The making of a lipid biochemist

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Patrick obtained his undergraduate degree at McGill University, and received his graduate training in Medicine and Biochemistry at the University of North Dakota. His postdoctoral work was conducted at the University of British Columbia. He joined the Faculty of Medicine, University of Manitoba as an Assistant Professor of Biochemistry in 1979. He was the recipient of a New Investigator Award (1979–85) from the Heart and Stroke Foundation of Canada, followed by a Scientist Award (1985–90) from the MRC/CIHR. These career awards allowed him to devote the majority of his time to research. He was promoted to Associate Professor in 1983 and Full Professor in 1986. He was cross-appointed as Professor of Pathology and participated in the teaching of General Pathology to residents. He was the founding Director of the Centre for Research and Treatment of Atherosclerosis at the University of Manitoba and the Winnipeg Health Sciences Centre. Administratively, he was appointed as the Professor and Head of Biochemistry and Medical Genetics (1992–1999), and an Associate Dean in the Faculty of Medicine in 1999 until his retirement in 2010.

Professionally, Patrick served as President of the Canadian Biochemical Society, Chair of the Medical Research Council/Canadian Institute of Health Research New Investigator Awards Committee, Chair of the Alberta Heritage Foundation Senior Investigator Awards Committee, Vice-President of the Heart and Stroke Foundation of Manitoba, and Secretary/Treasurer of the Canadian Society for Clinical Investigation. He continues to serve on Editorial Boards of scientific journals, including the *Journal of Clinical and Investigative Medicine*. He was invested into the Order of Manitoba in 2011.

In the beginning

Research in lipid biochemistry was not a popular topic in the 1970s. Lipids were thought to be just building blocks for biological membranes. In addition, these molecules are difficult to work with since they are water-insoluble, and organic solvents must be employed for their extraction and isolation. Interestingly, research in this area was rejuvenated in the 1980s by the discovery that some lipid molecules are involved in signal transduction.

My doctoral training in pathological biochemistry provided me with the basics in proteomics and immunology. For fellowship training, I decided to go into an area of biochemistry that I knew the least—lipid biochemistry. It was pure serendipity that I stumbled into this area of research, which in hindsight was both exciting and challenging. I was fortunate to have a postdoctoral supervisor (Dr. Dennis Vance) who gave me a lot

of freedom to do my work. Subsequently, I joined the University of Manitoba as a new faculty member in July 1979.

Phospholipid metabolism in the mammalian heart

The objective of my research was to examine the control of cardiac phospholipid metabolism in health and disease. In the early 1970s, the dogma was that phospholipids were synthesized in the liver, and then transported to target organs via lipoproteins. Using the Langendorff heart perfusion model, with labelled choline and ethanolamine, we demonstrated that cardiac phospholipids were actively synthesized *de novo* in the heart, and the rate of synthesis could be determined by the pool size of a precursor in the last step of its biosynthetic pathway. A manuscript of the work was submitted to the *Journal of*

Biological Chemistry nine months after the commencement of the project and, to our surprise, was accepted by the journal without revision. It was the highlight of my career, and we were ecstatic to learn that the paper was cited over 100 times in the following years. By employing various pathological animal models and cardiac myocytes, we were able to identify control mechanisms for the synthesis of phospholipids in the mammalian heart. Based on these studies, we postulated that the cytidine triphosphate level is a common denominator for the control of phospholipid biosynthesis in the heart, and may be a universal signal for the synthesis of lipids in other organs.

Phospholipids and cardiac arrhythmias

Another research project in my lab was to investigate the catabolism of phospholipids and the aberrant production of phospholipid metabolites in cardiac arrhythmias. Phospholipids are metabolized by enzymes called phospholipases, and these enzymes were reported to be tightly bound to membrane fractions. The study on phospholipases was initially fraught with difficulty until we identified and isolated a form of soluble (cytosolic) phospholipase A in the heart. The soluble phospholipase A has the ability to hydrolyze phospholipids at both the sn-1 and sn-2 positions and its levels are modified under several pathological conditions. Since the existence of a soluble phospholipase A was still under debate in the early 1980s, our work was under close scrutiny. It took us more than a year and three revisions before the manuscript was accepted for publication.

One metabolite produced by phospholipase A is lysophospholipid, which is a powerful detergent and causes hemolysis in erythrocytes. Under hypoxic conditions, there was accumulation of lysophospholipids in the heart prior to the production of arrhythmias. We were able to induce arrhythmias in the isolated perfused heart with the addition of various lysophospholipids. The presence of lysophospholipids also caused distinct changes in membrane potentials and other electrophysiological parameters similar to those observed in arrhythmias. Although we were very excited about the finding, we were not able to hypothesize a specific mechanism for lysophospholipid-induced arrhythmia. The work did not generate a lot of attention at that time

but after a hiatus of more than 20 years, it is satisfying to see that this subject has re-emerged and is currently under active investigation.

A new pathway for phospholipid biosynthesis?

An enigma in lipid biosynthesis was that esterification of the fatty acyl group to the glycerol moiety requires glycerol-3-phosphate instead of glycerol. Although the direct acylation of glycerol had been postulated, the existence of such a pathway had not been explored. Using myoblasts and hepatocytes as models, we demonstrated that glycerol could be directly acylated to form monoacylglycerol and subsequently diacylglycerol and triacylglycerol. The direct acylation pathway became more prominent when the glycerol-3-phosphate level was reduced or when exogenous glycerol levels became elevated. We were very excited by the discovery of this pathway, and proceeded to clone and characterize the enzyme involved. After many attempts, we ended up cloning an acyltransferase which could acylate both glycerol and glycerol-3-phosphate. Hence the importance of the direct acylation pathway has yet to be explored.

Clinical trials

Beyond basic research in phospholipid metabolism, we were also interested in the role of phospholipids in hyperlipidemia. In a post-market clinical trial, we studied the action of Lipitor (atorvastatin) on hyperlipidemic patients. The ability of oxidized LDL from hyperlipidemic patients to impair endothelium-dependent relaxation was significantly reduced after atorvastatin treatment. Such reduction resulted in part from an 11% reduction of lysophosphatidylcholine levels in these patients when compared with another group that received only dietary counseling. The work provided us with additional evidence that lysophospholipids may be important factors in the pathological conditions observed in hyperlipidemic patients.

Research vignettes

I was blessed with continuous support from Medical Research Council/Canadian Institutes of Health Research throughout my career, but there never seemed to be enough funds to go around, especially during the early days of establishment of my lab. When I started my

research program, we needed an anti-vibration table to enable the insertion of microelectrodes into cardiac cells. As we did not have funds to buy one, we had to make one ourselves. In collaboration with a colleague, we decided to get a slab of stone from a memorial headstone maker and place the slab, supported by four large rubber stoppers, on a sturdy table. The weight of the stone mounted on rubber stoppers would dampen the vibration from the environment. We were flabbergasted when the owner of the headstone firm asked us if we wanted a new or a “used” one (the used one was cheaper). He later explained that the used one was not actually used, but was designated as such due to errors in the engraving. Although neither of us is superstitious, still we insisted buying a new one (without the engraving).

A key element for a successful research program is the ability to recruit a team of high quality trainees. By aggressively marketing my research program, I was fortunate to recruit superb students and postdoctoral fellows, many of whom have gone on to become Professors, Deans and Canada Research Chairs.

Research collaboration was an important facet of my career. I was extremely fortunate to forge collaborations outside my own expertise, with an electrophysiologist (Dr. Ricky Man), a cardiologist (Dr. David Mymin), a vascular physiologist (Dr. Edwin Kroeger) and others. Our common interest in lipids and lipoproteins gained us the moniker “Grease Group”. In 1999, the Grease Group was successful in obtaining a \$500,000 development grant from an insurance company, plus matching funds from the province. This support allowed us to establish the Centre for Research and Treatment of Atherosclerosis, and to carry out many clinical trials through the Lipid Clinic.

The development of new molecular biology techniques provided a useful platform to advance our work, but it was a challenge to me as a classical biochemist. To acquire some knowledge in this area, I enrolled as an auditor in a colleague’s graduate course in molecular biology. I learned quite a bit from the course but, having missed many lectures in deference to administrative obligations, I would likely have failed the exam.

Success and “failure”

Looking back, the decision to become a researcher was

certainly a rewarding one, with the satisfaction of clinically-relevant discoveries and having gained meaningful friendships internationally. My success as a researcher was facilitated by excellent collaborators, a dedicated team of research trainees and technicians, and the support of the university. We were able to publish over 130 full length peer-reviewed papers, including a paper in *Nature*, several books and many book chapters.

In the latter part of my career, I accepted several administrative duties in, including those as Department Head and then Associate Dean of the Faculty. I was naïve to think that I would be able to reach a productive balance between my research program and my administrative duties, and inevitably I lost focus with my research. Eventually I retired from my research career in 2010 to spend more time with my family and do volunteer work within my community. My interest in science has not diminished since retirement, and I am still serving on editorial boards of several journals, including the *Journal of Clinical and Investigative Medicine*.

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