Reflections on my Family History, my Youth, and my Professional Career

Gabriele M. Zu Rhein, MD

Editors’ Introduction

The following reminiscence by Gabriele M. Zu Rhein is the seventh autobiography in a series published in the Journal of Neuropathology and Experimental Neurology. These have been solicited from senior members of the neuropathology community who have been noted leaders and contributors to neuroscience and to the American Association of Neuropathologists (AANP) and have a historical perspective of the importance of neuropathology in diagnosis, education, and research. It is hoped that this series will entertain, enlighten, and present members of the AANP with a better sense of the legacy that we have inherited, as well as reintroduce our respected neuroscientists as humans having interesting lives filled with joys and sorrows and allowing them to present their lives in their own way.

Dr. Zu Rhein is a special member of our association, a special scientist and a special person. In 1966 she resolved the cause of an enigmatic and important disease (PML), and, as if that were not enough to solidify her position amongst the most elite in our profession, in collaboration with two other neuropathologists, she described a new disease in 2007. Her biography highlights an intellectual life of achievement pulsed with a unique sense of humor.

M.N.H., R.A.S.

FAMILY HISTORY

Our family on my father’s side was first documented in Basel, in 1164, with the two Ritter (Knights) Hugo and Kuno de Reno. Latin family names were common at that time and often reflected the geographical location: de Reno – from the Rhine. German language changes later on resulted in Zu Rhein. Our earliest family residence was the Salzturm (salt tower) (Fig. 1), named for the traffic of salt across the Rhine from mines in the Alps that were started by the Celts. We collected customs for the transfer of this precious commodity, which was stored in the Salzhaus (salt house) next to the tower, prior to overland distribution.

Before the year 1500, three Ritter Zu Rhein were Mayors of Basel, and two Zu Rheins were Prince Bishops, which were territorial rulers. Friedrich hosted the Concilium of Basel (1431–1449) of the Roman Catholic Church and Kaspar acted as the second Chancellor of the University of Basel (1460–1461). Basel was one of the earliest cultural centers in Europe.

In 2005, my brother “discovered” in an art exhibit in Munich an illuminated medieval codex, which contains a miniature painting on parchment of Prince Bishop Friedrich as he bestows a fiefdom on a local knight. This is the first pictorial documentation of a family member.

Prior to the start of the Protestant Reformation, the family moved to the Alsace. A seven volume family history, written in French, was long preserved in the Castle at Dornach, once our property. In anticipation of the French Revolution, the last remaining family members moved to Germany. In the early 19th century, my great-great-grandfather obtained a law degree and served as Governor of Franconia. He established the first chair for Psychiatry at the University of Würzburg, and founded an academic art museum. Titles of nobility were officially acknowledged in France (Baron), and subsequently in Germany (Freiherr).

My father, Ludwig, who became fully orphaned at the age of 8½ years, received a special education for gifted boys, supported by the Bavarian Government, in Munich. This Gymnasium (High School with Latin and Greek) was called the Pagerie since the students also served as Pages at the Royal Court. The diverse instructions included fencing, dancing and horseback riding. Subsequently, my father attended a military academy and after this he studied law. He held a position in the Foreign Service of the State of Bavaria, but was called to active duty as an officer during WWI.

In 1918, he married Maria Therese von Bleul, my mother, and started in the service of the Royal Family of Bavaria, at first as a legal advisor, and then as the principal administrator of the property of Crown Prince Rupprecht. The Royal Wittelsbach family could not accept the Nazi ideology, and several members were held in concentration camps, while Rupprecht temporarily escaped to Italy. My father was called several times to the Gestapo headquarters in Munich for interrogation. In 1944, our family was bombed out in Munich, and my parents, and my younger brother, Hans-Herterich, found temporary refuge in a castle of the von Stauffenberg family, relatives on my mother’s side, before finding a final shelter at a friend’s castle in Franconia.

My mother’s family, on her maternal side, also came from the Rhine, with documentation in Speyer, in 1200. One great grandfather emigrated from Franconia (Schloss MainzHITE) to the USA where he perished in a natural disaster in Galveston, Texas, in 1883.

My mother grew up in Wiesbaden, in an officer’s family, chiefly in the care of French governesses. A devout Catholic, she attended a high school run by nuns, before obtaining a Teaching Diploma for French and English issued by the government. During WWI, she served as a nurse in the Red Cross.
EARLY CHILDHOOD AND ADOLESCENCE (1920–1939)

I was born in Munich, on April 5, 1920, and raised essentially as a single child; a sister, who had Down’s syndrome, died in infancy and my brother arrived only when I started in Gymnasium. My mother loved children and I had a very happy childhood due to her devotion. She supported early friendships. I much enjoyed operating on dolls under the guidance of a neighbor girl, the daughter of a surgeon. My mother also taught me early on about poverty, and death, by visiting with me our sickly, poor shoemaker with gifts, and by taking me to the cemetery where recently deceased people were presented, (behind huge glass windows), in their open coffins, prior to interment.

My father fit the image of an ‘‘authoritarian German father’’, which precluded a closer relationship. From age 6–10 years, I attended a private elementary school together with the children of 2 Nobel Prize winners (Karl van Frisch, Zoology, and Heinrich Wieland, Chemistry), among others. Discipline was very strict. When I offered to replace the old frayed Tatzenstock (spanking rod) with a freshly cut willow branch, however, the teacher did not react very favorably. Our very British English instruction started with ‘‘Humpty Dumpty sat on a wall...’’. From 1930–1939, I attended the Gymnasium of the Englische Fräulein, in Munich. This Catholic teaching order for girls had been founded by Maria Ward in England, in the 17th century, and members had been called by King Ludwig I of Bavaria to Munich in 1835, to settle in a wing of the Castle of Nymphenburg. My first year almost ended in disaster after my teacher found a drawing I had made inspired by my baby brother. It looked like a hermaphrodite, however, somebody who had “everything”. My diplomatic mother had to come to my rescue by explaining to the red-faced nun that I was a curious observer, rather than a sinner, and should not be dismissed.

Challenged by excellent teachers, I successfully and happily pursued my studies. An early medical interest was perhaps reflected in 2 essays, of my choice, one on the activity of leukocytes, the other on phrenology, the observation of special skull features and deduction from them of personality traits. To my father’s delight, I was awarded the Reichs-sport-abzeichen für Frauen, an athletic distinction created by the Nazi regime. Fortunately, however, the intrusion of Nazi ideology was minimal in our school. The required swastika was mounted below a larger cross in each classroom, and daily hand raising for the “Heil Hitler” salute was abortive. I never had to join the BDM (Bund deutscher Mädchen, analogue to Hitler Jugend for boys).

Long vacations, in the company of friends, invited by my parents, were spent chiefly in Schloss Hohenschwangau, on an Alpine lake. It was owned in part by the Bavarian Government, and in part by the Royal family. Sports dominated during the day (swimming, rowing, hiking, bicycling), and reading in the evening (Sherlock Holmes, Agatha Christie). Nose-diving bats in the hallways had to be accepted.

ADULT LIFE IN GERMANY (1939–1953)

My parents were most supportive of my decision to study Medicine at the Ludwig-Maximilians Universität in Munich (1939–1945). Prior to registering at a University, however, each student had to perform Landdienst, helping farmers during the summer months. Thanks to skillful negotiations by my parents, I spent this time at a von Stauffenberg castle tending to chickens and arranging flowers for rooms and hallways. During a brief visit, I met my hosts’ cousin Claus, an officer, who later paid with his life for his attempt to kill Hitler.

My start in the preclinical years coincided with the outbreak of WWII. However, classes proceeded unimpaired until the onset of Allied bombings, in 1943. My favorite Professor, early on, was Karl von Frisch, who described the ‘‘Schwanzeltanz’’ of honeybees, the dance-like movements of scout bees to communicate to the hive the location of pollen-bearing plants. I was fascinated by this silent language of invertebrates.

In the company of new friends, I enjoyed sailing on the Starnberger See, and long skiing vacations in the mountains I loved. Throughout the clinical years, it was more the etiology and pathogenesis of diseases than their treatment that interested me. Professor Ludwig Singer’s discussions during Gross Pathology Conferences were my favorite instructions. My father bought me a microscope to facilitate the study of glass slides throughout the weekends. They had a special appeal for me, as a visually oriented person.

My musical education profited from my father’s position, since a seat in the Royal box at the opera was available to me, in the absence of Royal visitors. Then, in 1943, life changed quite abruptly and became survival oriented. A detonation bomb totally destroyed a big air raid shelter that we had used for...
many months, killing everybody during “carpet bombing”. We survived in our own much smaller one, an attack with phosphorous bombs. However, the fires left us time only to save bare essentials, such as bedding, chairs, a table, my textbooks, and my father’s bicycle. I found refuge in a friend’s home in the Isar Valley, South of Munich, where an engineer had drilled a mineshaft into a slope as a shelter. On daily bicycle trips to the city I tried to continue my clinical studies, finding the building destroyed, however, with increasing frequency. The final examinations extended over several months, with several Professors having been evacuated into the countryside. In April 1945, I received the Approbation, a document certifying my readiness to work as a physician. A doctoral thesis, however, was finished only prior to my emigration, in 1953.

Professor Ludwig Singer hired me immediately after graduation to be trained as a Pathologist in the Laboratory of the Schwabinger Krankenhaus, the only large, still intact hospital in Munich. It became a short interlude. Units of the 6th U.S. Army marched into Munich in May, and in July the Medical Corps occupied our hospital with the exception of the Pediatric unit. Dr. Maurice Lev, the first Chief of Laboratory Service of the new 98th General Hospital offered me to join him since I was conversant in English and politically “clean”. With my father’s approval, I accepted. The German Government paid my salary, as it did for other German Physicians and Lab Technicians, in a contract to assist U.S. Medical Units. My affiliation as a Pathologist (“Assistant Chief of Laboratory Service”) persisted for 8 years. During this period of post-graduate education, I received personal instruction, and guidance from a succession of outstanding teachers who had been University Professors, or Directors of Laboratories prior to overseas military duty. My special gratitude extends to Dr. (Col.) Maurice Lev, the widely known former Cardiac Pathologist at the Hektoen Institute in Chicago, and to Dr (Col.) Helmuth Sprinz formerly of Walter Reed Army Medical Center. My duties were to perform autopsies, read surgicals, and give clinical-pathologic conferences (CPC’s). Professor Ludwig Burkhardt, from the University of Munich, came as a consultant to teach me the Pediatric Pathology I needed. Professor Willibald Scholz (Fig. 2) was the Director of the Max Planck Institute of Psychiatry, located just outside of the 98th General Hospital. Dr. Scholz, the successor to Drs. Alois Alzheimer and Walter Spielmeyer, conducted an introductory course in neuropathology biannually for U.S. army medical officers; and I became an additional beneficiary. During these years I crossed paths with two lifelong Neuropathology friends: Wolfgang Zeman, who studied with Dr. Scholz, and Elias Manueledis, who was part of a Yale University Epidemiology team accommodated at the 98th General Hospital to study Teschen disease (porcine encephalomyelitis). Prior to a commanding officer’s inspection, however, these pigs had to be amply fed, and scrubbed, and their basement doors camouflaged, to avoid detection. Important for my education from the start was the small but well-stocked library at the 98th General Hospital, which carried the latest editions of textbooks and many journals, which I needed to familiarize myself with the medical language in English. A special bonus, however, were volumes of Elizabethan poetry. These were happy years, without discrimination, or resentment against me as a woman, or a German. However, when our hospital got scaled down to the 2nd Field Hospital, the volume of work abruptly decreased, and I got bored.

My parents had died by then and my brother was engaged. Job offerings in Germany lacked challenge. One pertained to a woman’s psychiatric ward where, in this pre-tranquilizer era, the hallways were penetrated with constant shrieks and screams. In a fortunate turn of events, Dr. Alfred Evans, a former member of the Yale Epidemiology team, wrote to me about a job opening at the University of Wisconsin in Madison, whose faculty he had recently joined. He thought I would be an effective teacher, and offered to “sponsor” my visa application. I did not hesitate, and accepted gratefully a subsequent offer by Dr. D. Murray Angevine, Chairman of the Department of Pathology, to start there as an instructor. From my mother’s inheritance, I bought a Leitz Ortholux microscope, the best on the German market, to join me in my venture. It started with our SS OLYMPIA, sailing under a Greek flag, getting stuck on a sandbank in the English Channel. The damaged screw had to be repaired in Glasgow while we waited, illegally, in drydock for 2 weeks. We reached New York on New Year’s Eve 1953,

FIGURE 2. Dr. Willibald Scholz and Gabriele Zu Rhein with staff members at the Max Planck Institute of Psychiatry in Munich, ca. 1947.
escaping customs inspection. An old friend from the Gymnasium, Dr. Renate Dische, welcomed me at the pier to the U.S.A.

**ADULT LIFE AND ACADEMIC ACTIVITIES IN USA (1954-PRESENT)**

Madison, located on an isthmus between 2 lakes, is one of the most beautiful cities of this country. The surrounding scenery with its rivers, woods, and pastures reminded me so much of Bavaria. My friends, Dr. Alfred Evans and his German wife, Brigitte Evans (Fig. 3), found an apartment for me on the smaller lake, ideal later on for swimming far out into the distance, connected to my raft by a rope.

The small Pathology Department faculty was foreign born (Canada, Chile, Holland, Ireland, Norway, Yugoslavia), with one exception. I remained the only woman for 17 years. Dr. Angevine, a president of the Federation of American Societies for Experimental Biology favored animal experimentation as the dominant pursuit among the faculty. I was assigned to the autopsy service and to teaching medical students. Shortly after my arrival, the professor who had cut the brains, departed. No faculty member with an education in Neuropathology had been in our department since 1943. Dr. John McCarter had received training at McGill University in Montreal and gave a course in Neuropathology for Med III from 1940 to 1943. My greatest academic challenge came when Dr. Angevine stated very casually “The Germans have a good reputation for Neuropathology. Why don’t you do it?” The implication was not only an extra load of service work for me, but a teaching obligation for the coming fall. An early attempt to educate me at the AFIP for 2 weeks failed because, just then, Dr. Webb Haymaker happened to launch a NASA balloon with mice to study the effects of cosmic radiation. I found stacks of “backlog” cases to be worked up, but nobody to consult with.

So, I returned home to become self-taught, “on the go”, recalling Prof. Scholz’s introductions, and later, discovering in our Medical School library the seven big volumes (over 10,000 pages!) on diseases of the nervous system, edited by Prof. Scholz, and published by Springer Verlag, Berlin, from 1955–1958. Because 95% of the contributions were written in German, nobody else ever requested them and they remained on my desk, officially marked “discarded”.

The first comprehensive texts, written in English, became available only in 1958 and 1959, respectively: Neuropathology
by J.G. Greenfield et al., and Russell and Rubinstein’s *Pathology of Tumors of the Nervous System*. The Chair of the Department of Neurology, Dr. Hans Reese (Fig. 4), a native of Germany, became a most supportive friend who gave me books and journals, and permitted his lab technician to help me with the many special stains necessary for microscopy. The Pathology Department had a senior tissue technician who, however, lacked such expertise. He was one of a trio of older male civil service employees who disliked me from the start. He enjoyed turning the radio on to full volume listening to the Army vs. McCarthy hearings in the room we shared. The photographer, who provided prints and Kodachromes for teaching, considered me the last person to be helped; and the autopsy diener refused to leave the lunch room, whose built-in shower area I had to use for cooling off in summer when the temperature in the autopsy room reached 90°F and no fans were permitted. All this behavior was in marked contrast to that of my colleagues who, throughout my career in the USA, have been friendly, or even cordial, with the attendance at annual meetings. I found the Diagnostic Slide Sessions of the AANP, so cheerfully and ably managed by Dr. Leroy Sharer, and most effectively conducted by Dr. Lucien Rubinstein, followed by Drs. Lucy Rorke, Dick Davis, and Tessa Hedley-Whyte, particularly stimulating and informative. The University granted me a one year’s Leave of Absence which I spent in Dr. Harry Zimmerman’s Laboratory, at Montefiore Hospital (Fig. 5), in New York, engaged chiefly in electron microscopy (see below). This fateful assignment had been brokered for me by Dr. Reese, in 1964. During my first years in the Pathology Department I had to develop the skill of academic lecturing, and found my inspiration in Dr. John Harman, the Irish colleague. He combined basic and clinical science information, with much humor, and excellent blackboard drawings. The students followed easily and happily. What I did not anticipate was the linguistic problem with Neuropathology. With so many names of Latin or Greek derivation, I had to compose a glossary to be added to each student’s course book. The collection of teaching material became a difficult, drawn-out affair. With the help of an autopsy technician, I started to build a Museum of formalin fixed, sliced CNS specimens, mounted in plastic casings. We aimed at several hundred to be used for discussions in 4 parallel teaching labs. An initial set of a dozen Neuropathology glass slides per student was collected from surgical and from autopsy pathology, and from a few outside University labs. I did “inherit” a small number of Kodachromes for lecturing from Dr. Angevine. The vast majority, however, I had to contribute myself over the years.

The context for the teaching of Neuropathology to Med II students varied. From 1954–1970 Neuropathology was part of a “Systemic Pathology” course in our department; from 1971–1976 it was part of an interdisciplinary “Central Nervous System” course, directed by a Neurophysiologist, and from 1977–1994 it was a 32 hour, 2-credit course taught in Pathology, under my direction, with faculty participation from Neuroanatomy, Neurology, Neurosurgery, Neuroradiology, and the Veterinary Medical School. The students enjoyed this more meaningful approach, with the inclusion of clinicians; they proposed me as a candidate for the “Award for Distinguished Teaching”, which was granted to me in 1989 by our Medical Alumni Association. I cherished this honor, and took the criticism that “the tail is wagging the dog (Pathology)” with good humor.

After my official retirement in 1995, Pathology Chairman Dr. Michael Hart became the Director of a new course that included recently arrived Neuropathologist Dr. Shahriar Salamat among its faculty. I was permitted to continue teaching “Infectious Diseases” for 7 additional years (1998–2005). One of the final student evaluations contained the phrases “good lecturer”, “animated speaker”, “good sense of humor”, “nice old lady, very pleasant”. What more could I hope for?
My Neuropathology colleague Dr. Khang-Cheng Ho, an enthusiastic and much beloved teacher, invited me to contribute “Infectious Diseases” and “Tumors” to his Med II course at the Medical College of Wisconsin, in Milwaukee, from 1977 to 1992. I found the students there very inquisitive and strongly motivated. The course faculty always looked forward to the final dinner at the 4 star Pfister Hotel, a joyous affair enhanced by German food and beverages. Neuropathology was a component of many additional teaching activities at our University. In some of these efforts, I was joined by our faculty member Dr. Henrik Hartmann whose chief expertise lay in Neurochemistry. He and his Graduate Students focused on quantitative RNA analysis from individual neurons in various pathologic states.

Graduate Students in Veterinary Science received up to 18 hours of lectures and labs in a “Comparative System Pathology” course, and brain diseases were a component of a Graduate course entitled “Ultrastructural and Cytochemical Pathology”. Scattered throughout the year were gross and/or microscopic teaching conferences for Residents, Staff, local hospital Pathologists, and Veterinary School Pathologists. Nursing school students received gross demonstrations of the Anatomy, and selected Pathology of brain and spinal cord. A special challenge were the “Allied Heath” students from Pharmacy, Physiotherapy, and Occupational Therapy, since they needed an initial basic exposure to Neuroanatomy and Histology. Residents in Pathology and Neurosurgery rotated through Neuropathology for several months. The Neurology Residents, however, had to prepare CPC’s and had to take an evening course in microscopic Neuropathology with about 25 sessions per year. The very affable and effective chairman of Neurology, and successor to Dr. Hans Reese, was Dr. Francis Forster, one of the founders of the American Academy of Neurology. I held a joint appointment in his department from 1959 to 1968. Dr. Forster was keenly interested in the basic science training of his Residents. He bought me a Scopicon, an excellent small group-teaching device in which teacher and students sit at a round table. The teacher projects the glass slides onto a central white surface, and each trainee has a dirigible pointer to help initiate a discussion. In the darkened room, the ambiance was that of a séance. One appreciative participant, and good friend later on, was Dr. Mary M. Herman, later married to...

FIGURE 5. Dr. Harry Zimmerman with Gabriele Zu Rhein and Montefiore Hospital trainees at the AANP meeting in St. Louis, in June 1992.
Dr. Lucien J. Rubinstein, and a member of the National Institute of Mental Health at the NIH in Bethesda, Maryland.

My academic clock, however, kept ticking, and with sadness, I had to give up my Associate Professor position in the Department of Neurology, to gain more time for research. My first project (including all of its ramifications, and aided by a series of collaborators) extended over approximately two decades, and started in 1962. A brain consultation case, submitted by local Pathologist Dr. Etheldred Schafer, became for me a major cytopathologic challenge in a demyelinating disease involving the cerebellum. I showed the slides to Dr. Hartmann’s post-doctoral student Sam Chou, who was supported by the Multiple Sclerosis Society. He brought to my attention a paper by Åström, Mancall, and Richardson from 1958 describing “Progressive Multifocal Leukoencephalopathy” (PML). I agreed that the cytopathology of this chiefly cerebral disease matched ours. By lucky coincidence, I found another case just 2 months later, among our University Hospital autopsies, this time with lesions in the cerebrum. Only 30 previous cases of PML were known by then. Our Department acquired an electron microscope (EM), in 1963, and I intended to test the PML tissue for its suitability for taking along as a project for my sabbatical at Montefiore Hospital, in 1964. Dr. Chou had learned the EM technique and we used the originally formalin-fixed tissue to gain a first impression. The first ultrathin section gave me a déjà vu experience. I had seen such intranuclear crystalloid aggregates of virions just 3 months before when reading the thesis of a Veterinary Pathology student entitled “The Cytology of Canine Oral Papilloma”. It referenced a paper in Science (1962), by Dr. Joseph L. Melnick entitled “Papova Virus Group”. This combines the oncogenic Papilloma and slightly smaller Polyoma DNA viruses. Our measurements pointed to the latter in PML, but no virus of this kind was previously known to infect humans.

When I arrived in New York in September armed with Epon blocks and the first EM prints, Dr. Zimmerman, then the President of the Association for Research in Nervous and Mental Diseases (ARNMD), quickly added me as a speaker...
to the symposium of “Infections of the Nervous System”. I had 3 months to cram for this by reading on the topics of Virology, Cell Biology and Cancer Research, and to continue the PML-EM studies. Most helpful information came from the book “Oncogenic Viruses” by Ludwik Gross (1961), the discoverer of the polyoma virus in mice. The ARNMD meeting brought a most unpleasant confrontation with panel member Dr. Albert Sabin. I shared his ire with Dr. Rubinstein, who reported a recent PML case, coming to the same conclusions as we did. Dr. Sabin lacked appreciation of the electron microscope and considered the findings as artifacts, and of no use for a virologist. Later on, he remarked to Dr. Richard Johnson, with disdain, “she (ZR) thinks there are warts in the brain!”

Relations improved quickly after this nadir. On visits to Dr. Gross at the Bronx VA Hospital, and to Dr. Richard Shope, the discoverer of the Rabbit Papilloma virus, at the Rockefeller University, I found great interest, and encouragement, and true belief. An invitation to visit came from Dr. W. Bernhard, Director of the Cancer Research Institute in Villejuif, France. His own experience with the ultrastructure of Polyoma viruses led him to a complete acceptance of our own interpretation. He commented with surprise on the clarity of images, obtained in the EM, of initially formalin-fixed autopsy tissues, a so far shunned procedure.

Anticipating a delay (eventually 4 years!) in the publication of the Proceedings of the ARNMD meeting, Dr. Zimmerman urged me to save time. Mr. Dembitzer, the manager of the Montefiore EM suite, guided me in how to arrange a research paper suitable for Science. It was published in 1965, 4 months after submission, and without request for changes. Early that year Dr. Allan F. Howatson, from the Ontario Cancer Institute, became a most welcome collaborator. His interest centered on the ultrastructure of wart and polyoma viruses. He succeeded in analyzing details of the assumed viral capsids. The results clearly pointed to a polyoma virus. To get the message out to clinicians, Dr. Chou prepared an elaborate poster exhibit for the Annual Meeting of the American Academy of Neurology, in 1966 (Fig. 6). In it, our electron micrographs were juxtaposed to those of Dr. Howatson, obtained from polyoma virus-infected mouse kidneys. Clinical and microscopic data derived also from a case I had found at autopsy at Montefiore Hospital. Also in 1966, I received an invitation by the National Multiple Sclerosis Society to participate in a workshop on “slow virus” diseases at the USPHS Rocky Mountain Laboratory. Diseases
under discussion, in addition to PML, were rabies (Hilary Kopolowski), and spongiform encephalopathies (William Hadlow, scrapie; Carleton Gajdusek and Clarence Gibbs, Kuru). As advised by Dr. Bernhard, I referred to a recent paper in the French literature by M. Bouteille et al (1965) with images of paramyxovirus-like particles in subacute sclerosing panencephalitis. Increasing use of the electron microscope seemed to augment the list of “slow virus” candidates.

A decisive event (see below) in the unfolding PML “story” was the presentation of a paper on glial cell cultures by Dr. Harvey M. Shein (Harvard Medical School) at the Annual Meeting of the AANP, in 1965. In it, he described a dual cytopathic effect of Simian Virus 40 (SV40), a polyoma group virus, on cell cultures of human fetal spongioblasts and astrocytes, a system he had described that year in Experimental Cell Research. I approached Dr. Shein and told him how much his findings of infection and destruction of spongioblasts and transformation of astrocytes reminded me of the cytopathology of PML. Dr. Shein expressed a strong desire to collaborate in the future.

The pressure was on to obtain fresh PML tissues for testing in cultures or in animal systems. In 1968, a Hematology Resident familiar with our Science paper called from the VA Hospital in East Orange, New Jersey about a suspected PML patient, who, however, refused a brain biopsy. Dr. Aurelia del Rosario later succeeded in securing a brain autopsy permit from the patient’s mother, declaring me as recipient. On a turnaround aerial trip to East Orange, I cut the brain, found evidence of PML, and came back with a copious harvest of non-fragmented tissue. During a midnight transfer, Dr. Duard L. Walker, Professor of Medical Microbiology (Fig. 7), accepted the ice bucket, and with it the challenge. I had known Dr. Walker since 1956, when we collaborated on a Coxsackie virus paper. He showed great interest in PML, and I had informed him about Dr. Shein’s paper, and the sudden East Orange challenge.

In 1969, Dr. Walker, as Principal Investigator, received an NIH (NIAID) R01 grant entitled “Chronic Viral Infections of the Nervous System”, for a 5 year period. Included as co-investigators, aside from myself, were Professor Robert P. Hanson, and Dr. Richard T. Marsh, both from the Department of Veterinary Science, and both involved in slow virus research (transmissible mink encephalopathy (TME) and scrapie). Our slow virus group developed a ritual, followed for 10 years: twice a month the Medical School members marched in a file, with Dr. Walker as the leader (reminiscent of a famous Snoopy boy scout cartoon by Charles Schulz), from our building to Veterinary Science, accompanied by graduate students, and technicians, for progress reports, literature review, discussions and planning. During the first years, the East Orange tissue yielded no virus. Dr. Walker had insisted on the use of standard culture systems in which a “decent” virus should grow.

In June 1970, Dr. Bertram Dessel, Chief of Hematology at the VA Hospital in Wood, Wisconsin, forwarded to me a brain biopsy slide of John F. Cunningham, a patient with Hodgkin’s disease and progressing CNS symptoms. I could confirm his suspected diagnosis of PML. When he explained the situation to Mr. Cunningham the latter wished that his brain should aid our research related to this fatal disease. Dr. Dessel’s call reached me on a Sunday in the laboratory, and Dr. Walker drove us to the Milwaukee suburb, where I cut the much lesioned brain and sampled it for culturing and tissue studies. Dr. Dessel indicated that he would send a frozen sample to Dr. Gajdusek, at the NIH, since he had advertised his interest. Our electron microscopy revealed polyoma-like virions chiefly in oligodendroglial nuclei, as it had done in the East Orange case, and in astrocytes with atypical nuclei.

Meanwhile Dr. Walker had added a Research Associate to his laboratory team. Dr. Billie Padgett, who had obtained her PhD degree with him, but had spent 2 recent years in Canberra, Australia with Dr. Frank Fenner, a pioneer in the study of mammalian poxviruses. Dr. Walker presented her with the double challenge of isolating a suspected new human virus in an unfamiliar culture system employed for SV40. Dr. Padgett accepted: and I was very happy to assist the Microbiologists in their transition to Neuropathology by explaining brain terminology and furnishing illustrations. Dr. Walker made the necessary efforts to obtain a steady supply of human fetal brain tissue from local sources. With infinite patience and consummate skill, Dr. Padgett modified Dr. Shein’s culture system to increase the yield of spongioblasts, the oligodendrocyte precursors.

Exposure to PML brain extract resulted in the first viral isolation on March 24, 1971. I ran to get some champagne and we had an impromptu birthday party for the JC virus (JCV), named in gratitude after John Cunningham. We also thought of Dr. Sabin. Lancet published the isolation paper in June, 1971. Dr. Padgett later succeeded in also culturing JCV from the East Orange brain tissue using the fetal glial cell system.

During the mid-and late 1960s a few laboratories in the USA and Canada used brain suspensions to expose cells in standard cultures and/or to inoculate rodents, or primates by various routes. Neither cytopathology, nor diseases were observed during follow-up periods of up to 32 months. PML, however, received not only the attention of virologists. In 1967, Professor Edith Pette (Hamburg, Germany) invited me to give a paper on PML for the international symposium, “Pathogenesis and Etiology of Demyelinating Diseases” to be held in Locarno, Switzerland. The vast majority of speakers presented data on multiple sclerosis or experimental allergic encephalomyelitis (EAE). However, PML fit in particularly well, with a putative virus targeting oligodendocytes for replication followed by cell destruction, and, with this, causing unraveling and loss of myelin. The oligodendrocyte-myelin relationship had been explored by Drs. Mary B. and Richard P. Bunge, and visualized by electron microscopy only a few years earlier. Support for our observations came at the meeting from Dr. Fusahiro Ikuta of the Brain Research Institute in Niigata, Japan. He had collected 4 cases of PML and indicated similarity of the virions to the papova group of viruses. All tissues had initially been in fixatives. The interest in PML aroused at the meeting, led to a very enjoyable dance with Dr. Jones Salk, one of the invited discussants, during the social events at the end.

Following the JCV isolation, it was decided that Drs. Walker and Padgett would concentrate their efforts on viral propagation and on sero-epidemiological studies and that I should concentrate on viral pathogenicity in animals. To augment our funds, I applied for, and received an NIH R01 grant that eventually ran from 1973 to 1986. It gave me, as Principal Investigator, salaries for technicians, and for Project Deputy, as well as for research equipment.
In the following, I will comment on certain aspects of our diverse JCV oncogenesis experiments, which encompassed about 800 virus-inoculated and 500 control hamsters. The age at inoculation varied from late-fetal, to newborn, 3 days, 19 days, 22 days (weanlings) and 30 days (adults). The experiments were concluded with the deaths of the last JCV hamsters, up to 26½ months after inoculation, marking almost the lifespan of the species. JCV proved to be a “slow virus” indeed.

Inoculation sites included i.e., s.c. + i.c., s.c. + intraperitoneal (i.p.), intracocular (i.o.), and intravenous (i.v.). With suitable tail veins lacking in hamsters, Dr. Ohashi, a trained Neurosurgeon, used his nimble fingers to inject into the sublingual veins of male weanlings. Drs. Walker and Padgett supplied principally the MAD (Madison)-1 prototypical strain of JCV, and rarely, strains of later isolates. One of these, MAD-4, led for the first time in an experimental animal, to the induction of pineocytomas. The yield was 45% in one experiment. John Varakis and I enjoyed doing the EM studies, which revealed features of photoreceptor differentiation, reminiscent of a “Third Eye”. JC virus proved to be of unmatched poly- onogenicity in its mammalian host. The complete tumor spectrum included: medulloblastomas, ependymomas, grade 3–4 astrocytomas, gliomatosis of cerebrum and spinal cord, central and peripheral neuroblastomas, olfactory neuroblastomas, reti- noblastomas, pineocytomas, pituitary carcinomas, malignant schwannomas, meningeal sarcomas, and angiosarcomas of the heart and testes (unpublished, i.v. exp.) and leiomyosarcomas of the stomach (unpubl.). Tumors of oligodendrocytic lineage were not observed.

Some tumor phenotypes varied with the age at inoculation. In perinatal experiments, tumors resembling human childhood tumors dominated. After the age of 3 days, medulloblastomas ceased to appear. Perinatal i.o. inoculations resulted in retinoblastomas and paravertebral neuroblastomas, with metastases to viscera and long bones. In contrast, pituitary car- cinomas were obtained only in hamsters injected as adults. The tumor induction rate varied from 10% after parenteral inocu- lation to 80–95% after i.c. inoculation of newborns. A few older control hamsters died with benign visceral angiomias. Such occurrences are known from the literature, however.

Some of the above results were incorporated in my (unpublished) Presidential Address for the AANP in 1977, ent- titled “Papovaviruses and Neuropathology”. It earned me a warm hug from Dr. Richardson and some other audience members. I greatly enjoyed the honor of being the first woman president. Dr. Helena Riggs, however, from Philadelphia General Hospital, 10 years earlier, had declined acceptance of her nomination without an explanation.

During the decade form 1969–1979, my research interest was captivated also by a disease of commercially bred mink on farms in Wisconsin, which had caused three outbreaks since 1947. This fatal nervous system disease named “Transmissible Mink Encephalopathy” (TME), was assumed to have its origin from scrapie-contaminated sheep products in the feed. Mem- bers of our Department of Veterinary Science had brought the subject to my attention (see above) and we then formed a subgroup in the NIH supported study of “Chronic Viral Infec- tions of the Nervous System”. The final members comprised Drs. Robert J. Eckroade, Robert P. Hanson, Richard F. March, and myself. Dr. Jack Grabow, formerly of the Neurology Department of our Medical School, conducted prolonged electron encephalographic studies of Rhesus monkeys injected with TME, after a move to the Mayo Clinic in Minnesota. All autopsy tissues were transferred to Madison for analysis,
including electron microscopy. Due to the partial overlapping of the TME with PML/JCV studies, this became one of the most hectic times of my life. A colleague once commented, “You are burning the candle at both ends!” while we ran across the tarmac to catch a plane.

Most of the animals involved in our TME experiments were kept at the previously mentioned Charmany Farms facility, which temporarily evolved into a mini zoo with monkeys, raccoons, ferrets, and skunks. My first encounter, as a non-veterinary Pathologist, with a skunk, later on, almost prevented my re-entry into society. I will never forget those glands of ill repute lurking in the path of the scalpel!

Dr. Eckroade, working for a PhD degree in Pathology and in Veterinary Science (Fig. 8), with me and Professor Hanson, respectively, made many contributions to the clinical observations and the Pathology work-up of our mixed patients, several of whom he had trapped himself. One result was a detailed mapping, on coronal diagrams, of the location and intensity of the lesions of spongiform encephalopathy at the late stage of disease in mink. Our combined studies revealed a definite susceptibility of mammalian systems for the TME agent.

In 1973, in preparation for a meeting, I teamed up with Mr. Jack Lund of the Department of Photography-Cinema, to record certain aspects of the physical decline of two monkeys with advanced TME. The movies focused on the motor weakness apparent while negotiating obstacles and later on the non-reactivity to olfactory and visual stimuli. Segments of these movies were shown during my presentation of “Pathobiology of Transmissible Mink Encephalopathy” at the meeting of the American Association of Pathologists and Bacteriologists, in Washington, DC, in 1973. My strongest commitment in regard to the TME studies related to electron microscopy, however. The time allotted for these were the evening hours, often until close to midnight, when eyes and back finally gave up. The final tally indicates that I took 2256 electron micrographs from specimen of mink, monkeys, hamsters, and raccoons, 10% of which were from control animals. The Mink was the most extensively studied animal with EM sections being derived from 35 dispersed cortical samples.

In tissues obtained weeks prior to illness, and when light microscopy was still negative, the EM revealed enlargement of dendritic portions of synapses with dispersion of normal cytoplasmic components. Later on, the plasma membranes of synapses showed focal breaks. In vacuolar spaces, some of them interconnected, collections of curled membranes were assembled, or delicate threads connected to ribosome-like particles.

TME became a major intellectual challenge for me. What went on in this tissue? I could not recall having ever seen a similar process in Pathology. Synaptic cell membranes seemed to be cut by invisible scissors, and the injury did not evoke a cellular host response?

It had never been an aim of our Madison research group to check for infectious organisms in culture, and my “hunting” in the electron microscope brought only very rarely clusters of particles resembling small viruses in neuronal perikarya. None were located at the synaptic site of initial injury. At the conclusion of the TME project I remained mystified. Dr. Peter Lampert, La Jolla, California, who in the early 1970s was associated with the slow virus (spongiform encephalopathy) group at the NIH (Drs. C.J. Gibbs and D.C. Gajdusek) found in his EM studies of sick infected mice, (and of a chimpanzee), a few particles suggestive of viruses in cellular processes. Lampert did not speculate on their potential importance for this disease process. However, in a note to me, he did retract his comparison of the virus-like particles in the chimpanzee with papova virus(es). “This is nonsense P.L.”

After a leap of several decades, the PML/JCV project brought me the chance for another, and final, study of CNS pathogenesis. In 1990, Dr. Gary Ludwig of Neenah, Wisconsin sent me a consultation case hoping for confirmation of his diagnosis of PML. It was not. The process was one I had never seen or heard of. There were disseminated foci of demyelination and capillary abnormalities such as focal piling up of endothelial nuclei with compromise of the lumina. When first EM studies revealed intracytoplasmic inclusions, I sent a letter to Dr. Michael Hart, a connoisseur of endothelial cells, at the University of Iowa, with the question “Are these ‘Bodies’ mycoplasmas, or peroxisomes, or both, or a mutant of the Andromeda strain?” Dr. Hart forwarded my material to his friend Dr. James Powers at the University of Rochester, who agreed with the likelihood of mycoplasmas. My literature
FIGURE 9. Gabriele Zu Rhein enjoying the work on her last research project in 2006. Pleomorphic Mycoplasma-like bodies are displayed on the screen.
search had led me to Dr. Shyh-Ching Lo at the NIH, who had very recently documented a new species of this smallest bacterial, parasitic organism in human tissues, including the brain, of patients with AIDS.

I felt that it was not prudent to try to publish my single case (Fig. 9), and it went on the “back burner”, waiting for confirmation. Within a few years, Dr. Powers was able to send me 2 additional cases for confirmation by electron microscopy. Subsequently, he arranged from our combined materials, derived from 3 adult patients, a poster exhibit for the 2007 meeting of the American Association of Neuropathologists, in Washington, D.C., where it was awarded an Honorable Mention for the Moore Award. This prompted an old friend to comment that I had now advanced from a “late bloomer” to a “poster-child for Geriatrics.”

By 2011, in a “Letter to the Editor” of the JNEN, Dr. Powers and myself could refer to two additional pertinent cases. Dr. Jose Ferreira (Quebec, Canada), reported almost identical brain findings to ours at the autopsy of a middle-aged woman with motor deficits and Dr. Roy Rhodes (New Brunswick, NJ) et al found bacteria without cell walls in capillary endothelial cells in the brain biopsy of an elderly ataxic man. Immunostaining was positive for *Mycoplasma pneumonia* and subsequent immunostaining of Ferreira’s case and our case I also revealed positivity for *Mycoplasma pneumoniae* in capillary endothelial cells. In 2008, I briefly corresponded with Pathologist Dr. Bernhard Stamm, at the Kantonsspital in Aarau, Switzerland, who was the senior author of a paper on Mycoplasma pneumonia encephalomyelitis published, fittingly, in *Emerging Infectious Diseases*. To my great surprise the adult patient succumbed to an acute illness. There were no capillary lesions such as in our cases. Severe edema dominated. Macrophages contained antigens to *M. pneumoniae*. There were no ultrastructural studies.

A few months later, in 2008, Dr. Jim Powers sent me for consultation glass slides and electron micrographs of brain tissue of a 3-year-old boy with an acute fatal encephalitis and severe brain edema. I thought there could be mycoplasmas present in the somewhat autolytic tissue since suspicious “bodies” fit into their size range. Drs. Powers and Mahlon Johnson published the pertinent paper with documentation by immunolabeling of *M. pneumoniae* in glial cells, macrophages, and neurons, in *Acta Neuropathologica* in 2012. The range of mycoplasmal brain disease has kept expanding.

Before concluding my story, I would like to comment on the most enjoyable aspects of my academic life, and on some of the most challenging. The participation in International Congresses of Neuropathology, Virology, and Neurology opened my eyes for the culture, customs, art, and architecture of various countries outside of the USA. The efforts of host...
cities to showcase their assets were unrivaled in London, in 1955. At the initial official reception at St. James Palace, heralds in Beefsteak uniform announced each participant by name, and with stomping of a staff, before we ascended an elegant flight of stairs to reach the Lord Mayor’s buffet. In stark contrast, my other favorite type of professional get-together were workshops, assembled by invitation only, and held at secluded locations of scenic beauty like in Montana. Our common interest comprised “show virus” diseases and chronic transmissible diseases, later called Prion diseases (see above). Participants came from research centers such as the NIH, the Wistar Institute, and Johns Hopkins.

My longstanding active interest in comparative neuropathology got me on the speaker list for “Histopathology Seminars of the Nervous System of Laboratory Animals” sponsored by ILSI, the International Life Science Institute, in Washington, DC. In Nara, Japan, in the spring of 1988, it allowed time to stroll in the beautiful deer park where some over-friendly “locals” kept insisting to “goose” unprepared visitors, like me.

At our Medical School I particularly enjoyed participating in Dean Philip Farrell’s “Beautification Program” (Fig. 10). During a visit in Tampa, Florida, in 1997, I had bought a 6-foot tall, multtiered Ebony sculpture depicting village life, by a Maconde native artist from Tanzania. It eventually arrived in a custom made crate, in Madison. Dean Farrell was most appreciative and installed it at the Health Sciences Learning Center, for effective exposure.

The Medical School, and the Pathology Department, however, were the reason for the worst years of my academic life. A major construction project, right next to my office and lab, extending over almost 2 years (1980–1981), made my work almost impossible, and quite unbearable. The Dean could not offer me some “escape” facilities. Turned off lights, or water, intense vibrations, billowing clouds of dust, to which I am allergic, and disconnected telephones could be expected any time. My academic achievements became so meager that I had to petition the NIH to expand the running time of my R01 grant. During the same period, I was asked to move the EM suite twice, to different buildings, and there were meetings with contractors, engineers, and local managers. Then my EM technician one day just disappeared. She was found by police, several weeks later, in a Hippie camp in the Rocky Mountains, unwilling to return.

About ten years prior to my “Annus Horribilis,” our campus had been in an uproar against the Vietnam war with many clashes between students and tear gas-dispensing police, and National Guard. In August 1970, an Army Research Center across the street from our EM suite was bombed at night. Our shattered windows were then boarded up for many weeks. Once I saw in the same street soldiers with bayonet-mounted rifles lined up against a long row of “flower children” (students) holding bouquets to make their point. The scene remains indelible in my mind.

A final statement: My aims have been to be a competent Neuropathologist, a worthy member of my family, and a loyal, caring friend. I hope I did not fail.

REFERENCES
5. Eckroade RJ, Zu Rhein GM, Marsh RF, Hanson RP. Transmissible mink encephalopathy: Experimental transmission to the squirrel monkey. Science 1970;169:1088–90

© 2012 American Association of Neuropathologists, Inc.