GENERATIONS OF BIOPHYSICS: FROM NUCLEOTIDES TO NUCLEAR MAGNETIC RESONANCE

BY LINDA J. JOHNSTON

Over the past century, biophysics has led to game-changing discoveries that are now part of mainstream vocabulary. Research in this field has led to the creation of diagnostic imaging tools that are in high demand today, although often taken for granted, such as the MRI, CT and PET scans. It is also the foundational science allowing the popular discussion of pros and cons of antioxidants.

An interdisciplinary science, biophysics has changed how people view the functioning of biological systems, and has helped gain a better understanding in health, disease-prevention and the creation of cures. It applies the principles of physics, chemistry, mathematics and computational modelling to studies of life at every level, from atoms and molecules to cells, organisms and environments [1]. The National Research Council of Canada (NRC) began recognizing biophysics' uniqueness as early as 1929, promoting the field and helping people understand its place among the established disciplines of physics, engineering, biology and medicine.

Perhaps one of the greatest and most known examples of biophysic's impact comes from the discovery of deoxyribonucleic acid (DNA), which we now understand as having an influence in countless areas of daily life. Beginning in the 1940s with experiments pointing out that genes are made of a simple chemical now called DNA, subsequent studies led to the identification of the now commonly-known double helix in 1953. Using X-ray crystallography, it is possible to obtain the structural identification of a complex biomolecule. From that point on, we understood that DNA was comprised of only four individual bases, whose pairing is responsible for who we are as biologically unique individuals. For the double helix finding, Francis Crick, James Watson—who had done virus research at NRC as a Merck Fellow—and Maurice Wilkins were awarded the Nobel Prize. The discovery of the DNA double helix laid the foundation for many DNA based technologies that are widely used today.

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SUMMARY

From DNA, to antioxidants, Biophysics discovers the biological cycles of heat, light, water, carbon, nitrogen, oxygen, and organisms throughout our planet, and has been recognized for its importance by NRC since 1929.

In the 2000s, biophysical research, coupled with the vastly improved available technology, finally decoded all the genes in a human being. It was further possible to identify nearly 200 different complete species, and map a portion of the genetic material of over 100,000 others. DNA's discovery has been immensely significant, and has changed much of how we view the world. It is now taught in early education, almost as innocuously as algebra, a testament to its ubiquitous nature. It is used in paternity testing, forensic analysis, agriculture, and has obviously sparked a medical revolution in many circles.

NRC'S COBALT-60 FIGHTS CANCER

In the mid-20th century, only a few highly trained physicists were undertaking radiological work. In general, they had been trained in medical sciences rather than in the fundamental physical laws necessary for the proper understanding of radiology. The NRC Associate Committee for Biophysics therefore recommended that NRC work on radium standards.

Since Marie Curie's 1898 radium discovery, radioactive sources have been used for multiple purposes, including the treatment of various forms of cancer. While these were promising for some localized cancers, they could not, at the time, reach deep tumours. In 1947, NRC opened NRX – the world's most powerful research reactor in Chalk River, Ontario, leading the way to improved treatment.

NRX was a heavy-water-moderated, light-water-cooled, multipurpose research reactor used to develop new isotopes, test materials and fuels, and produce neutron radiation beams that became an indispensable tool for condensed matter physics.

The nuclear physics design of NRX emerged from NRC's laboratory at the University of Montreal, where during World War II a team of Canadian, British and other European scientists had engaged in top-secret heavy-water reactor research. Among these were Canadian

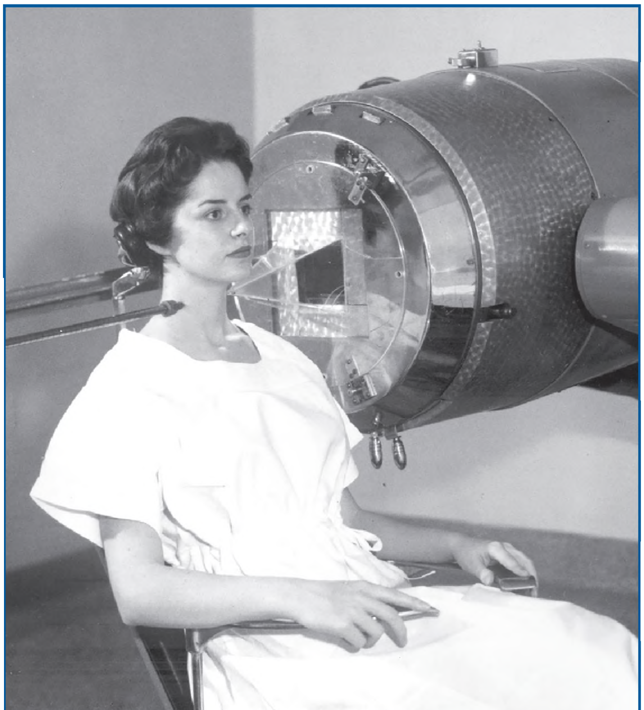

Fig. 1 Cobalt-60 and other isotopes used to combat cancer.

Bertram Brockhouse, whose work at NRX in the 1950s advanced the detection and analysis techniques used in the field of neutron scattering for condensed matter research. In 1994, he won the Nobel Prize in Physics, sharing it with American Clifford Shull.

In the 1950s, NRC began mass-producing Cobalt-60 and developed dozens of different isotopes at NRX. To ensure that radiation beams hit previously unreachable tumours, researchers increased Cobalt-60's (Fig. 1) strength and concentration by thousands of times. The "cobalt bomb" soon boosted patient 5-year survival rates by over 75 per cent in cancers that previously saw just one in five people pull through. Eventually, NRC medical physicists invented technology for targeting and destroying cancerous tissue only, and for many years Canada was a world leader in the area of medical isotopes, developing new uses and approaches to their production.

While NRC has licensed the technology to Nordion Canada Inc. for use at cancer clinics worldwide, Co-60 is still the main tool used in the Measurement Science and Standards division for calibrating radiation therapy instruments used at all cancer centres across Canada.

NRC EXPLAINS NUCLEAR MAGNETIC RESONANCE (NMR) SPECTROSCOPY

A research technique that exploits the magnetic properties of certain atomic nuclei, NMR spectroscopy is used by chemists and biochemists to investigate the properties of organic molecules. While relatively standard today, it required a certain degree of explanation when it first emerged in the 1950s.

Addressing both theory and application, the first authoritative and comprehensive book, *High-resolution Nuclear Magnetic Resonance,* was published in 1959 by former NRC president William G. Schneider and two colleagues, John A. Pope and Harold J. Bernstein. The book remained the definitive reference for the following decade on matters relating to high-resolution NMR spectroscopy. In its function as a textbook in graduate and senior undergraduate courses, it instructed students in the intricacies of spectroscopy and illustrated the practical applications of quantum mechanics as applied to spin systems. By providing a far-reaching, comprehensive, and elegant overview of the field at a critical time, this treatise laid the groundwork for much of the impact that NMR spectroscopy now has on science, medicine and industry.

The NMR work pioneered at NRC influenced many researchers. Among these was chemist Ian Smith, who began his career as one of Schneider's summer students in the 1950s and went on to specialize in magnetic resonance spectroscopy. In 1987, he was appointed Director General of the NRC Institute for Biological Sciences in Ottawa, and in 1992 founded NRC's Institute for Biodiagnostics (NRC-IBD) in Winnipeg. One of NRC-IBD's most notable achievements was the development

Fig. 2 900 MHz spectrometer at NRC's W. G. Schneider Building.

of a movable MRI system that allows non-invasive scans of patients before, during and after surgery.

In 2006, the Government of Canada, in partnership with the University of Ottawa and the provinces of Quebec and Ontario, opened the NRC W.G. Schneider Building, which houses five spectrometers. Such spectrometers (e.g., Fig. 2) have helped scientists develop new battery composites, nano-materials for electronics, plastic polymers for vehicles, glasses for more sensitive sensors and faster computer processors, new materials for hydrogen storage and health-enhancing antibiotics.

BIOPHYSICS MEETS CARBOHYDRATE CHEMISTRY

In the early 1970s, NRC chemist Harry Jennings devised a solution for successfully vaccinating children with meningococcal meningitis caused by three major strains of *Neisseria meningitidis*. At the time the vaccine – composed of purified sugars from the bacteria surface – was ineffective in children because the contents did not stimulate the immune system.

Jennings had heard about this problem from a chance encounter with a U.S. scientist working on meningitis who wanted to collaborate on NRC's NMR methods. As Jennings considered the problem, he realized it was possible to overcome the limitations of the existing vaccine by linking the polysaccharide to a protein to create a conjugate vaccine. Ian Smith was involved in the NMR work that laid the foundation for the meningitis vaccine.

While Jennings encountered a number of roadblocks in finding an industrial partner that would take the vaccine candidate forward, he persevered. However, it was not until 30 years after joining NRC that he found an industrial partner to commercialize it in 1996. The first major test of the vaccine against meningitis C was in 1999 against an outbreak among hundreds of British children.

This success led to a renaissance in vaccine research, opening up a number of possibilities for diseases that were not thought to be amenable to vaccines. Since the first conjugate vaccine patent for Meningitis C in 1982, the Institute for Biological Sciences has solidified a family of meningitis patents and continues to explore vaccines for a number of other diseases, including cancer.

PIONEERING STUDIES OF FREE RADICALS AND ANTIOXIDANTS

Studies of free radicals and antioxidants was an area of extensive work at NRC for almost 60 years in the Division of Chemistry, and then the Steacie Institute for Molecular Science. Chemist Keith Ingold, who joined NRC in 1955, is a pioneer in understanding the role of oxidation in the aging process and the role of Vitamin E in medicine and health as an antioxidant. When he began his research, the chemistry of intermediates such as free radicals was unknown.

Ingold's mandate was to examine the degradation of engine oils and learn how to prevent it. This launched his lifelong love affair with free radical chemistry (Fig. 3). Applying the chemistry of free radicals to living organisms – specifically the human body, which produces about 10 kilograms of superoxides every year – is the area for which his research is best known. He also demonstrated the role of antioxidants in preventing degradation of a wide range of materials and helped to redefine the petroleum and plastics industry.

In the early 1980s, Ingold turned his attention to the role of oxidation in aging and the development of cancer and atherosclerosis – two leading causes of mortality in industrialized society. His team's pioneering work proved that vitamin E behaves as an antioxidant in living animals. NRC's game-changing findings on vitamin E led to a variety of medical breakthroughs, including improved treatments for patients awaiting heart surgery and a greater understanding of certain diseases involving vitamin E deficiency. However, vitamin E can actually promote oxidation in some cases, so there is still much to learn, and the field remains active.

The benefits of antioxidants in food and various products have been widely publicized, analyzed and debated, yet the general public believes they prevent the harmful effects of free radicals. Antioxidants are supposed to keep us healthy, allow us not to feel guilty for having that glass of wine in the evening and keep us from looking our age. However, a definitive review of the area of oxidants, published by Ingold and Derek A. Pratt in 2014, reports that while antioxidants are probably preventative, they are unlikely to be therapeutic.

Ingold continues to investigate the relationship between oxidation and atherosclerosis and cancer, the two leading causes of mortality in North America and Europe. In 2016, he was

Initiation

$$\longrightarrow In^{\bullet} \xrightarrow{O_2} InOO^{\bullet} \xrightarrow{RH} InOOH + R^{\bullet} \qquad (1)$$

Propagation

$$R^{\bullet} + O_2 \xrightarrow{\text{fast}} ROO^{\bullet} \qquad (2)$$

$$ROO^{\bullet} + RH \xrightarrow{\text{slow}} ROOH + R^{\bullet} \qquad (3)$$

Termination

$$\left.\begin{array}{l} R^{\bullet} + R^{\bullet} \xrightarrow{\text{fast}} \quad (4) \\ ROO^{\bullet} + R^{\bullet} \xrightarrow{\text{fast}} \quad (5) \\ ROO^{\bullet} + ROO^{\bullet} \xrightarrow{\text{slow}} \quad (6) \end{array}\right\} \text{non-radical products}$$

Fig. 3 Ingold was a leader in developing methods to study free radical reactions, among them quantitative measurement of reaction rates (ESR) [2].

awarded the Royal Society of Chemistry's Sir Derek Barton Gold Medal for his "fundamental contributions to the understanding of free radical chemistry."

THE RISING IMPORTANCE OF BIOPHYSICS

The world is facing unprecedented physical and biological problems. Many aspects of daily life we tend to take for granted, such as energy, food and clean water, are in danger. Solving such crises requires scientific insight and innovation – and biophysics is an important key since it discovers the biological cycles of heat, light, water, carbon, nitrogen, oxygen, and organisms throughout our planet.

While there is much yet to be learned about the universe we inhabit, each new day brings us closer to understanding it. NRC researchers are at the forefront of biological sciences and physics, and continue their daily work towards discoveries that forever alter and enhance our lives.

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