Australian Academy of Science – Science education
Interview with Professor Donald Metcalf

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Professor Donald Metcalf, physiologist, was interviewed for the Australian Academy of Science's *Video Histories of Australian Scientists* program in 1998. The interview was conducted by Dr Max Blythe of the Medical Sciences Video-archive of the Royal College of Physicians and Oxford Brookes University in the United Kingdom. Here is an edited transcript.

You can order the videotape from us for $65.50 (including GST).

List of edited transcripts.

An itinerant country childhood

Professor Metcalf, it is good to be interviewing you in Melbourne, where most of your research career has been based. You were born in 1929, and in your early years you trailed around schools because your father was with the New South Wales teaching department.

Well, it's one way to grow up. You classify yourself as a country boy, an itinerant – the family moved every two or three years from one small country town to the next. (When my father was still low down on the ladder, the 'town' might be just five houses in a couple of square miles.) You never made any lasting friendships, because every time you moved to a different part of the country you started all over again. I suppose it bred a certain independence and stubbornness. I was in effect an enforced loner; I had to get used to the fact that my friends were going to keep changing.

Tell me about your parents. Did they encourage you towards medicine?

My father, Donald Davidson Metcalf, was the son of a Scottish migrant. Education was everything to him, not only as his profession but also as his route to promotion: he was always doing degrees at night-time by correspondence. I had an older sister and a younger one, and we all gained the ethos of keeping your nose to the grindstone and paying attention to your education.

My mother, in her younger days, was one of the teachers in the school, and even when she was mainly a homemaker she taught dressmaking to the girls. (In a single-class school that had to be done by the wife of the teacher.) Our family background encouraged attention to study, but it didn't in any specific way push us into one career path or another.

The idea of doing medicine came about halfway through high school, where I had to wait and repeat two years. That was partly to get a high enough pass for a scholarship that would get you to university. But the other reason was that because I had gone to school at the age of three – put in the back of the classroom, with the class acting as a babysitter, I learnt to read and write at an appallingly early age – and I would have finished high school at 14. I could hardly go to university then, so I had to wait until I was old enough. In fact, I think I was still not ready at 16½.

Besides schoolwork did you do other things, perhaps sport?

I played sport – and some exercise was unexpected, like if you were kept in after school and had to walk home nine miles. We were running a small farm as well, so there were cows to milk and poultry to feed. You got your exercise one way or the other!

I suppose you went to more than one high school. Did you have any teachers of special significance?

I went to four high schools. The classes were up and down, as you might imagine. My teachers ranged from the Latin teacher, in a mining town, who was the local Communist candidate for election – whether or not he sparked political views in me, he certainly didn't spark my Latin course – to one or two

who were very good. Sooner or later you run into an excellent teacher, and an exposure to somebody like that for a year or two makes an enormous difference.

Powerful discussions in medical science

You went off to university in Sydney.

Yes. At that time the only medical school in the State was at Sydney University. We were the first class to go through after the Second World War – 650 in a class, no textbooks, very few pieces of equipment (we shared a skeleton which was chained to a post in the middle of the museum), staff that was run down from war years. It was survival of the fittest, a tough way to do medicine, and doesn't compare at all favourably with today's mollycoddling of students. Nevertheless, out of that hopeless zoo of students quite a number of scientists did emerge. So one wonders whether a well-equipped university department is what actually breeds a desire to do science.

I gather that in your clinical programs you would often be unable to get to a patient because there were 40 or 50 students round the bed.

It is tough if the class size is about three or four hundred, which it still was at that stage. But from time to time, almost by random chance, some of the lectures were superbly good. What made the biggest single impact, though, was the development of a new research training course at Sydney University so that at the end of second, third or fourth year the medical course was broken; you joined a laboratory and for a year you worked full-time in research, eventually ending up with a BSc in medical science.

I was the first student to do this course, in 1950 – the beginning of the Korean War. I took it at the end of third year, in the Department of Bacteriology. The department was rundown but the professor and assistant professor were quite exceptional. They had had interesting training and backgrounds, and were prepared to sit and talk to a young person of 20 and discuss science as though you were actually a human, not a number, and had something worth saying. After a year's exposure to that, you at least knew what research life could be like. You finished your medical course, you did your training in hospital and then you could make an informed choice to continue with research or go into clinical medicine.

Professor Hugh Ward was a quite eccentric lecturer who would walk out of the lecture hall to the corridor and then in again to complete what he was saying – he had been a prisoner-of-war in the Balkans Campaign (in 1912!) and could never stand to be in a room with the door shut. As a post-doc he had been trained at Harvard by Zinsser, at that time the most famous bacteriologist in the United States, so there is almost a direct lineal descent from Zinsser to Ward and his protégé Patrick de Burgh, and then to Metcalf.

Patrick de Burgh was an equally eccentric scientist teacher who succeeded Ward as professor. Neither of them was at all creative in the sense of writing scientific papers, but they were very influential on everyone in their department. (Three Fellows of the Royal Society came out of that one little unequipped department.) They did essentially no research work, but they would sit and discuss and argue things with you, which is very powerful. I promised that I would always do this with my students – and forgot. Instead I decided to teach by example and do the experiments myself, and if my students watched and decided the idea was good, then so be it, that's the way they learnt.

Through virology to a fascination with leukaemia

You told me once, in relation to that medical science research, that you were 'just allowed to have a room and get on with it'.

That was after removing the junk from the room and making a bit of bench space! There were two of us as BMed Science students and we got by pretty well, working on ectromelia virus (a cousin of smallpox virus). It was straight virology, looking at the basis for the hepatitis that the mice were getting. It would never be permitted today, because it is a highly infectious agent that institutes live in terror of having within their four walls. We were let loose on it with no training, but although it is harmful to mice it's not harmful to humans.

Did that work contribute in any way to your deep interest in blood diseases, especially leukaemia?

During this research year haematology became fascinating, and cancer in general was fascinating, so that narrowed the field down to leukaemia. There was a certain amount of evidence that viruses would cause leukaemia in animals, particularly in mice and chickens. Nothing was known about the disease in humans except that irradiation would cause it, and the possibility of a virus cause was still viable. So it seemed a good time and a good subject to be in. Leukaemia was an incurable disease – 100 per cent fatal – but if it was virus induced, maybe you could develop a vaccine. That was the hope until the late '70s, I think, when people decided that humans were probably unlike other animal species and possibly did not harbour leukaemia viruses. This conclusion is still somewhat dubious, I'm sure.

Gaining a good clinical grounding

In about 1953 you got your MB BS and went into a residency. Although you had decided some time previously that you weren't going to practise clinically, I know that you like people and you care about patients. Did your residency experience have any effect on your research commitment?

They were not comfortable times, because you realised that despite your training you knew nothing. When suddenly you were faced with a ward full of patients, your inadequacies became very obvious very fast. But everyone was in the same boat.

Of the various types of clinical training I had, nothing was terribly relevant for later life: casualty surgery is not training you to be a cancer research worker, nor is orthopaedics, nor is chest surgery. But it is good general grounding, which you need to even recognise in later life that there are medical problems that should – or could – be tackled. Science graduates who have never had that exposure to real-life disease are quite capable of posing questions, but they don't know that particular diseases exist so they are at a disadvantage. Technically they are superior to medical graduates, but they just don't have that breadth of experience.

You kept some exciting research links with clinical practice and eventually, 50 years on, we get you doing clinical trials.

It was episodic, I think. You learn that most medical research will never have a direct application in clinical medicine. Everybody gets used to spending decades working at the bench in the knowledge that only a very exceptional body of research will lead to some change in treatment of actual patients. So you don't see patients very often. It's a mistake to think you can be a superior clinician and do superior research work simultaneously. To extend that argument a little: every hour you spend on clinical work reduces your ability to do anything creative in the laboratory. Early on you just have to make up your mind: is it going to be a clinical career or research? A lot of medical graduates have great difficulty reaching a decision, but I didn't. I was quite happy to drop the clinical work, to say, 'Okay, I've done that. I know the sorts of problems that are out there,' and just to get on with it in the laboratory.

Entering Burnet's realm

You came to the Walter and Eliza Hall Institute, here in Melbourne, virtually a year after qualifying. How did that happen?

Well, I was in the middle of an operation with a particularly unpleasant surgeon when somebody came in with a telegram offering me a Carden Fellowship in cancer research, and I said, 'Right! I'll take it.'

Australia was a small academic community in those days, and my opportunity to join the Hall Institute resulted from contact made by my professor with the Anti-Cancer Council of Victoria and then with the Institute. That type of personal arrangement no longer happens. For a youngster these days entry into a research career can involve a tortuous route. In retrospect you have to say you've been lucky to have been able to have a fellowship that took you to the

best research institute in the country and, after a little bit of huffing and puffing, to do pretty much what you felt should be done.

I had to come down to Melbourne to be interviewed. I worried the hell out of them by saying that I wasn't experienced enough to be appointed, and so I would take a fraction of the salary on offer. I'd never do that again.

Were you interviewed by Sir Macfarlane Burnet himself?

No, but I had visited the Institute as a student. I was thrust on Burnet by the Anti-Cancer Council as a paid Fellow with some research support, but he wasn't particularly enamoured of cancer research, which he saw as a pointless exercise. To him, cancer was an inevitable disease of ageing and therefore neither preventable nor curable. He couldn't grasp the concept that the occurrence of such a disease may be brought forward by other agencies so that it becomes a major problem. For example, if we all lived to 500, probably all of us would get lung cancer. But by smoking cigarettes you bring that curve back into your own lifespan. It becomes almost plus and minus: smoking equals lung cancer, but no smoking, no lung cancer. His attitude was correct, and to this day most known mutations are occurring either at random – spontaneously – or for reasons that nobody knows. Nevertheless, at the practical level there is such a thing as finding a cause that will accelerate a disease process and therefore developing preventive measures of value.

Burnet said, 'Okay, I will take you, but to prove you are a genuine scientist I want you to work for two years as a virologist.' This was a virus institute, so I was put to work on vaccinia, which was a kissing-cousin of ectromelia that I had been working on. I did that for two years – I was allowed in the main building so long as I was doing virology – and then slowly I began other experiments and deviated off into my own area. (Eventually I was put into the animal house for eight years to do my work.)

Burnet was then a major figure in Australian science. Did you find him impressive?

Yes. He was a formidable exponent of virology. You'd have to say that in the late 1940s, mid-'50s, he was at the top of his powers as a working scientist who knew virology backwards and was making important contributions. It just happened that he didn't know anything about cells or the blood-forming system, so he had no experience or detailed knowledge that I could gain from. And, obviously, he found it hard to interest himself in any discussion on that subject.

What could I learn from somebody like that? I think the first thing you learn is a way of working. He would insist that you began writing a paper when you were halfway through the experiments – which, as he said, points out like no other method what's missing from the study while you're doing it and you've

still got all your reagents. I still teach that to my students, because that is good technique. There were general things like that, but it was no use looking to Burnet for a sophisticated discussion on leukaemia or blood cell formation. And it is true for many of us that our interests become focused so much that we really are quite ignorant of other particular areas. I learned by general observation of him how somebody who was successful manages their scientific career, even if he was – like all of us – a little idiosyncratic in handling interpersonal relationships. He was certainly a plus to have around, but not in my particular area.

Lessons from chicken blood and mouse thymus

I suspect that you found a way of getting back from the vaccinia bench onto blood, which you were more enthusiastic about, by working on chicken leukaemia.

Well, it was a one-step retreat from the injunction to work on viruses, because chicken leukaemia is caused by viruses. So by going out and collecting chickens that had leukaemia, and starting to look at their blood, I could be doing haematology – studying blood cell formation – at the same time as carrying out various experiments that you could label 'virology'. I ended up taking chicken blood and putting it on the membranes of chick embryos, and then you could get quite large pox developing. Were these due to a virus?

Those were dismissed by Burnet as being somewhat uninteresting, but always, sooner or later, something unexpected turns up. When I returned to the Institute a couple of years later, almost everyone in the building was working on the so-called Simonson phenomenon, which was a reaction of lymphocytes against the host embryo. I hadn't realised what my original experiments were showing, but pox development became a central area of study in immunology – and everyone was working on what I had stumbled across by accident some years before Simonson.

What led to your involvement with the thymus?

I began to inject extracts of tissues into baby mice, hoping for evidence that they contained something that would stimulate blood cell formation. I kept getting answers that if you injected into mice an extract of the thymus, you could change lymphocyte levels in their blood. To this day, however, nobody has been able to repeat those experiments. Goodness knows whether they were true or not. They seemed to be at the time.

I think you found thymectomy affected peripheral lymphocyte distribution and growth.

That was the extension of this work. If the only extract that would produce this effect was the thymus, then why not take out the thymus and see whether

things reversed? And yes, the lymphoid tissues in the rest of the body did regress some. Those studies of mine in Boston preceded the formal observation that taking out the thymus of a newborn animal has a dramatic impact on the immune system. It's the same mechanism, just a little less dramatic, if you take out the thymus in adult life.

Tissue and hormone balances, in times of great scientific passion

Don, you mentioned Boston. Didn't you go there because of that remarkable character called Jacob Furth?

Yes. He had made the remarkable observation that if you were a mouse and somebody took out your thymus, you would not develop lymphoid leukaemia. And it made no difference whether irradiation or oestrogens were used to cause leukaemia, the answer was the same. He seemed to be the only person working on the thymus, and also it was a good idea to go to a large research centre like Boston, so I went there to the Harvard Medical School for two years (1956–58) as a post-doc. I forget whether I wrote to them or Burnet did, but as long as I came armed with my own fellowship, I was accepted.

That is how I fell under the influence of Furth, a Hungarian-born scientist with a remarkable flow of ideas and creativity on many aspects of cancer research. He was iconoclastic, never afraid to come up with 12 theories by tomorrow morning about how things might work. He wasn't very good at executing them, because he'd have another 12 the next morning, but if you ignored those and got on with the first 12, then often interesting things happened.

Furth was an incredibly seminal figure, but I don't believe he ever got the credit that his work deserved.

I agree. I later tried very hard to have him awarded the highest American prize for cancer research, when I was on the selection committee, but I was told, 'No, he is now 80 and not active. That previous work doesn't count.' Well, the rules have now changed a little!

I think the single most important aspect of Furth's work was the documentation that the development of many tumours, particularly tumours of endocrine target tissues, occurred because of an imbalance in the regulators controlling the tissue. So you could make tumours by creating regulator imbalance. The second part of that story is that for a time the tumours only behaved as cancers if you continued that regulator imbalance. If that was to have any meaning at all for leukaemia, which was my bag, I would have to find the regulators controlling blood cell formation, try to develop a system where they were out of balance, favouring cell proliferation, and see whether that causes leukaemia.

Actually, the mid- and late '50s was the time when viruses were flavour of the

month, with new mouse tumour viruses being found almost every few weeks. In Boston we were working most of the time with people developing tumour viruses, like Ludwig Gross and Charlotte Friend, both in New York. This was the era of the discovery of the big card-carrying cancer-inducing viruses, and if anyone said, 'Hey, wait a minute, maybe there's another contributing cause of leukaemia. Maybe it's a hormonal imbalance,' they were regarded as slightly wacky, out in left field. So you kept one foot in the virus camp to be conventional, and with the other foot you tested the water outside.

It was a good post-doc period for me. Although only three papers were published – few enough to earn you bad marks these days for a two-year postdoctoral period – it provided a lot of training and a lot of experience in animal pathology, which is hard to acquire.

Charlotte Friend, as a woman in science, was also not being recognised. But she was a fascinating person. I did enjoy reading your memorial lecture.

They were times of great passion. There would be furious fights during meetings and people would abuse each other publicly. During one leukaemia meeting I was at, the main speaker had a heart attack and was dragged out feet-first while the argument continued. These days, too much money and too many post-docs' careers are at stake for such public dissent. You do not get up and tell the speaker he is an idiot. But you did in the '50s. Different days.

The strangeness of the thymus

After those two golden years in America, you come back to the Hall Institute.

Yes, I came back with my own little group and my own series of mini-laboratories – but as payment I had to work in the animal house, surrounded by 10,000 mice, to which I am allergic, so my nose ran for the next eight years. But it was my own little laboratory, so we could do what we liked. We began with one technician, moving to one Japanese post-doc (probably the first in this country) and ending up with about three scientists and four or five technicians. We were not considered part of the Institute, but were just listed as 'visiting and attached'.

We were still working on the thymus, trying to figure out what controlled its growth. It is a very strange organ. For one thing, it is completely autonomous: it just follows its own rules. When we are young, the thymus grows to a very large size – so large that surgeons used to take it out, calling the 'disease' thymic hyperplasia when this was really a normal young thymus growing. As we get to adolescence it begins to shrivel up. In advanced age, it is quite a tiny, withered-up organ. In other words, there's a time clock in the behaviour of the thymus. But, extraordinarily, that time clock is within the organ, it is fixed. If you take a baby thymus and put it into an old mouse, it will still go through exactly that same size change, on time.

The main thing that came out of our study was something else very strange about the thymus: it made lymphocytes at an astonishing rate (it replaced itself every three days) yet very few of them seemed to get out into the rest of the body. That did capture Burnet's interest, because by then there were more immunologists in the Institute and his attention and enthusiasm had switched to immunology. By that time also, two members of our Institute had discovered that there were two sorts of lymphocytes, T lymphocytes made in the thymus and B lymphocytes made in the bone marrow. So why the devil did the body make 99 times too many cells in the thymus and promptly kill most of them?

At that time there were still very few people working on the thymus, and our very careful findings that there was little export of cells out of the thymus were just regarded as crazy. But that is now well established. Our work was the origin of what has become an almost religious dogma that the self-reactive cells have to be eliminated in favour of the ones that have rearranged their genes correctly, which are then the few that get out and are used.

So you were very early with that. But you have said in one of your papers, 'I went from the whole animal to chemist, in a way – to growth factors.'

Oh yes. But our work on the thymus was getting nowhere. You could do all those experiments but you couldn't actually penetrate to find out what made cells divide so quickly in the thymus. You could observe that they did divide, but as long as you were stuck working with the whole animal you couldn't really get ahead. You had to go to tissue culture.

Shifting emphases at the Hall Institute

Before we move on, Don, could you talk a little about the Hall Institute? You said Burnet became interested in the immunological side because the Institute's direction had massively shifted. Burnet says in his own writings, though, that he changed the direction of the Institute.

Both statements are probably true. There's no doubt that some of the existing virologists were encouraged, rather forcibly, to move elsewhere. But it did coincide with the arrival, almost by happenstance, of people who were interested in immune cells. So the two notions came together. And I think Burnet had always, since pre-war, had an interest in tolerance as he encountered it with virus infections but thinking laterally to immune responses. In the early '60s, such a thing as an antibody-forming cell was quite unknown. So, at this time, the nature of these cells was being discovered by Gowans at Oxford and by the young people in our Institute.

Gus Nossal was trying to prove formally (and eventually did) the correctness of Burnet's theoretical postulate that one cell made only one sort of antibody.

Jacques Miller had done the first work removing the thymus from neonatal animals, with obviously a dramatic impact on the immune system. And a younger person, Noel Warner, and an older Polish visiting scientist, Alexander Szoenberg, working with chickens, figured out that the cells from the Bursa of Fabricius seemed to be making antibody and that the cells from the thymus were engaged in cell-mediated responses. Now, it happens by sheer fluke that B for 'bursa' is also B for 'bone marrow', which in mammals is the equivalent of the bursa. So by the mid-'60s, which marked the end of my involvement with lymphoid cells, T and B lymphocytes were the star turn, the centre of all attention.

By that time Burnet had retired. The emergence of immunology as a cell science began in the late '50s and early '60s, and probably coincided with his feeling that the Institute's techniques had gone as far as they could go with viruses and that it was time to change. I think the way it's recorded is partly right, and in part the change would have happened anyway – a sort of a revolution.

Did Miller's work on thymus bring you close to him in your work?

Not really. He is a loner – unlike me, who won't admit to that. And immunologists were extremely arrogant in the '60s. They had a cell science that technically put them beyond workers with other tissues: with exquisite specificity you could take single cells and actually measure the amount of antibody they were making. So to the immunologists anyone working on any other cell system was barely worth talking to, let alone collaborating with.

You have said to me that your seminars in your field were rather poorly attended in that period.

That's true. Even in an institute so highly focused on immunology, there were other people – like us. Whether or not what we were working on was very scientific, it was certainly of little interest. Probably every day there are scientists who can say, 'Nobody pays attention to my work.' It's a scary life being a scientist.

Goodbye thymus, hello CSF

The great watershed year was 1965, wasn't it? Something quite dramatic changed it for all of you.

Yes. It arose from the phenomenon that individual cells in a culture of bone marrow cells growing in semi-solid medium, agar, could generate enormous colonies. Now, that technique was discovered by accident by Ray Bradley, a scientist working in the University of Melbourne with whom I had collaborated over the years. Two things became pretty obvious. For the first time in history, people could grow blood-forming cells as colonies. It turned

out that (as had seemed likely) they were clones, each one coming from a single cell – and they made a colony of daughter cells during a week of incubation. But unless you added something to the medium in the culture, colonies would not grow. That something we called colony stimulating factor, CSF.

The point about the cultures was that they gave you a technique for measuring CSF concentrations, because the number of colonies that develop reflects the concentration of CSF. So we had a way of doing three things: working in tissue culture, which I knew we needed; detecting some factor that, hopefully, was a regulator of the sort we had been seeking for a decade; and measuring it. So yes, almost overnight all work on the thymus stopped.

It wasn't that we immediately rushed over to Ray Bradley and taught ourselves how to culture colonies. We worked for the next year as a team, in which I continued to do the formal haematology and general cell biology, but eventually we did teach ourselves how to do the technique and take the next logical steps. Every so often there is an accidental occurrence like that, when you would have to be blind not to realise that here is something astonishing that warranted a few decades' work – and so it proved.

In the 1960s, at the time when you came into this culture work with the Bradley culture technique, there were suggestions that ideas might have been pirated from Israel. Would you like to tell us about that?

Well, it's a phenomenon that we're all familiar with now: it keeps happening that two quite separate groups, by accident, stumble on the same observation at about the same time. Why did it happen at that time? Maybe there were just the beginnings of tissue culture in many parts of the world. We had never had tissue culture in the Institute until then. Maybe it's just a fluke. But there have since been many examples of quite injured feelings with the parties concerned saying, 'Hey, you stole my technique.' On this occasion the senior Israeli scientist was convinced that we had read about his technique and copied it without ever quoting it – but that in fact wasn't true. It was a sheer coincidence.

Interestingly enough, these colonies were at first misidentified by the Israeli group. Growing in agar, which is metachromatic, the cells phagocytose lumps of agar and then have purple granules in their cytoplasm. That's what mast cells look like, so their first two papers described colonies of mast cells. But we met in Philadelphia – at a dinner in honour of Ludwig Gross, of all people – and when he said, 'We've been doing funny little cultures, growing colonies of mast cells,' I told him, 'That's funny, we've been growing colonies of granulocytes and macrophages.' I can take you to the spot in the Grand Ballroom at the Sheraton Hotel where that conversation happened. So yes, it was a simultaneous discovery.

So you were both growing neutrophil granulocytes and macrophages.

Yes, but until we fed them things for them to phagocytose, it took us a long time to figure out – rather grudgingly – that these cells were actually macrophages.

It's good to have an episode like that Israeli one on the record, to be openly addressed. If you believed everything you read in scientific papers, might get a mistaken idea about the history of this field – and you'd be an idiot anyway, because so many things written in scientific papers aren't very accurate.

Early steps toward purifying CSF

So what to do about that watershed in the mid-'60s? It's no good simply believing that you have a technique for discovering your favourite unknown hormone-regulating blood cells. You're working with cells in a culture dish, artefacts abound, maybe colony formation was all just an artefact. To get further forward, several things were needed. The first was to be able to show that CSF was detectable in the serum and hopefully in the urine. Why? Because it would make sense if it's a regulator that detectable levels of CSF should be present in the serum and urine. It would be nice also if you found that there were CSFs to be detected in tissues. It would make sense if you had an infection and needed to make extra protective white cells (granulocytes and macrophages) that CSF levels should go up, otherwise it would not be a good candidate for a regulator.

We spent about three years surveying patients with infections, looking at CSF levels in their urine and serum and looking at different tissues to see which had the greatest content of CSF – assaying all the time by the culture method, which was the only one available to us. And by, perhaps, late 1968 it was obvious that there was enough indirect evidence to support the notion, 'Yes, CSF is a good candidate for a regulator. Let's spend some time purifying it and putting a biochemical basis to it.'

I put a poor unfortunate PhD student, Richard Stanley, onto this 'simple' job of purifying CSF. (He is now a distinguished professor in New York; his photograph was on last month's issue of *Cancer Research*.) We started with human urine because it was a good, cheap starting material. We had buckets for collection of urine in the Institute. First you had to take the cigarette butts out of it – these were the days when you could smoke in a research institute – and then you had to dialyse it in great evil-smelling tanks in 50 litre batches. Great stuff! It took nine years to purify CSF from human urine. Richard did not complete the job until he was in Toronto working as a post-doc.

So many CSFs!

This must have been getting into the early 1970s, was it?

Yes. Meanwhile, the situation was becoming a little bit murky and uncomfortable. The CSF from urine did not stimulate colony formation all that well. In particular, mostly we got only small macrophage colonies, not the large beautiful granulocytic macrophage colonies seen with the original Bradley technique. Clearly things were a bit more complicated than we had thought. There must be more than one type of CSF. When we began to analyse what type of CSF was being made by different tissues, it became appallingly obvious that lung tissue was making a CSF that had no chemical relationship whatsoever with urine CSF (which was now being called M-CSF because it pretty much only stimulated macrophage colony formation). Lung CSF was a much smaller molecule and it stimulated the formation of beautiful granulocyte macrophage colonies, so we called it GM-CSF.

It also became obvious that if you took lymphocytes and stimulated them with mitogens they produced another type of CSF with some remarkable properties. While all this had been going on, we and others had developed culture techniques that would grow colonies of other types of blood cell. (There are eight major families of blood cells.) CSF made by activated T lymphocytes could stimulate the formation of red cell or megakaryocyte colonies. Urine CSF or lung CSF could not do this. So there appeared to be yet another CSF.

It took us quite a while to realise there was yet another, fourth CSF. This turned out to be the most famous CSF of all – G-CSF. For two years I had missed the fact that there were miserable little colonies developing in certain culture dishes. I thought they were merely dead colonies! But the CSF causing the formation of these small granulocytic colonies came to be known as G-CSF, and it's the one that is making mega-millions for drug companies.

So everything was happening simultaneously. You might say we were very slow to purify the CSFs, but the project had become four times more complicated. This is partly why the project took fifteen years to complete. Other sorts of assays were being developed all the time, we had to figure out all the novel biology behind why one type of colony was being made and why another, and we ended up with a project that needed four different purifications (for four different CSFs) and a much broader range of assays to be done.

Don, to recap: in just three or four years from the late 1960s into the '70s, using a whole range of different culturing methods, you went from the initial discovery to the conclusion that there must be four factors that related to your field?

Yes. This came partly from the development of different assays, but also from the fact that when you take an impure preparation and start to break it up into fractions on a column or use some other separative procedure, you get multiple peaks of activity. Then, if you look carefully, you will find the biological activity of the material in the various peaks is not quite the same. So you say,

'Uh, up here we're getting all granulocyte colonies. Whoops, down here we're getting all macrophage. Maybe these two peaks of active material are different.' Maybe they're not, because these molecules have a lot of carbohydrate on them and that can make enormous differences to their physical properties, and it might just be that some molecules are made in a sloppy way and have variable amounts of carbohydrate on them. That possibility was always a pain in the neck. But eventually you couldn't escape the fact that biochemically there had to be at least four different CSFs. And so the project began to get out of hand.

How pure is pure?

By now we had a group of nine or 10 scientists, and we were beginning to work as a tight team. Biologists like me concentrated on cultures and bioassays; biochemists concentrated on purifying the CSFs. And this was a tough slog. We are talking about purifications of one million fold – never before achieved – because there's only one molecule in a million of that type in tissues or in your serum. The techniques of high performance liquid chromatography that permitted such enormous purification had not been developed, and weren't until the late 1970s.

So there was a continuous battle going on about what 'pure' means. Take the cigarette butts out; is that purified urine? Well, it is one definition of purified. When do we stop? When do we say we have now purified CSF? The first definition of purity, in retrospect, was pathetic: a single band in a gel that would stain with Coomassie blue dye. Absolute rubbish – such a single band might contain dozens of different proteins in it. Then we got more clever and said no, the analysis had to be in a reducing gel and the material had to produce a very narrow silver staining band. Slowly during the '70s things got tougher, and with the invention of the amino acid sequencing machine here in Melbourne you could say, 'No, I want material that, when you start sequencing it, gives you just a single amino acid sequence. That's pure.' And then with the development of molecular biology, at the beginning of the '80s, people said, 'No way. You must make an artificial gene based on that sequence, then use that to pull out the corresponding gene, and when that gene is expressed and the product sequenced, it's the same sequence as the one you started with.'

We wrote any number of papers – as did everyone else – describing the 'purification' of CSF or saying, 'This is now purer CSF'. But nobody knew the acceptable definition of purity. In fact, it is impossible to purify anything absolutely. Nothing is ever 'pure'. Even the purest, purest, purest preparation may still have 10 molecules of Socrates' hemlock in it. But there is now a working definition: it must be sequence grade purity producing only one sequence, and you'd better pull out a cDNA whose sequence agrees and will encode the production of material with the same sequence. Then you can talk about purity.

That learning period in the mid-1970s was also disaster time, because incredibly minute amounts of material were involved as the end product – a few millionths of a gram extracted from a quarter of a million mice. Hard slog. A quarter of a million assay cultures and you end up with 10 micrograms of pure material that then sticks to the tube and you lose it, so you do it all again. We had to repeat parts of the whole six-stage purification sequence for G-CSF 100 times. Not every batch needed to go through all stages, but you kept running into dead ends that you couldn't then get out of with another purification technique. You can write a paper based on the first two or three purifications – 'Great, purification of CSF' – but then you've got to do parts of it another hundred times with no publication, just to get enough material to sequence. You can't write a paper about that. This is real grunt work.

The uncertain emergence of clinical goals

And beyond that distant horizon of purification it would be clear for you, a clinical person, why these factors – once you've got enough – are important?

If only that were so. It would be a lovely story to tell that way, so logical. I recently went back through all our publications on CSF, expecting to see sooner or later a discussion of how this could be used in the clinic. There is not one word about it until the late 1980s. There we were, gritting our teeth, slogging on with a project for 20 years, yet according to the written record there was no notion that the CSFs might ever be used clinically. This mystifies all of us. We can't remember whether this was so obvious, such common knowledge, so much part of our daily ethos, that we never ever bothered to write it down. Or were we damn stupid and blinker-visioned?

Certainly we knew we had to purify these things and make enough to inject into animals to see if they'd even work. Did we actually think that once we had done that, we might as well inject humans? Remember we'd been working with mouse tissues, and mouse hormones won't work in humans. Is it possible we never thought in clinical terms?

What did start to get talked about was the possibility of using CSFs to treat leukaemic patients. We had originally got into looking for growth factors because I felt they had something to do with the development of leukaemia (as it turns out they do) and so that story had been going along in parallel. Everything we did on normal tissues, we looked at also using leukaemic cells. And we ran into a very strange phenomenon.

By the early 1980s we could draw a little diagram that we used to use as a standard lecture summary. There were four different sorts of CSFs, and when they hit the common cell ancestors these cells started to divide and to make their progeny. So you started with quite immature cells that divided to make nice adult-type, mature cells that you would be proud to call your own: these mature cells will kill bacteria and protect you against infections. It turned out

that life wasn't quite so simple. We had discovered these CSFs because they were absolutely needed to make the cells divide. So they were mitotic stimuli. But the very same molecules proved to have all sorts of additional actions on those subfamilies of white cells. They could tell cells to start maturing, or to stop thinking about ever forming other sorts of progeny – or they could act on mature cells and say, 'Work harder. Eat more bacteria. Kill them more quickly.' That is, they could functionally activate them.

This notion that a regulator or a growth hormone might have multiple actions was not well received. Nonetheless, it turns out to be a principle that's true of all growth regulators, for any tissue. But as part of working on this bewildering pleomorphism of the actions, we had observed that the purified CSFs could make some leukaemic cells mature well enough. They would stop dividing and make a fairish attempt at becoming mature cells.

So if you sufficiently regulate a leukaemic line by CSFs, it will specialise enough not to go into division?

Right. The cells will take an irreversible decision not to reproduce themselves any more, not to display that characteristic of a cancer cell population, but to go down the pathway of maturation.

That suggested itself as a possible treatment for leukaemia. If you had a bottle of CSF and you had a patient with myeloid leukaemia – and the patient behaved the same way as the cell lines – hey, you could stop that leukaemia cold, using a natural body hormone instead of cytotoxic drugs. And that was discussed in our writings as a clinical application. Curiously, though, it's not actually in any of the papers prior to doing clinical trials. I, like you, assume in retrospect that we knew what we were doing, that we did have the big goal in front of us. But there's no written record of it.

Fishing out the genes for CSF

Don, you have talked about going from animal research to tissue culture. Now we've got you deeply into biochemistry and molecular biology.

Yes. For us the early '80s was a time of depression: we had figured out how to work as a team but we had an enormous logistical problem. We now recognised that we could never extract enough native CSF out of the richest tissue source to inject into one mouse – and to get enough material for one patient we would have had to work for 250 years. We had purified CSF, we had done elegant tissue culture experiments, but now we're into logistics and were facing a big black hole.

By this time we had another collaborative arm to our team, the molecular biologists in the Ludwig Institute for Cancer Research next door. So they took the bold step, 'Let's go for the CSF genes.' Doesn't sound a big deal now, but

by then very few mammalian genes had been cloned, and fewer still had been shown to be able to go on and generate their protein product. Looking back, we were probably pretty innovative to go for the CSF genes. But we were desperate. We were getting nowhere with purification. Even I, workaholic that I was, had begun to hate doing assays!

So in 1983 the decision was made to try to use what little sequence data we had from purified CSF to develop an artificial gene with which to fish out the gene for GM-CSF – the one we had sequence data for. And we had a pretty hairy time getting the gene out, because there were probably only two copies in our entire library and both of them were incomplete. We managed, by stitching together fragments, to get a complete gene out by '84. But then competition became fierce, and the remaining three CSFs were cloned by other workers. Companies were now beginning to get into the act, so some were cloned by company scientists.

Within two years (1984–86) genes for all four CSFs from the mouse and man had been cloned and with more or less tolerable difficulty you could mass-produce CSF, for example by using bacteria. The world's first vial of bacterially generated recombinant CSF, made by putting a GM-CSF gene into bacteria to make the recombinant product, probably contained $2 million worth of CSF. It would certainly have cost us $2 million to purify that amount. The cost now, to buy it off the pharmacist's shelf? $160. Cost to make now? Perhaps $2.

So this was the logistical breakthrough. Now we literally did have in our hands enough material to inject into a large number of mice, to ask the question after 20 years: does this stuff really work in an animal? It's great in tissue culture but can you now stimulate an animal to make more mature white blood cells? So you inject CSF into a mouse. Does the peripheral blood and other populations now look the way you hope? The answer was yes, they do – and the moment that answer came through, in 1986, it was all over. I clearly remember, after getting a positive answer in mice, saying, 'Okay, there is going to be a human with a disease where CSFs will be used.' It was evident that patients would be found whose white cell production could be stimulated by CSFs and the function of their white cells increased, improving their resistance to infections. And that is the way it's turned out.

How to optimise CSF use

You must be pleased that CSFs are actually being used now in cancer patients.

Well, CSFs themselves do not have anything to do with cancer, but patients who have cancer are the ones most often treated with CSF. Those patients have heavy chemotherapy to destroy their cancer cells, but this also damages the bone marrow so the patients often end up with no white cells, and then they need transplants of bone marrow to try and regrow the white cell population.

That's a slow, difficult business, but if you treat the patient with CSF for a short period you can accelerate this recovery.

You don't give any cells, you just treat with the CSF?

Yes, just CSF. It is a case of using the body's own product. The body does make CSF in increased amounts when faced with this emergency, but by giving more CSF you get a quicker recovery. And that saves time in hospital for the patients. For reasons like this, the CSFs became licensed for use and have now been used in more than three million patients.

It is probably not the best use for the CSFs and it's certainly not the way the body does it, but because of the way these CSFs were developed you're now talking about biotechnology companies that have licence or patent positions: one has GM-CSF, another has G-CSF, and another has M-CSF. The companies don't talk to each other and they don't permit clinical trials that would allow me, say, to combine GM-CSF with G-CSF. It's no good for me to say, 'The body uses combinations of CSFs, because it's more efficient, you get synergy. Why don't you do that in patients?' To this day it's never been done in patients, simply because each company is an empire to itself. So you can't do the clinical trials and therefore, you can't get licences for their combined use. A decade after the first use of CSFs, they are still not being used correctly – in combination with one another – nor are they being used extensively for probably the ideal situation, patients with infections.

If you were smashed up in a car accident and had compound fractures, pounds to peanuts you would get an infection. Wouldn't it make sense to start having shots of CSF right now, to crank up production of new white cells and make them work harder to stop any possibility of getting an infection? It's not done. First of all, the clinical trial hasn't been done, proving it. Why not? Because it's too hard to round up 200 car accident patients; it's easier to round up 200 cancer patients. What's more, the company would charge you $160-odd per ampoule, and in this era of economics there may be no cost-benefit to the hospital. Much better you get the infection and then be treated with penicillin, which will cost $2. So it's a competition between good medical biology and *Realpolitik*: it's cheaper for you to get antibiotics. And if you ask, 'Well, you can make that CSF for $2. Why can't I have it for $2? Then it would be okay,' you don't get an answer. The company simply says, 'Ah, but we need the money to do research' – which it does by duplicating what is done in the universities. So the public pays twice.

For whatever reason, these CSFs, which are very powerful and do work as single agents, are not yet being used in the right context. They are not being used for the right sorts of disease or in any combination that makes much sense. So it's still early days after a 30-year history of development, figuring out how to get round the problems, finding out to our delight that they are effective and that they're not particularly toxic. You can't assume that a

natural body product is not toxic – some of them are the most toxic agents known – so we had a bit of good luck there. And they do work. But I'm sure they can work much better.

Hastening slowly

You must be keen to reduce the distance from the bench to the bed, from the research unit to the patient. These things could have come on stream 10 or 12 years ago, but they haven't yet. Does that frustrate you?

They do take time, yes. On the other hand, you don't want to make mistakes. One or two growth factors have proved to be lethal – literally – and if you were one of the first patients in a clinical trial, you would not like to be dead now. So sometimes hastening slowly is an inevitable part of the equation. The CSFs were licensed within about five years, which was some sort of record. But things are a bit slow, and it must be tough for a patient to see a drug coming along but not yet ready.

What's the role of pharmaceutical and biotech companies? Are they the good guys or the bad guys? It costs $200 million to get a drug into the ward, and you can't afford too many mistakes. You will go broke if you spend your $200 million on a drug that's the perfect treatment for one of only six people in the world who have the disease. So there are constraints. Maybe it will be possible to develop simpler clinical testing programs that still have safeguards in them and are ethical, but it is a problem. And it's slowing down. I have talked about four CSFs. We ourselves have discovered other growth factors and meantime the rest of the field has discovered maybe 20, so they're all available now for potential use in patients – but some of them are not yet under test and some may never be, for economic reasons.

Can virtually all the steps in haemopoietic development be accounted for by factors that have now been isolated?

The factors known now may not be the best, or the final ones, but for four of the eight blood cell families a clinically used agent is now available – erythropoietin for red cells; two CSFs for white cells, and thrombopoietin, which may be licensed soon, for platelets. But companies are taking the view, 'Well, you've got one good agent for each of those families. What more do you want? There's nothing in it for us to spend money developing a second agent active on one of these families.' Well, it's a little more complicated than that, but there are many other agents available and some still to be discovered.

CSFs, receptor chains and cells with a mind of their own

We've talked about the purification of these substances, the cloning, the preparation. But these are also fascinating glycoproteins with interesting binding sites, and they can bind on multiple receptors in cells very widely

around the body. Perhaps you would just take me into that biochemistry.

CSFs are quite complicated, large molecules. They are big because they have two working faces that are going to make contact with the two receptor chains on the cell surface, which are some distance apart, and you've got to have a scaffolding that will hold those two working faces apart.

All of the growth factors work on the basis that there are at least two receptor chains. Sometimes the two chains are identical; sometimes, like the GM-CSF receptor, there's one little chain and one long one. Contact is made first with the little chain and then the whole complex makes a further complex with the big chain, and it's the tail of the big chain that sends out the different instructions to the cell – 'Divide,' 'Mature,' 'Do something,' 'Work harder' – coming from different parts of the receptor chain.

I think that in the early days you were scorned for suggesting that there were multiple messages, controlling so many actions.

Well, there is a physical basis for it. Our ability to say anything sensible about receptors depended on our developing techniques to clone the genes for these receptors – which we did – and then to make mutations along the receptor chain and cut out or change bits, showing that one or other function is lost. There is still a lot we don't understand about the details of this, but in principle, yes, the different sorts of signal are coming from different regions of the receptor. And the astonishing thing is that on any one blood cell there would only be about 300 of those receptors. That sounds a lot, but it means one here and one about half a mile away, and they need to talk to each other, actually to bind to each other. So they're probably occurring in clusters on the membrane – and that's not understood, either.

Okay, suppose you're a cell. Unless you have a receptor for a particular hormone or growth factor on your surface, you have no interest in it. Control by hormones is very much a passive thing. You, as a cell, make your own decision: 'I'm going to make a receptor for that hormone, and then I will listen to whatever it's telling me. What I choose to do when I hear the voice of the hormone is my business. I might choose to divide, or I might choose to make more lysozyme to kill bacteria. I'm going to think about that.' So it's a passive control system. The hormone has come along and locked on to the receptor (it can't choose to lock on to some other cell where there's no receptor) and what the cell does following that is it's own business. That's what we don't understand very well.

We know a lot about the hormones that control the cells and how they act through the receptor, but we know very little after that. We know some of the biochemistry but we don't really have a good handle on the basic ground rules of what's allowing the cell to do this or that. Plenty still to be done there, and that's part of what we're engaged on now. This problem of receptor signalling

is a real nightmare – conflicting data which all has to add together somehow.

Cells learning to behave badly: can leukaemias be suppressed?

Perhaps we should return to the 1980s and the leukaemia story. By then you must have been paid very much better at the Hall Institute than when you went there on your Carden Fellowship.

I've been on the same Fellowship for 43 years now, and they are bitterly regretting not writing in a termination date on it! However, I earn them more in royalties than they pay me.

Anyway, I was paid to do cancer research and was supposed to be finding out the cause of leukaemia and doing something about it. It turned out that my original idea that got me into this field was correct: just as Jacob Furth had shown with his endocrine tumours, if there is an imbalance in the control of blood-forming cells, if you are being driven too hard to divide, that's one of the abnormalities that will lead to leukaemia development. It's more subtle than that. The blood cell itself has to learn how to make its own growth factor, so-called autocrine growth factor production. Why, I don't know. You can surround a cell in a sea of growth factors and it won't behave as a leukaemic cell. But the minute it learns how to make its own growth factor, it's somehow different. That's one of the two big changes it needs for transformation from a normal to a leukaemic cell.

All of that was being worked out by us in model systems during the CSF development period, and so we ended up – at about the same time as we had the first recombinant CSF available to inject – actually showing by a formal model that you could transform certain cells to leukaemic cells simply by putting that CSF gene into them. Once they made their own CSF, suddenly they were leukaemic cells.

It also turned out when we were studying receptor function and the multiple actions of CSFs that you could suppress leukaemias. And which part of the receptor is issuing that instruction? It's the same part that says, 'Differentiate.' But that's still unfinished business. How do you control the decisions a cell is making about whether to self-generate – to make two daughters who are like the parent – or to have one or other of the daughters say, 'No, I will now mature'? It takes us back to the problem that we don't understand the molecular control of which genes are allowed to respond when a signal comes to them.

So leukaemia development is in a quite complicated state at the moment. Others have found specific genes that are associated with different types of leukaemia. Every one of them has to be fitted into this basic model of learning how to make your own growth stimulus and getting perturbed in the way you strike a balance between self-generation and differentiation. At least, that is

what I think – but all of those statements are not yet watertight. So part of my head still works on leukaemia development and part is still working on the biology of blood cell formation.

No more bone marrow transplants

We made an interesting observation that, when we started to inject patients with G-CSF, instead of all the ancestral cells living in the bone marrow, many turned up in the circulating blood. So it occurred to our clinical colleagues that if you were having chemotherapy and then needed a transplantation, instead of painfully having a litre of bone marrow sucked out you might just collect the cells from the peripheral blood after some days of injection of CSFs. Those cells have turned out to work much better than bone marrow cells. They regenerate much more quickly, and it's a much simpler technique – no anaesthetic, the patient reads a book, cells are collected, the rest of the cells are returned to the patient, and that's it. As a by-product of using these colony stimulating factors, bone marrow transplantation is now an obsolete procedure. Nor is it a big deal in a hospital. You don't always need a special ward set aside for bone marrow transplants; you can now often have the transplant as an out-patient procedure because it's fast and it's simple. That has had an impact on the way cancer patients are treated, because now with high doses of chemotherapy they can now have their bone marrow restored with peripheral blood stem cells.

So unexpected things turn up all the time. But a major problem persists: we still don't really understand how blood cell formation is controlled, although we've got clinical agents that are highly effective. This is similar to giving diabetics an injection of insulin without understanding that diabetes is an auto-immune disease and this is not ideal. We'll get there. It just takes time.

You say that you can provide a whole range of stimulating factors, with clear properties, but that the cell makes the final decision, based on its genome, of how it responds to the information.

Yes. Fortunately, it's not too common for the cell to act bizarrely, but leukaemia is one example of a cell population that is misbehaving – probably misbehaving because of genes that should have been turned on or off. One day perhaps cells can be made to behave instead of just being killed with a shotgun-like chemotherapy.

On the bottom line, though, is any of the material that you've developed being used well in leukaemia?

It's being used most commonly in leukaemias where the cells don't have receptors for the CSFs. If a child has got lymphoid leukaemia, the lymphoid cells don't have receptors for the CSFs and you can stimulate the rest of the marrow to your heart's content – no worries there for the clinician about

stimulating leukaemic cell proliferation. But the ability of growth factors to actually suppress leukaemic cells is so subtle that once we figure out why leukaemic cells don't always listen to those signals and behave the right way, using that ability might turn out to be a much more elegant way to treat leukaemia.

Another mysterious multiple-action growth factor

What about leukaemia inhibitory factor, LIF?

What's outstanding about it is that it was discovered and developed because it was a hormone that could make leukaemic cells suppress themselves. This work was done in the late '80s, in an attempt to resolve a controversy (this time between Japanese workers, Israeli workers and us) as to which was the real CSF that suppressed leukaemic cells. It turned out that everyone was right, because different cell lines responded to different agents, but in the hassle we came up with a novel factor that the Japanese had described but not managed to purify. We bulldozed our way through, purified and cloned it first, calling it LIF. It turned out to be a most mysterious molecule – a major player in regulating brain function, how the pituitary produces hormones, how fat cells take in lipids, how muscle cells regenerate.

And even the performance of gonads.

It has been in clinical trial now for stimulating repair of neurons. But it illustrates a problem we are finding more and more, that the body is using control chemicals that don't make sense to us, that are able to influence too many tissues. There are no diseases where you have something wrong with your brain, and your bones, and your blood cell formation, and your liver. There is no disease that combines those four different tissues, and there's no stage in development where it would make any sense at all for the same agent to control all four. Yet that's what the body is doing. That tells us, I think, that we don't understand too much about the body. LIF is itself an interesting factor but it's pointing to a phenomenon which, if you think about it, make you very uneasy that you don't really understand tissue and organ biology.

Lessons from the past

We've gone right through the 1980s. LIF is obviously a part of the '90s. What now?

Most of the '90s we've spent discovering different receptors and finding the genes encoding different receptors. There turns out to be a little region just on the outer edge of the cell membrane that is common amongst a large number of these receptors. This allowed us to recognise that in fact there is a big family of receptors that are obviously all related. Way back when we were a single-cell organism, we probably had only one hormone and one receptor system. Now

it's got a little fancy, but you can use that common region to pull out other receptors, and we've done a lot of this type of work.

A major activity now is that, for one reason or another, we've had to go back to whole animals, to establish just what role each of these many different hormones plays in the body. We know what they do in a tissue culture dish, we know they work in an animal, but how does it all fit together in the animal? Which ones overlap in their actions? Which do you really need? So you have to build mice from which you have knocked out the gene for one or other of these hormones. Each one of these can be a $2 million experiment, unless you're lucky, and it can take decades to figure out then what's happened in the animal. But this is the era of knockout mice or knockin mice, where you put another gene back into the space that one used to occupy. We have gone back 30 years, if you like, to working with animals again – but armed with very sexy *in vitro* assays or molecular probes that are, hopefully, helping us through the wilderness.

It's amusing that there are now very few old-timers like me who can work with animals and animal pathology, and so our Institute is full of beautiful models not being worked up properly because too few trained biologists or a pathologists are around. If you have a young son and you want to give him good advice, tell him to become a biologist or a pathologist and he will be in eager demand anywhere. These things are cyclic. We went through a phase where biochemistry was the glamour science, then we had molecular biology – and it still is glamorous, but even molecular biologists are realising that they're into big trouble unless somebody comes and tells them what they've just done.

We have talked about the evolutionary background of receptors. Are the CSFs directly related through evolution?

I wish I could tell you. We once studied kangaroo urine. We were so determined to write a paper that the Israelis could not accuse us of stealing that we decided to look at quokka urine from Rottnest Island. That was pretty distinguished starting material but it was not a great experiment. (Don't knock kangaroos, though. They're different from us – they've only got seven chromosomes.)

But if you are asking how far back CSF genes of this type go, I don't know. People haven't really looked. Has *Drosophila* got CSF genes? I think it might, but I wouldn't swear to it. Do bacteria have what amounts to one master CSF gene? How do they kill their own parasites? I don't know.

The standard party line is that there is probably some divergence in CSF evolution. If you find a whole bunch of receptors that have the same spaced structure, you say, 'Ah-ha, these guys have to be related, so probably they have all come from one master type of receptor.' The rest is guessing. Did the dinosaur only have one sort of CSF? One assumes it may well have done so,

but all you're saying is they are related, therefore ancestrally they may be derived from a common source. That's probably safe – the details could be worked out but it's a slightly boring taxonomical problem and nobody would get a grant to do it. Perhaps in my retirement I'll go and look at *Drosophila.*

Keeping administration and finance in their place

The Hall Institute has been the backdrop to all this research. As deputy head of the Institute for some 30 years, did you find your role as an administrator a comfortable one, or did you tend to keep it strictly to one side?

I wasn't plagued too much by administration. Anything I had to do, I did with dispatch. I'm not greatly in favour of democratic ways, so I'd rather do it myself and get it over with. But I was lucky: Gus Nossal, my Director, was talented and hard-working, enjoyed the publicity, and therefore did not put any great burden on me. In all truth, it was not a problem. We had an arrangement that if he was out of the country I would run the Institute; otherwise, he would run it. And he wouldn't offload scut-work onto me – everybody had some, but I had no more than most unit heads. For the couple of years when I was Acting Director, it was still possible to get a day's work done as well.

Such a large institute must need considerable funds. Has your work put significant new life into it through royalties?

You raise that question on an interesting day – royalty payment day. The answer is no, most institutes don't significantly benefit financially. The amount of royalties we get is about five per cent of our total budget. It's useful to have, it's a nice little safety reserve, but it does not make or break us. You can argue incessantly whether, if we'd played our cards better, we would now have mega-million dollar royalties, as is theoretically postulated. I think the answer for us in Australia was that one way or another we were going to lose things to the US or the Japanese and never make those mega-million dollars. Much to everyone's slight dismay, institutes now do have to watch what they're doing in terms of royalties and patenting. That's a fact of life. But does it provide an important of funds? No, it's the icing on the cake.

A time for impatience

Whether or not you decide to look at Drosophila, *I don't see you being retired, Don.*

Well, I am retired but it means working about three times harder, with about a third of the pairs of hands to help me. Research in retirement is not a game for the weak-willed, but it seems to me that I've finally figured out how to do research and now might as well capitalise on it. I've already made all the mistakes – or most of them, I hope.

Do you find now that the young minds around you play a critical part in drawing you into new areas, away from a fixation on a particular line of research?

That was always true. I am a workaholic: if you present me with 1000 culture dishes I'll sit here till I have counted all the colonies on them. Will I say, 'Hey, this is getting me nowhere. I think I'll clone a gene'? No, I won't. I have always depended on being in a team of colleagues who say, 'Listen, this is stupid. It makes no sense. Let's do that instead.' And I go along with it and say, 'I'll do my part of it; you do your part.' So now they say, 'Let's look at signalling genes,' or something else, and unless I think it's too outrageous I go along with it.

With time you tend to develop a very restricted knowledge base. I know what I've done and what I'm doing, and I may know what you have done because you're doing something related. But I don't have time to read the literature – I have no interest in finding out what Joe Blow did – and therefore I'm very ignorant and dependent on my colleagues for news of new things now able to be done. I may well be able to think of how to do them properly. Restricted knowledge, I think, gets worse with age. You tend to become impatient. I dislike refereeing manuscripts that journals send me. I can't be bothered reading what somebody else did. I'm realising that I am running out of time: I've only got time for a few more experiments, and I want to do them and not be stuffed around and deviated onto other projects. So I can no longer be bothered to read the literature or to write reviews.

I want to do my own experiments, which I feel are probably going to be novel enough that I won't accidentally repeat what somebody else has done. There's a bigger risk that I will accidentally do something I've done previously but forgotten. My colleagues take great joy from the fact that I forget so much. On four occasions now I've actually done experiments, repeated them with growing excitement, drawn up figures and got ready to write them up – only to discover that not only had I done the experiments before but that I had already written them up and published them! I have no memory – I can read a novel and three weeks later not remember the plot at all. So while someone else might have said, 'Hey, why don't we do this? Oh, but we already did it,' I don't have that cut-off and I'm at risk of doing the same experiment again. But is it exactly the same experiment that someone else is going to do? Not likely.

For a non-existent memory, yours has done remarkably well this afternoon.

Selective memory is a wonderful thing.

Personal impacts

Could we look now, just briefly, at some of your relationships with your family and your patients? I think that sometimes you have an opportunity to see what

you have achieved for your patients.

Yes. It happens in a number of ways. I have a photograph of a young lad who is now able to deal with a disease in which his white cell production stops every 18 days, so infections occur. The family could never go on holidays, because – predictably – the child was always sick. He now injects his G-CSF daily, just as a diabetic would inject insulin, and is essentially in normal health.

It's interesting; you do run into patients in the supermarket who say, 'Oh, I have had CSF treatment.' (They may feel it did them more good than it really did.) You need to keep in mind when giving public lectures that there's probably somebody in the audience who has had that treatment, or whose relative has. That is an uncommon thing to happen in medical research. Most research workers spend their life knowing that their work is not likely to have a direct impact on clinical medicine.

I don't visit the wards, even though you might think that's strange for a medical graduate. For whatever reason, I don't go, and so I don't see the patients in action. I suppose I am miserly of my time. I think I could do a few more experiments, rather than indulge in a bit of self-gratification.

Does that miserliness with your time affect your family life? Do you see your family?

Well, if you start work at 7 and don't get home till 7, and then work 5½ days a week you are sometimes not too popular. I admit that it's a big problem. Families of research workers can have difficulties that way, but then the spin-offs like going on sabbatical leave and living in other countries partly balance it.

What of the wife who has been such a supporter over the years, Don?

Josephine was a nurse who trained in Sydney, worked in Melbourne and raised four children. The children moved as far away from science as they could think of, becoming lawyers and teachers and painters. It is difficult to know, isn't it? You could say, 'Why don't you play chess at home?' but your daily scientific problems make a chess game look so infantile that there is no appeal. Maybe this is being selfish and you should play chess even though it's not quite as interesting as what you were doing. It's difficult to know sometimes how best to divide your time between your family and your research.

On that note, then, I'll say good afternoon to you, and thank you very much for such an enormous amount of information about your career.

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