Good Science, Strong Bones, and the Case for Supporting Discovery

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By

David M. Findlay

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INTRODUCTION

I am a working scientist. I have a Bachelor's degree in biochemistry, followed by a Master's degree in endocrinology (the study of hormones) and a PhD in bone biology. I have worked in these areas as a researcher for more than 45 years. I am passionate about the wonderful, complicated world of biology, and I would love to share some of that world with people who do not have science training. My friends and family sometimes ask "What are you doing now?" and I find it challenging to give them an answer that they find satisfying. Many times I'm sure they regret asking the question because my answer comes across as "Why do you ask? You won't understand my answer anyway. My work is way too complicated to give you a short one sentence answer." Such responses on my part are completely unacceptable. It is, after all, the tax-paying public who have paid for my work over decades. They have every right to know how this money is being spent, and as a scientist I should work hard to make my work intelligible, meaningful, compelling and exciting, regardless of people's education or background. I should be able to explain how my work fits into a bigger picture and why scientific research takes time.

Perhaps I am embarrassed that my work has not resulted in a major discovery. I should not be, because most scientists do not make major discoveries in their whole career. What a waste of time and money, you might think. Well, perhaps, but an analogy might be the building of a house- most of the construction time is spent on parts that are not visible in the finished product. Similarly, most scientific research is not 'visible', but contributes to a huge body of work, from which discoveries arise. My job as a scientist is to put pieces into a very large jigsaw, and every so often some of the jigsaw will have enough pieces in place to discern an important part of a much bigger picture. At that point discoveries happen, be they a new treatment for a disease, or a new test that leads to diagnosis, or a new management

approach that changes the course of a disease. Such discoveries are happening all the time, but for those outside of science they are only visible when they impact in a tangible way. I have been privileged to witness this process during my career- pieces being placed into a jigsaw until a part of the picture was clear enough to make sense of it. An idea going from nothing to a very useful, and in some applications lifesaving, drug. Now, towards the end of my career, I want to tell this story, with the hope that if I tell it well enough you too will be excited by this specific story, and by the scientific process more generally.

The story I wanted to tell is of the discovery of a protein called RANKL. RANKL turns out to do lots of things in the body, but I will focus on its role in bone and cancer. By telling this story, I want to show the way that science works, who does it, under what circumstances discoveries can happen, how difficult it is and how exciting it can be when it all works out. I wanted to hear from the people who contributed to the story in their own words, and I am very grateful to the scientists and doctors who have made this possible by sharing their stories with me. These are real warts-and-all people, who in all cases got involved with research because of the thrill of the chase. They are all smart people, who could have done many other, probably less frustrating, things. But they are too curious, too interested, too sleuth-like, too ornery to do repetitive jobs.

Today, many of the ways of doing the research described here would be different, easier, more automated, faster, and more high-tech. But regardless of the different methods and instruments that come along, discovery still comes to those with good enquiring minds, people with strong powers of observation, people who go with the evidence, people who read the literature, people who reach out to other researchers and share ideas, and people who are brave enough to take a chance. This book is dedicated to good scientists- the brave, smart, altruistic, honest people who work to overcome disease and to improve the world. And to you the reader as you encounter the wonderful world of scientific research, and a discovery of significance that emerged from it.

CHAPTER 1

SCIENCE: WHAT'S TO LIKE?

"I'd like to put in a vote for the intrinsic fascination of science."
[Joshua Lederberg, American biologist who won the Nobel Prize in Physiology or Medicine at the age of 33]

There are some things I would really like. I would like for people- for you, the reader- to enjoy science. To be curious. To thrill to scientific discovery. Because I am a biological scientist, I would particularly like for you to enjoy the branch of science called biology. I would like this because I'm convinced that being more aware of the complexity and awesomeness and improbability of the world of plants and animals and insects would help people to delight more in the living world and in life itself. I would also like it because it would help people to understand how their (your) own body works, how to look after it to keep it working, and why it goes wrong in disease. I would like this because I'm (almost) sure that this understanding would lead to better choices, both to stay healthy and to respond to disease when it happens.

Maybe I can't come up with a way for you to get interested in biology if you are not already, but I do have a cunning plan. It goes like this. We recently went on holiday with a friend who is an avid bird observer. Not a casual bird observer, but the type who will go to extraordinary lengths to observe all possible bird species in a given location. Absorbed. Obsessive. Binoculars at the ready. Bird book frequently referred to. Car brakes suddenly applied because of a possible sighting. Extreme disappointment at missing out. Exaggerated satisfaction at finding. Now, the two possible ways to respond to this behaviour, at close quarters, on a holiday, are (a) to go mad or (b) to join in and also start to observe the birds. You can

regret ever agreeing to the holiday, or you can start putting binoculars to your own eyes and seeing the birds yourself. I chose the latter, and the more I saw, and the more I learnt from my fount-of-knowledge friend, the more enjoyable it became. So, my 'cunning plan' for you to start enjoying the living world more is to ask you to begin actually looking at it. Closely. Often. In the places where you live.

Here's what I mean.

Look at those ants crawling on the kitchen bench. Maybe you've always seen them as pests and sources of annoyance. But what if you were to see them as little marvels? Then you might be full of questions, like the kid you once were. How do the ants know that you've left food on the bench? How can they walk so far from where they live to get it and how can they carry so much? In fact, how can they even walk with such small legs? Where are their muscles? Where does their energy come from? Do they have bones (kind of but their 'skeleton' is on the outside)? So you follow the ants down the bench along the floor, and out the door to where they're coming up from a hole between the pavers in the patio. How do they live down there? What does the colony look like? How is it organised? Fascinating!

What about those parrots that have descended on your apricot tree and are helping themselves to your not-quite-ripe fruit. You might be irritated that those birds are disrupting 'your' world. But think about parrots. They build nests and lay eggs and hatch and rear their young in complicated domestic arrangements. They all look the same and sound the same but they seem to know who is who. The parents seem to recognise their young. The young quickly grow feathers. Have you looked closely at a feather lately? Incredible structures that are different in different parts of the bird and that serve multiple functions. Insulation, to keep the birds warm or cool, or dry; display feathers to impress a mate; feathers that enable the bird to fly. And this whole flying thing. How do they do that? How do the young birds learn to do it so quickly and with such a high success rate? And how do they navigate- maybe just a few kilometres to your apricot tree or

maybe migrating across hundreds or thousands of kilometres? Intriguing!

And then there's the apricot tree. You planted it as a small tree- just a twig really. And in the spring that seemingly dead twig sprang to life. Buds on the single wooden twig swelled and leaves grew out of them. The leaves were completely different from the wood. The next year the tree was twice as big and some of the buds produced flowers. Explain that! A year or two later, the flowers somehow turned into fruit, which were complicated structures containing delicious juicy flesh with a hard wooden nut inside. The nut was capable of producing a whole new tree, and therefore seemed to have all the information in it for wooden branches, leaves, flowers and fruit. You see- if you walk around with your eyes open (observing) and your brain switched on (making interpretations based on those observations), the world is really quite incredible!

And then there's you. What do you know about your own body? Or maybe the question should be- what don't you know about your own body? How does it do what it does? Why does it go wrong? Why does it work less well when you get older? Why do bones break more often in older people? Many people hardly think in any detail about their body until something does go wrong. But life is much more fascinating and makes a whole lot more sense if you do try to understand why and how your body does what it does. Take just two bodily systems. The digestive system, all the way from eating food, to digesting it, to taking nutrients from it into the blood, to excreting the waste- how does it work? How do you swallow? It's kind of conscious and kind of not. What makes the stomach produce acid when you eat? Why does the acid not destroy the stomach? What controls the valves at each end of the stomach that prevent you bringing up acid into your oesophagus at one end and enable release of your food for further processing at the other end? What makes the pancreas release digestive enzymes into your gut to digest the food? How do the nutrients from the food get into the blood? Why does the pancreas release insulin and other hormones after you eat? Why do you feel hungry before a meal but not afterward? How do the parts of the digestive system 'know' to do what they do? (What happens if

they don't?) What controls the waves of muscle contraction that push the food through the gut? What do all those bacteria in your gut do? What controls the expulsion of waste from your gut? What goes wrong when you have diarrhoea, or inflammatory bowel syndrome, or gluten intolerance? Why are some people allergic to various kinds of food?

What about your reproductive system. You are either a parent or you had a parent, so how did you or your child come about? Sure- there's the easy obvious answer. But think about the components of sexual reproduction- the male components and the female components. You think you know what happens, but what controls what? How do people produce sperm and eggs, dividing their DNA by two in the process? How do these amazing cells get together, now with a half the mother's and half the father's DNA? How do fertilised eggs divide to produce more and more cells and then attach to the womb inside the mother, who somehow produces a placenta to nourish the growing embryo? How do all the different parts of the body arise from just two cells? What is the stimulus for birth? Why do things go wrong? Are you curious about this? Do you think it's amazing? Do you wonder how people know about these processes?

For me, the whole of life, be it human or any living thing, is inherently absolutely fascinating. But to really make it fascinating, you need some knowledge of it. And I mean knowledge of real facts about it and not some half-baked, poorly informed version of it. And how would we know about it? Well, of course knowledge comes from all kinds of places and all kinds of people, and has built up across historical time. But the people who find real knowledge have one thing in common. They look. They see. They observe with an open mind. They check their observations. They make cautious interpretations about what they see and they accept that more information might change those interpretations. The heavy-duty knowledge these days usually (but not always) comes from scientists, who are actually paid to observe in a rigorous and objective way. Good scientists produce reliable knowledge because their studies are performed and analysed rigorously and are subject to the highest level of scrutiny by other scientists. I think we kind of know this but

what most non-scientists do not know is this: how does science work? How are discoveries made? What is an example of a discovery from start to finish? From an idea to something useful at the end. Actually, what is 'the end' for a scientist, or does one thing always lead to another?

The following chapters will trace a discovery that has had a profound effect on the treatment of several human diseases and explores how that discovery came about. What kind of people were involved? Where and how and when (and over what period of time) did it happen? What were the contributions of cleverness, hard work and sheer luck? What was the environment for discovery? What resourcing was needed for discovery? The discovery described here relates to a single molecule produced in bone (among other tissues), the identification of which led recently to the development of an important new way to manage diseases of bone loss, such as osteoporosis and cancers involving bone. In addition to these practical outcomes, this discovery has helped to make sense of a whole lot of biology, from bone to the immune system, to cancer, to the brain, to the breast, to the gut, and more. And importantly, although the details are different, the pathways of discovery of other things, whether they be in biology or in other sciences, share many commonalities. In particular, almost every medical discovery has involved a pathway of chance findings, doubt, brilliant intellect, blockages, leaps forward, acceptance, uptake.

In this book, I want to persuade you that biology is something to be excited by. To do so, I have sought to make the process of scientific research and discovery accessible to everyone, regardless of education or prior knowledge. I have taken as an example a discovery that I am most familiar with. My aim is to open the door into a part of our society that is not only exciting and wonderful, but also incredibly important to every one of us. Unfortunately, it is a part that too often remains unnecessarily obscure.

Anyway, let's go to the beginning.

CHAPTER 2

CURIOSITY

"Curiosity is one of the great secrets of happiness".
[Bryant H. McGill, thought leader and best-selling author]

I'm not sure where it all started. But for me it started when I turned up to begin a new job at the Repatriation General Hospital in Heidelberg, Victoria, Australia. The old mortuary had been converted into our 'new' (rather rudimentary) laboratory, and the work to convert what had been a mattress sterilising room (from when tuberculosis was a big problem) into part of the lab had yet to be done. To get to the door to the lab, I had to make my way through the water and suds of Commonwealth cars being washed by their drivers, whose job it was to bring old Diggers (Australian war veterans) to hospital for their appointments. I had, against the advice of saner friends, resigned from my secure hospital scientist job to enter the precarious world of research. Typically, my decision to do so was based on flimsy advice. In this case, I was persuaded by the larger-than-life, very Italian, Valdo Michelangeli. Valdo had been employed by a brilliant young Australian doctor-researcher, Jack Martin, to work with him in Sheffield in England. Jack had been appointed Professor of Chemical Pathology in Sheffield, while still in his 30s. Then, as now, that was remarkable. Valdo had worked as a researcher at the Austin Hospital in their hometown of Melbourne, Australia, at the same time as Jack and had accepted Jack's invitation to help him set up and run a lab in Sheffield. This worked well for Jack and for Valdo- for Valdo because it enabled him to buy a Rover, an unusually prestigious car for an Australian researcher, and to travel around Europe. But after his stint in the UK, Jack wanted to bring his family of six kids back to Melbourne, where he had been appointed to set up an outpost of the University of Melbourne at the 'Repat'. This appointment involved organising medical student

teaching in the hospital, seeing patients, and doing the thing that excited him most- research.

Valdo returned to Melbourne ahead of Jack and worked at St Vincent's Hospital, where I was employed in the Endocrine lab. We shared a tea-room, so I got to know and like big Valdo, who somehow convinced me over time that I should join what he assured me would be an exciting new enterprise at the Repat, with Jack at the helm. I suppose I was ripe for the picking because my mentor, the amazing Don Chisholm, had given me a taste of research, within an otherwise fairly routine lab job at St Vincent's. Finding out new things and being on the cutting edge of knowledge appealed to me much more than the sameness of diagnostic work. And the timing was good- Jack's return date would give me a chance to bundle my research into a Master of Science degree before starting something new. In addition, Don Chisholm was returning to Sydney, where his family had lived from the time of his ancestor, the famous Caroline Chisholm of the original Australian $5 note. I therefore convinced myself that now was a good time to make a move. I do not remember having an interview- I think Valdo's say-so was good enough for Jack, who disliked 'procedure'. So, suddenly, and without really thinking it through, or having an appreciation of the downside of research- the uncertainty of funding and the ever-present possibility of ending up without a job- I found myself leaving the world of diagnostics, and of diabetes research, and a secure hospital pay cheque, and entering the world of bone research in a University of Melbourne lab at the Repat, where my future employment would be dependent on grant funding. However, my new job gave me a front row seat to watch one of the great stories in medical research unfold in front of me.

That was 1978. Not much was known about bone then, certainly when compared to today. And I suppose the key question was and is: why would you want to know about bone? Isn't all the interesting biology, isn't all the important research, aren't all the real medical questions about cancer and diabetes, heart and immunology, and reproduction and brain? Well, obviously I'm going to say no. Fairly emphatically no. If you have ever had a fracture (I've had four and

my sister recently had one while out on a hike with me!) you might share my view that bone is important. You might agree that fracture, and the pain and disability that suddenly engulfs and consumes you, focuses your attention like few other things can. You want it fixed and you don't want it to happen again. But fractures do happen with monotonous regularity- the numbers show that there is one fracture every 3 minutes or so in Australia! And many of these occur in older people, due to a condition known as osteoporosis. You will almost certainly know an older person who has had an 'osteoporotic' fracture. So, in 1978, and equally today, you wanted to study bones so that you could understand enough about them to prevent as many fractures as possible. Simple as that.

I guess I knew the basics- that bones are made of hard stuff, and are essential for muscles to attach to, so that we can move around. I knew that the hard stuff was made of mineral, which was a combination of calcium and phosphorus' and proteins (mainly collagen), running through the mineral to give the material strength without it being brittle (a bit like strong and flexible reinforced concrete compared to brittle china crockery). But how was the bone formed? How did bones get bigger during growth? How did bones repair after fracture or surgery? And what happened to bone to make it more likely to fracture as we grow older? Well, when bone was examined down the microscope it was clear that bones were not at all like china crockery. Bone contained blood vessels and nerves running through it and was both covered with, and full of, living cells. But what did these cells do and how did their presence relate to my questions about growth and repair? What might they have to do with the increasing fragility of the skeleton in older age? And, importantly, what regulates all of this? If you knew what regulated it, could you use that knowledge to intervene to make bones stronger, to prevent fractures?

Let me insert at this point enough information about bone cells so that the rest of the story makes sense. There are three main types of bone cells, which have been given the names osteoblasts, osteoclasts and osteocytes. The 'osteo' bit in each case comes from the Greek and means relating to bones. 'Blast' signifies growth or formation, so that osteoblasts are the cells responsible for the building/forming/growing

of bone. They do so by laying down a soft material called osteoid, which then becomes mineralised to form the hard tissue that we know as bone. During this process, while most osteoblasts remain on the bone surface, some are incorporated into the osteoid and then the mineralised tissue, where they are now called osteocytes ('cyte' simply meaning cell, or mature cell). During this incorporation process, osteocytes undergo a remarkable change of their shape and function: they develop long cell extensions, like skinny arms that reach out to surrounding osteocytes and to cells on the bone surface and to blood vessels. These cells have numerous functions, some of which are still being discovered, but that include the ability to detect and respond to loads placed on the bone. Osteoclasts are the cells (the 'clast' part describes cells that destroy the material outside of cells, called extra-cellular matrix; this extra-cellular matrix includes the material of bone), whose job is to break down or resorb bone, in a process known as bone resorption or osteolysis.

There were many questions about bone when I came on the scene. Importantly, 'we' did not know very much about why older people had so many fractures, and I in particular knew almost nothing. So to find out what was known before 1980, I asked two people who were really paying attention at that time- the aforementioned Jack Martin in Melbourne, and Timothy Chambers in London. I had left Jack's research group many years before, eager to test myself independently after Jack's excellent mentoring and example. Now in 2018, I arranged to visit Jack and found him in his office at St Vincent's Institute of Medical Research in Melbourne, just a few days after celebrating his 80th birthday. It says a lot about the man that almost everyone who had worked with Jack turned up at that celebration. Many of them had become heads of hospital and research departments, and had themselves made major contributions to medical science. Jack was working on in his 'retirement', freed of the responsibilities of running things and able to concentrate his formidable mind on research. He was still getting grants to fund his research, still writing papers, attending conferences and receiving invitations to speak at meetings around the globe. He was still able to get excited by new bits of information that helped to fill out the huge complicated jigsaw that would eventually describe how bone

worked. To Jack and many of his contemporaries, research was not work- it was indulgence. It was fun. It was a never-ending series of problems to be solved. And retirement in the normal sense was therefore rejected because of the need to keep feeding that curiosity (and perhaps ego!).

Jack started doing research as a young doctor and has never stopped. He was influenced by a clinical biochemist at St Vincent's Hospital in Melbourne, a Scot named John Owen, who was interested in the biochemical mechanisms of disease, and Jack thought that was really interesting too. He was under the impression that he also needed to become a chemical pathologist to do this kind of work and started to go down that path, a course of action he now describes as being due to naivety. But he soon realised that if you were a chemical pathologist the work was far too repetitive for him- he described it as "running 1000 marmites a day"! But taking this track was not all bad. In fact it resulted in the opportunity for Jack to undertake a stint at the Hammersmith Hospital in London, where, in the middle of the "seriously boring" project he was given, he met the ebullient Professor Iain MacIntyre, who was a co-discoverer of the hormone calcitonin. MacIntyre's influence on Jack was long-lasting, and had spill-over effects on me, since it led eventually to me embarking on a PhD to study the action of calcitonin. When Jack returned to Melbourne, his mentor at the Royal Melbourne Hospital was Roger Melick, who had been trained in endocrinology (the study of hormones), in Boston, by the very famous Fuller Albright. Albright was a true pioneer in this discipline and made major contributions to the understanding of diseases due to hormonal problems, to the extent that some are named after him. Among the diseases that he managed to make sense of, were those due to abnormalities of parathyroid hormone (PTH) secretion, and after a stint with Albright, Roger knew as much as anyone at the time about PTH. The Albright/Melick/Martin lineage is typical in medical science, where bright and productive people often 'stand on the shoulders' of the previous generation of bright and productive people (1). When I think about it, all the key people mentioned in this book belong in such lineages. They all acknowledge the training and mentorship of their mentor, and the previous body of knowledge that was foundational to their own work.

PTH had been identified early in the 20th century as a product of the parathyroid glands, and the unravelling of that discovery is itself a great example of the scientific process [2]. It had been shown that tumours of the parathyroid gland produce too much PTH, and that these tumours result in a disease condition that has one of those typical medical names, osteitis fibrosa cystica. The symptoms of this condition, which include the loss of bone material, sometimes to the extent of spontaneous fracture, bone pain, nausea, constipation, frequent urination, fatigue and weakness, were largely due to too much calcium in the blood. (As I write this, a nephew has just had a large parathyroid tumour removed, having suffered many of the above symptoms before his condition was diagnosed.) The thing is that calcium levels in the blood need to be very precisely controlled, and PTH has a central role in this control. Too much PTH secreted into the blood drives calcium out of the bone and into the blood and urine. On the other hand, removal of the parathyroid glands results in blood calcium levels that are too low, and this produces tetany, where the muscles contract involuntarily (thus the name Tetanus for the bacterial disease, a major symptom of which is muscle spasm). Tetany is a serious condition and needs to be treated urgently. Fortunately for my nephew, there are four parathyroid glands, so that the removal of one has no functional consequences.

Calcitonin was discovered more recently, in the early 1960s, as a factor that lowers blood calcium levels. It is largely produced by special ‘C’ cells in the thyroid gland (in which other types of cells produce thyroid hormone), in response to elevated blood levels of calcium. The actions of calcitonin, to reduce the concentration of calcium in the blood, are most obvious in young animals and humans. Unlike PTH, whose role to regulate calcium levels in the blood is now well understood, there is still debate about the physiological role of calcitonin in the body, and some people even describe it as a ‘vestigial’ hormone, kind of left over from the past, and without an important function now. One argument for this is that when people develop C-cell tumours, which produce large amounts of calcitonin, they do not seem to suffer untoward effects in terms of their blood calcium levels.

So Jack became fascinated by hormones, particularly PTH and calcitonin, because these were known at the time to have effects on bone, but it was not at all clear how they worked. Jack wanted to find out and thought of ways to do that. At the time, there were no good ways to study bone itself. To get around this, he ended up injecting rats with radioactive phosphorus, which had been shown previously to induce bone tumours, called osteogenic sarcomas (or osteosarcomas). The name for these tumours related to them being found in bone and sometimes looking a bit like bone. Therefore, Jack thought, they could be used as experimental models of bone, in which he could do things such as test their response to hormones that affect bone. Jack's aim was to try to make a bone tumour that would respond to calcitonin, although there was no particular reason to think that this method would achieve that. When I asked him what the rationale was for making osteogenic sarcomas, he described it as 'an idiotic rationale'. Today, researchers need a really well thought-out strategy to do experiments. There is no possibility of getting funding unless ideas are based on previous excellent research, and there needs to be a very compelling reason to perform the work. Even with that, there is around a 1/10 chance of getting funding, certainly in Australia. However, in the 1970s doctors who wanted to do research could often cobble together sufficient funds to check out an idea without having to convince someone else about the value of doing so. Research for curiosity's sake is called 'blue-sky' research, which Wikipedia defines as:

> "*scientific research in domains where 'real-world' applications are not immediately apparent… 'research without a clear goal' and 'curiosity-driven' science*''.

Well, how did it work out for Jack? His 'naïve aim' was to

> "get a calcitonin response in cancer"

but in fact he

> "got a PTH response and so that's what [he] worked on".

You could say he got lucky, because this PTH-responsive tumour was used in Jack's lab for years and an enormous amount was learnt

from it. But Jack's hunches and 'idiotic' reasons for doing things had a habit of paying off, and from these tumour experiments came the nucleus of an idea, from which big things would come. The seed, from which a huge tree would grow. But this development would depend on Jack's curiosity and powers of observation, and his imagination and ability to think new thoughts.

As summarised above, bone is made and formed by cells called osteoblasts. To understand anything about bone it is important to find out what makes osteoblasts do what they do. To ask these questions, Jack started with osteosarcoma tumours and osteosarcoma cells because normal osteoblasts were not available. But over time, researchers began to work out how to isolate and study real osteoblasts from bone, rather than cancer cell look-alikes. Jack and his group soon realised that the PTH-responsive osteosarcoma cells were behaving similarly to these 'primary' osteoblasts. This was wonderful because getting the real osteoblasts out of bone was much more difficult and tedious than using the tumour cells. There was another family of molecules that had become very interesting to Jack, and this was the prostaglandins, so named because they were first discovered (in 1935) in semen and were thought at the time to come from the prostate gland [3]. There was a rush of new knowledge about prostaglandins in the late 1960s, and they were shown to have important actions in almost all tissues. Jack was able to show that prostaglandins acted on the osteosarcoma cells in a similar way to PTH, although the actual response depended on the particular prostaglandin.

By the late 1970s, when I joined Jack's group, he was trying to make sense of an intriguing observation that he had made back in Sheffield. This is really important. He had seen that the order of potency of molecules such as PTH and prostaglandins to produce measurable changes in osteosarcoma cells (and also in normal osteoblastic cells) was spookily similar to their order of potency in causing the release of calcium from mouse bones in organ culture. This means that if, say, PTH, and prostaglandins E, I, H gave a response of 10, 5, 2 and 1 units, respectively, in osteosarcoma cells, they also gave responses of about 10, 5, 2 and 1 units in releasing calcium from bone. Now, at

first glance these data did not make sense. Osteoblasts were the cells that made bone; the opposite process of breaking down (or resorbing) bones was thought to be performed by osteoclasts. The release of calcium from bone implied that the bone was being broken down. Why then would bone-forming cells respond to molecules like PTH and a bunch of prostaglandins to the same extent as the bone resorbing process? Jack wrestled with this puzzle long and hard and presented his observations at a meeting in London in 1979. In the manuscript of his presentation at the meeting [4], and then in a paper published in the journal 'Endocrinology' in 1981 [5], he made what turned out to be an amazingly prophetic proposal:

> "*that the osteoblast may be a primary site of action of PTH and prostaglandins, which may cause this cell to produce some other substance which would then act on the osteoclast to elicit bone resorption".*

In other words, Jack proposed that PTH and prostaglandins acted directly on osteoblasts, and that osteoblasts responded by producing something that then caused osteoclasts to resorb bone.

Jack's presentation at the 1979 London meeting began a chain of events, which was to continue for two decades. In response to his talk at the meeting, he was contacted by Gideon Rodan, with whom he remained close friends until Gideon's untimely death in 2006. Gideon was at the time Professor of Dental Medicine at the University of Connecticut (UConn), in Farmington, where he was steadily becoming an important member of the fledgling bone research community. Remarkably, he was also working with osteogenic sarcoma cells, and had made similar observations to Jack. Fortuitously, Gideon worked in the same building at UConn as the larger-than-life Larry Raisz, another of the pioneers of the bone field. Larry had developed an assay, which measured the opposite of bone formation, that is, the process of bone resorption. Larry's assay involved incorporating radioactive calcium into rat bones so that when the bones were isolated and placed in a culture dish and exposed to various substances, the effect of those substances on bone 'resorption' could be measured by the release from the bone of radioactive calcium. The cells that were responsible for the

breakdown of bone (resorption) were thought to be osteoclasts but these cells were poorly understood at the time.

Because Gideon was a close colleague of Larry, he knew first-hand what Jack had learned second-hand from Larry's publications, that the order of potency of molecules such as prostaglandins on osteosarcoma cells matched their order of potency in Larry's assay. Eventually, Jack and Gideon met and discussed their data, in particular the puzzling observation of the equal activity of prostaglandins and other molecules on cells that seemed to have opposite activities- osteoblasts, which made bone and osteoclasts, which destroyed bone. Jack had thought this interesting, but, as he said to me;

> "it didn't really occur to me then that there was some great significance of it out there."

Gideon had also been thinking about osteoblasts maybe influencing osteoclasts because of work by an English microscopist called Alan Boyd. Boyd's work suggested that when you treated bone with PTH, the cells covering the surface of the bone (lining cells, 'inactive' osteoblasts) contracted, perhaps making the bone surface available to osteoclasts. In other words, this was also evidence that PTH did not directly affect the cells that 'digested'/resorbed/removed bone but rather acted on osteoblasts and that these cells somehow influenced the bone-removing cells. It was really only in discussion with Gideon that Jack started to see how important his observations might be in understanding bone biology. As so often happens in science, discussion between investigators leads to thinking and ideas that would probably not occur while those investigators sat isolated in their offices. Both Jack and Gideon made it their career-long practice to engage with all the top bone researchers, and continually expanded their networks of colleagues and collaborators. This meant that their research findings and ideas were constantly subjected to interrogation and refinement, in an environment where people were very happy to speak their mind. Anyway, Jack and Gideon's conversations were extremely productive and led to them writing a paper together in 1981. This publication would become a seminal paper in the bone field, and was entitled:

> *"Role of osteoblasts in hormonal control of bone resorption- a hypothesis" [6].*

This publication was very controversial at the time but, as we shall see, it drove a great deal of research in many labs in the years to come. And subsequent research has overwhelmingly supported this hypothesis!

Half a world away from Jack's lab in Melbourne, and across the Atlantic from Gideon's lab in Farmington, another key player in this story was thinking a lot about osteoclasts and how they worked and what regulated them. It's a fascinating and even slightly spooky thing that time and time again when someone is thinking new thoughts in one part of the world, someone else starts to think similar thoughts somewhere else! It's not quite the quote that I misremember about a butterfly flapping in the Amazon setting off a tornado somewhere else, but there are overtones of brainwaves travelling around the world. However, as pointed out to me by my less hysterical colleagues, it is more likely that a platform of knowledge is built that can increasingly support certain new ideas, and in the global industry of biomedical research, these ideas can spring up anywhere. Anyway, in 1978, at St Bartholomew's Hospital in London, a young pathologist called Timothy Chambers had just completed a PhD in Professor Wally Spector's department. Wally was an academic clinical pathologist, and one of the pioneers in understanding a type of cell known as the macrophage. Macrophages are cells that work in the immune system and have a special role in 'eating' and getting rid of foreign material from the body, for example removing particles of carbon or TB bacteria from the lungs. Tim recounted his story by Skype from his London apartment, poking his computer out the window to show me that he overlooks Court Number 1 at Wimbledon! Tim is a gracious, well-spoken example of an English gentleman, who is now living in retirement, believing that his research has been brought to its logical conclusion. He is entitled to be satisfied with his career, given the importance of his discoveries.

After Tim was awarded his PhD, Wally said:

> "well why don't you [Tim] write a review, which includes all multinucleated cells".

Just to explain, cells in the body mostly have a single nucleus, the part of the cell that contains the DNA and tells the cell what to do. But macrophages are actually made up of several cells (sometimes a lot of cells) that fuse together, each one adding its nucleus, so they are 'multinucleated'. To write this review, Tim told me, he had to learn about osteoclasts because they are also a type of multinucleated cell, which are only found in bone. He told me:

> "I was working away as a diagnostic pathologist, looking at biopsies and doing post mortems and teaching pathology and I must say that I really cut myself off quite a bit (from) looking into bone because…. it wasn't much use to my clinical career.. I was only interested in it from the point of view of scientific interest, not in my clinical interest. But it did seem a nice thing to look at."

So Tim did look at it. More than look at it, he cut through the accumulated body of knowledge like a knife through butter. He came into a field that was foreign to him and masterfully assimilated what was known at the time, generating hypotheses that were prescient and that not only drove his own research for many years, but influenced the whole bone field, as perhaps no one else did. In March 1980, he submitted a second review, focusing on osteoclasts, with the lofty title:

> *"The Cellular Basis of Bone Resorption" (7).*

Published later that same year in an orthopaedic research journal, it is interesting to speculate on how the article was received. Here was someone, unknown in the bone field, publishing the most comprehensive paper on osteoclasts to that time, and more than that, making prognostications that many workers in the field would not (yet) have agreed with. The article extensively mined the osteoclast literature and brought the reader up-to-date with the very latest thinking on this fascinating cell type. It described the osteoclast as the cell responsible for removing bone, and cited evidence that the

condition known as osteopetrosis, in which there is too much bone, is due to the absence of osteoclasts in those individuals.

At that time, it was just becoming clear that osteoclasts derived from cells that circulated in the blood. Before that, the osteoclast had been thought of as being a resident bone cell and Tim's review described the elegant experiments, which conclusively showed that the cells that would become osteoclasts in the bone actually arrived in bone via the blood vessels. The review thoughtfully considered what those cells in the blood could be, and concluded from the available evidence that they were mono-nuclear phagocytes (macrophages with a single nucleus), and that these cells fuse together after arriving in the bone. The paper wrestled with the observation that at any moment in time, osteoclasts are only active in particular parts of the skeleton- not everywhere. It stated:

> "*During growth, bone is laid down in some areas and removed in others to result in the largely genetically determined final anatomy of bones. …. To accomplish this, the osteoclastic resorption of unwanted bone during morphogenesis* [the development of shape of an organ] *must be under very careful spatial control. Now…. I find it inconceivable that circulating cells can be genetically programmed to remove the appropriate areas of bone without instruction from the bone itself. A .. likely model would be that the resident bone cells cause a local change in the properties of the bone, which result in its recognition by … macrophages*."

The review spoke at length about PTH, and how it might influence osteoclastic bone resorption indirectly because it seemed that it could not do so directly. By now it was known that macrophages could not bind to or respond to PTH but that osteoblasts could, as Jack Martin had shown very clearly. With beautiful logic, Tim concluded that:

> "*Since it seems likely that the recruitment of osteoclasts* (to bone) *by PTH is mediated by bone-lining cells* (osteoblastic cells)*, the simplest model for the effects of PTH on bone resorption would be that bone lining cells both recruit osteoclasts and continue to control their activity once formed*."