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Australian Academy of Science - Science education
Interview with Professor Gordon Ada

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Professor Gordon Ada, microbiologist, was interviewed for the Australian Academy of Science's *Video Histories of Australian Scientists* program in 1993. The interview was conducted by Professor Frank Fenner. Here is an edited transcript.

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List of edited transcripts.

In 1993 with wife Jean, children Ian (B.Ag.Sc), Andrew (MB, BS), Louise (PhD), Neil (PhD), and grandchildren Tim, David and Benji.

The fascinating word 'biochemistry'

Gordon, I have quite clear memories of meeting you 45 years ago, when you had just joined the staff of the Walter and Eliza Hall Institute to set up a new biochemistry unit. How did your upbringing lead to such an interest in science?

I grew up during the Depression. My father, who was one of six children, was an engineer. He had had a Peter Nicol Russell scholarship through the University of Sydney, and he was very keen for at least one or two of his children to get to university. But although I looked the most promising candidate in some respects, I didn't really know what I wanted to do. I was not particularly interested in mathematics at school, but rather in what I thought

was biology. We had very little of that at school, but a year or so before I was due to finish school with my matriculation I received as a Christmas present *The Science of Life*, by Huxley, Wells and Huxley. The term 'biochemistry' in this book fascinated me.

I talked about this with my parents. At one stage it was thought I might become a doctor, as that was one of the ways to get into this area, but in my ignorance I thought doctors just made other people well and didn't do very much medical research. My father took me to be interviewed by a senior person at the University of Sydney, and it was decided I might go into a science course and specialise in biochemistry. And so, at the University of Sydney, I did the classical four subjects for the first year – chemistry, physics, botany and zoology. I had some superb teachers in those days, especially people like Bob (later Sir Rutherford) Robertson and Eric Ashby. The chemistry lectures of Francis Lyons, who introduced me to organic chemistry, were a great experience.

But when I got to biochemistry, in my second year, it was a disappointment. The professor of the department was Henry Priestley, who had worked on vitamins at the Lister Institute – but well and truly in the distant past. He gave very little encouragement or excitement at all to his students. It wasn't until Dr Jack Still, who had worked under Gowland Hopkins at the University of Cambridge until early in the war, came back to Australia that I began to get some real feeling for what biochemistry was about. He was largely responsible for my becoming interested enough in the subject to stay on to do an honours degree after my Bachelor of Science.

Blood serum opacity

Then two things happened. The first was in early 1944. Dr Bob Walsh, who was then director of the Red Cross Blood Unit at Sydney Hospital (it was sometimes called the 'New South Walsh' blood unit) visited the department to see if he could interest Professor Priestley in putting one of his students to work on a very worrying problem associated with the blood supply at that stage. It was wartime, and serum sent to the front line often deteriorated, developed a precipitate, so it was not very obvious to the people who had to transfuse the serum to the troops whether it had gone off, might have become contaminated, or was safe to use. Dr Walsh thought someone might work on how the blood unit could treat the serum in order to prevent this opacity. So I started that work early in 1944.

The second event which made a difference to me was that Dr Val Bazeley, who was then in charge of the production of penicillin at the Commonwealth Serum Laboratories, paid a visit to the school. When he heard what I was doing, he expressed a keen desire for me to work at the laboratories because there were many more facilities there. So about mid-1944 I left Sydney, and I was then at the Serum Laboratories for almost two years.

I tried to work at the laboratories but it wasn't very satisfying, because in those days they were very run-down, with little modern equipment. I did actually develop a system for extracting serum with ether to get rid of lipid, because it was fairly obvious that the trouble was probably that lipoproteins were dissociating and causing the opacity. But at that time a group at the Lister Institute in London, under Ralph Kekwick, were doing the same sort of thing and they beat me to publication, in a paper in the *Lancet* describing what was happening. Both of us, however, were overtaken in due course by the method of alcohol fractionation of serum which was being developed by E J Cohn at Harvard University and which became the worldwide process for fractionating serum and getting out its different components.

Moving on: serum proteins

I decided there wasn't very much future for me at the Serum Laboratories – I needed to go abroad to get further experience. I had by now become interested in the different proteins known to be present in serum. At that time there were two major physical techniques of looking at serum proteins: the moving boundary electrophoresis apparatus and the ultracentrifuge for separating out proteins according to their size. Both these techniques had been developed to their current level in Sweden but A S McFarlane – an English scientist who had worked with people like The Svedberg and Kai Pedersen, and Arne Tiselius – had mainly developed these technologies. He was now working at the Lister Institute, where Kekwick was, so I thought perhaps I could go and work with those two. When I wrote and asked about it they said yes, I would be very welcome.

My application for leave to go there was supported by the director of the Commonwealth Serum Laboratories, Dr Morgan. But about three weeks before my boat was due to leave, the answer came back: no, I couldn't be supported by the Health Department because I wasn't a permanent member of the staff. Maybe rashly – but I think it turned out right in the end – I gave up my job and went under my own steam to work in England, hoping I would get a position once I was there. I worked in England without any salary for the first few months, until they realised that I was serious about it and gave me a salary.

While this was going on, A S McFarlane changed his position and went to work at the National Institute for Medical Research, then at Hampstead, in London. So I had to decide whether to go to work with Kekwick at the Lister or with A S McFarlane at Hampstead. I decided on the latter, and in many ways it was probably the best decision because that was a very go-ahead place. I wouldn't like to say the Lister was not like that, but perhaps it didn't have the same sort of drive to it.

I worked at Hampstead for nearly two years and liked it. I did some interesting work, but A S McFarlane, though a very fine person on the technical side,

wasn't an outstanding research scientist as such. He was more interested in technology than the results that you got using the technical equipment. Dr George Popják, however – a man of Hungarian origin – came to work with Dr John Cornforth, the Australian scientist who later got the Nobel Prize for working out the synthesis for cholesterol. I learnt more from George Popják in three or four months at the National Institute than I did from anybody else there. So that was a positive thing.

Joining Burnet and Holden at the Hall Institute

I was one of four young people in the Hampstead unit. Most of the scientists there were, if not middle-aged, certainly far older than I was. But about a year after I went there, out of the blue I received a letter from Dr Burnet, as he was in those days, the director of the Walter and Eliza Hall Institute.

While I was at the Commonwealth Serum Laboratories I had felt greatly the need for outside contact. There weren't many biochemists there, or people I could talk to, who could interest and excite me in the work I was trying to do. So I made arrangements to meet Mr Henry Holden, the very interesting biochemist at the Hall Institute, who had done some nice work in Cambridge on haemoglobin before coming out to live in Australia and work in the Hall Institute. I used to go and see him quite regularly and we got to know one another quite well. At about that time Burnet was offered a chair at Harvard, and it seems he was making up his mind whether to take it. What came out of this – by what means I don't know – was that he wished to expand the institute in Melbourne and to start up a biochemistry and biophysics unit there (with a special grant of £20,000 which he received from the Commonwealth Government).

So, about a year after I arrived at Hampstead, I had this letter from Burnet which started along the lines, 'Circumstances have arisen recently which enable us to set up at the Hall Institute a special new section employing new biophysical techniques. I am writing to ask whether you would be interested in coming back to work at the Hall Institute in association with Mr Holden to set up this new unit of biochemistry and biophysics.' I decided that I should do so.

I remember Macfarlane Burnet bringing round this young man who had just joined the staff of the Hall Institute. It was the month, August 1948, when my wife and I were leaving for the United States. You were coming to set up biochemistry, other than the Gottschalk type, within the institute.

Yes. I came back to Australia and we ran into one another, as it were, at the Institute. The moment you vacated your laboratory, I took it over. I remained in that laboratory until just before you came back in 1950 after your year in the USA.

Virus agglutination of red cells

By the time I came back, you had been brought into the influenza team.

Yes. I met Burnet at his little office, where he told me the background of the work they were doing. He was a chain-smoker in those days, as you may remember. The thing that sticks in my mind is how many cigarettes he got through in the half hour or three-quarters of an hour I talked with him there.

A phenomenon called agglutination had been discovered by Dr George Hirst, in New York, and Burnet had become very interested in the agglutination of red cells by influenza viruses. He had noticed two things. First, influenza viruses of different strains varied in their ability to agglutinate red cells. If you treated red cells with one strain of virus, that virus would no longer glutinate the red cells when it had finished, as it were, but they were still agglutinated by another strain of influenza virus. So he could make a gradient of influenza viruses. But above all he had found an enzyme called the receptor destroying enzyme, RDE. If you treated red cells first with this soluble enzyme, those red cells could no longer be agglutinated by the influenza viruses. And this enzyme was found in the culture fluid from growing *Vibrio cholerae*. So he was able to have a gradient of influenza viruses and the RDE right at the very end.

He asked me whether I could do something to find out what was happening in this process, and I came up with the idea that if you were splitting a particular bond and releasing molecules from the red cells, it might make a change in the isoelectric point of the red cells. I talked about this with Henry Holden, and between us – and largely due to his efforts – we made a little micro-electrophoresis equipment whereby we could follow the movement of red cells under an electric current and measure their mobility. Much to my joy, the first experiment I did showed that if you treated red cells with a particular virus, it changed the electrophoretic mobility. But one day Stephen Fazekas, another worker at the Hall Institute, came down and said, 'Gordon, somebody's beaten you to the post.' Somebody else had made a similar finding and it had now been published. At any rate, I went ahead and we spent some years working on finding an explanation for why this happened.

Crystallising the cholera enzyme

You did actually crystallise the cholera enzyme, didn't you?

Yes. I really hesitate to say this, but it took something like 10 years from the time I started the work to when I actually got the crystals.

Obviously this was an enzyme of some interest. Dr Gottschalk, the other biochemist working at the Hall Institute, was working on what this enzyme did, and isolating and characterising the neuraminic acid which was a product of the reaction of the substrate with the enzyme. Some time later, though, Dr

Josh Lederberg came to visit and work in Melbourne. He said to me, 'Gordon, you won't get very far working with a crude extract of the broth that you grow the *Vibrio cholerae* in. You'll have to have a completely synthetic medium to grow the *V. cholerae* in', one which didn't contain all these other proteins. 'Then you'll have a chance to crystallise the enzyme.' And actually that is what happened.

Following Lederberg's wise advice, I developed with some difficulty a completely synthetic medium, but although the bacteria grew well in this synthetic medium, very little RDE was produced. It was clearly an inducible enzyme, but where would I find a specific inducer? For a long time, I had no luck.

Some time later, my wife was breastfeeding our fourth (and last) child and I began to think – well, if a child can live for a few months on breast milk, would it contain an inducer? So I added some of my wife's milk to the synthetic medium. The assays for RDE activity were being done by Dr Eric French, who was very painstaking in his work so that one could completely trust his results. On measuring the RDE level in this particular culture, he became very excited, saying that the level of RDE activity had increased by 20-30 fold. Then we had two more pieces of luck. Bovine colostrum, which was more readily available than human breast milk, was also active, and we found that the inducer was dialysable. The active factor turned out to be sialyl lactose.

It was fairly straightforward to purify the enzyme after that. It was a very pleasant occasion when we saw crystals for the first time. We were still excited about it quite a few months later when we learnt that Burnet had been awarded the Nobel Prize for Medicine or Physiology for 1960.

The nucleic acid of influenza virus

When did you begin to get interested in the nucleic acid of influenza virus?

While I was doing this work on the electrophoretic mobility of virus and RDE-treated red blood cells, I started of course to read a lot about the virus. There was a big discussion at that time, in the early 1950s, as to the nature of viruses and what was the important genetic molecule. Amongst other things it was postulated by some people that plant viruses had RNA as their nucleic acid, animal viruses had DNA. I don't think anybody ever took this terribly seriously; we just didn't have the analyses of all that many viruses. But when I looked up the situation with influenza virus, one person who seemed to be an authority was Dr C.A. Knight, who worked with Wendell Stanley in California. He had claimed that the influenza virus contained both RNA and DNA. It seemed to me this was rather unusual and perhaps it should be looked at. They were using colour reactions in those days to measure the sugar moiety of the nucleic acid, and these could be interfered with in many ways by other

carbohydrates present in the virus particle. I thought one had to get away from these influences of the other sugars, so I worked on trying to extract all the nucleic acid from the virus in such a way that it wouldn't bring out the other components of the virus. And when I did this, it came out that the virus only contained RNA, and rather a small amount of RNA. We published that the influenza virus only contained RNA, and so it turned out to be.

The next thing was that we began to characterise this RNA. There was a lot of interest: the structure of nucleic acid had just been elucidated by Watson and Crick in these years, and we wondered whether we could find anything special about this nucleic acid. We looked at the base ratios of both influenza A and B viruses, using a technique devised by Dr Roy Markham, in Cambridge, using chromatography to measure the ratios of different nucleic acid bases. We were able to show that influenza A viruses fell within one particular pattern; influenza B viruses within another pattern. So we published this.

Also, at that time, when you grew human influenza viruses for the first time in the chick embryo you could get what was called an incomplete virus – large virus particles, which were poorly infectious compared with the small virus particles. We found they contained less nucleic acid per particle of virus than did the purified viruses that had been adapted to grow in the chick embryo.

In 1956 Burnet was invited to attend a meeting in London on the nature of viruses, organised by the Ciba Foundation. He managed to get me an invitation to present my work, and the meeting was fascinating. People like Watson and Crick were there. At the time there was still very great discussion about the importance of nucleic acid and what role it really had. Bawden and Pirie, for example, still maintained that protein was the important part of the virus particle but others said no, that the nucleic acid was the crucial ingredient. Roy Markham in Cambridge had shown that you could divide the plant virus he was working with into non-infectious particles and infectious particles, and the non-infectious particles didn't contain any ribonucleic acid.

But the most interesting thing was the description by Fraenkel-Conrat, from California, and by Gierer and Schramm, from Tübingen, that they could make infectious RNA from tobacco mosaic virus. If you isolated the nucleic acid very freshly – as Fraenkel-Conrat put it, if you ran up to the fourth floor where the herbarium was, and inoculated the plants immediately with this nucleic acid, instead of going up slowly by the stairs or lift – it retained its infectivity. You could reproduce the virus particle via the nucleic acid. It was tremendous to be at that meeting where this very exciting discovery was announced for the first time.

At Hawaii airport in 1956, after attending a Ciba Foundation meeting in London – my first trip around the world by plane.

No infectious RNA from influenza virus

Then you had a go at that with influenza but failed. Your control at that time was Murray Valley encephalitis virus, wasn't it?

Yes. When we came back we thought, 'Will this work with influenza virus?' And, by a quirk of fate, the first experiment was positive – to this day I don't know why. I tried for six months to repeat the experiment, and to see if I could get something else to work with. I worked with Gray Anderson, with Murray Valley encephalitis virus. We could make infectious RNA from that virus but we couldn't make it from influenza virus, no matter how hard we tried. I talked to Burnet about how I could get it to work, and we tried to get recombination between the nucleic acid from one strain and a whole virus particle of another strain. But we still couldn't get it to work. So, for the first time in all the 20 years I was at the Hall Institute, I published a paper with Burnet. It appeared in *Nature* with the title, 'Failure to recover infectious RNA from influenza virus'. It's probably the only paper that has been written for *Nature* about a failure!

I think the advantage of having Burnet as the co-author is that Nature *would publish a negative paper like that.*

Absolutely. The worrying thing was that in the next two years there were about six reports that you could get infectious RNA from influenza virus. We now know they were false, because it's a negative-strand virus and it's just impossible to get infectious RNA.

And also it is fragmentary.

Yes. But you look back and you wonder if you did the right thing. I should really have asked, 'Why can't you get it from 'flu? What is there about 'flu

that you can't get infectious RNA from it?' Probably the reason was that the Hall Institute was such a small place in those days. There were things happening in America with new technologies, looking at nucleic acids and so forth, and if I had been working in a big institute with a lot of other biochemists, such as the one at Hampstead or one of those in America, perhaps I would have been able to get somewhere. But the Hall Institute was an isolated place, with a very small staff.

It must have been about the size of your department, later, at the John Curtin School.

That's right, yes. The Hall Institute was a great place to work, very exciting. People like Stephen Fazekas and Joyce Stone were there, and Alick Isaacs and John Cairns came out to work there. It was a terrific time and it was great with that small band of people, but you missed a lot of ancillary aspects that were going on in the rest of the world. So I look back on those days thinking that it was good but it might have been better.

Immunology: a tempting new theory

Eventually, Burnet made a dramatic transition from virology, which was the sole occupation of the Hall Institute when I knew it, to immunology. How did that happen?

I think it happened for two main reasons. Burnet had done fantastic work on viruses in the 1930s and 1940s, initially with bacterial viruses, and many of today's findings in this area follow from his early work. But he had never tried to work on the genetics of these viruses. He was now studying the genetics of influenza virus, but although he was partially successful – he was able to show recombination and things of this nature – the tools, the end-points of the techniques he was using, really were not accurate enough for him to get very far. I think by the mid-1950s he was feeling frustrated.

He wouldn't get into tissue culture, would he?

No. All his work was done with the embryonated egg. I don't know what decided him finally not to go into tissue culture – perhaps the fact that you had this going superbly in Canberra with your Department of Microbiology – but I think it was a critical decision. It meant that he wasn't going to keep up with the rest of the world in his virus work.

The second thing was that, as you would know better than I do, he had been interested in immunology, and particularly the formation of antibodies. The current way of approaching this was by what we called the instructive theories, that the antigen acted as a template inside the antibody-forming cell. The antibody molecule moulded itself around this template and in so doing obtained the specificity to react with the antigen. This was a concept put

forward by the chemists, particularly. I don't really blame them for thinking like this because, in the 1930s, people like Karl Landsteiner found they could make antibodies to substances that did not occur in nature: that chemical compounds when attached to proteins could act as haptens. How could the body have the information to make antibodies against such structures when they didn't occur in nature? So it was a not completely irrational way to think about things. But Burnet said that the instructive theory just could not explain other aspects, particularly the nature of tolerance and the finding that you got an enhanced secondary response to an antigen. Burnet was convinced this had to be associated with a property of cells in some way.

Then, in 1955–56, two papers appeared. In one, Niels Jerne showed that natural antibodies occurred, of all different specificities. They were present there all the time, essentially. He got the idea that they were made in cells like macrophages, but Burnet wouldn't agree with this. And then, in a paper by David Talmage in America, one paragraph in a review about the structure of antibodies said that the selection of cells by antigens must be an important component. This fitted in completely with Burnet's ideas. He decided that he would push this idea and he came up with the concept of clonal selection theory by antigen, that antigens selected out particular cells which already had receptors on their surface of a specificity matching that of the antigen.

Burnet had published earlier some work trying to tie in the formation of antibodies with the properties of enzymes, but it didn't go down at all well, as you know.

It was his least successful book, I think.

Yes. He was very frightened that if he put forward this clonal selection theory – that antigens selected pre-existing cells which made antibodies of a particular specificity, and one cell only made an antibody of one specificity – and it turned out to be wrong, it might be rubbished. So he decided he would publish this first paper in the rather obscure *Australian Journal of Science*. If anybody wants lessons on how to write a three-page paper, it is a superb paper. But he published it in an obscure journal which he hoped nobody outside Australia would see if it turned out to be wrong! The more he got into it, however, the more he realised that the clonal selection theory had to be right, so he decided to 'market' the theory – to push and push it.

Antigens, labels and immune responses

Burnet's interests had swung almost completely from virology to immunology and he decided that from now on, people coming to the Hall Institute would work not in virology but immunology. It was a gradual process. For example, Eric French had been attracted to the CSIRO, so Burnet had lost a senior virologist. Gus Nossal, who had done his PhD at the Walter and Eliza Hall Institute earlier and had gone to work with Josh Lederberg over in Stanford,

had come back to work at the institute as a post-doc. Slowly the pattern of life within the institute changed, and I found myself out on a limb as to whether I should switch over to immunology or keep on in virology. One possibility was to come to work in Canberra in your department, but for a number of reasons I decided to look at immunology.

So I began to read about immunology. I had been used to working with very small numbers – 10^6 or 10^7 – of virus particles, which might be nanograms or micrograms of virus. It struck me that the immunologists, when they looked at what happened to antigen when they injected it into animals, used very large amounts of antigens. In fact, one of the antigens they were using was a product called ferritin, which had an iron core. When they isolated or made sections of lymphoid tissue, which had antibody-secreting cells present in these sections, they found that this ferritin appeared to be present in these cells. That was very much in line with the instructive theory.

I decided that it would be nice if one could do experiments with an antigen which was so powerful that you only needed to inject very small amounts of it – not milligrams, but micrograms or nanograms or even less. But if this was the case, you had to follow it in some way. We initially used an isotope of iodine called iodine-131. It had a short half-life, therefore you could have a highly specific activity of the protein. Then, after we had been working about a year, a carrier-free preparation of iodine-125 came on the market. It had a half-life of about 60 days, but as it was carrier-free every molecule was active and you could label proteins with it.

But I really couldn't go into this on my own. I was trained as a biochemist; I had no experience of dealing with tissues to any great extent. At that time Gus Nossal was doing very interesting work on the tolerance to antigens. I asked him whether he would help me to try to find out what happened to this antigen when we injected it, and very kindly he agreed to collaborate with me. We had to decide what to use as the antigen. One possibility was to use influenza virus: inactivate it and see what happened to it. But Gus had worked with a particle, the flagella, which are on the outside of *Salmonella* bacteria and are the mechanism for their motility. They are made up of sub-unit proteins called flagellin, and he was working with these two preparations. Unknown to him, the preparation of flagellin he was using could polymerise into a structure very similar to a flagella, so most of the time he was probably working not with a soluble sub-unit but with a polymer of it. But this was a very active antigen, very powerful. You could get an antibody response in rats by giving micrograms or nanograms of this material. So we decided this would be the best thing to use: we knew something about its structure, its properties and so forth, and it was a much simpler product to work with than influenza virus.

This began a very active collaboration with Gus for about five years on determining what happened to the labelled material when we injected into rats.

It was one of the best experiences of my life, because Gus was an ideal person to work with, very considerate, and we got on very well together. I think he would look back on that time as a very nice period in his life as well.

Suicidal cells: support for the clonal selection theory

Then came the 'suicide experiment', one of the most famous experiments.

Yes. After I had been working on this area for a couple of years I decided, because I hadn't had a sabbatical period of any significance in all the 15 years I had been in the institute, that I should have a period abroad. I was switching from virology to immunology, so I went to work with John Humphrey at the National Institute for Medical Research, which was then at Mill Hill. That was a great year: I met a whole lot of people such as Avrion (Av) Mitchison and Ita Askonas – it was really the world centre of immunology in those days.

One day after I came back, in about 1967, Gus came down to me and said he had just read a paper published by two Israelis, David Naor and Dov Sulitzeanu, in which they labelled antigen, bovine serum albumin, with iodine-125 and took a suspension of spleen cells, reacted it with this labelled antigen in the cold, washed away any antigen that was bound, made a smear of the cells and did radioautography of this smear of cells. When they did that, they found that some of the cells were heavily labelled but the great majority were not labelled. And of those that were labelled, there was a variation: some were more heavily labelled than others. This is what you would expect if the clonal selection theory was right. The clonal selection theory at that stage was still being contested, particularly by the people who still favoured the instructive theory of antibody formation.

Gus said, 'I think it would be a good idea, Gordon, if you repeated this work to see what happens, and particularly use some of the antigens that we have been working with.' So we labelled flagellin and some of the others, and repeated the work. We found we got a very similar pattern, and we extended the work by showing that if you treated the cells first with antibody to the immunoglobulin receptor for the antigen, you no longer got this binding pattern. So there was evidence to think that it was really binding to a proper receptor on these cells. But a couple of years later I was still thinking, 'Well, this is interesting, but what does it mean?' We needed a functional test to show beyond doubt that it strongly supported the Clonal Selection Theory.

The first Coldspring Harbor Symposium devoted to immunological topics took place in 1967, and I was invited to talk about another aspect of the flagellin molecule, the fact that by splitting the protein into smaller pieces gave a product which more readily induced tolerance. At that meeting Dick Dutton, an English scientist working in the States, showed that if you took cells which were about to become antibody-secreting cells and fed tritiated thymidine to them, you destroyed their ability to proliferate and differentiate. The reason for

this was that the radiation given off by the tritiated thymidine was sufficient to damage the cell so it could no longer differentiate and proliferate to become an antibody-secreting cell. For some reason or other this stuck in my head and one night, when I was driving home, I suddenly thought, 'There should be some sort of connection between these two things. Here am I binding antigen with a radioactive label attached to it to a cell, and here's this other guy who stops cells from working with a radioactive tracer.' I think I went through a couple of red lights, while mulling it over in my head. It struck me that maybe the radiation given off by the radioactive iodine would also damage the cells it was binding to. So when you later challenged them with the same antigen, they couldn't convert into antibody-secreting cells. I think it was a good reason to drive through a couple of red lights, actually.

At any rate, we went back and did the experiment. I labelled the cells with radioactive flagellin and let them cook like that for about an hour. The technique was that once you had labelled your cells, you washed away the unbound material and then injected the cell suspension into an X-irradiated animal which you challenged with the same antigen. Over a period of days, if the cells were still active they would become antibody-secreting cells and form antibody. As a control, you injected the animal with another, different antigen, because that should result in the production of antibody. It didn't work the first time, and I puzzled, 'Why didn't this work? Something's gone wrong.' I worked out that perhaps I wasn't giving it enough time for the radiation damage to occur. So I decided, 'Okay, I'll treat them with labelled antigen and let them sit for 24 or 48 hours before I inject it into the recipient animal.' When I did this, it worked beautifully.

Gus Nossal was the first to show some years earlier that individual antibody-secreting cells made antibody of a single specificity. My experiment showed that B cells, even before they responded to antigen, were programmed in this way. Av Mitchison coined the phrase 'hot-antigen suicide' to describe the experiment. The cells essentially suicided by taking up and binding this radioactive labelled antigen. That was great – it was nice work.

A fruitful conjunction of virology and immunology

You left the Hall Institute and came to the John Curtin School in 1968, as I remember.

Yes, at the end of '68. You had become director of the school, and I think the ruling was that you couldn't be director and also head of a department, so you interested me in this and I was appointed. The reasons for this seemed obvious to me at the time. First of all, I had been at the Hall Institute for 20 years, I was in the mid-time of my life, the mid-40s, and I felt I needed a new experience. I had been in charge of a small group of about seven or eight people at the Hall Institute but I felt I could take on a large responsibility. Secondly, you had built up an absolutely first-class department in virology. I had a virological

background but I was now an immunologist, essentially. This seemed to me to offer an opportunity to introduce into that department some people doing work in immunology, bringing together the two groups, the virologists and the immunologists. That had not been done elsewhere, to the best of my knowledge. The third thing was that by that time I had been living on a two-year appointment for some considerable time, and coming to the John Curtin School gave one tenure. Moreover, it enabled one to plan ahead for a considerable time. So I think they were the attractive features of coming to Canberra.

We were able to bring immunologists and virologists together. Before I came to the John Curtin School, cellular immunology had blossomed. T- and B-lymphocytes had been discovered – Jacques Miller had been able to show the different roles for each type of cell – and this offered an opportunity. We knew a lot about how antibody to viruses was produced but very little about the cellular immune response to viruses. Bob Blanden helped particularly and I was able to bring one of my students, Chris Parish, to Canberra with me. This gave me the opportunity to work on cellular immune responses to influenza viruses, or to viruses in general.

We were tremendously lucky, almost by chance again, to have two young scientists come and work with us. One was Peter Doherty, an Australian who had been working in Scotland. Cedric Mims had suggested we bring him back as a post-doctoral fellow. And then Rolf Zinkernagel came out under rather unusual circumstances from Switzerland. Rolf initially worked with Bob Blanden who was studying the role of cytotoxic T cells in viral infections. Then, when Bob needed space in his laboratory for somebody else, I had to decide what to do with these two people. I put them in a laboratory together and they came up with H2 restriction (for mice) or more generally now called MHC restriction. It was one of the major discoveries in immunology in that decade, that the cellular immune response to the viruses was restricted in this way.

They carried out their work with LCM, lymphocytic choriomeningitis virus, while Bob Blanden worked with ectromelia virus, one of the poxviruses. They tried it with influenza virus but it didn't work. I didn't see any reason why it shouldn't work so I put a new student, Leong Yap, onto it, and it worked the first time with Leon. And so I got back into virology from immunology and could go ahead working with influenza viruses again.

It was a very fruitful conjunction, I think, of virology and immunology.

There is no doubt about that. The study of MHC restriction rapidly became very popular. A review on the topic written in 1979 by Peter and Rolf, only a few years after their discovery, contains over 500 references.

There were two crucial aspects. Firstly, how important was this response in

controlling different infections, especially in humans, and might it also be important in the control of some non-communicable diseases, such as cancer? Secondly, how did MHC molecules combine with foreign antigens to form a structure which was recognised by the receptor on the T cell?

With respect to the first question, it was soon found that cytotoxic T lymphocytes (CTLs) were formed following infection of a host by viruses, and by intracellular bacteria or parasites. Using different model systems, but especially mice, it became clear that the early formation of CTLs was the primary mechanism for clearing many infections, especially acute infections (those that the host usually clears). A similar situation is found with human immunodeficiency virus (HIV) in humans where CTLs control the infection in the early stages. Additional data now shows that in three quite different disease situations, individuals who possess certain class I MHC specificities (called HLA specificities in humans) are more protected than others:

- Individuals possessing HLA B27 and A32 are less likely to develop AIDS and die after infection with HIV – they become long-term non-progressors.
- In the Gambia, individuals possessing HLA Bw53 are less likely to experience repeated bouts of malaria (following infection by the parasite, *Plasmodium*). In sub-Saharan Africa, Bw53 occurs in 15-40 per cent of the population, compared to less than 1 per cent in Caucasians and Asians. This strongly suggests that people with this HLA specificity have had a higher survival rate in Africa over time because of the greater protection from early death due to repeated malaria infections.
- Individuals possessing HLA B8 and B35 did not suffer repeated eye infections by Chlamydia bacteria, which can result in trachoma (blindness).

In the 1960s, Macfarlane Burnet coined the term 'immunosurveillance', which suggested that the immune system could prevent tumours from arising. This stimulated a great amount of research which indicated this can happen but that most tumours arose because they evaded the immune system. However, the possibility arose – could an immune response be induced against an existing tumour and cause its destruction? This is now a very active field. In model systems, induction of a CTL response has been found to destroy an existing tumour and new tumours have been prevented from developing. Using the same approach, the destruction of an existing tumour in humans has not been so readily achieved though there are some encouraging results in clearing melanomas in a small number of humans.

With respect to the second question, for some time after the initial discovery by Zinkernagel and Doherty, it was unclear whether the T cell receptor recognised a complex between the MHC molecule and the foreign antigen at the surface of the infected cell, or that the T cell had two receptors, one recognising the MHC molecule and the other, the foreign antigen. But in the

early 1990s, first Emile Unanue (St Louis) and shortly afterwards Alain Townsend (Oxford) showed that only a peptide (a breakdown product) from the foreign antigen bound to the MHC. The peptide binding to class I MHC molecules averaged 9 amino acids in size, and Pam Bjorkman, Jack Strominger and Don Wiley and colleagues set about crystallising such a complex. Once achieved, the crystals were examined by X-ray crystallography and showed that the antigenic peptide was held in a groove at the tip of the MHC molecule. Thus, a single T cell receptor recognised an 'area' formed by the peptide and the tip of the MHC molecule.

The following paragraph was added by Professor Ada in 1999:

Peter and Rolf's findings were recognised by the award of several prizes, but in 1995, they with Unanue, Strominger and Wiley, shared the 50th Lasker Prize, which is the most prestigious award for biomedical research in the USA. Not infrequently, recipients of this award go on to receive a Nobel Prize, but a maximum of only three individuals can share this award. In 1996, Peter and Rolf were awarded the Nobel Prize in Physiology or Medicine, in recognition of their original discovery of the role of the MHC antigens in immune recognition. The Nobel Committee rightly considered that recognition of a novel and biologically crucial functional activity was of prime importance. Frank Fenner, Gordon Ada and Robert Blanden, the first three chairmen of the John Curtin School's Microbiology Department, attended the Nobel Award ceremony in Stockholm in December 1996. In 1999, Strominger and Wiley shared the Japan Prize in further recognition of their contribution.

From cancer epidemiology to tropical diseases

When did you start to get involved in international health through the World Health Organization?

It happened shortly after I came to Canberra. The WHO had an agency in Lyon, the International Agency for Research on Cancer (IARC), which had been set up in the mid-1960s to carry out research work on epidemiology of cancer. They were offering fellowships in cancer research to people all over the world who could go and work particularly on aspects of epidemiology of cancer, and to monitor this they had formed a scholarship selection committee. Neville Stanley, I think, had been the first member on this committee. He was in those days Professor of Microbiology in Perth, and he had finished a three-year time with the IARC. Out of the blue I got a letter from the agency, inviting me to become a member of the fellowship selection committee. For three years this involved me in going round to countries in the Pacific Rim, particularly – not America, of course, but on the other side of the Pacific Rim – interviewing candidates for scholarships, after which all the members of the committee would meet in Lyon and decide who would get the fellowships.

This was like a special program of WHO. It was funded separately from the main budget by supporting nations, of which Australia was one. As well as the fellowship selection committee, they had a scientific council which oversaw the scientific work of the agency. Some members of the scientific council were people from the supporting nations, but others came from nations who weren't supporting nations. I was asked to become a member of the council for a four-year term, and in due course I became the chairman, which was wonderful

for me. In contrast to your experience with ectromelia, I had no real experience with epidemiology at all, but the main thrust of this agency was epidemiology work. They were interested in Burkitt's lymphoma, for example, and whether there was another factor: why was it that only a very small proportion of children infected with Epstein-Barr virus got Burkitt's lymphoma? They thought for a while that malaria was a co-factor, and certainly a lot of the data seemed to support this. They were interested in whether there was an association between the ingestion of aflatoxin and liver cancer. After a while they were able to show that there was a connection: those countries where there was a high intake of aflatoxin had higher levels of liver cancer. There were studies on breast cancer going on in Iceland, and so forth. So this really opened a new part of science to me which I hadn't been exposed to before. I thoroughly enjoyed that. Of course, I met a lot of other scientists as well, which was great.

Did the TDR follow directly from that?

Yes, pretty well. This is a special program which has been set up by the World Health Organization to sponsor research and training in tropical diseases. Gus Nossal, during a sabbatical year at WHO in 1976, had a very big role in setting up an overseeing committee which in due course became the Scientific and Technical Advisory Committee. I was asked to join that committee and I was a member for seven years. That introduced me to more work of the World Health Organization. The committee had a very big say, not so much in how much money came into the program but in how the money was spent in the different sub-components: the malaria component, schistosomiasis, leprosy and so forth. These programs had to be reviewed every four years, and as a member of the STAC committee I became chairman of some of these review committees. Reviewing the progress made by the different steering committees was a very great experience as well.

A growing interest in vaccines

So that introduced you to a group of infectious diseases, the parasitic diseases, that you had had no contact with, and those fascinating diseases led to other things in the vaccine development?

Yes. Firstly, the senior advisory body to WHO was the Global Advisory Committee on Medical Research. Australia, as a member of the United Nations, could nominate a member for a four-year period every eight years. Burnet was the first member to represent Australia, Eccles the second and Nossal the third. I became the fourth. This was a very different sort of committee – a discussion committee, a talking committee, essentially. It came up with ideas rather than controlling any specific program. I was asked to chair a small ad hoc committee to discuss the ways that modern techniques in molecular biology and immunology can contribute to the work of WHO. We wrote a report on it, and this body was expanded to become a rather major

subcommittee of the Global Advisory Committee over a period of eight years.

What led from that was the opportunity for Dr Asaad, who was then head of the Communicable Diseases Section, to form a new in-house committee on the Programme for Vaccine Development, and I was asked to be Chairman of the Committee which oversaw this Programme, the Scientific Advisory Group of Experts, SAGE. (It was first of all called the Scientific Advisory Group, but the acronym for that was SAG and it wasn't very wonderful, so we added the word 'Experts' at the end to make it SAGE.) I was chairman for six years. The program tried to bring together different vaccine components in WHO and provide a focus for vaccine development. I got involved with the human reproduction program in the same way, because they had a program to develop a vaccine to control human fertility. So I got a very broad exposure, coming into contact with almost every part of WHO. My interest in vaccines grew enormously because of that.

I think the thing that stood out most for me was that, although the efficacy of a vaccine depends on the immune response it generates, immunologists were not involved in vaccine development at all. There were microbiologists, molecular biologists and so forth, but no immunologists. So, as I was getting closer and closer to retiring age, I saw this as something I could get into: to start to talk about the immune responses that were made to vaccines and how you could generate these immune responses. In my last half dozen years or so at the John Curtin School I devoted my experimental work to using a model system which I picked – the influenza virus in the mouse. It was very good because it only infected one organ, the lung, and you could follow everything that had happened within the lung. And so all my last years were spent on working out what did happen when the virus infected the mouse lung: what immune responses were generated, what determined those immune responses, how long they lasted, why they lasted as long as that and so forth.

And you were able, with the newer techniques, to dissect this in terms of each protein molecule?

Absolutely, yes. You could quantitate things, you see: the action of antibody-secreting cells, the number of B and T memory cells, what determined how long they lasted and what happened. You could dissect the influenza virus – which proteins were recognised best by cytotoxic T-cells, for example. And they turned out to be the internal rather than the external proteins. It was really great.

With Dr Bob Chanock (National Institutes of Health, Washington) at the international meeting to honour Professor Frank Fenner on the occasion of his 80th birthday, December 1995.

Becoming a trans-Pacific commuter

Your retirement years, I think, brought an even deeper involvement in that subject, first with the human reproduction program and then at Johns Hopkins.

Yes. You're responsible for what happened in that, as in so many parts of my life. D A Henderson was in charge of the WHO program for smallpox eradication, but afterwards he became dean of the School of Hygiene and Public Health at Johns Hopkins University, in Baltimore. I had met him when we were both members of STAC but I never got to know him well. Then, about two years before I was due to retire, I was present in Geneva at the same time as you and D A – you were very close friends, having known one another for a long time. We went out together to dinner, during which you said, 'D A, do you realise Gordon is retiring in a couple of years' time?' D A looked up and said, 'Is he? That's interesting.' Nothing else happened. But about two months later I got a letter from Noel Rose, the head of one of his divisions in the school, asking me to spend my retirement at Johns Hopkins.

I suppose the thing that really made a difference was that just before I retired I was invited to give the plenary lecture on the prospects of an AIDS vaccine, at the forthcoming International Congress on AIDS, in Stockholm in 1988. This came out of the blue, but I decided to accept it because I was interested in that subject. It so happened that the common feeling at that time – promoted by a very eminent US researcher two years earlier – was that within a few years there would be a vaccine against human immunodeficiency virus, HIV. But I had discussed with you all the associated difficulties and the more I looked into it, the more I realised it was extremely unlikely because we had to find out so much more about the virus, and the amount of antigen variation was turning out to be a very great factor. So I got up in front of these 8,000 people in Stockholm in the middle of 1988 and said, 'There will not be a vaccine against HIV for some time.'

This had a tremendous influence on what I did when I went to Baltimore, where a lot of AIDS work was being done. The National Institutes of Health had decided they would form Centres for AIDS Research, CFARs, to bring together the different parts of the program – filling in the gaps, as it were. You had to be already receiving about half a million dollars in AIDS research work

to get extra money of about a million dollars a year to set up a centre. I was asked to be an associate director for this because my reputation from the talk had gone ahead of me. After a while the director – the deputy dean of Johns Hopkins – took up another position and they made me the director of the centre. So, in the American scene, I got into AIDS to a very large extent. I don't know what would have happened if it hadn't been for AIDS, but it was a key to open many doors in America.

All your previous sabbaticals had been spent in Britain. American science must have been a new book to you.

Yes. I had visited America many times but never worked there. The thing that struck me most, Frank, was that in America I was accepted almost from the word go. People would say, 'Oh, Gordon's here. Let's get him to come down to this meeting,' or 'to talk at this meeting,' and so forth. That's one of the very great strengths of American science. I suppose people might not be accepted, but I was lucky. Fred Brown had exactly the same experience of being accepted there; they want you to help them do things. So, almost from the beginning, I became involved very much with the National Institutes of Health. I guess they recognised me as having no axe to grind: I could give up at any time and go back to Australia, and I would give my very best opinion as to what I thought the situation was.

On two occasions I was asked to speak about vaccines before the council – which met a couple of times a year – of the National Institute for Allergy and Infectious Disease, NIAID. Half of the work of the AIDS division of the institute was to promote the development of AIDS vaccines. When manufacturers of candidate vaccines wanted to have these tested in a phase 1 clinical trial, they would come to the vaccine selection committee of the division with their information and ask for approval to go into clinical trials. The selection committee would decide yes, it was at the stage for that, or no, it wasn't. And I was appointed as a member of that committee.

It is a big decision, because the costs and difficulties involved are substantial.

Oh, absolutely. There is a lot of money involved in testing vaccines. So I felt particularly honoured. I still am a member of the committee, even though I don't attend every meeting these days.

I came back to work in Australia. But, because of the continuing problems that were associated with carrying out efficacy trials with any particular vaccine against AIDS, in the second half of last year they formed a special working group to promote the development and assessment of HIV vaccines. And much to my astonishment they invited me to become the only non-American member of it. So here am I commuting between Australia and the USA four times a year just to attend meetings of this committee – which has got a tremendous job to do.

Why an HIV vaccine is needed

To carry out an efficacy trial of an HIV vaccine is going to cost about $30 million, so you've got to be sure you're doing the right thing, you've looked at all the aspects.

It's not only the money, because the places and groups in which you can do this are rather limited. And you can't just go on doing it as you would do an experiment with mice or rats, say. You're dealing with human beings.

That's right. One of the difficulties is that HIV is a sexually transmitted disease. The only similar disease we have handled is hepatitis B, but developing the vaccine for that took 15 years. Looking back on it, it was a cinch compared with HIV, an absolute walkover.

Excellent immunity.

Yes. The chimpanzee was the only animal model for it, which was a restriction, but it was relatively straightforward compared with this virus. And the trouble is that, worldwide, the major means of transmission is by the sexual route, whether vaginal or via the rectum; it is a mucosal surface. You can't mimic the sexual behaviour of people with animals. Mating monkeys, for example, behave – I would like to think – very differently from most humans. They fight and bite and scratch, and it's a free-for-all. I'd like to think that generally that is not the way humans act when they mate. So you've got to go into people sooner or later. The main thing is to be as sure as you possibly can be that it's safe. You can't always guarantee that, but safety is an ever-increasingly important aspect of vaccine development. You hope that it's going to have a chance of working.

There are a couple of things which are hopeful, in a sense. One is that the transmission in normal circumstances, where people are not suffering from other sexually transmitted diseases, is very low – 1 per cent at the most. If sex weren't so popular, this and most other sexually transmitted disease would not be so serious. But it means that on many occasions the infectious dose is probably not all that high – probably two or three infectious doses, rather than 100 infectious doses. There are some men, supertransmitters, who infect every woman they sleep with the first time, but fortunately it's not like that most of the time. So it may not require the world's best vaccine to have some effect. That's perhaps a reasonably optimistic side of things.

There is a terrific amount of study of this virus going on, far more than with any other virus, I think. The amount of money being spent is really enormous. But it is a major problem: it's devastating some communities. In the belt on the east coast of Africa, people in small villages are dying every week from AIDS. A township I went to, Bukoba, on the shores of Lake Victoria, had 40,000

people. Two years ago, 28 per cent of people were infected with HIV. You can imagine what this is going to do to their community life. It's going to destroy communities.

That 28 per cent would include most of the people active supporting their community. Not the kids, not the old ones, but the people that the village depends on.

Absolutely. And the trouble is that a man doesn't know whether he's infected, whether his wife's infected. She doesn't know whether her husband's infected, whether she's infected. They can't go and get tested as we can in this country, for example. People in these countries are fatalistic to some extent, because they're used to losing one in every four or five children from malaria and so on, but this is of an order of magnitude greater still.

Things have changed, for example in India and Thailand. India used to be said to have no prostitution. Film stars weren't even allowed to kiss on the screen. Now we know that there are 200,000 prostitutes in Bombay alone, and up to 50 per cent of them are infected. About a year ago I saw the first film on prostitution in India – and with an Indian lady as director. This wouldn't have happened five years ago. What HIV has done, more than anything else, is to bring these things out into the open. It has had a tremendous influence. When I was awarded the AO this year, I talked about this at a dinner that night at the Governor-General's residence! It would have been impossible to do that five years ago, but now it was accepted as quite normal. I regard it as a positive thing that people are facing up to reality about such matters now.

Retirement: putting indefinite tenure to good use

Well, Gordon, like me you are past what in Australia is called the official retiring age of 65, but the United States doesn't recognise that barrier at all and would have kept you on there in an active role forever, if you'd wanted to stay. I think they are doing even better by using you as the uncommitted outside expert who will go over there whenever given a reasonable...

...excuse! (laughs)

No, a reasonable request. It would be too exhausting to go over every time you could. Fortunately, within the John Curtin School you are able to have indefinite tenure, as long as you feel active enough to do the job. That will be many years from now.

Hopefully. It is good that you set the pattern for that at the John Curtin School by coming back to work for the school after you had finished your job at CRES. I felt that it was a good thing for me to get out of the department for a while – to let my successor, Bob Blanden, take over completely and not to interfere in the slightest way. Being out of the place was a good way to do that.

And in the process you had some marvellous experience in the United States.

Yes. It was the best thing I could have done. I don't say that everything about American science is wonderful, but I really do appreciate the attitude over there of making visitors such as myself so welcome.

And using them to the maximum.

Yes. Just before I came back, a meeting was held at the Hamilton laboratories in Montana to look at the future work of NIAID. One of the six groups was our group on vaccines, in which initially there were myself, yourself and Fred Brown: half of the six members were visitors. That's fantastic. I don't know whether NIH is different in this from other scientific set-ups in the States, but I doubt it. Then they have the Fogarty Fellowships – you've been a Fogarty Fellow – as another way of bringing in very good outside people to work there. And both the visitors and American scientific institutions benefit. It's been a very positive thing.

That the John Curtin School allows people like me to come back and work is good for both of us, essentially. I can take part in the life of the school again, be a sort of consultant, be an adviser on PhD programs, give seminars and what I used to call my old Bible classes every now and then within the school, without feeling I am taking anything away from the school. I'm not occupying a position which a younger person could occupy. I like to think that they benefit from the association just as much as I do.

Well, Gordon, even though we have had to leave out such things as your spell as Foreign Secretary of the Academy we'll have to close this discussion, which has been fascinating. Thank you very much indeed.

Thank you, Frank.
