How animals have contributed to our understanding and treatment of respiratory diseases - 2017 Paget lecture given by Professor Clive Page.

Thank you very much Jeremy for that kind introduction and I have so that it is an absolute honour to be standing here this evening as someone who started life as a lowly post-doctoral fellow junior lecturer on the RDS council as it used to be when it was chaired by Sir David Jack. I'll mention David again later in my lecture because he has had profound influence on my own research activities and I think he has been outstanding as a person contributing to this area. As Jeremy said my whole career has really been looking at understanding lung disease and particularly asthma and COPD and I wanted to concentrate on that this evening and talk about how animals have helped us develop the drugs that we use and how they continue to help us understand both the pathogeneses of disease but also in finding new treatments.

0:58. Lest you think that we don't need to work on lung disease anymore, this is *The European Lung White Book* from 2013 showing you that one in eight deaths in the year are caused by respiratory disease and a considerable number of people die every year from respiratory diseases and indeed they occupy obviously a lot of hospital beds and take a great part of the healthcare budget particularly this time of year with people getting chest infections. So this is an area that costs society a lot of money and whilst we have made significant progress, as I will hopefully show you, I think there is still more to do. Now I realise that we have got a very mixed audience and just really to say that if you look at someone undergoing an asthma attack, and this is a picture of someone undergoing a bronchoscopy, you can see without being an expert that there is profound inflammation, oedema, fluid occurring in the airway lumen that brings out this wheezing and coughing that is seen in asthmatics and for many years we have used drugs to relive this by relaxing the airways smooth muscle but over the last 20 years particularly we have recognised that this is a complex inflammatory condition.

02:10. Sadly people, particularly under the age of 30, still die from asthma. Several thousand people died in the UK last year from asthma, mainly under the age of 30 and this is because as you can see here, if they do not treat that underlying inflammatory response properly we end up with a very thick mucus plug obstructing the airway lumen and preventing gas exchange. No one in 2017 should be dying of asthma but unfortunately we still have this situation going on. If we look at this histologically it is very clear that this is indeed something that is due to inflammation, we can see inflammation into the airway wall and you can see here the airway lumen which is absolutely covered or is filed with mucus and this mucus I can tell you from experience of working with airways like this is actually like cement. To get this out is really difficult because the inflammatory cells coming into the lung release enzymes and mediators that change the composition of the mucus, and again, we now recognise that this inflammation contributes to this mucus plug-in.

3:20. If we turn to COPD, and I started going to respiratory meetings in the 1980's and COPD was almost never talked about because it was a disease associated with smoking and everyone said let us give up smoking, and clearly that is very sound advice, however, I've put up there air pollution because we now recognise and I think in the next 20 years we are going to see more and more younger patients developing COPD having never seen a cigarette in their life because they have exposure to air pollution and the air pollution in turn is producing inflammatory changes in the lung that are different to asthma but nonetheless are causing profound changes in disease and in particular it leads to bronchitis early on and then in a longer period of time we get alveolar wall destruction and emphysema.

4:11. If we look histologically we can see on the left a healthy normal lung parenchyma and you can see your airways are there for gas exchange, they are the size of a tennis court in most of us. In the middle we have an early inflammatory response to the former bronchitis and on the other side we have the extreme form when we get alveolar destruction. Of course, this is not something we can reverse we cannot rebuild airways but we can I think in the longer term hopefully prevent this. If we look at this in a different level you can see compared to lungs from someone who does not have emphysema from somebody that has, you can see the profound pathological changes that we are up against and trying to prevent. Now actually the pharmacology of asthma and COPD is relatively simple, we use bronchodilator drugs now and anti-inflammatory drugs in both conditions. We have the short acting β-agonists and I will come back to this shortly, such as Salbutamol or for those of you who have asthma or know people who have asthma, the blue inhaler and more recently we have longer acting β-agonists but most importantly in the last 15 years there has been the introduction of fixed combinations of taking bronchodilators and anti-inflammatory drugs together.

5:32. Because physicians want the patients to have the anti-inflammatory drug primarily glucocorticosteroids whereas the patients have what I call the McDonald effect, they like this acute bronchodilation and symptom relief. We have some very, very successful drug combinations both in terms of medical care and also commercial success. So, the formoterol, budesonide and salmeterol, fluticasone, between them generate about 11 billion dollars a year from sales to people who for treating asthma. If we look at the β-agonists there is actually a very long history going back to 3,000 BC and the discovery of ephedra, and ephedra and epinephrine as we know now as adrenaline of course are all very successful at causing the airways to open and people to feel better but they have the problem of also increasing heart rate and blood pressure. Over the years we have made this progressive improvement in these drugs and particularly the ability to deliver these drugs directly into the lungs through aerosols or powders right up today where we have drugs that actually one puff can last for three days and that is a profound improvement in terms of both selectivity of the drug but also the duration of action to make it easier for patients to comply with. Everyone in this room is familiar with this particular blue inhaler which

contains salbutamol, it is used by approximately 90% of the worlds' asthmatics but no one gives a second thought really as to how it got there.

7:07. I think it is only fitting this evening that I pay tribute today to David because David chaired the Research Defence Society council when I joined. David is the man, if we go back, who really discovered β-agonists and particularly salbutamol but also his team went on to discover salmeterol; the long acting β-agonist, and very importantly as I will come on to; topical corticosteroids and I think the world owes this man a lot and I think he should really should have deserved to have a Nobel prize, and sadly David passed away a few years ago but he has certainly been a fantastic mentor to me, and I will come back to a project that I worked on prior to him passing.

7:55 I want to take you back to 1969 because this is actually the first demonstration that salbutamol was a new class of drug, it was a beta-adrenoceptive stimulant but unlike adrenaline that also stimulates heart rate and blood pressure, this drug at the right doses did not. This experiment was initially done in guinea pigs as you see here, so this is actually showing you the overflow of air and giving salbutamol to show that you can suppress the effect of 5HT in causing bronchoconstriction. He then went on to show this is through a beta-receptor because if we blocked with a beta-blocker the response disappeared. You can also see that when you aerosolise this to guinea pigs, and the important thing here is that he did all of his work early on in guinea pigs, you can see that there is this very clear dose related effect when we inhale salbutamol in guinea pigs to reduce bronchoconstriction, and the number of people this very minute taking salbutamol somewhere on the planet have no idea that this all started with dosing guinea pigs.

9:04. In fact David really was I think important in going from what was adrenaline that affected both alpha and beta receptors, isoprenaline which was a beta but non selected β-agonist to salbutamol shown there. At the bottom is salmeterol, and salmeterol is a drug that instead of taking it four to six times a day, he put this long lipid tale on it to produce a drug that is actually active by bronchodilation twice a day. Now how did he do that was very simple and I think unfortunately we have forgotten how we have discovered many of the drugs that actually work and this is actually a series of experiments that have been done by taking an isolated guinea pig trachea, electrically stimulating it and the different spikes you see there are actually the contraction of the tissue. If you look at what happens when we put isoprenaline on there is a very transient reduction in the contraction of the airway smooth muscle, the same is true with salbutemol, you can see it reverses, it has got a very short half-life but then look at what happens when he puts this new drug salmeterol on the guinea pig trachea, you can see it is supressed and seven hours later it stays supressed and that is translated into the clinic as a drug that we now have twice a day and this very same assay is being used to now as I say discover drugs that are active for once a day or in some cases even longer. So the guinea pig has been profoundly useful in helping us discover a very, very effective clinical bronchodilator for treating asthma and COPD.

10:43 The other thing and this is again taken from one of David's early studies, he actually at the same time as doing these experiments in guinea pig trachea, also took pieces from the heart for example, to actually show that you have also got very selective effects in the airway but you do not have the same effect on receptors in the heart. The other major class of drugs that are used today not just for asthma and COPD but a range of inflammatory conditions of course are glucocorticosteroids, and animals have helped us really profoundly in getting to the drugs that we have got today that again we take for granted. So we have to go back to the 1940's when cortisone was extracted from the adrenal glands and used as an anti-inflammatory drug but of course cortisone whilst effective also supresses your hypothalamic pituitary axis and it leads to all sorts of changes in the endocrine system that are unwanted in people with inflammatory conditions. It was David's work that led to the discovery of beclomethasone diproprionate and please if you ever write beclomethasone down in any article make sure you put the diproprionate on it. David once said to me when I left it off, it wouldn't do the same thing without the diproprionate, it is there to give it the activity locally in the tissue. This is the first steroid that was developed for the treatment of asthma in 1972 where we could inhale the beclomethasone, have a very pronounce anti-inflammatory effect in the lung but it was poorly bio-available and therefore you did not have the same degree of suppression of the hypothalamic pituitary axis and this drug and subsequently the discovery of other topical steroids such as budesonide have really revolutionised the treatment of asthma and COPD because we can now give large doses locally to get a very clear anti-inflammatory effect without the systemic problems associated with oral or systemic steroids.

12:53. I just wanted you to go back to, this is a patent actually for fluticasone, another steroid but the same is true of beclomethasone, of how this drug was actually shown to be anti-inflammatory. It was nothing to do with molecular biology, it was nothing to do with cellular biology, it was a very simple test called the croton oil test, and you rub croton oil o the ear of an animal and you can compare the activity to supress inflammation locally in the ear whilst measuring levels in the blood and changes in the hypothalamic pituitary axis, it is as simple as that. This test has been used widely to discover nearly all the steroids that we currently have in clinical practice. This can be done in rats, it can be done in mice, and you can see here by rubbing the croton oil onto the ear we end up with a local inflammatory response and we can then add a topically active anti-inflammatory drug to see if we get suppression.

13:53. The reason that I show you this is and people forget this, is that the activity of all the currently topically active steroids actually were not developed for the lung, they were developed in the skin. They use something called the McKenzie Skin Blanching Test, that is, the light is probably not conducive to seeing it properly here but if you put steroids topically on the skin you get blanching and the blanching is a feature of a vasoconstriction caused by

the steroid reducing blood flow and why steroids actually have an acute anti-inflammatory effect. One of the reasons in my opinion we have not managed to find so-called 'soft' or safer steroids beyond the ones we have is because this is a non-genomic effect and we have spent our entire life trying to find effects of drugs that affect the genome around steroids and that has not proved very successful to date.

14:48. The reason I show you this is that if you go back to the potency of these corticosteroids in this rodent test in the air against the ability to be anti-inflammatory compared to their ability to supress the hypothalamic pituitary axis you can get a therapeutic index, and as was reviewed by Phillips and colleagues some years ago that therapeutic index, the potency of these steroids absolutely predicts their potency in their nose and also in the lung. So they were never ever developed just for the lung, they were developed topically for a whole range of things which of course is why we can use corticosteroids topically in other tissues. What happened in the rat and the mouse absolutely bore out what happened when we did this blanching test in human skin, it predicted, so a very simple assay in-vivo predicted the potency of these steroids in man. Now a third class of drug that has been introduced for the treatment of asthma was really something that I started my PHD on which was the so called leukotrienes receptor antagonists, they were proved in 1998, Montelukast is the first tablet that actually acts as an antagonist for leukotrienes. I'm often asked how we got there, and you have to go back to the 1940's from experiments taking venom, from snake venom, adding it to guinea pig lung, you can see on the left from the original paper in the 1940's, getting contraction of the tissue that was not due to histamine.

16:22. We are thinking now, in the mind-set of the 1940's, that was a very novel observation, the contraction was long lasting and it was actually the day that I got my BSE degree, the paper appeared in Nature from Priscila Piper and Howard Morris and I got asked about this at my viva which I though t was really unfriendly given that it came out in the morning, my viva was at 2pm in the afternoon but to identify SRS-A as leukotrienes C4 and D4 that being these very powerful lipids produce arachidonic acid that would distinct from the prostaglandin blocked by aspirin and then receptors appeared, we could find drugs that targeted this and montelukast being the first. Now I say this because this is a tablet, it is very widely used in certain parts of the world particularly the US because it is not a steroid and it is actually something that has generated commercially a lot money, four billion dollars a year for MERCK since it was actually approved. You might think it is all good, we have got some very effective drugs but actually respiratory this is a recent study from Tufts University with the FDA and actually respiratories have a lot of failures and the question is why have we had lots of failures and I may come back to that later if we have time but partly I think is that we have become too reliant on the mouse and it is a discussion we might have later on but clearly we still have quite a lot of failures in the respiratory area compared to other therapeutic areas.

17:59. What is absolutely clear, particularly for COPD is that we still need new drugs and particularly new anti-inflammatory drugs, we have very good bronchodilators and I say that because this is one of three very large studies in the literature showing that if you give inhaled steroids everyday over many, many years it has no impact in the majority of people in the decline of lung function. That means that whilst they are being used for the majority of people, it may not be best thing to do. We also now know that many of these patients have a bigger risk of pneumonia and in fact there is a very recent study in the *New England Journal of Medicine* that if you withdraw steroids the majority of the patients with COPD nothing happens. Now that means given that I have shown you it is an inflammatory condition there is a need for novel anti-inflammatory drugs in this disease, and so with David, I set about trying to say can we find new drugs that were alternative to steroids that actually could improve on some of the therapies that we have because each of them whilst they are effective does also have significant short comings.

19:09. And for that we turn to a group of enzymes called phosphodiesterase, a very complex set of enzymes that are in fact 11 families of phosphodiesterase enzymes that actually are involved in modulating second messengers in cells, the cyclic nucleotides, cyclic GMP and cyclic AMP. Now I don't need to tell most of you in this room that if we inhibit PD5, it is the basis for why we have the effect of sildenafil or viagra and many others that followed that actually bring about vasodilation and an erectile dysfunction. We have used inhibitors of PD3 such as milrinone for heart failure, for claudication. The question is, given we now know that PD4 is found in inflammatory cells and PD3 is found in airways smooth muscle, could we actually target this to produce new drugs for asthma and COPD.

20:03. I am pleased to say that one of these, roflumilast, has been approved for the treatment of COPD as a PD4 inhibitor and also another one called apremilast has recently been found for the treatment of psoriatic arthritis. What David and I set out to do, I'm pleased to say I think we have succeeded in doing this, is to really find a class of drug that is both bronchodilator and anti-inflammatory in a single molecule but is not a β-agonists and is not a steroid, and I want to acknowledge particularly Alec Oxford who is a Royal Society medallist in chemistry for his discovery and synthesis of sumatriptan but also David who I have mentioned and my own group, particularly Victoria Boswell-Smith who did the initial experiments and my late friend Don Spinner who died almost exactly a year ago today and I want to pay tribute to Don because without him most of the work that I am going to talk about now would not have happened.

21:04. So we set about trying to find something that has never been done before was to find a drug that was a bronchodilator and an anti-inflammatory in a single molecule, and to do this we found a drug that actually inhibited phosphodiesterase-3 in airways smooth muscle and it inhibited phosphodiesterase-4 in inflammatory cells, and at the same dose when we give this we can have both acute bronchodilation as you have seen with a β-agonist and a clear anti-inflammatory effect with the 4 inhibitor and I want to take you back to this very

familiar picture I showed you earlier because when we sat down, I said to David, what do we start? How do we start going about finding a drug, from a man who has done it multiple times? And there are very few people on the planet who have done that and he said that it is obvious, you take a piece of guinea pig trachea, you electrically stimulate it, and I will just show you here the same piece of guinea pig trachea in an organ bath is electrically stimulated every single second over a period of six hours and you can see that it is a beautifully robust preparation you are stimulating the parasympathetic nerves, releasing acetylcholine, contracting the tissue.

22:12 If we add the vehicle for the drug on this you can see that nothing happens, we made a 180 molecules with Alex Oxford and David and one of them just shown here as 554 forget anything else about it, you can see it almost immediately supresses the bronchoconstriction just as we have seen with salmeterol and over the next three to six hours you can see this suppression continued. I said to David what should we do next? He said put it into a dry powder, blow it into the lungs of guinea pigs, challenge them with something that constricts their airways and if it bronchodilates you will pick it up, and I want to just show you one of the first experiments we did where in the upper panel we are measuring increasing doses of histamine given to an anaesthetised guinea pig to measure airways resistance and you can see a beautiful increase in resistance.

23:07. If we give them lactose powder which is the carrier for the drug there is no suppression whatsoever over in this case three and a half hours, whereas in the lower panel we have added the 554 drug and you can see by powder inhaled by the guinea pig, we got complete suppression, and that lasted as it did in-vitro over many hours so we knew we had a drug, we were looking for something we could give that had a very long duration of action. You can see very good suppression and what was interesting is that we achieved that without changing blood pressure to any dramatic effect, so we knew we could get a local effect in the airway. Then the second bit is, was this drug that causes bronchodilation capable of anti-inflammatory activity and again just some summary of work that we did over a number of years, we can show that it supresses the eosinophils, the main inflammatory cell present in allergic disease, the allergen causes the eosinophils to come in the lung, that is supressed by the same dose of the drug that we get at inhibition of the bronchoconstriction, and you can see other markers of inflammation that are reduced.

24:16. If you compare that to now two existing anti-inflammatory drugs that are in the clinic, fluticasone propionate which is a very, very effective steroid, roflumilast I have already mentioned to you is very recently been approved for COPD, you can see that compared to this, this drug when inhaled at doses that bronchodilate also caused equivalent anti-inflammatory effect to the steroid or the PD4-inhibitor. So then I said about a long journey around the planet, finding some money and because to go from there through toxicology to put this into people when you are not black, so you are not Astra (AstraZeneca) takes a lot of effort. I have to say it has been a real journey that I have

enjoyed doing but it has taken a lot of time and effort but the bottom line is we got through toxicology we used dogs, we used rats for 28 days, all of the things that any drug has to go through and I think it is why I was happy to talk to *The Sun* journalist not so long ago about the use of dogs because we just finished the study with dogs that allowed us to pick the dose that we then put into human beings for the first time. I show you this because the very first patient that inhaled this drug just absolutely told us they felt better, and you can see here that the extent of bronchodilation is profound. It is a very marked changed in FEV1 compare to placebo. We did that in a group of patients with asthma and we were very good, the MHRA allowed us to go immediately to asthmatics because they said no healthy person is going to help tell us whether or not this drug effective.

25:58. So, when we then went on, we published this work in the end of 2013, a single inhalation in patients with COPD, we got actually very good bronchodilation that lasted over many hours and more recently the company that has taken this forward have got a new formulation that this now I think very clearly a twice a day drug which is exactly what we wanted to try and do but at the same dose that cause the bronchodilation in people, working with professor Dave Singh's group in the University of Manchester, we actually did something fairly heroic that other people have done taking lipopolysaccharide, getting animals to inhale it, we knew that it was blocking neutrophil infiltration in the animal, we then did exactly the same experiment in people.

26:44. So in this study in Manchester, they inhaled lipopolysaccharide, you then ask the patient to cough up sputum and you can see at different time points afterwards the sputum is full of neutrophils, and if we give this drug at the same dose as bronchodilation you can see the very clear statistically significant inhibition of the infiltration of cells in the airway of people, now the interesting thing of why people use this model is it is not sensitive to prednisone and steroids, and so if we are thinking about a disease like COPD where steroids for many people are not effective, clearly a model that is insensitive to steroids but works we know to drugs that block phosphodiesterase we thought was a useful way of checking whether this was truly anti-inflammatory in people.

27:30. Now one of the exciting things and we had first of all done this in guinea pig trachea, we then were very fortunate to get access to human bronchial smooth muscle. I want you just to look at the left here is what happened if we take a submaximal dose of this drug, we cause it to relax airways smooth muscle, we take a muscarinic receptor antagonist like a glycopyrrolate against submaximal, the third column is what you would expect to get and if you put the two drugs together and actually what happens, you get profound, really pronounced synergy in terms of greater bronchodilation. Now this is important because it means we can lower the doses of existing drugs whilst not compromising on efficacy.

28:14. We now know from recent work again done by Professor Singh's group in Manchester that if we take a low dose of this drug and we take a low dose of a muscarinic receptor antagonist that is widely used in the treatment of COPD namely tiotropium, you

can see here that if you add the drug you get a much faster onset on bronchodilation, so instead of having to wait 40 minutes before you see a change in lung function, you are seeing it in four to five. So this is a really clear indication that there is increase benefit of having this drug to anticholinergic. It's not just on smooth muscle, people with these disease have hyperinflation and gas trapping, you can see here again combining this drug with 554 in people also reduces gas trapping.

29:04. So everything that we have done in the guinea pig and I say here and now, we never did an experiment in the mouse, it was all done in the guinea pig because it was David that was mentoring me to do this, has ended up with a drug as you can see here that has clinical benefit. I am pleased to say that this is now currently in phase2b in the United States and has been in more than 600 people now. There is no nausea, we have got no major cardiovascular effects but it is clearly a novel class of bronchodilator that also has anti-inflammatory properties. My good friend, Wisia Wedzicha, who works at Imperial in London and I know nothing about this when it came out, we talk about media but *The Lancet Respiratory Medicine* wanted to make a lot of noise about this study because it was so new, and I think what Wisia has said in this editorial is that this actually could turn out to be one of the most substantial advances in the management of patients with chronic airway obstruction because there is very little coming along that is novel but again none of this would have happened without guinea pigs, none of it would have happened without the dog.

30:14. So, the question is do we need further anti-inflammatory drugs? We have now got a very good understanding of how cells recruit into tissues and we clearly know that we have got lots of drugs that have been through this kind of cascade and not done very well, and one of the things that have been done for many years is to try and measure inflammatory cells into the airway as I have shown you both in animals and in people we could wash the lungs out we can collect sputum, we can quantify inflammatory cells but we have all been taught from school about keeping chemotraction and a single mediator causing particular cell to migrate into a tissue and I think our concepts of how inflammatory cells come into tissue is very simple.

31:00. With my colleagues at Kings, particularly Simon Pitchford who is now back at Kings as a lecturer but when he was a PhD student with me actually made some interesting observations that many of the leukocytes that migrate out into the tissue such as the lung often have inflammatory cells attached to them and the platelet which is a cell that we are considering as something that plugs up holes, and Jeremy Pearson knows very well, he spent his career looking at platelets, this is something that has nothing to do with haemostasis, these are a platelet leukocyte interactions rolling on the endothelium prior to these cells coming out into the tissue such as the lung.

31:40. Why is this important, well we did a very simple experiment one day, we took neutrophils up there, PMNs, we incubate them with endothelium and you can see that if nothing happens a few of them sticks to the surface of the blood vessel. If you put platelets back in as you find them in the circulation you get lots of leukocytes attaching but more importantly if we then took animals, we have done this now in rabbits and we have done it in guinea pigs, we have done it in mice. If you actually remove all their circulating platelets by destroying them so these are animals without most of their circulating platelets and we then look at the ability of lipopolysaccharide on the left which I have already shown you, causes neutrophils to come into the lung, in the animals without platelets they do not show up.

32:27 So, our concept of a chemical causing one cell type to migrate, in-vivo is far more complex and it is very clear both in the lungs and other anatomical areas that the platelets are necessary to optimally recruit leukocytes into the tissue. This is not something I can do in people. Furthermore, others have come up with some really interesting examples of microscopy where you can label these very different cell types, the endothelium here is labelled green, you can see that the neutrophils are in blue, the platelets are in red and these are actually cooperating and we can look at that over time and see how these cells are actually migrating into tissues and if the video works which it did earlier, I just wanted to show you an example which is not my work but is a paper I would have love to have been the author of because this shows you, using intravital microscopy by looking at the cremaster muscle, and you can see here in green the leukocytes and the platelets are actually sitting at either end of them, they are polarised and you can see them there prior to them sticking to the blood vessel and getting into the tissue and that's partly why when we can take these platelets away, the leukocytes don't show up, they need platelets to cooperate in terms of moving them into the lung.

33:52. Another very, very interesting, and Jeremy knows from my time early on in my career that I have been interested in platelets in the context of lung disease and this is a beautiful study, my PhD student Simon Cleary who generated this data at UCSF in San Francisco with Mark Luny's group, this is based on a previous work from Luny's study recently that shows that actually megakaryocytes that go into the lung, they are breaking up and you are getting platelet formulation and you can actually watch this happen. We have got adhesive platelets in blue, non-adhesive platelets in green and then we can actually study those that are activated or not in real time in the lungs on animal. Again if we have got any volunteers we want to have some kind of thoracic window put in to allow us to do microscopy on their lung I'm happy to talk to you but this is a good example of why we have to continue to do in-vivo work to understand more about how leukocytes behave in the lung and platelets are necessary for that.

35:01. The other area and again another one of my PhD students Blaze O'Shaughnessy has very recently done similar experiments with something that we have often neglected in this area is that there are key pathogens in respiratory diseases, they lead to exacerbations, they can lead to pneumonia and again we have very little understanding, we have all heard about leukocytes phagocytosis bacteria but less is understood about how those leukocytes appear and from our work showing that platelets are necessary for leukocytes to come into tissue and inflammatory diseases the obvious thing was, were platelets is necessary for host defence.

35:38. Just really wanted to show you a few slides here to say that we think they are. So on the left here is a strain of pseudomonas put into the lung attached to beads into the lungs of animals, you can see a profound inflammatory response associated with neutrophils coming into the lung compared to sham animals or the negatively untreated controls. What we then also saw is that we could find platelets coming in at the same time as leukocytes in response to this infection, and very important when we took the platelets away not only did the bacterial load go up but the bacterial load, this is a mild form of infection that is restricted to the lung, suddenly you got lots of bugs going into the blood and you get death from septicaemia.

36:28. Now we have spent many, many years trying to stop leukocytes coming into the lung to treat acute lung injury. What this tells you is if you get rid of the leukocytes by removing the platelets, we have a self-contained infection in the lung that actually goes systemic and increases your risk of death and I think that this is a paper – we have just had it accepted in an American journal, I think it is a really profound observation that says that platelets are absolutely necessary for host offence in the lung and that is not something that has been generally accepted up until now.

37:04. Finally, we need new drugs- where are we going to find them from? I had the pleasure of giving Rachel a prize earlier on for her work with primates and Fergus Walsh but one of the interesting projects that she knows that I have been involved with over the years started life here working with some marine biologists up in Oban in Scotland, this is their lab view and they are marine biologists who are interested in why these guys and things smaller than this and oil rigs – things stick to them we call it biofouling, and they ask a very simple question, what is it that allows things to stick to these artificial structures we put in marine environments whereas the things that live there nothing sticks to them. This has been a very interesting story, it's me slightly younger with Rebecca Leaver, some of you will know Rebecca she is at UCL in London and we took the NERC sea vessel out into the Minch and started dredging for these guys and these guys are echinoderms, all sorts of different echinoderms, and one of the things about these echinoderms is nothing sticks to their surface, and the question is why because they are reasonable sedentary compared to fish or other things that are in the marine environment.

38:25. To cut a very, very, very long story short, we published actually last year the identification of polysaccharides from the surface of these marine organisms that are anti-inflammatory in-vivo in experimental animals and the thing is they look a bit like heparin but they are not anticoagulant because they are far enough back in evolution where we don't have a pressurised cardiovascular system, so we don't probably need anticoagulants, and so these molecules are very interesting because they are completely novel structures that are found on the surface and it looks very similar to heparin sulphate that we find on the surface of our own vascular endothelium which I like to think is a bit like Teflon and anything we do to disturb that Teflon surface, it's a bit like me trying to poach an egg on the saucepan that has damage surface you get it sticking, the same is true here if you think about metastasis of tumours, thrombosis, inflammatory responses, it is all happening at the level of the endothelium, so if we can find things in nature that are actually able to restore the negativity of the endothelium we may have a whole new class of drug, and to that end, working with my good friend Charlie Bavington in Scotland, John Hogwood and Barbara Mulloy who are the National Institute of Biological Standards and Control which now part of the MHRA, and others working in Edinburgh – David, you will be pleased to know, we have identified a whole series of new chemicals from these sugar-like structures on the body wall in this case of a cucumber that actually have very selective binding for some of the adhesion molecules that are involved in leukocytes recruited into tissue.

40:10. There is a whole family of anti-inflammatory drugs that I'm convinced are out there because we already know that heparin has a very good anti-inflammatory effect it's just that it suffers from being an anticoagulant. This is just an example from our recent biological chemistry paper of the ability of some of these materials that we have identified for being anti-inflammatory. You might sit here and think well we have not really made any progress, I just wanted to take you back to 1684 if you had asthma, a physician from Oxford defined asthma as a difficult frequent breathing with a great shaking of the breast without any fever, the organs are breathing which are the pillars of life are shaken by this disease as if by an earthquake, the treatment then before David and others came along, was sleeping on a chair powdered millipedes, and volatile salts, and I think we have made some progress even as I said to you, not always.

41:06. I recently had the great privilege of going to Honiara in the Solomon Islands and I took this because this is actually in the last three of four years, their approach to respiratory diseases is putting a big sign up in the market and it says "stop spitting about places, always cover your mouth and nose when sneezing and keep children safe at home" which is another approach, and then if we go back not very far we had this, Potter's asthma smoking mixture, which this is an early form of what David Jack I think has improved on greatly is inhalation of drugs that actually lead to improvement of symptoms. So there is no question that we have made a lot of progress but as I have said I think there is still more to do. I wanted to leave you ladies and gentlemen with a book that one of my postdocs found some years ago in Bath and some of you have heard me speak before have probably seen this, it is

such a beautiful example of how we have moved forward. This was A.J.D Cameron, for some of you who don't know him, he was a general practitioner in Tunbridge Wells and this was a book about the treatment of asthma and this is an absolute quote from the book "*I was amazed to find cutaneous reactions could change or disappear entirely by detoxification. From that I was bound to conclude that whatever part allergy played, it was certainly not a fundamental one…*". That is very interesting because we are beginning to realise than allergy and asthma often coexist but one does not necessarily lead to the other.

42.38. *"…A secondary condition of affairs in the syndrome, a condition which departed with adequate detoxification*". So what is active detoxification if you are patient in Tunbridge Wells in 193, it included colon irrigation and the method of irrigation which I will go with you because you are experimentalists most of you and you will appreciate this – it is *"...a stand with a two gallon container which can be raised or lowered is used. The container is connected by a rubber tube to one of the arms of a Y-shaped glass, to the stem of which an ordinary soft oesophageal tube in which an extra aperture is cut is passed into the rectum…*". So far so good, *"…the water at body temperature is run into the bowels, stopped before discomfort is caused, and then allowed to drain off. This procedure is repeated again and again until the prescribed quantity, anything from two to eight gallons…*" I have never worked out whether it is Imperial or the US *"… has been passed through…*".

43:38. "*…the pressure employed is determined by the height of the container above the couch and I find as a rule a height of 12 inches is most satisfactory…",* listen to this bit carefully,*"… it is dangerous to use too much pressure, the optimum for each patient is learnt by experience…*". Now ladies and gentlemen we may sometimes think, are we doing the right thing with our experiments, I think that I would not want to be a volunteer in this particular experiment but it is an absolute, this is a real quote from what was done in Tunbridge Wells in 1933. Now I presented this at the Edinburgh science festival some years ago and a lady came running up to me and said I hope you are not really laughing at colon irrigation I get it every week and my asthma improves. I believe actually IBD is basically asthma with a different tube and a different smooth muscle, maybe there is something in here but again I think that you would find that most people would consider the treatment we have got now, however it could be improved, much improved over this or inhaling millipedes.

44:47. This is the final bit, I will leave you that before you go to your canapes, so finally ladies and gentlemen, thank you for your attention, I would like to thank my current group, many of which have contributed to the work, I certainly want to thank my colleague Michael Walker and Lui Franciosi in Vancouver who did a lot of the work, the early work on RPL554, David without whom I think we would not have most of the drugs we have currently got and I think as I have said previously, previous chairman of RDS, this man has had a profound influence on this area and he has certainly had a profound influence on me. Charlie

Bavington in Scotland, Rebecca Lever in UCL who is a former PhD student of mine and also I would like to acknowledge Mario Cazzola and Luigi Calzetta who have done a lot of the human airway work and human studies in Tor Vergata University in Rome and very much I would like to stand here and acknowledge Dom, at the back there with the glasses because Dom has been an absolutely profound influence on my career, and helping as I say, tomorrow is the anniversary of him dying prematurely and he is sorely missed by all of us who knew him. I will leave you with this because I think without the dog, without the guinea pig we would not have any of the drugs that certainly I have talked about this evening and I think we will continue to need animal experimentation to help us understand some of the complex things such as beta leukocytes interactions as I have shown you this evening and to help us find new classes of drug going forward because we still need them. I think anybody who tell you otherwise, as I once famously said on the platform like this to Caroline Flint when she told me that I should start working in sub-culture, I said you find me the cell that coughs and I will use it and with that thank you very much for your attention.