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Obituary

Myron Kendall Brakke: 1921 to 2007

Karen-Beth G. Scholthof, Andrew O. Jackson, and James L. Van Etten

Plant pathology lost a distinguished pioneer in plant virology with the death of Dr. Myron K. Brakke on 15 June 2007. Those who were his close colleagues also lost a valued mentor, an irreplaceable friend, and a trusted adviser. Brakke’s most notable accomplishment was the development of sucrose density gradient centrifugation for the purification and characterization of viruses and macromolecules, which led to major advances in biochemistry and molecular biology. He also was responsible for introducing numerous techniques and equipment for fractionation of macromolecules that were crucial for the development of virology, biochemistry, and molecular biology. In the early stages of his career as a member of Lindsay Black’s group, Brakke had a major impact on the understanding of insect-transmitted viruses including Wound tumor virus (WTV), Potato yellow dwarf virus (PYDV), and Tomato spotted wilt virus (TSWV). In addition, Brakke collaborated with other members of the Black group to make significant contributions to tissue culture research by identifying an α-amylase that was secreted from plant cells.

Myron Brakke was born 23 October 1921, near Rochester, in Fillmore County, Minnesota. He was the son of John and Hulda Brakke. He grew up on a farm and attended a one-room country school through the 8th grade. In 1938, he graduated from Rochester High School and attended Rochester Junior College from 1939 to 1940. At the University of Minnesota, Brakke completed a B.S. degree in agricultural biochemistry in 1943 and earned a Ph.D. in agricultural biochemistry in 1947.

In 1947, Brakke accepted a postdoctoral fellowship with Lindsay M. 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 (17). Black had determined that leaffoppers could transmit an unknown agent that caused tumors in some plant species, and Brakke thought that identification of the agent might be an “applied protein chemistry” problem (17). As outlined below, this work was the basis for Brakke’s life-long interest in the development of analytical techniques for virus studies, which he continued as an independent scientist at the University of Nebraska-Lincoln (UNL). The early work in Black’s lab culminated in a landmark contribution to science: the invention of density gradient centrifugation (5,6). This technique allowed for improved capacity to purify proteins, nucleic acids, and viruses and to separate cellular organelles, such as mitochondria and nuclei. In his groundbreaking paper on density gradient centrifugation in the Journal of the American Chemical Society, Brakke reported the “basic procedure can be modified for application to many different problems involving particles and large molecules of either biological or non-biological origin” (5). Yet, the utility of Brakke’s invention as a “separation procedure” and “as a criterion of purity, or as a technique for measuring densities of particles or large molecules” (5), did not become widely recognized for nearly 10 years. Ultimately sucrose density gradient centrifugation became commonly used in a wide range of biological sciences and was a key tool in the development of modern virology and molecular biology. Indeed, many advances in biology and biomedical fields could not have been made 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 early work provided the foundation for a more profound understanding of gene expression, and the synthesis and structure of proteins and nucleic acids.

While working at the Brooklyn Botanic Garden, Brakke developed zonal sedimentation using PYDV (5,6). As Black wrote in 1981, “no economic motive initiated the research on Brakke’s invention of density gradient centrifugation. It resulted in 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” (2).

The development of Brakke’s method was predicated on work initiated in the mid-1920s, involving the advancement of new techniques 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 quantitatively study the biological, physical, and chemical properties of viruses. His use of sucrose, in conjunction with his contributions to development of a horizontal (swinging bucket) rotor, essentially eliminated mixing and had the effect of ‘stabilizing’ the sample. These improvements, i.e., a linear gradient and the swinging bucket rotor, provided a truly preparative method for the nondestructive isolation of large amounts of virus that was suitable for biochemical, serological, molecular, and crystallographic studies.

Brakke often mentioned that 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.” However, in reviewing his publications, 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 WTV. After several false starts with WTV, he and Black decided to use PYDV to develop a purification protocol. PYDV had the advantage of inducing chlorotic lesions on Nicotiana rustica, thereby providing an infectivity assay to monitor the purification protocol. Brakke’s first successful experiment using PYDV was performed by centrifugation at...
3,100 rpm for 5 h in 150 mM NaCl and 10 mM neutral potassium phosphate buffer, not a sucrose gradient, for determination of the sedimentation rate (21,22). The buoyant density of the virus was then determined with a range of sucrose solutions. Using centrifugation, Brakke determined that the upper boundary of a light scattering band was associated with infectivity on N. rustica plants (21,22). This band was not observed in healthy extracts prepared under the same conditions. Examination of the light-scattering fraction by electron microscopy revealed membrane-containing particles that were similar to those observed earlier by Black et al. (4).

These early centrifugation experiments, as summarized by Brakke, resulted in three important advances: (i) the virus could be visualized by light scattering, (ii) the sedimentation value could be calculated, and (iii) the buoyant density of a virus could be determined (5,6,22). A second critical advance by Brakke was the development of the swinging bucket rotor for gradient centrifugation (6) and, of course, rate zonal density gradient centrifugation. In using angle rotors, particles can 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, the wall effect and “nonideal” sedimentation is reduced greatly, especially when care is taken in the early steps of purification to reduce or eliminate aggregate (13,17). In collaboration with Josef Blum, then head instrument maker at the Rockefeller Institute of Medical Research, Brakke designed the first high-speed swinging bucket rotor for density gradient centrifugation (17). This invention was described in a manuscript published in 1953 (6).

As early as 1953, Brakke developed a modification of a Beckman model DU spectrophotometer to “scan” the density gradient tubes following centrifugation by modifying the test tube holder (6). By doing so, “the tube could be raised by known increments and the absorbance determined at each depth” (6). Brakke used this strategy to illustrate how to determine sedimentation coefficients of viruses by density gradient centrifugation (8). Later, after moving to Lincoln, he greatly simplified the fractionation procedure by collaborating with Robert W. Allington to design and build a density gradient column fractionator and flow densitometer to scan and sample sucrose gradients directly (11). The outstanding success of this venture led to the formation of Instrumentation Specialty Corporation (ISCO) by Allington. As was typical of Brakke, when asked why ISCO held the patent, he commented in his true generous way, that “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 macro-molecules (7), and he later collaborated with Allington to develop an apparatus for this purpose (19). Brakke’s intent in using gradient electrophoresis was to eliminate several problems previously encountered with electrophoresis in solutions, including mixing and poorly resolved boundaries.

The general principle behind rate zonal centrifugation is to separate particles based on their size, shape, and density (5). Although Brakke was attempting to develop a method to purify WTV, a number of problems including low WTV concentrations in tumors and inefficient infectivity assays in leaffoppers hindered rapid progress on the methodology. Brakke therefore turned to PYDV, which also was a subject of Black’s research, for most of the exploratory experiments. PYDV was a fortuitous choice. It is a plant rhabdovirus that consists of a large membrane containing virion with a uniform shape and light scattering properties, which permit easy visualization during rate zonal centrifugation. These features initially made it possible to centrifuge PYDV and determine its sedimentation coefficient (~1,150 S) using a small, slow-speed centrifuge. The fact that the virus could be transmitted mechanically also allowed for the 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 closely followed by application of the method to WTV (510 S). The 1953 report (35) on the purification of WTV also represented the first demonstration that a virus had the same physicochemical properties when purified from the plant and insect (leafhopper) vector. These results implied that the virus multiplied in the insect host and that it could be characterized by a combination of density gradient centrifugation, zone electrophoresis, and electron microscopy (35). Brakke was at the forefront of the development of virus purification, stabilization, and assay methods. His success with PYDV, WTV (32), and TSWV (3) was followed by numerous reviews and also led to the application of this method to many viruses that had been particularly difficult to purify (12,14,19,27,29,32,33). The extent to which Brakke’s thinking was ahead of others is evidenced by the fact that it was nearly a decade before other virologists applied his methods for purification of other viruses with diverse physical and chemical properties.

Brakke moved with Black to the University of Illinois in 1952 and continued his work as a research associate in the Department of Botany. 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 UNL. Here he continued his work on the analytical aspects of density gradients and virus purification problems (9–11,30). At UNL, Brakke shifted his focus to cereal viruses, including *Barley stripe mosaic virus* (BSMV) (9,10,20,27,40,45,46), *Barley yellow dwarf virus* (BYDV) (29,48,49), *Soil-borne wheat mosaic virus* (SBWMMV) (16,23,24,28,37,39,47,53,54), *Wheat streak mosaic virus* (WSMV) (31,34), and *Maize chlorotic mottle virus* (38). His studies with these viruses represented notable advances in the fields of virology and plant breeding and included mathematically interesting demonstrations of how to use plants for systemic infectivity assays when local lesion hosts were not available (15). Notably, he determined that SBWMMV is transmitted by the soilborne fungus *Polymyxa graminis* (23,24,37,47). This work led to the establishment of the genus *Furovirus* (fungal-borne, rod-shaped virus) (54). Brakke, in collaboration with Ellen Moorhead Ball, developed leaf dip serology to provide a tool for identification of viruses by electron microscopy (1). This method led to the development of more refined serological techniques for the identification of viruses (42).

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. These methods also have helped establish the careers of several subsequent generations of scientists working on these viruses. In 1986, Brakke retired from the USDA-ARS and the Department of Plant Pathology. He then spent 2 years as a half-time Assistant to the Director of the Center for Biotechnology at UNL.

One of the most interesting biological phenomena characterized by Brakke was on the epigenetic effect of virus infection on host genetics, which is referred to as aberrant ratio (AR). In 1960, H. H. McKinney and George Sprague, both working at the USDA in Beltsville, Maryland, investigated “the possibility of virus induced genetic changes in maize” (18,41). After several years of experimentation, they reported that BSMV had a mutagenic effect in maize (55), and that virus infection was associated with an increased frequency of trait differences (1:108, about fivefold higher than in the control plants at 1:556). In the F2 progeny from virus-infected plants “abnormal segregation ratios” or an AR (18), were observed by McKinney who noted that “some disturbing influence is operative” in association with BSMV infections (55).
In a reanalysis of the data, Brakke asked, “did the virus infection [actually] cause mutations?” and the diverse AR phenotype? This was based on “two reservations” concerning the data. The results of McKinney and Sprague “prevent[ed] a firm conclusion” about whether there was a correlation between virus infection, mutation, and AR; he 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 AR. This reanalysis provided a particularly insightful look into Brakke’s demand for excellence in science, and his ability to analyze and critique intriguing results (30,50). He was open to pursuing the possibility that plant virus-associated AR was a real phenomenon, and he presented five mechanisms by which this phenomenon could be tested (18). Once it was established that virus could not be detected in the F1 generation and there was no evidence of BBSV-cDNA integration into the maize genome, three possibilities remained. First, virus infection stressed the plant to activate controlling elements (transposons). However, in this case, it remained to be proven that BBSV “per- se” was the stressor or if it was sufficient to cause the genotypic effects. Second, virus infection might affect nucleic acid repair or proof-reading to increase the rate of mutation; again, no data were available to support this possibility. Third, the virus might act as a vector, transferring a host factor via pseudovirions. He 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” (18). Brakke further suggested, “from an evolutionary viewpoint, high mutation rates associated with virus disease could increase the adaptability of plants and their survival under stress” (18). These ideas, with the emergence of our understanding of RNA silencing and suppressors (36,44,51), seem strikingly modern and worthwhile revisiting.

Brakke’s careful analysis of the AR 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. For example, his observations were confirmed with the identification of a retrotransposon that disrupted the Adh1 gene (43). Similarly, his finding that selfing AR plants could result in reversions, or “reversal of inactivation;” restoring aleurome color in progeny is now known to be due to Jittery, a unique transposon that can be excised, but not reinsert itself in the genome (58).

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

The second story involves the characterization of the unusual bacteriophage “φ6” which was discovered by Anne Vidaver in the UNL Plant Pathology department, who was working on biocontrol of phytopathogenic bacteria. Characterization of phage φ6 revealed several firsts: it was the first bacteriophage to be discovered with an external lipid envelope, the first with a dsRNA genome, and the first with a segmented genome (52,56,57). I (Van Etten) was helping Anne characterize the virus genome. However, at that time I had no experience with viruses and so I conferred with Myron about everything. Myron designed the perfect experiment for the initial physicochemical characterization of this bacteriophage using density gradients and it could be run 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 3-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 co-author 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 the more than 40 years at Nebraska, 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 is one of his most enduring personal characteristics.

Brakke’s creative and original research accomplishments were recognized by numerous awards and honors. He received the APS Ruth Allen Award (1968), was a Fellow of APS (1972) and received the APS Award of Distinction (1988). Dr. Brakke also was a Fellow of the American Association for the Advancement of Science (1976) and was elected to the National Academy of Sciences (1974). Brakke was the Regents Professor of Plant Pathology at UNL and he received the Outstanding Research and Creative Activity Award in 1982 from UNL and an Outstanding Achievement Award from the University of Minnesota (1977). The USDA also honored Brakke with a Certificate of Merit, and he is the only person to have received the USDA Superior Service Award twice. In 1987, he 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 Dr. 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.

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 13 March 1923 and 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, WA to be closer to Joan and her family. Myron and Betty were married for almost 60 years and she died just a month after Myron, on 16 July 2007.
LITERATURE CITED


