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Building a Cathedral

Neuroscience and the Legacy of Leon Wolfe

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Abstract

This article describes the scientific legacy of Dr. Leon Wolfe, with illustrations from his contributions to insect physiology, glycolipid, and eicosanoid biochemistry as well as to our understanding of neuronal ceroid lipofuscinoses and lysosomal storage diseases. In addition to the written record, Wolfe inspired all who knew him with his boundless imagination and enthusiasm for science and his ability to see the promise and potential of every experiment.

Index Entries: Insect physiology; gangliosides; eicosanoids; neuronal ceroid lipofuscinoses; lysosomal storage diseases; scientific legacy.

Introduction

It is daunting to attempt to capture something of the scientific legacy of a man who was interested in so many areas and who had a major influence on my own scientific career. When I first met Dr. Leon Wolfe in 1961, he was young, energetic, and excited and had dark hair! Figure 1 is very formal, but you do get a sense of the suppressed energy. Wolfe appeared to be just waiting to break into an animated conversation about the latest thing he had read or heard! Newly appointed as an assistant professor in the Department of Neurology and Neurosurgery at McGill University and as associate neurochemist at the Montreal Neurological Institute, Wolfe was embarking on his formal independent studies of brain biochemistry—an undertaking that would occupy the following four decades.

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Building a Cathedral

However, before describing some of these contributions, I should explain the title "Building a Cathedral: Neuroscience and the Legacy of Leon Wolfe." This title is drawn from an apocryphal story concerning Sir Christopher Wren, the architect of the magnificent St. Paul's Cathedral in London *(1)*. Briefly, the story is that Sir Christopher approached one of the bricklayers working on the project and asked him what he was doing. The man replied very matter-of-factly, "I am laying bricks." When Sir

Dr. Leonhard S. Wolfe, circa 1962.

Table 1
Major Research Interests of Leon Wolfe[a]

Insect physiology in biochemistry
Biochemistry of glycolipids and glycoproteins
Phospholipid metabolism
Essential fatty acids
Prostaglandins, thromboxanes, leukotrienes, and related compounds
Neurochemistry and neurochemical pathology
Inherited neurological diseases
Biochemistry of lysosomal storage diseases
Vitamin A and metabolites
Polyisoprenols and dolichols
Metabolic consequences following brain injury and ischemia
Metabolism of cerebral endothelial cells in culture
Age pigment formation in aging brain and Alzheimer's disease
Development of biochemical tests for neurological diseases
Medical consequences of chemical warfare agents
Long-term potentiation in the hippocampus, signal transduction mechanisms, guanylyl cyclase
Subunit C of the mitochondrial ATPase protein translocase mitochondrial proteolysis

[a]Prepared by Leon Wolfe in the 1990s.

Christopher approached a second workman and asked him what he was doing, the man said, "I am building a wall!" Finally, Sir Christopher approached a third workman and asked him what he was doing, and the man replied with great pride, "I am building a Cathedral!"

Wolfe and many of those who worked with him helped create the scientific evidence that formed some of the bricks of the Neuroscience Cathedral; indeed, some of us helped raise a small part of the wall. However, it was Wolfe's genius—a blend of fundamental knowledge, logic, and imagination—that allowed us to catch a glimpse of the cathedral we were trying to build. He could take the most meager brick, the smallest piece of knowledge, or the reading from a spectrophotometer or a liquid scintillation counter and build a hypothesis around it that helped one see how he or she might be contributing to the bigger picture. Wolfe did that not only for those of us who worked with him but also for those he met at meetings, in seminars, or in airport lounges. It was his imagination and thought (coming up with hypotheses and theories) that provided the excitement and inspiration that led to the next experiment; sometimes this thought was on the right track, and often it was on the wrong track, but Wolfe always had renewed enthusiasm and a wider comprehension of the possibilities before us.

Continuing with an architectural metaphor, what are some of the bricks, walls, cornices, and cupolas that Wolfe contributed to the field of neuroscience? Table 1 lists 15 areas that Wolfe considered some of his major research interests. Many who knew him might argue that the list is incomplete; however, it is his list, prepared by him at a later point in his career.

Wolfe and his colleagues made important observations in all these areas; some are explored later in this article or in the articles of other authors in this issue.

Insect Physiology

Wolfe's first forays in science and, indeed, his doctorate from Cambridge were in insect physiology. He studied with one of the great pioneers of insect endocrinology, Sir Vincent Wigglesworth. As a fresh young New Zealander in Cambridge, Wolfe examined the deposition of the third instar larval cuticle of Calliphora erythrocephala *(2,3)*, more commonly known as the blow fly. As an insect physiologist, Wolfe began his first contributions to the understanding of the neurosciences, examining cholinesterase activity in the brains of these insects *(4)*. The knowledge gained from this activity was important to the understanding of acetylcholinesterase in addition to the practical idea that was often the major focus of previous and subsequent work: the control of insect populations through the use of cholinesterase inhibitors as insecticides.

Wolfe's interests that arose from his doctorate studies were in the fundamental physiology of insect development. However, his contributions, as well as those of many of Wigglesworth's other students, helped provide the foundation for the understanding, tools, and technologies of insect development, on which the substantial and important field of insect genetics has been built. Wigglesworth's students laid some of the bricks for the cathedral of insect genetics, and insect genetics has become a powerful tool in the armamentarium of the neuroscientist. Perhaps best illustrated by the fruit fly (*Drosophila melanogaster*), there is now a host of genes that are implicated in the development and control of the nervous system *(5)* and whose gene products control the development, migration, and connectivity of complex neural networks. In an ingenious combination of novelty, informing, and marketing, these genes were given imaginative names such as big brain, Kuzbanian, and scabrous. Their homologs in mammals are being identified and characterized, providing major contributions to our understanding of mammalian neurosciences.

From Insects to Humans

Wolfe moved from insects to humans, preparing by attaining a degree in medicine at the University of Western Ontario and an internship at the Royal Victoria Hospital in Montreal. He then went to England to work with Professor Henry McIlwain at the Biochemistry Department of the Maudsley Hospital as well as the Institute of Psychiatry in London, England. This was a time of unprecedented growth in the traditional fields of neurophysiology, neuroanatomy, physiological psychology, and neurochemistry. The field of brain research was dominated by physiology, anatomy, neurosurgery, and neurology, but collectively, brain research demonstrated the gradual emergence of the new interdisciplinary approach to the study of the nervous system, which came to be known as neuroscience.

During his time at the Maudsley Hospital, Wolfe amplified and expanded what would become a career-long fascination with membrane lipids. He realized early that rather than being static structural elements, these membrane components played a role in energy metabolism as dynamic structural elements, solvents, and chemical messengers. He concentrated most of his attention on two roles: dynamic structural elements and chemical messengers.

Glycolipids

The first membrane lipids Wolfe studied were the gangliosides, members of a ubiquitously distributed group of acidic glycolipids *(6)*. This class of membrane lipids is characterized by a hydrophobic lipid group, to which a carbohydrate chain of varying complexity is attached. This structure confers on the molecule the ability to associate not only with the

hydrophobic membrane core but also with the hydrophilic, aqueous environment on both sides of the cell membrane. The gangliosides have glucose as the first sugar in the carbohydrate chain and have varying amounts of an acid sugar called sialic, or neuraminic acid *(7)*. They were particularly interesting, because they are highly concentrated on the surface of neurons *(8,9)*, but are also widely distributed among other cell types. Furthermore, members of this class of lipids accumulate in the cells of the central nervous system in Tay–Sach's disease, one of a family of diseases that would be known as the lysosomal storage diseases and that continued to fascinate Wolfe throughout his career *(10)*. The multiple molecular structures of these molecules and the potential paths of biosynthesis and metabolism were the subject of much speculation for Wolfe and some of his early students (myself included) and have been gradually elucidated over the years.

Their great structural complexity and remarkable patterns of distribution (marking stages of development and cell types) fostered the concept that these lipids play important roles in many biological processes. Synthesized in the cell interior and with completion in the Golgi, these glycolipids arrive at the plasma membrane, where they are organized into lipid-based microdomains known as rafts and caveolae *(11,12)*. The ability of the hydrophobic ceramide in sphingolipids to associate with cholesterol may assist the assembly of these structures. Because of their affinity for signaling proteins, the rafts may function as signaling platforms. The advent of targeted mutagenesis has provided some additional insights. In the mouse, the only known lethal mutation in the ganglioside biosynthetic pathway is the absence of the initial attachment of glucose to the hydrophobic sphingolipid, resulting in death during gastrulation *(13)*.

Elimination through mutation of the biosynthetic pathways of the more complex gangliosides structures demonstrates that most of these molecules are not necessary for the gross morphological development of the mammalian nervous system. However, there appears to be a requirement for these molecules for proper function of the nervous system. The ability to test the functioning phenotype in these animals and to look for subtle correlates of higher nervous system function has lagged behind our ability to create mutant models. Accordingly, many of the early theories from Wolfe and his colleagues concerning glycolipid structure and function remain to be tested.

Interestingly, because of subsequent developments, Wolfe and his colleagues paid little attention to the sphingolipid portion of the glycolipid molecule, generally postulating that it served as an anchor, holding the more important carbohydrate portion closer to the membrane. Subsequent work from many laboratories has demonstrated the central role of the lipid backbone and its components (ceramide, sphingosine, and sphingosine-1-phosphate) in intracellular signaling *(14)*. Wolfe might have looked at the ceramide portion of the glycolipid molecule if his attention had not turned to a whole new area of chemical messengers derived from the fatty acids of membrane lipids, the prostaglandins, and related compounds.

Eicosanoids

The prostaglandin or eicosanoid era of Wolfe's scientific career provides another opportunity to reflect on the advance of science in general as well as Wolfe's contribution in particular. Scientific revolutions are not only concept-driven but also technology-driven, or tool-driven. Tools are often the sparks that ignite scientific discovery. Such tool-driven revolutions can have profound societal consequences (e. g., the impact of the telescope on the medieval world). Restriction enzymes, Sanger sequencing, high-throughput robotics, and bioinfomatics are tools essential to the fundamental platform of the human genome project. Wolfe appreciated the importance of original thinking and concepts, the develop-

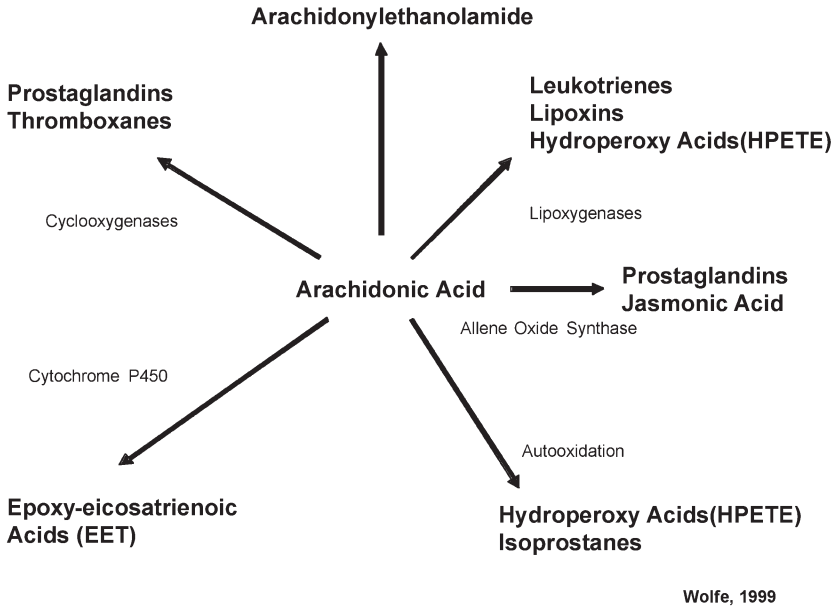

Fig. 2. Arachidonic acid cascade. (From ref. *17*, with permission from Elsevier.)

ment of theory and its modification and testing, and the continuous development of new science based on knowledge of the past. Simultaneously, however, Wolfe was a technology junkie, with a true appreciation of the importance of tools. His involvement in the eicosanoid era was an effective blend of "concepts and tools," from a few prostaglandins to the entire family of eicosanoids and from a bioassay with a kymograph to a gas chromatograph-mass spectrometer *(15,16)*.

Figure 2 is a diagram taken directly from one of the last reviews that written by Wolfe, for which the date of publication is 1999 *(17)*. The diagram is remarkable because it combines numerous potential pathways for the metabolism of arachidonic acid that are not often seen together. There are many diagrams illustrating the cyclooxygenase and lipoxygenase pathways *(18,19)*, but there are few regarding some of the other pathways. This provides another illustration of Wolfe's broad lateral thinking, thinking outside the box. The diagram and the relevant discussion stress the central role of free arachidonic acid normally esterified in complex lipids, whose levels in the free state are tightly controlled through a balance between release by phospholipases and esterification by acyl transferases. The release of free arachidonic acid follows a wide variety of stimuli to receptors: hormones; neurotransmitters; antibodies; peptides; toxins; and pathological conditions such as inflammation, vascular shock, burns, seizures, and immune reactions. The release of arachidonic acid, its metabolic transformation, and the physiological effects of the eicosanoid products all occur locally in both an autocrine and paracrine fashion. The eicosanoids formed from arachidonic acid are characterized by precise stereochemistry in formation and recognition, effects in the nanomolar range, and potent biological activities *(18,19)*.

In the past decade, the major focus has been the cyclooxygenase products (prostaglandins and thromboxanes) and the lipoxygenase products (leukotrienes and lipoxins) *(18,19)*. Both groups are characterized by the stereospecific addition of oxygen to a lipid molecule. A combination of this specific addition of oxygen and stereospecificity in subsequent

enzymatic steps provides a wide variety of products that differ in biological activity *(18,19)*. Although a large part of the genome is devoted to coding for kinases and phosphatases, the principal pathways of arachidonic acid metabolism are governed by only three classes of enzymes that initially add oxygen to the substrate: the cyclooxygenases, the lipoxygenases, and cytochrome P450.

A few comments on some of the other pathways that Wolfe included in this diagram are classical examples of his wide-ranging interest and thinking outside the box. The pathway on the right of the diagram is catalyzed by an allene oxide synthase, a cytochrome P450-type enzyme. The pathway is important in lower organisms and plants, leading to a family of potent regulators called the jasmonates. This family of oxygenated fatty acids has various roles in plants, including, but not confined to, plant immunity, the initiation and maintenance of long-distance signal transfer in response to wounding, and the regulation of fertility *(20)*.

Arachidonylethanolamide, the product of the pathway going toward the top of the diagram, is also known as anandamine and was recently shown to be the first endogenous ligand for the cannabinoid receptor. It has been reported to mimic the pharmacological effects of cannabinoids and, possibly, to play a role in endotoxic shock and inflammation *(21,22)*.

The products of oxygenation by cytochrome P450 play critical roles in the regulation of renal, pulmonary and cardiac function, and vascular tone. A series of auto-oxidation products formed by the free radical peroxidation of arachidonic acid are being investigated for their role in oxidant stress and injury and for their deleterious effects on the fluidity and integrity of membranes *(23)*.

Lysosomal Storage Diseases and the Neuronal Ceroid Lipofuscinoses

Finally, let me discuss another facet of this fascinating and complex man: his passionate interest in applying the tools of the basic scientist (i.e., chemistry, physics, and biology) to the elucidation of the etiology and pathogenesis of human disease. In part, this was driven by his own medical education, but it was also driven by the environment in which he found himself—the Montreal Neurological Institute, a hospital centered on neurological diseases with a strong clinical investigation tradition. Wolfe's translational work began with glycolipids (in general) and the gangliosides (in particular) and resulted in a series of contributions to the understanding of the gangliosidoses and other glycolipid storage disorders. With John Callahan, Wolfe ventured into GM1–gangliosidosis *(10)*; with Joe Clarke, Wolfe explored Fabry's disease *(24)*.

However, Wolfe's contribution to unraveling the basis of the neuronal ceroid lipofuscinoses (NCL; sometimes collectively called Batten's disease *[25]*) was arguably his longest and most difficult quest and one that he had to leave unfinished. Why did he explore this condition? There are probably many reasons, but clearly, one of them was the Willie Sutton Principal. Willie Sutton was a bank robber; when asked why he robbed banks, Sutton said because that is where the money is.

For Wolfe, this group of diseases was a clear challenge. They are a group of autosomal recessive inherited childhood disorders sharing numerous clinical and pathological features *(25–28)*. With an incidence of up to 1 in 12,500 live births, NCLs are collectively the most common childhood-onset neurodegenerative disorders. They are characterized by progressive blindness, neurodegeneration, and, perhaps most significantly, the accumulation of autofluorescent lipopigment material in neurons and other cell types. In the NCLs, the stored material appears to be lysosomal in origin and is apparently similar in composition to the aging pigments described by many pathologists as ceroid and lipofuscin.

Using the paradigm developed by many great clinicians and pathologists, Wolfe and others searched for initial biochemical clues by trying to characterize the accumulating mater-

Table 2
Neuronal Ceroid Lipofuscinosis Genes and Gene Products

Gene	Age of onset	Gene identified	Murine model	Protein	Function	Location
CLN1	Infantile	Yes	Knockout	Cln1p	Palmityl prot thioesterase	Soluble lysosome
CLN2	Late infantile	Yes	Knockout	Cln2p	Tripeptidyl peptidase	Soluble lysosome
CLN3	Juvenile	Yes	Knockout	Cln3p	?	Membrane ER/Mito/ lysosome
CLN4	Adult	No	Natural mutant	–	–	–
CLN5	Late infantile	Yes	No	Cln5p	?	Membrane
CLN6	Late infantile	Yes	nclf	Cln6p	?	Membrane
CLN7	Late infantile	*CLN8?*	?	–	–	–
CLN8	Late infantile	Yes	mnd	Cln8p	?	Membrane ER

(From ref *35*, with permission.)

ial in the central nervous system and in other organs and body fluids *(25,26,29)*. In a series of important contributions, he and others pointed to the long-chain polyisoprenoid alcohols known as dolichols *(30)*, a 10-fold increase in human cerebral cortex from age 5 to 80 yr, increased levels in NCL brain, and increased levels excreted in the urine of patients with NCL *(26,29–34)*. For a short time, this appeared to be an attractive diagnostic test, but it soon became clear that there were too many false-positives and false-negatives. It is likely that the accumulation of these materials, although important in the pathophysiology of the disease, is secondary to some other primary defect causing the condition.

Table 2 summarizes subsequent studies by others that used a wide variety of molecular techniques that were not available to Wolfe and his colleagues *(35)*. Basically, there are probably at least eight genes involved in this complex of diseases; the protein products of two of these genes are known, and the others remain undetermined *(26–28,35)*.

Wolfe's contributions will surely be integrated into this story in the future. There is no unifying hypothesis to explain the range of molecular mechanisms from defects in the spectrum of known NCL genes and proteins to what appears to be a relatively uniform cellular phenotype. Does the storage of the autofluorescent lipopigment (the hallmark of this group of diseases) reflect the ultimate involvement of a common biological process? The accumulation of storage material in many cell types suggests a defect in a ubiquitously distributed degradative pathway. However, the selective vulnerability of brain and eye in this group of diseases implies additional specialized pathways may be affected, either primarily or secondarily (*26*).

The autofluorescent pigments ceroid and lipofuschin have been studied for more than a century, but their molecular nature is only now being clarified (*36,37*). Lipofuscin, or age pigment, accumulates progressively in lysosomes of postmitotic cells such as neurons, cardiomyocytes, and retinal pigment epithelium. Ceroid

accumulates under pathological conditions in cells with mitotic capacity. Both pigments are produced from various biological molecules, including lipids, carbohydrates, and proteins. At least one mechanism by which these pigments may be generated is suggested by the fact that some of the NCLs are caused by defects in lysosomal enzymes involved in protein degradation.

Making a Difference

Did Wolfe and his science make a difference? Speaking personally, I can assure you that it does now, and it did when I first met him. I suspect it made a difference for everyone who had the opportunity to train with him and work with him in the laboratory. However, the greatest contribution was always his vision of the cathedral. He could take the simplest facts from your small experiment and extrapolate an entire scientific vista, a tantalizing lead to a magnificent journey! He did not only do this just for his trainees. I do not know how many times I ran into people at meetings who had just spent some time with Wolfe and who were dazzled, excited, and thought differently about their work. He had the capacity to get excited about almost anything, and it was contagious, infectious, and strengthening.

In seeking a closing statement, I was drawn to two terms to describe Leon Wolfe: one that is out of fashion, and one that is in fashion. The first term is "Renaissance man." Today, when people in college specialize in medicine and in science, calling someone a "jack of all trades" (which is what a "Renaissance man" implies) has become somewhat derogatory. However, for a few like Leon Wolfe, the term remains as a symbol of genius.

The second term is very fashionable: "multidisciplinary." This term is invoked by research institutions, research funding agencies, the health system, and countless other organizations. For me, Wolfe's wide-ranging approach embodied the very principles of multidisciplinary: knowing enough about something to be able to speak the jargon and to be able to bring the people with the different skill sets and tools together to tackle important scientific problems.

Finally, Emerson *(38)* wrote that "an institution is lengthened by the shadow of one man"—we who knew and worked with Leon Wolfe realize how long his shadow is, and hopefully, our contributions will continue to add to the shadow.

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