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MYRON KENDALL BRAKKE
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A Biographical Memoir by

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Biographical Memoir

Myron K. Baker

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THE NATIONAL ACADEMIES AND the biological sciences lost a distinguished pioneer with the death of Myron Kendall Brakke on June 15, 2007.[1] Those who were his close colleagues also lost a valued mentor, an irreplaceable friend, and a trusted adviser. Brakke's most notable accomplishment, which led to major advances in biochemistry and molecular biology, was the development of sucrose density-gradient centrifugation for purification and characterization of viruses and macromolecules. Brakke also was responsible for devising numerous virus purification techniques and for engineering equipment for fractionation of macromolecules crucial for the development of virology, biochemistry, and molecular biology.

From 1947 to 1955 Brakke was a postdoctoral fellow with Lindsay M. Black, where he had a major impact on the understanding of insect-transmitted viruses, including wound-tumor virus, potato yellow-dwarf virus, and tomato spotted-wilt virus. In addition, Brakke collaborated with other members of the Black group to make seminal contributions to early plant tissue culture research. These phases of Brakke's research were conducted from 1947 to 1952 at the Brooklyn Botanic Garden, and from 1952 to 1955 at the University of Illinois, where he had moved with Black.

In 1955 Brakke accepted a U.S. Department of Agriculture position at the University of Nebraska-Lincoln (UNL), where he remained until his retirement in 1989. During this period, Brakke became the world's foremost authority on purification, characterization, and ecology of cereal viruses. He also initiated a collaboration with Robert W. Allington (1935-2006) to develop instruments to facilitate fractionation of density gradients. As indicated below, these achievements greatly accentuated the application of macromolecular purification technologies by the scientific community and resulted in Allington's formation of Instrumentation Specialties Corporation (ISCO, now Teledyne) in Lincoln.

Myron Brakke was born on October 23, 1921, in Fillmore County near Rochester, Minnesota. He grew up on a farm owned by his parents, John and Hulda Brakke, and attended a one-room country school through the eighth grade. In 1938 he graduated from Rochester High School and attended Rochester Junior College from 1939 to 1940. He completed a B.S. degree with distinction in agricultural biochemistry at the University of Minnesota in 1943 and entered graduate school where he earned a Ph.D. in agricultural biochemistry in 1947. Brakke then accepted a postdoctoral fellowship with Black at the Brooklyn Botanic Garden to study "plant cancers" with funding from the American Cancer Society. The reason for taking this position, which might have seemed unusual for a protein chemist, was practical. "Jobs, at least interesting jobs, were scarce," Brakke later explained.[2] Black had determined that leafhoppers could transmit an uncharacterized agent that caused tumors in some plant species, and Brakke thought that identification of the agent might be a "good applied protein chemistry" problem. As outlined below, this work was the basis for Brakke's lifelong interest in the development of analytical techniques for virus studies.

Brakke's early work in Black's lab culminated in a landmark contribution to science: the invention of density-gradient centrifugation, which he used for the first time to purify potato yellow-dwarf virus. This technique provided an unparalleled capacity to purify viruses, separate nucleic acids and proteins, and fractionate cellular organelles. In his groundbreaking paper on density-gradient centrifugation in the *Journal of the American Chemical Society*, Brakke reported that the basic procedure could be modified for application to many different problems involving particles and large molecules of either biological or non-biological origin." Yet, the utility of Brakke's density-gradient centrifugation invention as a "separation procedure" and "as a criterion of purity, or as a technique for measuring densities of particles or large molecules," did not become widely applied for nearly 10 years.[3] Of course, ultimately sucrose density-gradient centrifugation became the most commonly used tool for a wide range of biological science applications and was key to the development of modern virology and molecular biology. Indeed, many advances in biology and the biomedical fields would not have been possible without this technique. By the latter half of the 20th century, density-gradient centrifugation was routinely used in nearly every biochemistry, molecular biology, cell biology, and virology laboratory in the world. Thus, Brakke's novel development provided the foundation for a more profound understanding of disease agents, and the synthesis and structure of proteins and nucleic acids. As Black wrote in 1981,

> No economic motive initiated the research on Brakke's invention of density gradient centrifugation. It arose from an effort to purify wound tumor virus, which had no economic importance, and the technique was worked out with a second virus, potato yellow dwarf, of minimal economic importance at the time, because it was thought to be better than wound tumor virus for the purpose.[4]

The foundations of Brakke's sucrose gradient procedure were predicated on work initiated in the mid-1920s to isolate and study proteins (or colloids). Brakke began to think about how the work on colloids and his training as a chemist could be combined to quantify the biological, physical, and chemical properties of viruses. His use of sucrose gradients, in conjunction with his contributions to the development of a horizontal (swinging bucket) rotor, essentially eliminated mixing and had the effect of stabilizing sedimenting macromolecules. These improvements provided a nondestructive and preparative method for isolation of large amounts of virus suitable for biochemical, serological, molecular, and crystallographic analyses.

Brakke often mentioned he was lucky that he was "given the time to put his feet up on his desk and think about the problem of how to purify the virus." In reviewing his publications during this period it is unlikely that much time was spent with his feet up; instead, it seems more likely he was mostly at the bench working on various aspects of wound-tumor virus (WTV). Because mechanical inoculation to plants was unsuccessful, a major problem was that the only biological assay for WTV was injection into leafhopper vectors and subsequent transmission to clover. A number of other problems, including low WTV concentrations in tumors and the lengthy periods required for infectivity assays by leafhoppers, hindered progress. After several false starts, Brakke and Black decided to use potato yellow-dwarf virus, a plant rhabdovirus that also was a subject of research in the Black lab, as an alternative to developing a purification protocol that might be suitable for WTV.

Potato yellow-dwarf virus had the advantage of inducing chlorotic lesions on *Nicotiana rustica*, thereby providing an infectivity assay to monitor enrichment. Although it was not known at the time, PYDV consists of rapidly sedimenting

membranous bacilliform particles with substantial light-scattering properties that are useful for visualization. Brakke's first successful experiment using potato yellow-dwarf virus (PYDV) was performed by centrifuging at 3100 rpm for 5 hours in 150 mM sodium chloride and 10 mM neutral potassium phosphate buffer for determination of the sedimentation rate. The buoyant density of the virus was then determined with a range of sucrose solutions and density gradients. Using centrifugation, Brakke determined that the upper boundary of a light-scattering band was associated with infectivity on *N. rustica* plants. This band was not observed in healthy extracts prepared under the same conditions. Electron microscopy examination of the light-scattering fraction revealed membrane-containing particles that were similar to those observed earlier in Black's lab in infected plant extracts.

These early centrifugation experiments, as summarized by Brakke, resulted in three important advances: (1) the virus could be visualized by light scattering, (2) the sedimentation value could be calculated, and (3) the buoyant density of a virus could be determined. A second critical advance by Brakke was development of the swinging bucket rotor to facilitate rate zonal density-gradient centrifugation. In angle rotors, particles accumulate along the wall of the tube and sediment rapidly to the bottom of the tube. By using a swinging bucket rotor at high centrifugal speed, wall effects and nonideal sedimentation conditions are reduced greatly, especially when care is taken early in purification to reduce or eliminate aggregation.

The general principle behind rate zonal centrifugation is to separate particles based on their size, shape, and density; PYDV was a fortuitous choice for developing this approach because of its large size, uniform shape, and easy visualization during rate zonal centrifugation. These

features initially made it possible to centrifuge PYDV and determine its sedimentation coefficient (~1150 S) using a small, slow-speed centrifuge. The fact that the virus could be transmitted mechanically also permitted use of infectivity assays to demonstrate that the infectious agent sedimented with the light-scattering band.

The success of density-gradient centrifugation with PYDV was followed closely by application of the method to the wound-tumor virus in 1953. The purification of wound-tumor virus (WTV) also represented the first demonstration that the virus had the same physicochemical properties when purified from a plant host and an insect (leafhopper) vector. These results also provided a gold standard to verify previous reports from the Black lab that WTV multiplied in the insect host and that it could be characterized by combinations of density-gradient centrifugation, zone electrophoresis, and electron microscopy. Brakke's successes with PYDV, WTV, and the tomato spotted-wilt virus were followed by numerous reviews describing virus purification, stabilization, and assay protocols that led to the application of these methods to many viruses that had been particularly difficult to purify. The extent to which Brakke's thinking was ahead of others is evidenced by the fact that it was nearly a decade before other biologists commonly used his methods for virus purification and enrichment of subcellular components (e.g., polyribosomes) with diverse physical and chemical properties.

In another collaboration with Josef Blum, then head instrument maker at the Rockefeller Institute for Medical Research, Brakke designed the first high-speed swinging-bucket rotor for density-gradient centrifugation, which was described in 1953. Simultaneously, Brakke developed a modification of a Beckman model DU spectrophotometer to scan the density-gradient tubes following centrifugation by modifying the test tube holder so "the tube could be raised by known

increments and the absorbance determined at each depth," as he reported in the *Archives of Biochemistry and Biophysics*. Brakke used this strategy to illustrate how sedimentation coefficients of viruses could be determined by density-gradient centrifugation. After moving to Lincoln, he greatly simplified the fractionation procedure by collaborating with Robert W. Allington to design and build a density-gradient fractionator and flow densitometer to scan and sample sucrose gradients directly. The outstanding success of this venture led to the formation of ISCO by Allington. As was typical of Brakke, when asked why ISCO held the patent, he commented in his truly generous way, "Well, I got the paper and Allington got the patent." Shortly after inventing density-gradient centrifugation, Brakke also developed zonal electrophoresis using sucrose gradients as the support medium for separation of macromolecules, and he later collaborated with Allington to develop an apparatus for this purpose. Brakke's intent in using gradient electrophoresis was to eliminate several problems previously encountered with electrophoresis in solutions, including mixing and poorly resolved boundaries.

In 1955 Brakke accepted a position with the U.S. Department of Agriculture, Agricultural Research Service (USDA-ARS) as a research chemist stationed at what was then the ARS Wheat and Sorghum Research Unit in Lincoln, Nebraska, and he joined the faculty of the Department of Plant Pathology at the University of Nebraska-Lincoln. While at Lincoln, Brakke continued his work on the analytical aspects of density gradients and virus purification problems. At UNL, Brakke shifted his focus to cereal viruses, including barley stripe-mosaic virus, barley yellow-dwarf virus, soilborne wheat mosaic virus, wheat streak-mosaic virus, and maize chlorotic-mottle virus. Brakke also mentored numerous young developing researchers at Nebraska and was instrumental in establishing the careers of several subsequent generations of scientists interested in

cereal viruses. In 1986 Brakke retired from the USDA-ARS and the Department of Plant Pathology. He then spent two years as a half-time assistant to the director of the Center for Biotechnology at UNL.

Each of the cereal viruses studied by Brakke presented unique problems in identifying purification methods, host range, vectors, and environmental conditions favorable for infection. Brakke repeatedly applied his ingenuity and persistence in developing new tools and techniques to solve these problems. His numerous seminal findings with each of these viruses provided notable advances in the fields of virology and plant breeding, and included mathematical demonstrations that systemic infections could be used to determine relative virus titer,[5] an idea proposed by H. H. McKinney in 1927, when local lesion hosts were not available.[6] Notably, Brakke determined that soilborne wheat mosaic virus is transmitted by the soilborne fungus *Polymyxa graminis*. This work led to the establishment of the genus *Furovirus* (fungalborne, rod-shaped virus). Brakke, in collaboration with Ellen Moorhead Ball, also developed leaf dip serology to provide a tool for the rapid identification of viruses by electron microscopy. This method led to the development of more refined serological techniques for the identification of viruses.

During the 1980s, Brakke clarified the mechanisms of an unusual anomaly referred to as aberrant ratio, a most interesting biological phenomenon in which barley stripe-mosaic virus infection exerts epigenetic effects on host genetics. This enigmatic phenomenon of the possibility of virus-induced genetic changes in maize was first reported in 1960 by McKinney and George Sprague, both working at the USDA in Beltsville, Maryland. After several years of experimentation, they reported that barley stripe-mosaic virus had a mutagenic effect in maize, and that virus infection was associated with an increased frequency of skewed segrega-

tion ratios over those of control plants. In the F2 progeny from virus-infected plants "abnormal segregation ratios" were observed by McKinney and Sprague, who noted that "some disturbing influence is operative" in association with the virus infections.[7] In his 1984 reanalysis and extensions of the data, Brakke asked, "Did the virus infection actually cause mutations and the diverse aberrant-ratio phenotype?"[8] This question was based on two reservations concerning the data. The results of McKinney and Sprague "prevented a firm conclusion" about whether there was a correlation between virus infection, mutation, and aberrant ratio; Brakke also noted that appropriate uninfected control stocks had not been carried along with the experimental stocks. Without such controls it could not be ruled out that environmental conditions or several generations of crosses could explain some of the perceived aberrant ratios.

This reanalysis provided a particularly insightful look into Brakke's demand for excellence in science, and his ability to analyze and critique intriguing results. He was open to pursuing the possibility that plant-virus-associated aberrant ratio was a real phenomenon, and he presented five mechanisms by which this phenomenon could be tested. Once it was established that virus could not be detected in the F1 generation and that there was no evidence of barley stripe-mosaic-virus cDNA integration into the maize genome, three possibilities remained. First, virus infection might stress the plant to activate controlling elements (transposons). In this case it remained to be proven whether barley stripe-mosaic virus per se was the stressor or if it was sufficient to cause the genotypic effects. Second, virus infection might affect nucleic acid repair or proofreading to increase the mutation rates; again, no data were available to support this possibility. Third, the virus might act as a vector, transferring a host factor with pseudovirions. Brakke worked through this latter

possibility with an idea that if "there is a host regulatory RNA replicated by a host RNA-dependent RNA polymerase, and if this regulatory RNA is transferred in a pseudovirion, then the stimulation of this enzyme by virus infection might explain why systemic invasion of the plant by the regulatory RNA would be synchronous with invasion by the virus." In this *Annual Review of Phytopathology* article Brakke further suggested that "from an evolutionary viewpoint, high mutation rates associated with virus disease could increase the adaptability of plants and their survival under stress."

These ideas, in concert with the recent emergence of our understanding of RNA silencing and suppressors, seem strikingly modern and worth reinvestigating. Brakke's careful analysis of the aberrant-ratio phenomenon, which was based upon field experiments and calculations, revealed his deep thinking about the problem and laid the foundation for research by a new generation of molecular geneticists. It is also worth noting that Brakke's observations were confirmed by the identification of a retrotransposon that disrupted the *Adh1* gene. Similarly, his finding that selfing aberrant-ratio plants could result in reversions, or reversal of inactivation; restoring aleurone color in progeny is now known to be due to *Jittery*, a unique transposon that can be excised but not reinserted into the genome.[9]

Brakke was an extremely kind-hearted, empathetic, and humble person who was never boastful. He exemplified the best personal characteristics of a scientist, and he had a positive influence on everyone who passed through the University of Nebraska-Lincoln, including graduate students, postdocs, and technicians. Two stories, elaborated by one of us (J.V.E.), illustrate these traits. The first involved Brakke's election to the National Academy of Sciences; he was the first person elected from The University of Nebraska. Myron did not tell anyone in the department on the day he received a

phone call indicating he was elected to the Academy. That evening my former technician Rex Koski and I were fishing at a lake on the Brakke farm. Myron and his wife, Betty, walked out to the lake and wanted to know how we were doing. I remember saying to Rex as we drove back to Lincoln that Myron seemed even more upbeat than usual that evening. When I arrived at the lab the next morning, the chair of our department, Mike Boosalis, said that he had just received a phone call from the University's information department indicating that Myron had been elected to the Academy and wanted to know what he should do. I suggested that we should verify the information by calling Washington. At that moment Myron arrived and I remember tactfully asking, "Myron, what is this stuff we are hearing about you?" Myron broke into a big smile and asked what I was talking about. In contrast, most people would have told everyone in the building within minutes of the honor of being elected to the National Academy of Sciences.

The second story involves the characterization of the unusual bacteriophage "ϕ6," which was discovered by Anne Vidaver, who was working on biocontrol of phytopathogenic bacteria in the UNL Plant Pathology Department. Characterization of ϕ6 revealed several firsts: the first bacteriophage to be discovered with an external lipid envelope, the first with a dsRNA genome, and the first with a segmented genome. I (J.V.E.) was helping Anne characterize the virus genome. At that time I had no experience with viruses, so I conferred with Myron about everything. Myron designed the perfect experiment for the initial physicochemical characterization of this bacteriophage using density gradients and running on a rotor with six swinging buckets. Myron watched me scan the gradients and immediately knew the significance of the results, which he explained to me over a three-day period. Day 1, "The virus has a dsRNA genome and I (Myron) do not

believe any other bacteriophages have such a genome." Day 2, "The dsRNA is segmented and I (Myron) do not believe any other bacteriophages have a segmented genome." Day 3, "dsRNA is probably a good interferon inducer." (This turned out to be an accurate prediction and was the basis of a patent.) By day 4, Anne and I were convinced that ϕ6 was a unique virus. Even though Myron deserved to be a coauthor on all of our ϕ6 papers, he never let us put his name on any of the papers, because he thought that the USDA might not approve.

During more than 40 years at Nebraska, Myron Brakke influenced the careers of numerous graduate students and faculty by giving selfless advice about their research and professional decisions. In most cases Brakke declined authorship on manuscripts that had benefited considerably from his intellectual input, simply by saying it's your research and I had only a few minor ideas. Indeed, this modest nature was one of his most enduring personal characteristics. Brakke's creative and original research accomplishments were recognized by numerous awards and honors. He was the third person to receive the American Phytopathological Society (APS) Ruth Allen Award (1968), was a fellow of APS (1972) and he received the APS Award of Distinction (1988), which has been presented to only 13 recipients since its inception 45 years ago. Brakke also was a fellow of the American Association for the Advancement of Science (1976) and was elected to membership in the National Academy of Sciences in 1974. Brakke was the Regents Professor of Plant Pathology at UNL, and he received UNL's Outstanding Research and Creative Activity Award in 1982. Brakke was the recipient of the Outstanding Achievement Award from the University of Minnesota (1977) and is listed as one of the University of Minnesota's Historical Greats. The USDA also honored Brakke with a Certificate of Merit, and he is the only person

to receive the USDA Superior Service Award twice. In 1987 Brakke was named a member of the Agricultural Research Service (ARS) Science Hall of Fame. In 2006 at the APS Annual Meeting in Quebec City, the Myron K. Brakke APS Student Travel Award was awarded for the first time. This award was established to thank Brakke for decades of friendship and valued advice he gave to former students and postdoctoral fellows and colleagues, and to honor his accomplishments in virology.

Above all, Myron Brakke was a family man who was devoted to his wife and four children. It was at Minnesota as a graduate student that Myron met Betty Jean Einbecker, who was a student in a chemistry lab that he was teaching. Betty was born in Highland Park, Illinois, on March 13, 1923; she completed a B.S. degree in nutrition at the University of Illinois (1944) and obtained an M.S. from the University of Minnesota in 1946. They married in 1947 and are survived by four children: Kenneth, Thomas, Joan Youngquist, and Karen Brakke Crompton, all of whom have completed doctoral degrees. In 2004 the Brakkes moved to Bellingham, Washington, to be closer to Joan and her family. Myron and Betty were married for almost 60 years; Betty died just a month after Myron, on July 16, 2007.

NOTES

1. Much of the material from this memoir was obtained from "Myron Kendall Brakke: 1921 to 2007" by K.-B. G. Scholthof, A. O. Jackson, and J. L. Van Etten. *Phytopathology* 98(2008):1056-1059.
2. M. K. Brakke. The origins of density gradient centrifugation. *Fractions* 1(1979):1-9.
3. Myron made the comment to one of us (J.V.E.) that the editor who handled his manuscript said in his acceptance letter that the method looked interesting but that it would not be very useful.
4. L. M. Black. Recollections and reflections. *Annu. Rev. Phytopathol*. 19(1981):1-19.
5. M. K. Brakke. Systemic infections for the assay of plant viruses. *Annu. Rev. Phytopathol*. 8(1970):61-84.
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7. G. F. Sprague, H. H. McKinney, and L. Greeley. Virus as a mutagenic agent in maize. *Science* 141(1963):1052-1053.
8. M. K. Brakke. Mutations, the abberant *[sic]* ratio phenomenon, and virus infection of maize. *Annu. Rev. Phytopathol*. 22(1984): 77-94.
9. Z. Xu, X. Yan, S. Maurais, H. Fu, D. G. O'Brien, J. Mottinger, and H. K. Dooner. *Jittery*, a *Mutator* distant relative with a paradoxical mobile behavior: Excision without reinsertion. *Plant Cell* 16(2004):1105-1114.

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