effect on the mucous-secreting tissue. As you know, isoprenaline, and similar drugs, are quite widely used clinically in the treatment of asthma; we don't yet know what the clinical significance of this finding is.

These findings also raise other questions to do with the fundamental aspects of control of mucous secretion in the airways. In the 30s, Florey and his colleagues investigated this in the rat and dog and concluded, from their studies, that the bronchial glands […]

<**Reid over diagram showing effect of vagus on gland secretion, then to camera**>

[…] are under motor control through the vagus; that there are sensory afferents from the surface epithelium so that stimulation either of the surface, or directly of the vagus, will produce active secretion from the gland. But recently, as I will show you, it

seems that there may be sensory nerves both or within the epithelium, as well as beneath it, and not only sensory nerves, but there may in fact be motor nerves also.

**00:30:04:00**

<**Reid over electron micrographs showing aspects of epithelium under control of motor nerves, then to camera**>

If you look here, you will see some pictures of electron micrographs, first of all, of the human bronchial gland. We weren't, of course, surprised to find this because you know from other evidence, which you'll hear about in the next session, that these glands are under nervous control.

This is the basement membrane of a gland, this the secreting cell, here its nucleus, and here, in the hollow of this cell is one nerve fibre including dense cord vesicles which suggest that that is a motor cell.

Recently, in man and in animals, nerves have been found within the surface epithelium, superficial to the basement membrane. This is part of an airway in the rat lung, here the lumen of the airway, here the basement membrane, here the secretory cell and in a hollow, in its base, is this small nerve fibre, probably a sensory nerve.

A little more surprising it was to find in another section from this animal, again, the lumen would be up here, here the basement membrane of the epithelium, here a cell within the epithelium and here, in this hollow, a group of nerve fibres – 1, 2, 3, 4, 5, 6 such fibres, several of which include dense cord vesicles suggesting that these are motor nerves.

So, although our organ culture work supports what Florey found that, in fact, the goblet cells do not seem to secrete in response to direct stimulation, these findings with the drugs and findings with the electron micrographs do suggest that there may, in fact, be degrees of nervous control of surface epithelium that we haven't previously suspected.

I've tried, in this session, to give you some idea of the fundamental changes of patients with chronic bronchitis, to indicate to you that the mucous gland hypertrophy and the increase in goblet cells and their extension to the periphery, these are features which we can produce experimentally and for which we now have several models. The one that we've had for the longest time is the one where the increasing mucous secreting tissue is being produced by irritants, but clearly with the increase in these cells and glands produced by drugs, we have a new field of investigation opening up.

One, of course, is concerned with prevention. And from what I've said to you this afternoon, there is the obvious question of prevention being better than cure when we consider the role of tobacco smoke. But, some experiments we're carrying out at the moment and, again, these are preliminary results of work in progress, suggest that certain anti-inflammatory agents may be able to prevent the response of the airways to irritation by this increase in goblet cells. And these experiments are certainly, at the moment, offering us some hope of control.

<**End credits**>