Russell F. Doolittle
1931–2019

BIOGRAPHICAL Memoirs

A Biographical Memoir by
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Russell Doolittle made extraordinary contributions in two important areas of biochemistry and molecular biology. He was one of the pioneers in the field of molecular evolution by being among the first to show that the history of biological speciation could be elucidated by aligning amino acid sequences of proteins and using that information to create phylogenetic trees. He also, almost single-handedly, provided the molecular explanation of how fibrin monomer polymerizes to form polymeric fibrin, the transformation responsible for the clotting of blood, a process of central importance in the practice of modern medicine. His detailed molecular description of the mechanism by which the polymerization of fibrin is initiated and propagated is also of great interest biochemically because of its elucidation of the capabilities demonstrated by the protein itself and the molecular strategies at work, and it is also of importance pharmaceutically because it suggests how the process can be controlled.

Russ was born in New Haven, Connecticut, in 1931. His father was employed in the municipal coking plant that provided the fuel to run the central heating system of the downtown. He attended Connecticut Wesleyan University from 1948 to 1952.

After graduation in 1952, at the height of the Korean War, Russ enlisted in the United States Army at the suggestion of a recruiter, who implied that this decision would lead to him becoming an officer. Russ’s inference never became a reality—he arrived in Korea a few days before the armistice in July of 1953 as an enlisted soldier in a combat unit. He was always grateful that he came to the battlefield when he did. He did not have fond memories of the boredom of his two years in the army. He enjoyed relating a story, perhaps apocryphal, about the day his platoon was taught how to put on long underwear: lessons on the tops in the morning and on the bottoms in the afternoon.
After his discharge from the Army in 1954 and his marriage to Frances Tynan in 1955, he completed a master’s degree in education, which he received from Trinity College in Hartford, Connecticut, in 1957 while teaching science at New Milford High School. Uninspired by a future in this career, in the fall of 1957 he entered the doctorate program of the Department of Biological Chemistry in the Harvard Medical School. He performed his doctoral research in the laboratory of J. Lawrence Oncley. It was as a graduate student that Russ became interested in the clotting of blood as well as the lamprey, the fibrinogen of which played a role in his doctoral studies (1). For the next thirty years, he returned regularly to Massachusetts to collect and bleed lampreys; later in life, his visits were scheduled to coincide with the Boston Marathon.

Following his graduation from the department of biological chemistry in 1961, he spent a year teaching at Amherst College. One of his anecdotes from this period was of a lecture on a Saturday morning. A student arrived quite late to class barely awake while Russ was mid lecture. After a few minutes, the student looked around the classroom and realized that he was the only attendee. In that year, the publications describing Nirenberg and Matthaei’s breakthrough experiments showing the translation of RNA into polypeptides appeared—results that grabbed Russ’s attention. He realized that the era of molecular biology had arrived. This realization and his love of scientific research, which would last a lifetime, drew Russ to the laboratory of Birger Blombäck at the Karolinska Institutet in 1962 on a postdoctoral fellowship from the National Institutes of Health. There, he and Professor Blombäck studied the amino acid sequences of fibrinopeptides, short fragments of fibrinogen released during clotting, and showed that the differences between animal species in the amino acid sequences of these short peptides, which could be readily determined with the methods available at the time, provided indications of evolutionary relationships (2).

In 1964, he accepted a position in the laboratory of S. Jonathan Singer as an assistant research biologist in the department of biology at the University of California, San Diego. After returning to native soil from Sweden, he and his wife drove themselves and their son across the country, from Connecticut to San Diego, in a Ford Falcon station wagon, filled with most of their worldly belongings. On arriving in San Diego over the Fourth of July weekend with only ten dollars in his pocket, he was dismayed to find a line of cars at the entrance to the campground in Palomar State Park. He made it in just before the campground reached capacity.
In 1965, Russ was appointed assistant professor in the department of chemistry at UC San Diego, beginning his more than fifty years as a member of the department, which included both an appointment in the School of Medicine and, later, an appointment in the department of biology. During this long period, he concentrated almost continuously on two distinct areas of research: the evolution of proteins and the molecular mechanism of the clotting of blood. He made major contributions in both of these disparate areas. His study of the evolution of proteins encompassed a huge collection of different proteins, which included fibrinogen, the protein responsible for clotting blood, so there was some overlap. His evolutionary studies involved the aligning of amino acid sequences of proteins and the construction of phylogenetic trees, tasks that are performed solely by computing. His elucidation of the molecular mechanism of clotting, however, involved a wide array of biochemical and biophysical techniques, all involving wet chemistry in the laboratory.

In addition to these main thrusts of his research, Russ also tossed off a couple of studies that led to a good deal of attention. He and I, in our spare time, with help from Russ’s son Larry who, unlike the two of us, understood how to write a computer program, developed a method for displaying a running average of the hydrophathy, the hydrophilicity and hydrophobicity, of the side chains in the sequence of amino acids in a protein (3). With Gernot Walter, he developed the procedure for eliciting antibodies against a synthetic peptide containing a sequence of amino acids that exists within a particular protein. This method could be used to make antibodies specific for only these sequences in that protein (4). The most novel aspect of this work, however, was the potential for antibodies to be generated against proteins that had never been isolated and were known only from their DNA sequences. The development of this technique had associated with it, regrettably, an intriguing history of subterfuge and intellectual theft of Russ’s ideas by others (5).

When he became a faculty member in the department of chemistry, Russ initiated his independent career by continuing his study of the amino acid sequences of fibrinopeptides. When he resumed these studies, he expanded his cohort of species by setting up a collaboration with the San Diego Zoo to obtain blood drawn from the wide range of animals in their collection. As one might imagine, his collaboration led to a cover story in C&E News about Dr. Doolittle at the zoo (6).

While in Sweden, he and Professor Blombäck had realized that the rate at which the sequence of amino acids in fibrinopeptides changed with time would provide infor-
mation about the history of speciation over tens of millions of years, the period of time over which the separation of species within a particular order of animals had occurred. By choosing animals in the collection at the zoo within the cetartiodactyla order, which encompasses the even-toed ungulates including pigs, mule deer, reindeer, muntjaks, sika deer, red deer, elk, pronghorn antelope, goats, sheep, water buffalo, bison, cattle, camels, llamas, and vicunas, and comparing the sequences of the fibrinopeptides from them, Russ was able to create a definitive phylogenetic for these species (7).

This tree greatly expanded his earlier efforts in Sweden. He then turned his attention to the sequences of fibrinopeptides of the primate order of macaque, green monkey, baboon, drill, gibbon, chimpanzee, and man, and he was able to produce a family tree of these primates (8). During these studies, he discovered that the fibrinopeptides from chimpanzees had the same sequence of amino acids as those from humans (9). This discovery was one of the first indications of the extremely close relationship between these two species. When Russ was in Sweden, he also sought to correlate the points at which the tree of the cetartiodactyla branched with the geologic times that had been assigned to the fossil record (2). This correlation was one of the first attempts to produce a “molecular clock,” a clock that Russ was continually expanding over his career. All of these efforts were seminal contributions to the nascent field of molecular evolution, which has exploded over the last fifty years.

Russ continued his study of molecular evolution even after his attention turned to fibrinogen itself rather than its fibrinopeptides. Frustrated with the printed versions of Dayhoff’s Atlas of Protein Sequence and Structure because the amino acid sequences of proteins were being published in the scientific literature so rapidly, he compiled his own computer file of all of the amino acid sequences available at the time, by the name of Newat (New Atlas), so that he could update the data regularly and have on hand all of the known sequences. Before the Internet existed, Russ instructed that, “Computer
printouts of Newat may be obtained by sending a self-addressed and suitably stamped manila envelope (10 by 13 inches) to me.” This project, run by a single scientist on his own, was one of the forerunners of the massive databases available online that now employ large staffs and are generously supported by the governments of the United States and the European Union. In those early days of computing, getting the protein data bank together, finding homologous sequences within it, and running comparisons came in fits and starts, with more than one blind alley. Nevertheless, Russ was gradually able to search this early library, align sequences, and produce phylogenetic trees with computer algorithms developed in his laboratory, at first by his two sons, Larry and Will, and then by Da-Fei Feng.

In the early days of cloning and sequencing of DNA, molecular biologists were getting short segments of sequences, but they had no idea which proteins might be encoded by these newly cloned pieces of DNA. Russ understood that by searching Newat with programs that identified similarities with known proteins he could provide the answers. In doing so, Russ allowed molecular biologists to understand the genes they were cloning as well as their functions. One of his favorite examples of such a collaboration was when he was able to show, by aligning the sequences of amino acids, that the oncogene for simian sarcoma virus was derived from the host platelet-derived growth factor, one of the first indications that alterations in growth factors are associated with the onset of cancer. He seldom failed to have the time to respond to a query, and they arrived from all over the world.

Russ also began studies of the amino acid sequence of the complete molecule of fibrinogen from the lamprey, the member of the phylum of chordates most distant from mammals. When sequencing of complementary DNA became available, he combined the results of those procedures applied to complementary DNA from the lamprey with his results from chemical sequencing to obtain the entire sequence of this fibrinogen. These sequences allowed him to extend his quest for the ancestors of fibrinogen into invertebrates. Eventually, using the techniques of molecular genetics, he was able to find a protein in sea cucumbers that shares a common ancestor with chordate fibrinogen thus extending the evolutionary history of this protein.

Russ always had associates in his group who spent their time working with him to align sequences and build phylogenetic trees. These efforts extended our understanding of the evolution of proteins, and hence the evolutionary history of the species from which they are isolated. Eventually, he was able to build a tree that set the time for the diver-
gence between plants, fungi, and animals at around one billion years ago, archaeabacteria and eukaryotes at around 1.8 billion years ago, and archaeabacteria and eubacteria at around two billion years ago (15). He also continued to advise others around the world who were doing similar work. This lifelong interest in evolution prompted him to engage in public debate with advocates of what has been wishfully called “creation science.” To the day he died, he retained the files in which he had consolidated his arguments relevant to those debates.

In the 1970s, the main emphasis in his research shifted from molecular evolution to fibrinogen, the molecule in the blood that produces the clots that staunch bleeding but also can accumulate in the wrong places, causing strokes and heart attacks. He set out to determine the sequences of the amino acids in the three polypeptides that comprise human fibrinogen, 1,810 amino acids in all. At the time, this task was a monumental undertaking that tested the limits of the existing techniques. After his work on the shortest of the three polypeptides was abandoned when its sequence was published by the Henschen laboratory, he succeeded in determining the complete sequences of the longest two of the three polypeptides that constitute fibrinogen (16, 17, 18). On the basis of these results he realized that the three polypeptides that compose fibrinogen were homologous in sequence and, consequently, shared a common ancestor.

Once the sequences of amino acids in the three polypeptides were in hand, Russ set out to discover clues to the three-dimensional structure of fibrinogen within the sequences, which at the time was considered a waste of time. It was already known that the entire molecule was comprised of two copies of each of the three polypeptides and there were suggestions that it contained three domains. The key to the elucidation of the structure was the recognition that the central portion of each polypeptide displayed a heptad repeat, and Russ predicted, correctly, that in this central region, the three polypeptides would wind around each other in a triply stranded $\alpha$-helical coiled coil (19). Based on an earlier conjecture about the structure of fibrinogen (20) he applied this new information to a more detailed proposal. The molecule had a central, globular domain containing the amino terminal portions of the six polypeptides that preceded the coiled coil. This central domain was arranged around a twofold rotational axis of symmetry relating the two identical halves. Connected to this central region by the two identical coiled coils were two identical carboxy-terminal domains (21).

This arrangement of domains was consistent with a proposal that Russ had already made for the mechanism by which the fibrin molecule, a molecule of fibrinogen from
which the amino-terminal fibrinopeptides were removed, could polymerize into the long fibers that cause the blood to clot (20). The mechanism of the polymerization would involve specific associations between the carboxy-terminal domains and amino-terminal sequences exposed upon the removal of the fibrinopeptides. His proposal for the structure of fibrinogen and the way in which it polymerized were soon validated when Robley Williams presented his dramatic electron micrographs revealing the steps in the assembly of fibrin monomers into fibrin oligomers (22).

These micrographs, however, were unable to validate his proposal that the polymerization resulted from the binding of the amino termini in monomeric fibrin that are exposed upon digestion of fibrinogen by thrombin to specific binding sites in the carboxy-terminal domains of neighboring molecules of monomeric fibrin in the polymer. To provide evidence for this proposal, he designed a series of chemical experiments the results of which were consistent with his hypothesis (23, 24).

Russ realized, however, that the ultimate validation of his mechanism would require a molecular model of fibrinogen at atomic resolution, which up to that time had defied the techniques of protein crystallography. These techniques require crystallizing molecules of the protein, exposing a crystal to a beam of X-rays, submitting the resulting pattern of diffraction to analysis, and inserting a molecular model into the resulting map of electron density. These are complex procedures, none of which he had ever performed.

Undaunted, he learned the methods and persevered until he obtained several crystallographic molecular models of individual domains isolated from fibrinogen and its clotted counterpart, fibrin, and, in the end, the entire molecule of fibrinogen. He first crystallized, with the assistance of Huguette Pelletier, a graduate student in the laboratory of Professor Joseph Kraut, the domain of fibrinogen formed from the carboxy-terminal portions of the three polypeptides (25) isolated from a digestion of fibrinogen. He then obtained a crystallographic molecular model of this domain at atomic resolution, as well as a crystallographic molecular model of this domain isolated from a digestion of fibrin, and located the binding site on the domain for the tripeptide glycylprolylarginine, the amino-terminal sequence of the \( \alpha \) polypeptide exposed on the digestion of fibrinogen with thrombin (26). He then located the binding site on the domain for the tetrapeptide glycylhisitinylargininylproline, the amino-terminal sequence of the \( \beta \) polypeptide exposed on the digestion of fibrinogen with thrombin (27). These crystallographic molecular models proved that the carboxy-terminal domain did indeed bind the amino-terminal ends of the two the polypeptides in the central domain and that the
carboxy-terminal sequences of fibrinogen do form a compact globular domain. Russ then was able to crystallize the intact molecule of fibrinogen and provide a crystallographic molecular model of the entire molecule at atomic resolution (28), a goal that had frustrated crystallographers for decades.

These crystallographic molecular models validated both his proposal for the structure of fibrinogen as well as his molecular mechanism for the polymerization of fibrin that produces a clot. Well into retirement, when he had closed up his large laboratory, Russ conned the department of chemistry into giving him a windowless room in which he, by himself, could continue to carry on experiments, a practice that had given him so much enjoyment over his long career. During that period, he was able to demonstrate that the enzyme papain is able to catalyze the formation of a peptide bond between the amino terminal tyrosine of a γ polypeptide in fibrin and the carboxy terminus of Glycine 403 in the γ polypeptide of another molecule of fibrin, but only when the polypeptides containing these amino acids are in a fibril of fibrin (29). In the detailed atomic model of a fibril of fibrin he had previously proposed by model building (30), these two amino acids are adjacent to each other. Consequently, this result was consistent with the model.

One of the amazing aspects of Russ’s scientific career was his insistence on being at the bench performing or directing the experiments himself, unlike many of his colleagues who directed their armies from behind the desks in their offices. He would sit for an hour or more at a time with his secretary double-checking the transcription between the original publication and Newat, his growing atlas of amino acid sequences. He performed, or directly supervised his assistants, in the thousands of digestions, chro-
matographic separations, chemical reactions, and analyses required to determine the amino acid sequences of the fibrinopeptides of the different species and then the polypeptides of fibrinogen. He himself mastered the novel, at the time far from routine, procedures of recombinant molecular genetics necessary to complete the sequence of lamprey fibrinogen and discover the ancestor of fibrinogen. He and his students together performed the synthesis of the peptides that he needed to provide evidence for his proposals that explained the polymerization of fibrinogen. When he realized that crystallography was needed to validate his proposals, techniques to which entire laboratories are exclusively devoted, he mastered them with the help of scientists in the laboratories of Joe Kraut and Xuong Nguyen-Huu at UC San Diego. Alongside his graduate students and his postdoctoral fellows, he was growing crystals, traveling north to the synchrotron at Berkeley to collect data from diffraction, applying the Fourier transforms, and, on the computer screen, inserting the molecular models into the electron densities that resulted.

This compulsion to do it himself extended to his home. He was always reluctant to hire someone to do a job he could do himself. At one time, for a couple of weeks, Russ was mostly absent from his laboratory, and as we later learned, he was reroofing his house. Soon after arriving in La Jolla, he ordered a telescope kit from Edmund Scientific, which consisted of several paper tubes, an eyepiece, and a cylindrical blank of optical glass. He then spent many evenings in his basement grinding the blank into a parabolic mirror. Later, when Haley’s Comet appeared, my wife and I were told by Russ to wake our two young children at 2 a.m. and drive with him into the mountains to view it with his homemade telescope because they, unlike the rest of us, would be alive when it returned.

Another intense interest—Russ always did things intensely—was running. In 1976, Russ enrolled in a study at the university documenting the health of its participants. At the time, the prognosis that he had a 50:50 chance of having a serious heart attack within the next five years was alarming enough that he took up running marathons. No reason to do anything halfway. There was little doubt as to his tenacity. Although it took him two years to work up to a full marathon, in at least one later year, he was listed in the top one hundred marathon runners in his age group in the State of California, and he annually ran the Boston Marathon. He was always so proud of his best time of three hours and twenty minutes.

Among one of the many unusual aspects of his life was running for the House of Representatives of the United States in the election of 1968. When the Democratic Committee of our district failed to find a candidate in what was then a deeply Republican area, Russ
volunteered. He received 47 percent of the vote in the Democratic primary but lost to a much more conservative Democrat in a district that, at the time, extended into Orange County. The most peculiar plank in his platform had to do with firearms. Although he conceded that the Constitution seemed to guarantee a right to bear arms, it said nothing about ammunition. Consequently, he proposed that there should be a number of rather strict regulations on its sale.

Russ had a number of admirable personal traits. He had high standards for scientific research and was willing to do the work to meet those standards, and he expected the same from his peers. When relaxing, he was an entertaining raconteur, who had many amusing stories that he was able to present in a way guaranteed to elicit happy laughter from his audience. He was also a mentor to younger scientists who were drawn to him and always found him willing to listen and give advice freely, and he usually had a good word to say about them later to his older colleagues. His younger colleagues remember fondly from their days in his group the weekly tea times where everyone would gather on the balcony at 3 or 4 in the afternoon. Often Russ would invite other scientists and old lab members who were in town to discuss their science and the science being pursued in Russ’s lab and remember old times. When he became a friend, he was usually a close friend for life.
REFERENCES


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