TODAY'S NEUROSCIENCE, TOMORROW'S HISTORY

A Video Archive Project

Professor Salvador Moncada

Interviewed by Richard Thomas

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Interview transcript

Growing up in Central America

I grew up really in El Salvador. I went to primary, secondary schooling in El Salvador, and later I went to study medicine at the University of El Salvador. So I grew up in Central America, specifically in El Salvador, although I was born in Honduras.

Well, my household was unusual for Central America - for the Central American cultural environment. It was a mixture of the European part of the family, my mother and her parents, and on the other side a very Central American part, which is my father's side of the family. So I grew up in a home which, in countries like Argentina or Chile, would be usual because there was a lot of immigration of Europeans, but in Central America was a very strange background. Well, my mother and her parents left Europe in, I think, 1938, just before the annexation of Austria to Germany. They managed to get out just in time. And they went to Honduras because it was one of the easiest countries to emigrate to. They left it late, there was no time to get visas to go into anywhere else, and they ended up in a country that my grandfather had never heard of, really. So it's an unusual situation - for them, was completely unusual. He told me once that he didn't know really where he was exactly going, and he bought himself a safari suit. So, he arrived to Honduras dressed up in a safari suit in 1937, '38. My father was a very strong influence in terms of leading me towards a thinking career, if you want; an academic career. He was very strong in that type of push. And somehow my Jewish European part of the family was much more relaxed about it than my father was.

Medical school and expulsion from El Salvador

As I progressed in the medical school, the first few years I was definitely interested in fundamental research and I took every opportunity to work in the laboratory. There was an

opportunity for the students of first, second and third year medical school to stay during the holidays and work on physiology or pharmacology, and I did every year that. But, as time progressed I became much more interested in social issues and political issues, and I became much more of a student activist in politics as I was finishing my medical career.

I was extremely lucky, because when I was expelled from El Salvador I couldn't come back to El Salvador to see my family at the time. So we would meet every month in Guatemala, which is the country between the two of us, and we wanted, of course, to be together. I had a small daughter and my wife, and this professor, Fernando Molina, of Guatemala, had been for a sabbatical in El Salvador when I was a student, so I knew him, and I decided to go and visit him. And I said, 'Look, I want to do physiology or pharmacology. I am much more interested in going to Europe than going to the United States. Do you see any possibility?' And he said to me, 'Well, seven or eight years ago I was in London, working at the Royal College of Surgeons, and I became good friends with a man called John Vane, who I haven't written to for the last six or seven years but he was very friendly. Why don't we write to him and see if he can suggest a place where to go?' We wrote, sent the letter; I went back to Honduras, my wife went back to El Salvador, and he said, 'Let's see when he replies.' Almost by return post John Vane wrote back and said, basically,'I don't know who you are. I don't know anything about your background, but if my good friend Fernando Molina is saying that you can come here, I will take you.' That was it.

Starting work in John Vane's laboratory, 1971

The laboratory of John Vane had done several things, and I arrived at the Royal College of Surgeons in February 1971, when some of the results had already started to appear. There were actually two groups in the same department, working on the problem, and they were independent, all right? John started to work on isolated … on lung homogenates, looking at aspirin inhibition of prostaglandins in the lung homogenates. But at the same time Brian Smith and Jim Willis were taking, themselves, aspirin, and taking blood samples and isolating the platelets and showing that their own platelets had the prostaglandins inhibited by injection. And the third paper is the one in which I was put on as a project by John. We collaborated, Ferreira, John and I, on the dog spleen - the release of prostaglandins from the dog spleen. Those three papers were the ones that were published together in 1971, side by side. It was a fantastic, beautiful work by Brian Smith and Jim Willis. They never got the recognition for that, although the papers were published - or the same level of recognition, let's say.

Prostaglandins and Aspirin – the importance of bioassay

Well, bioassay has the great advantage that it measures biological activity, and that sounds a bit tautological but that's the great thing about bioassay, and for a hundred years before that, people were cutting pieces of tissue, putting them in an organ bath and connecting them to a transducer and measuring contraction and relaxation as they injected things. Gaddum had the brilliant idea of not immersing the tissue in physiological solution but dripping it, dripping it, so it doesn't dilute the substance very much, and what John Vane added to that was the idea of putting several tissues in a cascade. So, if you put one tissue in a cascade and you drip a solution, you can use that solution to drip on a second tissue, on a third tissue and so on, and what that adds to the assay is the possibility of profiling. If you have three tissues, which respond in slightly different ways to different substances, you won't have just a measurement of a substance, you will have a profile of activity on a bank of tissues.

So - and I always thought that the amazing thing about that, is that when I arrived to the lab they had these maps of profiles of active substances on banks of tissue. So, if you wanted to measure bradykinin, you put these tissues, and if you wanted to measure angiotensin 2, you put the other tissues, and so on. What that gives you, if you are doing it well, is that if you ever come across a profile that you don't know what it is, you might have discovered something. You better stop and think again. And that's exactly what happened. We knew the profiles, we knew how each substance behaved on the different tissues, so when we came across profiles that we couldn't recognise, we were aware of the fact that we might have discovered something new. So that's the great thing about the bioassay.

Well, I started to discover the immense power of the technique when we were working with prostaglandins - the staple classic of prostaglandins - and I managed to demonstrate that tissues were able to differentiate between prostaglandin E2 and prostaglandin E1. The difference between these two substances is just one double bond, and the tissues, the profile, was able to tell you if you were working with either one or the other, which means that you have the ability to differentiate, very clearly, what you are looking for, and also what you are not expecting.

When we started there was very little known about the pathway of arachidonic acid metabolism. It was known that arachidonic acid was the precursor. It was known that prostaglandin E2 and F2 Alpha were formed; it was not very clear what were the enzymatic steps, and what we found with John was that aspirin is there. And that leads to inhibition of the formation of prostaglandins, which are present in tissues, and so on and so forth. The big question at the time was, 'So how does aspirin produce bleeding?' Everybody knew that if

you take an aspirin you bleed more, and there was no logical explanation for that. So Bengt Samuelsson at the Karolinska, and a man which I have just mentioned, Jim Willis, at the Royal College of Surgeons, started to look for intermediates in the pathway to the formation of the stable.

Group leader at Wellcome Research Laboratory

John Vane had decided to go to the Wellcome Research Laboratory. I went to Honduras and I kept in touch with John, and he said to me when Sergio left, 'Look, I need a group leader here. I will keep this position open for a year for you to come back. After a year, if you don't, then I will have to fill it up with somebody else.' So exactly eleven months and twenty days after, I phoned him back and I said, 'I'm coming back.' So I came back, and on the way back I attended the International Conference on Prostaglandins, where I heard Bengt Samuelsson presenting the results, and the first structure of thromboxane A2.

Blood platelet function - why aspirin causes bleeding

We're looking here at a blood vessel which has different types of cells including these small particles here, which are the platelets. The main function of the platelets are to produce what is called a haemostatic plug, which is a plug which is formed in an area where there is bleeding. We show here, for example, how it happens if there is a cut in the vessel. The platelets emit these protrusions, become sticky, and begin clumping together towards the area where the blood is coming out. And that produces this structure here, which is called a haemostatic plug. If thromboxane A2 is generated in enough quantities it will produce constriction of the vessel.

That discovery of thromboxane A2, which is made by the platelets as they are activated, explained for the first time why, if you take an aspirin, you bleed more, because the platelets don't clump so much because they don't have thromboxane. So, many tissues in the body make prostaglandin E2, prostaglandin A2 alpha, but the platelet made from arachidonic acid - thromboxane A2 - which helps them to clump. You take an aspirin, you block your platelets, you bleed more, and that was an important discovery because it explained not only why you bleed more, but it explains an interesting fact that aspirin, in 1954, had already been suggested that, because of that effect, it could be used to protect people against cardiovascular disease. Because what you get when you get a heart attack is a very similar mechanism than the one I just explained to you for stopping bleeding. Your platelets start clumping on a damaged surface of the vessel until they clog up to a point where they close the circulation.

Discovering thromboxane synthase, and a new enzyme

By the time I came back, Phil Needleman from the United States was spending a sabbatical and was starting to do experiments on platelets already. So we teamed up to look for the enzyme that converted the prostaglandin and endoperoxide - those substances I told you we injected in ourselves - into thromboxane A2, and we discovered thromboxane synthase.

I said, 'It could be that the vessels have thromboxane synthase also, because this immediate spasm might be that the vessels and the platelets are synergising to favour the formation of the plug.' So I discussed that with the people in the lab and I said, 'If we are going to map the thromboxane synthase, I would like to look specifically in the vessel wall.' And everybody agreed that we would directly go there, but that we would, in the process, do a mapping of tissues. and we did that when Richard Gryglewski was coming - after he came for a sabbatical. He used to come for short sabbaticals. He would bring a project, we would discuss it, and then we would agree which project. Everybody agreed that what I was suggesting was a great project and we should go for the vessel wall.

So we ended up buying hundreds of pig aortas and smashing them up to try to isolate the enzyme to look for the formation of thromboxane. I remember Richard doing the first experiment, coming back to me and saying, 'I cannot see anything. This is not worth doing because thromboxane is not made.' I said to him, 'Do it at least once more.' 'Yes,' he said, 'I'll do it once more but that will be the end.' I said, 'All right.' Next day or three days later, when he did it, he came back to me again and said, 'It doesn't work. Let's change project.' And we said, 'Okay, we'll change project.' And then came Stuart, who had been doing the experiment with him, and said, 'It's funny, I don't see thromboxane, but look, this tracing is … somehow, the endoperoxide activity is finishing. It's disappearing.' And I looked at it and was impressed. I said, 'It's completely gone.' I said, 'Why don't you boil the enzyme and see what happens?' I had to leave for a meeting, and the next morning when I came he said, 'Look, I boiled the enzyme and the endoperoxide is not disappearing so there must be an enzyme there.' So then we found that it was not an enzyme that was metabolizing anything further, so it became inactive. There was an enzyme that was converting the endoperoxide into something that we could not detect by our vascular tissues.

The Prostacyclin Discovery

We tried everything, and really we were close to desperation. We knew that the substance might have some vasodilator activity because sometimes when we put whatever was made, that we didn't know what it was, it relaxed the vascular tissue. So we were convinced that whatever it was, was a new substance, which we started calling PGX, and again, we worked

for several weeks without knowing what it could be. And one day, on a coffee break, with Stuart and with Richard Gryglewski, I said, ‘Look, we were looking for thromboxane E2, which is a vasoconstrictor and an aggregator of platelets. We have found a vasodilator. To complete the equation, this substance must inhibit platelet aggregation, so it’s exactly the biological…’ ‘Too much imagination!’ said Richard Gryglewski.

So that afternoon, Bunting and I did the experiment and we – it was a wow experiment, no doubt whatever – because the moment we tested it on platelets, platelets were completely obliterated in aggregate. We started to dilute, dilute, dilute, dilute, dilute, and we knew we had found probably the most potent inhibitor of platelet aggregation that was known - that very same afternoon. So the next day we, I went to John Vane and I said, ‘I think we have discovered something new.’ And he laughed and said, ‘You have to show me that.’ And for several weeks actually, I showed him the data, and he used to joke with me and said, ‘Have we still discovered something new?’ And I said, ‘Yes, we have.’ And that’s the story about prostacyclin.

Homeostatic functioning of the blood vessel wall

Arachidonic acid is converted in the platelets into thromboxane A2, a powerful vasoconstrictor and pro-aggregating agent, while in the vessel wall arachidonic acid is converted into prostacyclin, which is a powerful vasodilator and an anti-aggregating substance. Now, the conversion in the platelets and in the vessel wall takes place via the action of an enzyme called cycloxygenase. These findings led us to suggest that a balance between the generation and actions of thromboxin A2 and prostacyclin was important for the homeostatic functioning of the vessel wall, and in addition, that changes in this balance towards an increase in thromboxin A2 might be deleterious, while changes in the balance towards prostacylcin might be protective for the cardiovascular system.

You are talking about two enzymes, not one. You have one complete pathway in the vessel wall, from arachidonic acid to prostacyclin; and you have a whole pathway in the platelets, from arachidonic acid to thromboxane. So, you have two cyclooxygenases, and it’s the cyclooxygenase in the *platelet* which is so sensitive to aspirin. So you are not inhibiting one enzyme, you are inhibiting two, and one is more sensitive than the other. A very small dose of aspirin will be enough to block the platelets for a long time without touching the vessel wall, all right? And that was why smaller and smaller doses of aspirin started to be used, and we ended up with even 100 milligrams of aspirin, the baby aspirin, which is taken by many people, even without problems, to prevent cardiovascular disease.

Structure of prostacyclin, 1976: Nobel Prize, 1982

I organised for Stuart Bunting and a man called John Salmon, who was a chemist in my group, to go to the Upjohn Company to try to work with the Upjohn chemists to do the structure of prostacyclin, and I think it took them four or five weeks. My chaps were doing the biological experiment. They were taking the extracts and they had in the kitty some analogues of prostaglandin they had made, which were very useful to make this compound. And within six weeks, we got the structure of the compound. That must have been towards the end of August of 1976. And having the structure - we announced the structure with John Vane - we went to a meeting in Santa Monica in December that year, and we gave back-to-back lectures, John and I, announcing the structure of prostacyclin - 3 December 1976.

In 1982, the Nobel Prize of Medicine was given to Sune Bergström, Bengt Samuelsson, and to John Vane. Sune Bergström was well known because he did the first crystal structures of prostaglandin E2 and prostaglandin F2 alpha. He was working with Von Euler at the Karolinska, the man who discovered, or was co-discoverer of prostaglandins, and he did that. Bengt Samuelsson did, as I have described to you, the work on thromboxane, and later the work on leukotrienes, and John Vane was given the Nobel Prize for the discovery of prostacyclin, and the finding of the mechanism of action of aspirin-like drugs.

We knew that prostacyclin itself would have great difficulties in being a drug. Prostacyclin is unstable. It was a terrific difficult job to make it into vials to be used, and it's very unstable. Once you make a solution it starts decomposing. It's still being used also occasionally on cardiopulmonary bypass or even in organ transplantation but because of the difficulties of making it, because of the instability, because of the difficult formulation, it really never took off.

Discovery of EDRF (Endothelium Derived Relaxing Factor), 1980

Bob Furchgott published the famous paper in 1980. That is a fantastic example of a serendipitous discovery that could only be made by Bob Furchgott, because of his background. Bob had been working with strips of artery for a long time, and he has told his story many times, and sometimes, when you prepare a strip or a ring, and you contract it, and then you give acetylcholine, it doesn't relax as expected. And for fifteen years or even more, he knew that about 30-35 per cent of the tissues never relaxed to acetylcholine, and jokingly he said - I heard him saying - that he had come to the conclusion that there are good technicians, the ones that prepare tissues that relax to acetylcholine after a contraction, and bad technicians, the ones that don't. It turned out that the assumption was correct, because a bad technician, when he's preparing the tissue, takes the endothelium off, which is a very

thin layer, alright? It clicked onto him after many years – it took a long time – because he knew, he knew, he knew for about fifteen years that that was the case. Suddenly he said, 'Well, it could be true!' The bad tissues are the ones that don't have endothelium. So he took the endothelium on purpose and demonstrated that tissues with endothelium relaxed to acetylcholine and tissues without endothelium, don't. So that type of discovery was unique to him because of his background and because of the things he knew.

Discovering that EDRF is nitric oxide (NO), 1986

There was a conference in Rochester in Minnesota about endothelium derived relaxing factor, and other vasoactive substances to which I attended, and also Robert Furchgott was there. In that meeting, Bob speculated, because he had a CD5 sodium nitrite and produced a very sharp relaxation instead of the slow, long active relaxation of nitrite, that that relaxation was very similar to the one that EDRF produced. 'And it would be strange,' he said, 'but it could be that EDRF is NO, or something like it.' I was sitting next to John Pike, and John Pike, being a chemist – when Bob Furchgott said, 'No,' he (John Pike) said, 'Well, there are not too many analogues you can make of this molecule,' and closed the book. He was making notes. Different from that, I thought, if this is true, it's just unbelievable. So, when the conference finished at midday, I went out and I phoned Richard Palmer who was working in the lab with Gryglewski - no Gryglewski had left already. It was Richard Palmer, and I said to Richard, 'I just heard something unbelievable. If EDRF is NO, this is going to be a massive discovery. Can you buy a bottle of Nitric Oxide from British Oxygen Corporation and try to make solutions, and see if they produce relaxations similar to the EDRF we're producing from the cells.' Two days later, we knew that they were the same from a pharmacological point of view. Nitric oxide from the bottle, and EDRF from the cells, behave pharmacologically exactly the same.

Measuring nitric oxide produced by endothelial cells

Pharmacological identity is not good enough. If you have a new substance you better identify it properly. The reason why I wanted to measure nitric oxide directly [was] because it was so unusual, and so unbelievable at this stage that nitric oxide might be made by tissues. So I discussed with Richard Palmer - at that time we worked very closely together with Richard - and we decided that we should look for methods to measure nitric oxide directly, as a gas. There were hundreds of methods to measure nitrite or nitrate and convert it to nitric oxide, all sorts of possibilities, and he came up with this machine that was being used in the car industry, which is a machine that is based on the reaction that nitric oxide has with ozone, and you can measure by chemiluminescence the signal that develops.

But we could see the most amazing little bleeps there every time he stimulated the cells, or every time he injected the amounts of nitric oxide that we were supposed to be using. Obviously, that was the case because the amounts of nitric oxide which are measured in the car industry or the food industry are hundreds of times higher. So, with those little bleeps we said, 'We have to make a machine ourselves.' And, fortunately, we were in the drug industry and we had a very good department of electronics and they made machines, and we went to the engineers and said, ;This is what we want to do.' And they said, 'No problem. We will make your machine.' I said, 'Fifteen hundred times more sensitive.' 'We'll do it.' And they made a machine that was two thousand times more sensitive than the one we had used in Surrey. And, indeed, we connected the cells as I am describing, and there it was. You could measure nitric oxide coming from the endothelial cells, and the quantities were enough to explain endothelial independent relaxation. So we published in *Nature* in 1987 - myself, Richard Palmer and Tony Ferridge, who was the man who was mainly responsible for making the machine with which we measured nitric oxide.

Determining how nitric oxide is made

As soon as you find a new substance, you want to know how it's made. What is the biosynthetic process. So, we immediately went on to trying to elucidate the pathway. We had already seen a paper by John Hibbs showing that the nitrite and nitrate in macrophages might be coming from arginine. In fact, that was a good demonstration, and we said, 'This must be an intermediate in the formation of nitrite and nitrate, but we cannot make arginine work.' So I said to Richard, 'How much arginine the cell culture fluid has?' And he told me it was an amazing, massive amount. And I said, 'Why don't we try to make a culture without arginine and then we give arginine and see what happens.' So, we cultured the cells in the normal way, and then twenty-four or forty-eight hours before doing the experiment, we changed the culture medium for a medium without arginine, and then we prepared our cells, put them in a cascade and sure enough, when we gave arginine, EDRF came pouring out - nitric oxide. So we, a year later, in 1988 publish the finding, and then we did mass spectrometry experiments and so on, and publish in 1988 the fact that nitric oxide *was* a product of arginine metabolism, and that was specifically made from the guanidine nitrogen atoms of the molecule, because we had identified that by mass spectrometry. And that's the 1988 paper.

This is the metabolic pathway that leads to the synthesis of nitric oxide. Nitric oxide, we found, is specifically generated from the guanidino nitrogen atoms of L-arginine, which are shown here, through a series of transformations which involve the formation of these unstable intermediate N-hydroxy L-arginine. We found that the enzyme that we later called

nitric oxide synthase incorporates molecular oxygen into the formation of this intermediate, and later on into the formation of L-citrulline which is the co product of the reaction that generates nitric oxide. During *those* studies, we also identified the first inhibitor of this pathway, LNMMA, which is a monomethylated version of L-arginine and that blocks the pathway competitively with L-arginine at two specific states; one previous to the formation of N hydroxy L-arginine, and later on following the formation of this compound.

The biochemical pathway of nitric oxide in the brain

After we found that arginine was the precursor of the synthesis of nitric oxide, it became evident that what we had found was a whole biochemical pathway. And very early on, and that must have been in early 1988, I came to the conclusion that in order to have an idea where that pathway *might* be, we should look at all the literature related to actions of arginine in the body and, also, on actions of the soluble guaryl cyclate which is the end point. And Richard Palmer and I went through the literature of *everything* that had been published on the two subjects and we found a very intriguing piece of information, and that was that in the 1980s, a Japanese group had been working on arginine and showing that arginine activated the guaryl cyclate in the brain. And we came to the conclusion that the pathway must be present in the brain.

Many months after initiating the discussions, the paper of John Garthwaite appeared showing that an EDRF-like substance was present in the brain, something that we had already suspected for a long time, but could not convince people to do the work. As a result of all this work, three enzymes were identified. One in the vasculature, one in the macrophages, and one in the nervous system, which was first found in the brain and later in peripheral nerves which are now known as nitergic nerves. These three enzymes, although encoded by different genes, have basically the same structure with an oxygenase domain containing the end terminus and and a reductase domain which has a number of binding sites for co-factors, and basically is similar to the cytochrome B450 reductase.

Nobel Prize, 1998

It’s not for me to say, and I have always insisted that it’s not for me to say. The Nobel Committee can give the prize to whoever they want. And the work is there. The work can be analysed and the relative contribution of the players can be analysed, and I think that, historically, that will remain as, what was done, really. So, the decision of the Nobel Committee is something that obviously is important - obviously it would have been important for me - but I think the work is far more important than … How the field was made, and how the breakthroughs actually happened, is what matters.

Nitric oxide as regulator of blood pressure and blood flow

I think that the showing of the release of nitric oxide and the demonstration that EDRF was NO, was a very important finding. However, the most important findings were facilitated by the fact that we used LNMMA, the inhibitor of the synthesis of nitric oxide. Let me remind you that that compound had already been used by the macrophage people when they were working on nitrite and nitrate, to try to identify what was the enzyme that makes nitrite or nitrate. We picked it up and we tried it as an inhibitor of the synthesis of nitric oxide, and we were the first to show that. Now, being a medic and a pharmacologist as a background training, the first thing I wanted to know was, what's the kind of functionality I get if I use the inhibitor. It's the most powerful thing you have, if you are a pharmacologist, if you find a pharmacologically activation.

So, I always say that probably the most important experiment we did in the vascular area, conceptually, was not simply the demonstration that EDRF is NO (nitric oxide), but the fact that we show that if you inhibit, if you inject that compound into an animal intravenously, the blood pressure goes up. That has the most significant message you can imagine in relation to the concept of how the cardiovascular system works. Until *that* point, everybody who talked about blood pressure regulation, talked about constrictor influences mainly. This experiment was telling us that vasodilator influences in the form of NO was probably the most important regulator of blood flow and blood pressure.

The question was, does it exist also in humans? And then, a very good opportunity arose because Patrick Vallance who was finishing his medical studies, got in touch with me to see whether he could come and work as a post doc for a few months at the Wellcome Research Laboratories. And he came and he had this technique by which he could infuse things into either the arterial or the venous circulation of the forearm, and measure the responses to that. And we translated the experiment that we had done into humans with him, into our own forearms, and we found that, indeed, if you give the inhibitor of the nitric oxide synthesis into the forearm, which is closed, so it's not connected with the whole body, you get a very significant vasoconstriction.

Nitric Oxide in pregnancy

There is a very significant adaptation which is required in pregnancy as the fetus grows, in terms of vascular regional blood flow. There is a massive need and requirement for increasing it. It looks as if there is a significant part played by nitric oxide in adapting the vascular system of the woman to the fact that there is this extra requirement of blood. If that

is lacking, then you have different problems that might arise. First, hypertension in the woman. Secondly, probably a defective circulation to the placenta and an effect, obviously, in the growth of the foetus.

Nitric oxide, erectile dysfunction and Viagra

The corpus cavernosum in the penis of man and animals is full of nitrergic nerves, and if you do an immuno-histochemistry for nitric oxide synthase, for nNOS, those nerves lit up, showing that nitric oxide is produced in large quantities in the innervation of the penis. And it is now clear that the adaptation of the penis - the erection of the penis - which involves a significant dilation in the corpus cavernosum, is due precisely to the release of nitric oxide. And nitric oxide, as you know, acts on the soluble guanylyl cyclase, increases the cyclic GMP - and cyclic GMP is destroyed by an enzyme called phosphodiesterase. The compound which is now so famous, Viagra, or Sildenafil from Pfizer, what it does is to inhibit the breakdown of cyclic GMP. So the nitric oxide comes, the cyclic GMP is formed, and by inhibiting its breakdown, you enhance the action of nitric oxide. So, in erectile dysfunction, where there is a reduction of nitric oxide production, this will exaggerate the effect of the little NO which is produced. So that's the explanation.

Inflammation, rheumatoid arthritis and tissue damage

Inflammation is a response to injury, and it's supposed to have developed, evolutionarily, as a defence. The problem is that inflammation takes place often when we don't need it, and often in places where it's not required, and that leads to situations like rheumatoid arthritis and other inflammatory conditions. The second thing is that inflammation is a dynamic process. Many things that do not happen in physiology, are activated and happen in inflammation, including some inducible enzymes. We, physiologically, have a COX1 enzyme that makes prostaglandins, that play a role in the regulation of the mucosa - of the flow of the mucosa in the stomach - but when we get an inflammatory process, a COX2 appears, which is brought by the inflammatory cells that come into the area, and which is induced in the inflamed area.

The same happens with nitric oxide synthase. We have constitutive enzymes - the endothelium and the neurone - but if macrophages are activated, if other tissues are activated in inflammation, this third enzyme, inducible nitric oxide synthase, appears. And this inducible nitric oxide synthase is very different from the others from a functional point of view because it produces large quantities of nitric oxide for long periods. The other ones produce small amounts in response to receptor stimulation. This one, once activated in the cell, it will produce nitric oxide for many hours, and inflammation is an environment in which

nitric oxide, which is released by these cells, interacts with other molecules including free radicals, which are a well-known component of inflammation. And if nitric oxide interacts with free radicals, in which superoxide anion produces a compound called peroxynitrite, which is a highly oxidant, which probably plays a very significant role in the damage of tissue that occurs during inflammation.

Well, as happened with the inflammatory reaction, in general, is a defence mechanism gone wrong, the production of nitric oxide by macrophages probably developed evolutionarily to fight against invading micro-organisms. But in an arthritic joint, you don't have invading micro-organisms. Those macrophages which are releasing nitric oxide, which was supposed to have the purpose of defence against micro organisms, is damaging the tissues around the macrophage. The use of selective inhibitors of the inducible enzyme used in situations like rheumatoid arthritis will first tell us how important nitric oxide is in that type of inflammation, and secondly, whether we have a therapeutic window inhibiting the production of the large quantities of nitric oxide which are produced in chronic inflammation.

Septic shock

Well, septic shock is the result of invasion in the vasculature of, among other things, lipopolysaccaride. Lipopolysaccaride comes from the walls of bacteria, especially a gram negative bacteria. Once you have that and you are infected with that, the lipopolysaccaride produces this famous induction that we're talking about, of an inflammatory reaction, but because it's happening in the *whole* of your vascular system, it produces a very dramatic clinical outcome. The most significant part of it is a very dramatic decrease in blood pressure, which had never been explained. What we know now is that the inducible nitric oxide synthase, the one that produces very large quantities of nitric oxide, which is a powerful vasodilator, starts generating masses of nitric oxide, and the NO, which is released normally by the endothelial nitric oxide synthase and regulates our blood flow and our blood pressure physiologically, now producing in large quantities, just drops the blood pressure to the point in which it has significant effects on organ perfusion, and all the consequences. This is an emergency situation and the deaths from septic shock continue in the 50 per cent range. If anybody gets septic shock, 50 per cent of the people who get it will die because there is nothing we can do, and septic shock is increasing because people are living longer, and people are having more procedures, in terms of putting catheters and keeping them on infusion and things, and that's one way of getting infected.

The immediate thought was, 'Why don't we give an inhibitor of the nitric oxide synthase, LNMMA?' It turned out that, indeed, a big clinical trial was organised by GlaxoWellcome with

LNMMA, and there was, rather than protection, increased mortality. And I have always argued that the problem in septic shock is that what you have in septic shock is a mismatch circulation. You have a general vasodilatation, but in some areas the vasocontrictor influences still predominate, so some areas are very dilated, and some areas are partially constricted at the same time. It's a mad situation. If you then give something that takes nitric oxide out – the inhibitor of nitric oxide – the areas that were vasodilated will get better, but the areas that were partially constricted will close completely, and I think the bad outcome of the trial is due to the fact that too much LNMMA was given.

So, in the future, I think we will come back and clinically revisit septic shock, either with inhibitors of the nitric oxide synthase - giving less doses, smaller doses - or with the development of *selective* inhibitors for the inducible enzyme that will take away the inducible, take away the damage, and leave the endothelial, which regulates physiologically the blood pressure. That's probably for the future.

Mitochondria – nitric oxide as modulator of oxygen consumption

The reason why we are working on mitochondria is because mitochondria are probably the most significant organelle in the existence of eukaryotic cell. It uses oxygen to generate the ATP which is the source of most of the energy that is used in the body for all biochemical processes. So, oxygen is the key for the understanding of the bioenergetics of eukaryotic cells. When we found, in 1994, that nitric oxide itself was competing for the same enzyme in the mitochondria, which uses oxygen to create energy, I immediately was not only astonished, but fascinated by that finding. How is it possible that evolution has used two gases - one for energy, and one to control the use of the oxygen to generate energy? It is an incredible mechanism. So, rather than just doing something and leaving the field as I do very often, I have been hooked for the last twelve years, trying to understand what might be happening there, and why this gas, nitric oxide, which has the potential of garrotting the cell, because the enzyme which takes oxygen to generate the energy, has more sensitivity for nitric oxygen than for oxygen itself.

So you have the potential of your cells being garrotted by nitric oxide, and we have to understand what is happening there. It turns out that, probably, nitric oxide is a modulator of the consumption of oxygen, and by modulating *that* step, which is called Complex 4, which is the last step in the oxidative phosphorylation chain, what it's doing is changing the redox state of the chain - regulating oxygen consumption and using those very basic mechanisms for signalling, and also for regulating the bioenergetic status of cells. If that's true, as we are supposing, or we are hypothesising, it's going to be a very significant finding. Several of

these things I am telling you are still working hypotheses, and we haven't yet sorted it completely out, but it's looking very promising in terms of us having understood a little bit of what's going on.

Mitochondria – new ideas about the way cells use energy sources

When I moved out of the prostaglandins to work on the EDRF nitric oxide, I went to a completely new world, and now I'm meeting mitochondria people and I feel very shy and very humble because I don't know half as much as what they know about the mitochondria when I'm making some of the statements. But it's a very exciting situation because you go completely new. You don't have the weight of the excessive knowledge, and you can ask very naive questions some of which turn out to be interesting.

When I started to work on mitochondria, indeed I knew very little about mitochondria. I only knew that Complex 4 was inhibited by nitric oxide, and my earliest interactions with people who knew about mitochondria was for me to understand what the basic knowledge of mitochondria was, and to put to them some of these questions. And I had several experiences in which I said, 'I have done this experiment and this is the result I get.' And I had several experiences of people knowing a lot about mitochondria who said to me, 'That's not possible.' It turned out that the experiment was right, not the textbook, and that is the excitement of the, if you want, the naïve approach.

The work on mitochondria has been very interesting. As I said to you, it has taken me from one problem to another - to very fundamental questions that I never expected to get into. For example, one of the things that is very attractive to me at the moment, in terms of understanding, is how much we really depend on burning oxygen and how much we are using of other - which have been considered more primitive - ways of sources of energy like glycolysis. And whether our cells constantly live by using oxygen or sometimes use glycolysis, not as a backup, but as a balancing mechanism. We have no idea. So, we are going into very basic, very fundamental questions in terms of cell survival, and cell death, and cell defence, which have to do with the metabolic aspects of it. I think that what we are doing, which I think is very useful, is that there have been many, many years of molecular biology, mapping details of pathways, and we have, if you want, slightly forgotten functionality. I come from a functionality background, being a pharmacologist and a medic, and what we're trying to do is put molecular pharmacology together, with some biochemistry and function, to see, if by marrying the two, we can really understand cell physiology and pathophysiology in a modern way.

Wellcome's important characteristics

Wellcome was a very special place. It had two characteristics that were very unusual in the pharmaceutical industry. First, an enormous respect and tradition of very high quality science, and secondly, the idea that you could combine, in the same environment, very good science and science for drug discovery. For many years, it was believed that the two activities were very different. You do science in the universities, you do drug discovery in the pharmaceutical industry. The beauty of Wellcome was that the two co-existed in a perfectly natural environment: some people doing fundamental research, some people doing drug discovery, helping each other, working as a team, and a very, very well organised team. The work that we did when I was research director in the last ten years of the company, produced compounds that are some of the most significant compounds still of the GlaxoSmithKline pipeline of products. So, it was unusual, and I always thought that that environment had to be reproduced somewhere when I realised that the drug industry was under increased pressure to perform at a higher and higher rate, and that was starting to affect the fundamental research pace. When Wellcome was bought by Glaxo, its nature disappeared, was absorbed into the Glaxo environment, which was different, and I felt the urge to go somewhere else to create something similar to that environment, and that was the objective of this institute (The Wolfson Institute for Biomedical Research, UCL) - to create a place where, and I used to say that and I still say it, 'You could put the best of industry and the best of academia together in the same environment.'