Anybody Got Any Data?

This question is frequently asked in the James lab. David James describes his lifelong search for his true niche, which he found through his passion for experimental science and good data.

David James.
Photograph by Irene Dowdy, courtesy of the Australian Academy of Science.

Growing up in Sydney's St George district, I was embedded in a culture of Rugby League, cricket and surfing at Cronulla beach. As a 12-year-old, I was fortunate to have Brian Booth, an ex-captain of the Australian test side, as a cricket coach. Struggling to fine-tune my fast bowling skills, he told me "clip your ear as you bring your arm through" – a trick that transformed my ability to bowl swing. I tried other sports like golf, skiing and squash in the hopes of finding my true niche. Although I didn't realise it at the time, what this gave me was a keen sense of the importance of hard work, collaboration and mentorship.

Going Where No Man Had Been Before

Since my father died of cancer when I was five, my mum decided education was more important than sport, which was probably a good thing. Failing to get into Medicine, I enrolled in Science by default. First year was a nightmare because I found the didactic style of teaching laborious and I failed to see the relevance. In second year, I opted for Physiology since it seemed relevant to medicine, but I needed another major. A careers advisor suggested I had an aptitude for biochemistry. In the quest to find out what it was, I made an appointment with Ted Thompson (long time member of ASBMB) and, remarkably, he agreed to see me. Patiently, in his fatherly way, he explained how biochemistry had been good to him. Physiology and biochemistry turned out to be a terrific mix. In physiology, I was fortunate to be lectured by people like Peter Gage, who spent two entire lectures deriving the Hodgkin Huxley equations from first principles, and Ian McCloskey, who told us about Otto Loewi's discovery of neurotransmitters through a beautiful experiment that came to him in a dream. This got me fired up because all this time I had been dreaming about surf and there were people out there dreaming about experiments (some might call this growing up). Biochemistry was fascinating, particularly those areas that were at the interface with physiology. And so it was in third year during Phil Schofield's lectures that I became enamoured of metabolism. I enrolled with Phil to undertake a small research project in third year and my job was to prove the existence of one of the elusive steps in the pentose phosphate pathway. I vividly remember sitting in the university cafeteria staring into space trying to think of the key experiment that would enable me to prove what nobody had ever been able to do before. I felt very comfortable at that moment in my own space with my own ideas and the excitement of going somewhere where nobody had been before. I did Honours with Phil, ironically, working on sugar transport in rat intestine. Here, I met Arthur Jenkins, one of Phil's PhD students. Not wanting to pester Phil, I would often run ideas by Arthur. Arthur was the coolest guy I had ever known. He knew everything about metabolism, he was incredibly patient, he could explain the most difficult concepts and he loved to indulge in one or two of life's pleasures. In many ways, meeting Arthur was one of the most important things that had ever happened to me. I wanted to be just like this guy. In retrospect, I realise that a lab is bigger than any one individual.

Cartoon by Phil Schofield, David's Honours supervisor, depicting the rat glucose clamp.

As Luck Would Have It

As a PhD student, I was lucky to find Ted Kraegen and Don Chisholm from the Garvan as my advisors. They both heavily influenced the way I do science today. As a biophysicist with no real formal biological training, Ted regularly thought about physiological problems in black boxes connected

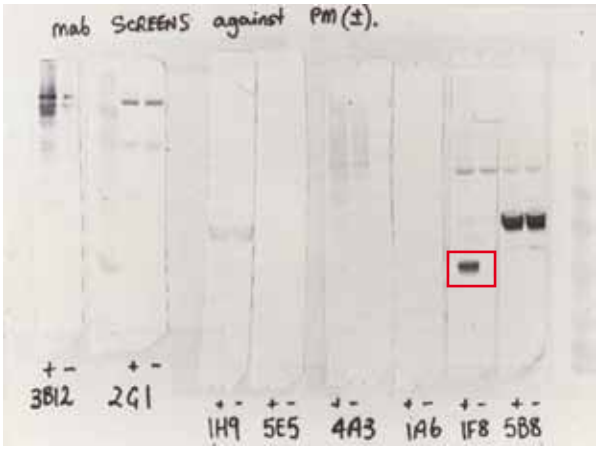
by arrows and rate constants. I have continued to do this throughout my career and this helped me overcome the semantics of complex cell biological problems, particularly those related to compartmental boundaries. Ted's influence has recently come back to roost with my desire to pursue interdisciplinary science and to bring a more mathematical focus to our research. Don was the stabilising force always reminding us of the clinical relevance and patiently teaching us endocrinology. At the time in the early '80s, diabetes research in humans relied heavily upon isotopes to measure glucose flux combined with steady state techniques such as the hyperinsulinemic glucose clamp. My PhD project was to develop this technique in rats – "but Ted, rats are bloody small, you know". The kicker came when Ted was reading one of Lou Sokoloff's papers on brain activity using tracer 2-deoxyglucose. It occurred to him that if combined with the clamp, this might be a unique way of dissecting *in vivo* metabolism in living animals. What a great idea and I was lucky enough to be the right guy in the right place at the right time.

Provocativeness Leads to Progress

This was an incredibly productive and fun time in large part because we were unrestrained due to our isolation – it used to take six months for *JBC* to arrive on a boat. Our work pointed to skeletal muscle as being a major determinant of whole body glucose metabolism. Right around that time, two papers were published showing that insulin-sensitive tissues, such as muscle and fat, increased their rate of glucose transport by moving a glucose transport activity protein from inside the cell to the plasma membrane, a phenomenon referred to as the 'translocation hypothesis'. This became one of the hot topics in the field, and like so many important observations, it was surrounded by controversy. Provocateurs, like Mike Czech, claimed insulin increased glucose transport by modifying the intrinsic activity of transporters, largely because insulin increased glucose transport by 30-fold, but the increase in transporter activity at the membrane was only about 4-fold. I became intrigued by this problem and went to postdoc with Paul Pilch at Boston University, again a chance meeting that would change my life.

Romance in the Number 1 Bus

During my PhD, I had focused on whole animal studies and, despite having been subjected to extensive undergraduate training in biochemistry, I had little exposure to cell biology, a rapidly emerging field. I attended the Whitehead symposium on endocytosis in October 1985 when two guys called Brown and Goldstein talked about clathrin. "What the hell is clathrin?" I asked of the guy next to me, who looked at me strangely. When it was announced the next day that these guys had won the Nobel Prize, I was shamed into buying my first cell biology text *Molecular Biology of the Cell*, from the Harvard Co-op, which possessed me for several months. In Boston, science took on a romantic feeling for me. It was about people, ideas, experiments and places. The best people from all over the world would come there and you could sit and listen to their ideas and their experiments for free. This was almost as good as a Led Zeppelin concert. I devoured this stuff and made it my business to know what everyone on my floor was working on at the time, and we would all sit on the floor in the corridor and talk about experiments. There were people doing all kinds of amazing things, and for the first six months, my head was spinning. The gap between the lab at Garvan and what was going on in Boston at that time was as big as the Grand Canyon. Strangely, this gap has narrowed considerably since then and I can imagine that postdocs today may not have the same dizzy feeling I had back then. Every day when I caught the Number 1 bus down Massachusetts Avenue, I would voraciously read papers on phosphorylation, receptor biology and protein biochemistry. In the beginning, it took hours per paper as the Methods were so foreign, but persistence paid off.



Discovery of GLUT4. *This is a copy of data from the original monoclonal antibody screen performed by David in Boston in 1987. GLUT4 is shown in the box as a protein that underwent a major insulin-dependent movement to the plasma membrane (PM).*

A Eureka Moment

My project was to understand the cell biology of the translocation phenomenon, as Paul believed this side of the argument on first principles, and I agreed with him (one of the few occasions). The approach I took was out there, as we decided to purify the intracellular pool of transporters, which at the time we measured as ^{3}H-cytochalasin B binding. I immunised mice with this fraction and made monoclonal antibodies against as many constituents as I could. This was one of those laborious tasks (see below). Around this time, Mike Mueckler reported the cloning of a mammalian glucose transporter in *Science*. Mindful of the fact that cloning was an emerging discipline and that few sequences had been described at that point, this paper led most people to conclude that this was the missing transporter we had all been looking for. Curiously, when antibodies specific for this protein were used to immunoblot fat cell membranes, insulin only had a minor (~2-fold) effect on this protein, and so this fuelled the fire of the opposing camp who believed the effect of insulin was on intrinsic activation. My monoclonal screen dragged on for over a year with many failed attempts. But one day in the summer of 1987, I returned from the developer with data that showed a band, recognised by one of my monoclonals, that was increased >10-fold by insulin.

The band had the right size for a transporter, but was it the long-sought-after insulin-responsive transporter? When our antibody immunoprecipitated glucose transporter activity and it recognised a band only in muscle and fat cells, the Eureka moment had come. This was what ultimately would be known as GLUT4! I remember saying to my wife, "I think I might have done something really important." Little did I know this was the beginning of what would become a life-long effort to figure out how this molecule works. But the field was sceptical. Gus Lienhard told one of my colleagues after he heard me present the work in Boston, "it's up to James now to prove it."

David with postdoc Kyle Hoehn livening up the conference dinner at the 2008 Queenstown Molecular Biology Meeting.

Real Men Do Molecular Biology and Share Their Wares

In the mid '80s, the biological research community was polarised with the molecular biology jocks, like John Shine, in one camp and the die-hard biochemists and cell biologists, like myself and Pilch, in the other. But I knew that if I was going to prove the existence of this transporter, there was no other option but to clone it. Just two years after becoming a self-taught cell biologist, I had to buy a copy of Maniatis and learn about phage, supercoiled DNA and restriction enzymes. This transition was made easy for me, though, as I made the decision to move to St Louis to work with Mike Mueckler, an expert molecular biologist. When papers came out reporting that liver cells (and similarly, brain) express a different transporter, we knew we were onto something, but we also knew that others would be nipping at our heels. Working day and night, we finally found what we were looking for – a new transporter expressed in muscle and fat cells only! We submitted the paper to *Nature* and waited. Meanwhile, Mike and I gave a joint presentation on the project at the International Diabetes Federation meeting in Sydney. We reported that unlike other transporters, the insulin-responsive transporter was a 10-transmembrane protein. After our talk, Dan Lane from Baltimore told us his lab had pulled out a clone from fat cells, but it was a 12-transmembrane spanner. Our hearts stopped! On returning to the lab, we rechecked the sequence of three independent clones and observed that all of them had an aberrant deletion that was not conserved between them, and this deletion encoded two transmembrane domains. We reluctantly withdrew our paper and got back to work. We corrected that mistake and eventually published the correct sequence in *Nature*. Thanks to the generosity and kindness of Dan Lane, a true but rare gentleman of science, we were spared this embarrassment.

The 'Diggers' of Science

After this, I took up independent positions at Washington University in St Louis, at the Institute for Molecular Bioscience in Brisbane, and at the Garvan Institute in Sydney, never staying more than eight years in each place. In retrospect, this was refreshing, allowing me to continuously update my science and meet and collaborate with entirely new people on a regular basis (not to mention the fact that after 5–8 years in one place, I had offended most people and had no one left to work with!). I have stories from each of these adventures that have impacted in various ways. I was always enchanted about how research used to be in the old days and hearing stories of science legends.

In St Louis, I struck up a friendship with Albert Roos, who had been on the faculty at Washington University since 1946. He overlapped with Gertrude and Carl Cori, who had trained some of the great names in biology, such as Earl Sutherland and Severo Ochoa. I remember the first time Albert and I met, he said to me "you're not related to that bastard Joe Bornstein are you?" Joe had worked with the Coris back in the '60s and Albert had a sharp tongue. After exchanging Bornstein stories, we became friends and talked for many hours about the Coris and what it was like doing research in the '50s and '60s. He told me the story of Rollo Park, a postdoc in the Cori lab, who had done elegant work to show that insulin increases the glucose space in muscle, but not in other tissues such as brain. Park suggested that insulin regulated the transport of sugar into the muscle cell and that this was a unique mechanism by which insulin regulates the post-meal absorption of food. But this contradicted a large body of work the Coris had done to show that the truly important regulatory step was hexokinase and not transport. When they saw Rollo's data, Carl Cori said, "I don't like these data," and Rollo put the data in a drawer – until he started his own lab at Vanderbilt several years later. Eventually he published the data in the *JBC* in the late '50s and this was heralded by many, even today (although incorrectly – see below), to be the first report of insulin-regulated glucose transport in muscle. Of course, as someone who had just discovered the molecular identity of this transporter, I found this story unforgettable as it had a certain scallywag irreverence about it that I found appealing. One year later in 1991, I was invited to Vanderbilt to give a seminar, and as I began my talk, I noticed 75-year-old Park, sitting in the front row. During my introduction, I recalled the story Albert had told me in a poetic but respectful way, acknowledging Rollo as the discoverer of insulin-regulated glucose transport. Later that day, as I was talking science to one of the younger faculty, a gentle knock on the door revealed Rollo, who quietly handed me an envelope and requested I read its contents on the way home, which I did. It turned out he wasn't the first to report the insulin regulation of glucose transport at all, as the letter

contained a Xerox copy of a paper published in an obscure German journal in 1939 describing the same phenomenon. This was an extremely humbling moment and I hope this story carries the same message for you as it did for me.

The Wild, Wild North

In 1993, I returned to Australia to join a vibrant crew of research cowboys at what would become one of the premier research institutes in the country, the Institute for Molecular Bioscience. Initially, this transition was filled with anguish and hand-wringing questions like "what have I done?" But I soon realised that now was my opportunity to do something completely different and help build something from the ground up. The place was filled with the pioneering spirit of people like John Mattick, Peter Andrews, John Hay, Paul Greenfield, Brandon Wainwright and Peter Koopman. My goal amidst this flurry of activity was to put cell biology on the map. This provided an intimate glimpse into the inner workings of a large Australian university, and unbelievably, it worked very well. In 7–8 years, what happened in Brisbane was extraordinary, largely because of the amazing cadre of people like Rob Parton, Jenny Stow and Toshi Yamada that went there to work because they all wanted the same thing – to be truly great, and great they were.

The Cell Biology of Diabetes

In Queensland, my research focused on basic cell biology of protein trafficking, particularly related to GLUT4. But I missed the ability to collaborate with people who worked on diabetes and so I returned to the Garvan in 2001. My lab in Brisbane at the time was so amazing that they all decided to come with me, which made setting up a new lab in a different city easy and we were doing experiments in weeks. The Garvan was completely different to working in a large university. There was less bureaucracy and things seemed to happen much faster. However, it's not without its challenges in that access to large equipment or other infrastructure pots of money is limited and we rely to a great extent on philanthropy for these kinds of things. The best part was that I now got to be the boss of my PhD advisor, Ted Kraegen, and there is an important lesson here for all of us. One of the great things about the Garvan, and institutes like it throughout the country, is that being embedded both in the academic and clinical environment affords many novel opportunities. It provides access to clinical material for sure, but more importantly, it enables people like myself with a PhD to work cheek to jowl with MDs, providing deeper insights into the clinical framework of what we are all trying to achieve in our lab test tubes.

The Recipe

Throughout my career, I have always had great expectations of myself and for those I have had the privilege of working with. For me, the script has been quite simple. Follow your instincts, have as much fun as you can, talk about science to as many people as you can as often as you can and always ask yourself "is what I am doing important?" At a recent careers talk, I told the students "don't be afraid to fail" and that made them feel safe. So I think we all have to be careful not to get onto the research roller coaster and lose track of why we are doing this. I have tried to reinvent myself every five years or so, and I am currently trying to become a Systems Biologist. I have, largely through collaboration, been able to see science through the eyes of some really great people and this has helped me see many new things. Many of these people have become lifelong friends. Like anything we do, research also has its drudgery, like filling out forms or repeating the same experiment six times to achieve statistical significance. But that pales into insignificance compared to those Eureka moments. There is no formula to success in research, as science is made up of all kinds, who each have their own stories to tell and their own operational styles and that's what makes it work. If I had to be pressed, I would say my career has been distinguished by a sprinkling (some would say an overdose) of irreverence. This often gets me into trouble, but it has enabled me to navigate my way through science and to always question everything, while having fun. In the end, it's not just about answers or publications, but rather the truth. If I had my time over, I would not change a thing. I feel content that I have done something important that I am pretty good at and that I have a group of colleagues all over the world who are quite special.