Gabriele Zu Rhein (1920-2016), a long-lived neuropathologist. From progressive multifocal leukoencephalopathy to dementia associated with *Mycoplasma pneumoniae*

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ABSTRACT

Shortly after the end of the Second World War, the German physician Gabriele Zu Rhein emigrated to the United States, where she developed a fruitful professional career as a neuropathologist at the University of Wisconsin-Madison. She was an autodidact who also benefited from the interdisciplinary collaboration of such other specialists as virologists, neurologists, paediatricians, and veterinarians. For more than 50 years, she tirelessly dedicated a significant amount of her time to research, taking advantage of her skills in the emerging field of electron microscopy and her interest in neurovirology. Her main professional achievements were her studies on progressive multifocal leukoencephalopathy and the discovery of the John Cunningham virus, the tumours associated with this virus in experimental animals, and transmissible mink encephalopathy; and her research into dementia associated with *Mycoplasma pneumoniae* in the final years of her professional career. She was also an outstanding general pathologist, conducting noteworthy research on congenital diseases that progress with psychomotor disability, and research into viral diseases of the nervous system.

KEYWORDS

Gabriele Zu Rhein, neuropathology, progressive multifocal leukoencephalopathy, JC virus, transmissible mink encephalopathy, dementia associated with *Mycoplasma pneumoniae*

Introduction

The German researcher Gabriele Zu Rhein (1920-2016), born into a noble family, was able to overcome the challenges of studying medicine during the Second World War and subsequently working in a devastated country. Aware of the lack of opportunities for professional development if she remained in her country, Zu Rhein emigrated to the United States. There, she began working at the University of Wisconsin-Madison as a neuropathologist, in a setting dominated by interest in animal experiments and interdisciplinary collaboration. Her main achievements were the study of progressive multifocal leukoencephalopathy (PML), transmissible mink encephalopathy (TME), and dementia associated with *Mycoplasma pneumoniae*, at the end of her professional career. Her expertise in electron microscopy, a technique in very early stages of development when she arrived in the United States, was essential to this work. Zu Rhein distinguished herself in the study of congenital diseases associated with impaired psychomotor development and viral infections of the nervous system. She worked in close collaboration with virologists, neurologists, paediatricians, and veterinarians from the same institution and other centres. She was always willing to interact with other neuropathologists, whom she consulted or who sought her advice.

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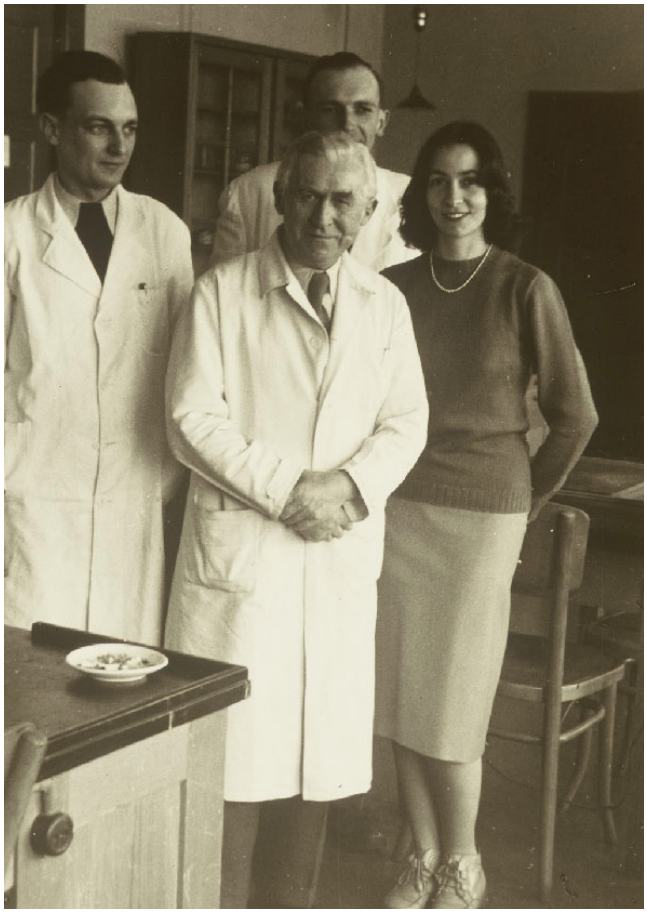

Figure 1. Gabriele Zu Rhein with Willibald Scholz (centre) and other members of the Max Planck Institute of Psychiatry, circa 1947.[1]

Material and methods

An extensive literature search was performed in different languages of the life and work of Gabriele Zu Rhein. Biographical aspects, the history of the discovery of PML and the John Cunningham virus (JCV), and her studies on spongiform encephalopathies were the main focus, without overlooking her professional and scientific career in general.

Development

A Bavarian baroness

The paternal family of Gabriele Zu Rhein originated from Basel, where her first known ancestors lived in the 12th century. This side of the family included knights, governors, and prince-bishops. They moved to Alsace before the Protestant Reformation and then to Germany before the French Revolution. Her father, a military man who studied law, worked at the service of the Royal House of Bavaria, which opposed the Nazi regime. Her mother was born into a Catholic family from the Rhine valley.[1]

Marie Gabriele Freiin Zu Rhein, daughter of Ludwig and Marie Therese, was born in Munich on 5 April 1920. She held the title of baroness (in German, *Freiin*) by birth. Zu Rhein had two siblings, Michele, who had Down syndrome and died during childhood in 1926, and Hans, who was 10 years older than her. Between 1930 and 1939, she attended the Gymnasium der Englischen Fräulein, in Munich, in which she learnt English from excellent teachers. There, her interest in medicine was born. She also received musical education and enjoyed playing sports.[1]

She studied medicine at Ludwig-Maximilians Universität in Munich (1939-1945). Her preclinical training coincided with the outbreak of the Second World War. Her favourite professor was the biologist Karl von Frisch. During her first years as a clinician, she became interested in disease aetiopathogenesis and enjoyed looking through the microscope that her father bought her.[1,2]

In 1943, when the Allies began bombing Munich, her life changed and focused on surviving. A bomb destroyed the air raid shelter in which her family was living, and they moved to a mineshaft close to the Isar valley, from where she commuted to university by bicycle. She graduated in April 1945, but could not obtain her doctorate degree until 1953, soon before she emigrated to the United States.[1]

After becoming a doctor, she was hired by her pathology professor, Ludwig Singer, to further her training as pathologist at the laboratory of the Schwabing Clinic, the only laboratory that remained intact in Munich after the war. In July 1945, her hospital was occupied by the medical corps of the US Sixth Army and Maurice Lev, head of the laboratory of the new 98th General Hospital, employed her as pathologist for eight years, with the title of assistant head of the laboratory service, thanks to her command of English and clean political records. There, she learnt from American and German professionals, including the neuropathologist Willibald Scholz

(1889-1971), head of the nearby Max Planck Institute of Psychiatry, the successor to Alzheimer and Spielmeyer (Figure 1).[3] Zu Rhein was responsible for autopsies and surgical reports, and participated in clinico-pathological meetings. The hospital had a small but well-sourced library that had the latest editions of medical textbooks and journals. During these happy years, she suffered no discrimination for being German or a woman.[1]

In 1953, when the category of the hospital was lowered and the workload decreased, Gabriele Zu Rhein decided to emigrate. Her parents had died and job offers in Germany were scarce. Her friend Alfred Evans, with whom she had worked in Munich, had just started working as a lecturer of epidemiology at the University of Wisconsin campus in Madison. He recommended her to Murray Angevine, head of the department of pathology, who offered Zu Rhein a job as an instructor. Zu Rhein purchased a Leitz Ortholux microscope and boarded the SS Olympia for New York, arriving on New Year's Eve of 1953.[1,2]

This small pathology department at the University of Wisconsin-Madison was mainly made up of foreign staff, and for 17 years she was the only woman in the group. Angevine's interest in experimental pathology was well-known. He considered the diagnosis of human diseases to be a secondary subject as it was not expected to provide significant scientific progress. Anybody not working with rats, Guinea pigs, or dogs was considered a member of a lower class. Zu Rhein was commissioned to perform autopsies and to teach medical students. Her great opportunity soon arrived, when the lecturer performing brain examinations resigned, and Angevine asked her to replace him. He told her: "The Germans have a good reputation for Neuropathology. Why don't you do it?"[1,2]

Neuropathology as subspecialty of pathology was something completely new in 1954 and so she had to turn to self-teaching. To do this, through the library of the School of Medicine, she accessed Scholz' treatise on diseases of the nervous system, divided into 7 volumes and published by Springer-Verlag in 1955-1958. The majority of the book was written in German, which only Zu Rhein was able to read. Soon she could also add the books *Neuropathology*, by J.G. Greenfield, and *Pathology of tumours of the nervous system*, by Russell and Rubinstein, which were published in 1958-1959. She was also supported by her friendship with the head of the Department of Neurology, Hans Reese, also German, who provided her with books and journals and permitted his laboratory technician to assist her with special staining of the nervous system. Rotating residents in pathology, neurology, and neurosurgery were under Zu Rhein's responsibility. Furthermore, she taught medical, veterinary, nursing, and pharmacy students. She worked at the university for 41 years, until her retirement in 1995.[1,2]

Autopsy material came from the University hospital, the Central Wisconsin Colony and Training School for the developmentally disabled in Madison, and from consultations at other centres across the state. She was not in charge of the surgical specimens, but was allowed to access them if necessary.[1] Zu Rhein also had a close relationship with Carl Olson's powerful Department of Animal Health and Biomedical Sciences, which had an electron microscope and research groups on papillomavirus, scrapie, and TME.[2]

In 1956, she met the microbiologist and veterinarian Duard Walker, with whom Zu Rhein would establish a long and fruitful partnership. That same year, they reported how prolonged exposure to cold made Coxsackie virus infection, which normally caused local effects, become generalised and have fatal outcomes in adult mice. They attributed this effect to the role of cold as a stressor.[4]

Zu Rhein soon showed a special interest in viral diseases, and her attention was drawn to acute necrotising encephalitis after the report of 3 patients between 1957 and 1960. The first case occurred after a head trauma, in which identification of the typical inclusion bodies led to the diagnosis of herpetic encephalitis.[5] This interest in viral diseases was also shared by Shi-Ming Chou (better known as Sam Chou), a young researcher supported by the National Multiple Sclerosis Society (NMSS) who in 1959 joined the Department of Pathology as postdoctoral student.[2]

Progressive multifocal leukoencephalopathy

In autumn 1962, Gabriele Zu Rhein was consulted by a pathologist from a hospital in the suburbs of Madison about the case of a 33-year-old woman with lupus erythematosus who died after several weeks due to a progressive neurological disease. The patient's brain showed multifocal demyelinating disease with presence of giant astrocytes of tumoural appearance and a

significant number of oligodendrocytes with enlarged nuclei. As she was unable to identify the disease, she showed the preparations to Sam Chou, and he in turn showed her articles on PML by Aström et al.[6] from 1958 and by Richardson from 1961.[7] They had no doubt that this patient was one of the fewer than 30 known reports of this disease. Just two months later, they performed the autopsy of a 67-year-old woman with a long history of bronchitis who developed a neurological disease that progressed for seven months, whose brain showed extensive demyelination and the characteristic cytopathological results of PML. By that time, Gabriele Zu Rhein worked with students in inducing brain tumours in chickens with the Rous sarcoma virus.[2]

In 1958, Aström, Mancall, and Richardson had reported three cases of progressive neurological disease, which they called PML, indicating its clinical progression, and the macroscopic findings.[6] They also identified another 5 previously published cases. Pathological findings in the oligodendrocyte nuclei led Cavanagh,[8] in 1959, and Richardson,[7] in 1961, to suggest a viral aetiology and underscore the need to study fresh autopsy material.[9,10] This phenomenon usually manifests in patients with chronic diseases that lead to a decreased immunological response of the body.[11]

Gabriele Zu Rhein and Sam Chou decided to study the enigma of abnormal oligodendrocytes in PML using the electron microscope purchased by the Department of Pathology in 1963, analysing tissues that they had fixed with formalin two years earlier. Chou, who had learnt to use the electron microscope at the Department of Animal Health and Biomedical Science, was one of the first to use it.[2,11] The first models of transmission and scanning electron microscopes were created in the 1930s, and became available commercially during the 1950s and early 1960s.

On 7 August 1964, they identified rounded and filamentous intranuclear particles that could be polyomavirus virions. No human polyomavirus was known at that time. These images reminded Zu Rhein of those she had seen in May 1964 during the presentation of the doctoral thesis of a student of Dr Olson, "The cytology of canine oral papillomavirus", which showed electron micrographs of cell nuclei with aggregated and disperse virions. In the list of references of the thesis, she found an article by Joseph Melnick, published in *Science* in 1962 under the title "Papova virus group,"[12] which combined papillomaviruses and the slightly smaller polyomas to define a group of oncogenic DNA viruses with the ability to cause latent infections. This oncogenic potential may explain the abnormal types of astrocytes and their penetration into the deeper layers of the brain, unlike papillomaviruses.[2,11]

Montefiore Einstein Medical Center

In 1964, Murray Angevine authorised Gabriele Zu Rhein to take a sabbatical year and Hans Reese secured her a stay at Harry Zimmerman's neuropathology laboratory at the Montefiore Einstein Medical Center in the Bronx. Before leaving, she taught neuropathology to Sam Chou so that he could substitute her, and she learnt electron microscopy from him.[2]

In early September, she arrived at Montefiore Einstein Medical Center carrying samples of an extensive oesophagitis with intranuclear inclusion bodies of possible herpetic origin, which was later confirmed, and PML samples. When Zimmerman saw these images, he rushed to include her as a speaker in a symposium on infections of the nervous system to be held in December by the Association for Research in Nervous and Mental Disease, of which he was coordinator as president of the institution. Gabriele had to study virology, cell biology, oncology, and what was known about PML in detail.[1,2]

On 5 December, with Albert Sabin, other distinguished virologists, and Sam Chou in the audience, Gabriele Zu Rhein presented her two cases, explaining that the virions had the same characteristics as polyomaviruses in the papovavirus group, suggesting that they were the cause of PML. She was followed by Lucien Rubinstein from Stanford, who presented a similar case. Sabin, who was one of the moderators, took control of the session and vigorously attacked them. He defined Zu Rhein's work as very regrettable and rejected the idea that a virus could be identified based on morphological evidence. "This is deplorable: everybody thinks everything is a virus. We cannot assert that there is morphological proof of a virus." With regard to electron micrographs, he stated: "This is a good way of not interesting a virologist." Rubinstein responded by stating that all electron microscopes should be thrown away if doctor Sabin was right. Sabin later stated: "She thinks there are warts in the brain!", as during that time, human wart virus was the only papovavirus known to cause a human disease. During the lunch, he also explained to another

Figure 2. Gabriele Zu Rhein and Sam Chou at the Annual Meeting of the American Academy of Neurology in 1966.[1]

guest: "Do you know, doctor, that if you take a piece of wood, carve it, paint it, and polish it you will eventually have an apple?" Despite this, after the session, Zu Rhein received shows of support from several scientists. When the proceedings of the symposium were published years later, Sabin's intervention appeared in softer terms.[2,10]

Anticipating a delay in the publication of the proceedings, Zimmerman urged Zu Rhein to send her study to *Science*, in which it was published under her name and Sam Chou's in March 1965,[11] four months after her presentation at the symposium. Allan Howatson, from the Ontario Cancer Institute, soon collaborated with them, thanks to his interest in the ultrastructure of wart virus and polyomaviridae. He had managed to analyse details of capsids using the negative staining method. The findings obtained in the three cases of PML suggested the presence of a polyomavirus similar to the simian virus 40 (SV40). They acknowledged that the term virion had been proposed to define entities with clearly defined structural characteristics of viruses, whether biologically active or inactive, thus reserving the term virus to cases when infectivity had been established.[9] Chou presented a poster communication at the 1966 annual meeting of the American Academy of Neurology, comparing the virions from his micrography studies of a patient with PML from the Montefiore Hospital with those obtained by Howatson of a polyomavirus from a mouse kidney, which were very similar (Figure 2).[1] Zu Rhein also found lesions characteristic of PML with presence of virions in subclinical cases (Figure 3).[13]

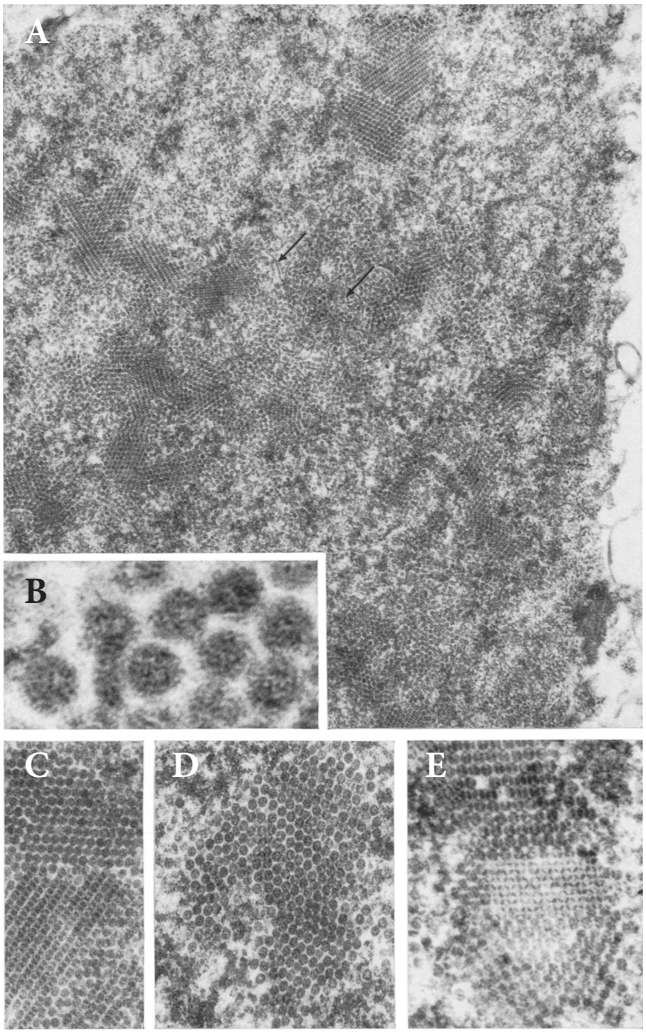

Figure 3. Electron micrographs of progressive multifocal leukoencephalopathy. A) Glial nucleus with most of its chromatin replaced by spherical virions, scattered or aggregated in crystals. Arrows point to occasional filamentous structures. B) Detail of the same nucleus with similar spherical and elongated patterns. C-E) Differently patterned crystals with focal blurring of the image due to overlapping of particles. Hexagonal contour and central lucency of some particles.[13]

She finally obtained national and international recognition for her findings, and Joseph Melnick requested that Zu Rhein write a review article for the journal *Progress in Medical Virology*, which was published in 1969. She gathered 27 cases of PML showing papovavirions.[14] At that time, data from biological studies on the disease were scarce as cases were rare.[2]

Discovery of the JC virus

During the annual meeting of the American Association of Neuropathologists (AANP) in June 1965, Harvey Shein presented his work on the growth of the SV40 in cell cultures of spongioblasts and human fetal astrocytes (primary human fetal glial cells [PHFG]). Zu Rhein became fascinated by the dual cytopathological effect of necrosis of spongioblasts and astrocyte transformation, which is similar to what happens in PML. She proposed using the same system to isolate the polyomavirus in this disease, which required fresh brain tissue from patients.[2,15] To do so, she collaborated with the virologist Duard Walker, whom she had known since 1956,[4] and Billie Padgett, an expert in poxviruses, using the negative staining method (Figure 4).[1]

In March 1967, a haematologist from the Veterans Administration Hospital in East Orange (New Jersey) consulted Gabriele Zu Rhein about the case of a patient with Hodgkin lymphoma and clinical symptoms of PML diagnosed after a brain biopsy. The patient died on a Sunday in January 1968, and Zu Rhein flew the same day to East Orange to perform the brain autopsy, finding lesions compatible with PML. She took samples for virological and pathological analysis and she arrived back in Madison at midnight, where Duard Walker was waiting for her in the autopsy room. With the tissue available, they soon showed that it contained the virions they had already identified, but cultures yielded negative results.[1]

In June 1970, Zu Rhein received a histological slice from the brain biopsy of the patient John F. Cunningham, who presented Hodgkin lymphoma and progressive neurological deficits. He was a patient of the haematologist Bertram Dessel of the Veterans Administration Hospital in Wood, in the Milwaukee area, who was aware of the neuropathologist's interest in PML. Cunningham died on a Sunday in June 1970 and Zu Rhein travelled there by car, accompanied by Walker, with the aim of obtaining fresh brain samples for tissue culture. Using the electron microscope, they once more observed polyomavirus virions, mainly in oligodendrocytes and astrocytes with atypical nuclei, as in the patient in East Orange. In this case, Padgett modified Shein's culture technique to increase the production of spongioblasts (oligodendrocyte precursors), which are the main target of the virus. Using this technique, the virus was cultured on 24 March 1971. The virus was named JC virus, in honour of John Cunningham. On that day, they had a

Figure 4. From left to right, Billie Padgett, Duard Walker, and Gabriele Zu Rhein, in 1988.[1]

special thought for Albert Sabin. Eventually, using the same method, Padgett also managed to isolate the virus in the brain of the patient from East Orange.[1,2,10,15]

They published their findings on the JC virus in a June issue of the *Lancet*,[15] the same in which Gardner et al.[16] reported the isolation of another human polyomavirus, BK, from the urine of a patient who had undergone kidney transplantation. Thus, they identified JC virus as a cause of PML, an obscure disease that became prevalent in the following decades due to the AIDS pandemic and the expansion of immunosuppressive treatments.[17]

Up to 1976, they isolated the virus in seven patients with PML. By that time, it had been detected in 20 cases, and was indistinguishable from the original virus, which could not be cultured in human culture media other than PHFG or in monkey cells.[18]

They also studied the antigenic and morphological properties of the JC, BK, and SV40 polyomaviridae. They compared the characteristics of the T antigen shared by these viruses, which are linked to their oncogenic potential.[19,20] Furthermore, they observed that all polyomaviridae had another common antigen located

on the major capsid protein VP1, which persists for several years; therefore, viruses could be routinely detected in tissue samples.[21]

The JC virus in oncology

After her success isolating the JC virus, Gabriele Zu Rhein focused on studying its pathogenicity in animals. She received a grant for this work from the National Institutes of Health (NIH) as principal investigator; other participating researchers included Robert Eckroade and John Varakis, among others. Bearing in mind the success previously achieved with SV40 in inducing brain tumours in hamsters, they decided to use this rodent. They enjoyed many resources at the University of Wisconsin, and especially its partnership with the Department of Animal Health, which had a network of farms and a primate research centre.[1]

In the 1970s, it has been already discovered that humans are natural hosts of two polyomaviruses, JC and BK, which cause cryptic infections during childhood. A variant of SV40 was also isolated in two cases of PML, which was named SV40-PML.[22] Tumours were induced in golden hamsters with the JC, BK, SV40, and SV40-PML viruses. The JC virus was particularly noteworthy as it was a potent inducer of different tumours of the nervous system, including medulloblastomas, undifferentiated neuroectodermal tumours, glioblastomas, ependymomas, pineocytomas, neuroblastomas, and meningiomas. In contrast, the BK and SV40-PML viruses only induced tumours on the ventricular surface, such as choroid plexus papilloma and ependymomas.[23,24]

In a study published in 1973 in *Science*,[23] they intracerebrally and subcutaneously injected the JC virus into newborn hamsters. At the end of the experiment, after six months of follow-up, 83% of animals had developed brain tumours, predominantly medulloblastomas and supratentorial glioblastomas, which were preferentially located on the cerebral cortex and thalamus, and two papillary ependymomas, from which they isolated the virus. They observed the copresence of several tumours in the majority of brains, frequently of different histological types. Many cultured tumour cells contained an intranuclear antigen similar to the papovavirus T antigen. They also presented their findings at the 1973 annual meeting of the AANP, and were awarded the prize for best communication.[1,23] Furthermore, they were the first to experimentally induce a pineocytoma, in this case in an adult hamster,[25] although later they also used newborn hamsters.[26]

Zu Rhein and Varakis concluded that in hamsters, the JC virus caused medulloblastomas, ependymomas, astrocytomas, gliomatosis, central and peripheral neuroblastomas, olfactory neuroblastomas, retinoblastomas, pineocytomas, pituitary carcinomas, malignant schwannomas, meningeal sarcomas, cardiac and testicular angiosarcomas, and gastric leiomyosarcomas. Some tumour phenotypes were associated with age at inoculation. The induction rate in newborn hamsters ranged from 10% when inoculation was parenteral to 80%-95% when inoculation was intracerebral. Zu Rhein included some of these findings in her presidential address to the AANP in 1977, with the subject "Papovavirus and neuropathology."[1] Today, the term papovavirus is obsolete, and has been replaced by two new families, *papillomaviridae* and *polyomaviridae*.[27]

They also studied the susceptibility of non-human primates to the JC virus, by inoculating it into the brains of four owl monkeys; two of them died due to astrocytoma after latencies of six and 25 months. They chose this New World monkey species because it exhibited low exposure to the JC virus, with low or undetectable levels of antibodies. They also induced astrocytomas in squirrel monkeys. No brain tumours had previously been induced in these species.[1,24,28]

In the case of hamsters, Zu Rhein and Varakis' hypothesis was that, during the neonatal period, mitotically active cells of the external granular layer of the cortex are infected by the virus, and subsequently emigrate to the internal granular layer with the viral genes integrated into their DNA; then, at the age of five months, tumoural transformation occurs. In this model, a high incidence of cerebellar medulloblastomas was observed.[29,30]

The JC virus became the only virus known to trigger multiple types of nervous system tumours and the first human virus capable of inducing solid tumours in primates. However, neither hamsters nor owl monkeys displayed any demyelinating disease resembling PML. Zu Rhein hoped that this research on the virus to contribute to deepening the knowledge of human brain tumours.[2]

Antibody studies suggest that JV virus infection is very frequent in humans, apparently occurring in early childhood. It is still debated whether such

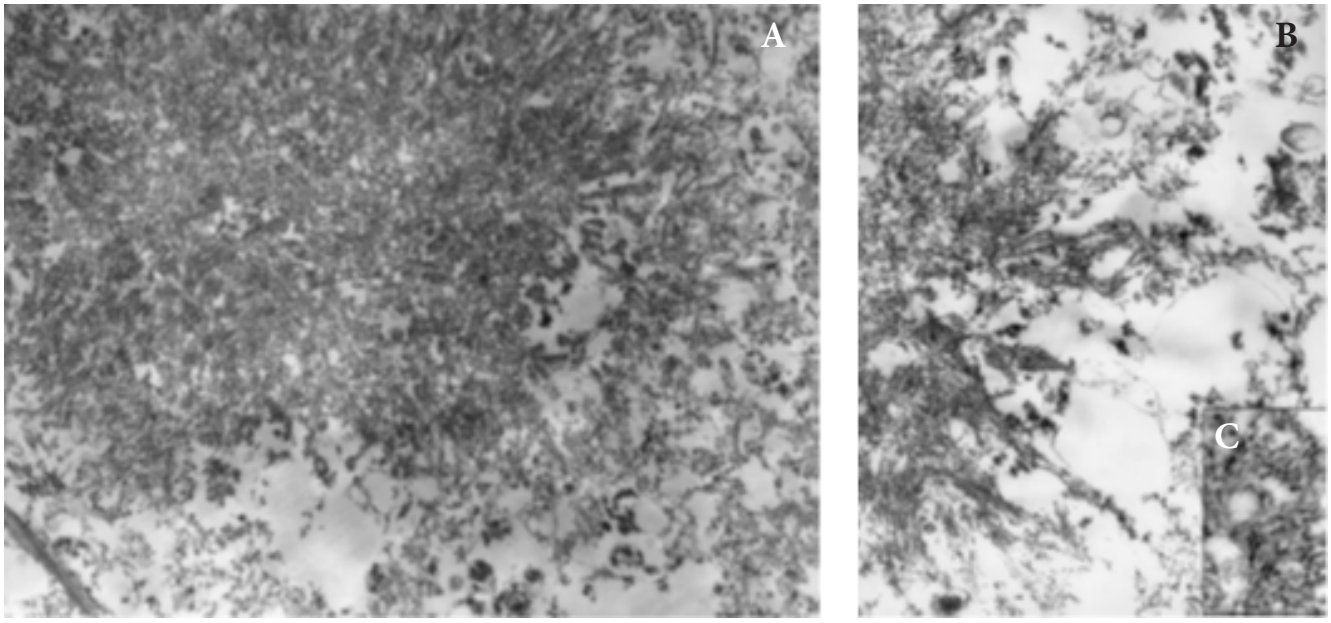

Figure 5. A) Major portion of a kuru plaque of the cerebellar cortex at a magnification of ×13 700. B) Higher magnification view (×48 200) of the peripheral part of a kuru plaque, revealing its fibrillar composition. C) Area with some cross-sectioned fibre bundles (×92 000).[32]

human tumours as medulloblastoma, glial tumours, and colon cancer are associated with the virus, whose oncogenicity has been linked to its T antigen, as well as their association with proteins regulating the cell cycle (eg, p53) and with the activation of antiapoptotic proteins. Agnoproteins, another viral component, have also been identified as a contributing factor. In 2019, 15 different polyomaviruses were known to affect the human population, with four of them causing diseases in immunocompromised patients. These include the JC virus, causing PML and granule cell neuronopathy, the BK virus, causing haemorrhagic cystitis and interstitial nephritis, and the Merkel cell carcinoma and trichodysplasia spinulosa polyomaviruses. All of these are transmitted independently, with different risks of exposure and re-exposure throughout life.[27,31]

Kuru

In autumn 1966, Gabriele Zu Rhein attended a symposium on slow virus diseases organised by the NMSS at the Rocky Mountain Laboratories in Hamilton, Montana. Virologists, pathologists, epidemiologists, and neurologists met to discuss diseases believed to be caused by slow viruses, such as subacute sclerosing panencephalitis, PML, scrapie, and kuru, at a time when the concepts of neurovirology and prions had not yet been developed. The recent development of electron microscopy expanded the list of potential slow viruses.[1,2,32]

In November 1966, the NIH researcher Michael Alpers sent Gabriele Zu Rhein samples of formaldehyde-fixed brain tissue from a 12-year-old girl with kuru, to be analysed by electron microscopy. She was interested in finding viral particles in this transmissible disease and describing the ultrastructure of kuru plaques. Since 1960, she had closely followed the literature on the subject.[32]

In May-June 1967, Zu Rhein attended the symposium "Pathogenesis and Etiology of Demyelinating Diseases," held in Locarno, Switzerland. At the symposium, she presented her findings on PML and kuru. She presented 189 electron micrographs in which she described the structure of kuru plaques in the cerebellum (Figure 5). She never collaborated again with kuru researchers,

turning her attention towards TME, another disease associated with non-conventional slow viruses.[32]

During a meeting held in London in 2007 to celebrate the 50th anniversary of the discovery of kuru, Gabriele Zu Rhein was recognised as the first person to perform electron micrographs of the neuropathology of this disease, in 1967.[33]

Transmissible mink encephalopathy

In the 1970s, Gabriele Zu Rhein became interested in mink encephalopathy, three epidemics of which hit Wiscosin in 1947, 1961, and 1963. It was assumed to be caused by the consumption of products derived from sheep with scrapie, a disease that resembled such human diseases as Creutzfeldt-Jakob disease and kuru. She collaborated with members of the Department of Animal Health and Biomedical Sciences, creating a study group on chronic viral infections of the nervous system, together with Robert Eckroade and other colleagues. TME was a great intellectual challenge for her, as she had never seen any similar pathology. Her mission was to perform neuropathological studies, in which she took thousands of micrographs of specimens from different animals, especially minks. During that time, she was performing this research simultaneously with her studies into PML.[1,34]

The morbidity of these epidemics was high, and limited to adult minks, which presented a progressive disorder exclusively affecting the central nervous system and associated with spongiform degeneration of the grey matter; the course was invariably fatal. The experimentally induced disease was preceded by a prolonged incubation period of several months and lasted from a few days to up to six weeks.[34-36]

The researchers induced a fatal, progressive spongiform encephalopathy in mink brains by inoculating a suspension of a sheep brain tissue infected with scrapie. The clinical symptoms and neuropathological lesions were indistinguishable from those typical of TME. The researchers suggested that this disease was a form of scrapie resulting from the consumption of infected sheep tissue by minks.[35]

Optic microscopy of the mink brain revealed a spongiform encephalopathy with vacuoles located mainly in the neuropil and occasionally in the body of nervous cells. Vacuoles were empty, although some contained amorphous remains. Lesions were predominantly located in the cerebral cortex, striatum, diencephalon, and midbrain. The disease also manifested with extensive astrocyte proliferation and hypertrophy in areas of intense vacuolation.[35]

Furthermore, they used several administration routes to inoculate the TME agent in several species of vertebrates, with different clinical and neuropathological results that are not comparable to those obtained with experimental scrapie, although the agent persisted for a long time in the lymphoid tissue of all specimens, with some of them not developing clinical manifestations.[37]

In 1970, they induced a fatal spongiform encephalopathy in three squirrel monkeys, 11 months after inoculating them with the mink encephalitis agent. This species could be used as a primate experimental model to study subacute spongiform encephalitis (Figure 6). They also induced the disease in rhesus monkeys and stumptail macaques.[38] The researchers performed serial electroencephalography studies in squirrel monkeys inoculated with this agent, thus showing that generalised slowing of background activity preceded the clinical signs of the disease. In the absence of an immunological response, this method may be used to detect the onset of preclinical encephalopathy in the study of animals susceptible to the infection.[39]

In 1973, they studied several species of small vertebrate carnivores inoculated with the TME agent, which triggered a condition indistinguishable from TME in raccoons and skunks. Although ferrets did not develop the clinical condition, they did show neuropathological changes, albeit with a different distribution than other species. This may have been due to the incubation period being longer than the life expectancy of some short-lived rodents, such as ferrets. The ease with which the disease was induced in minks, raccoons, and skunks suggested that it may occur naturally if tissues from infected animals were introduced in their diets. Unfortunately, behavioural alterations in these animals were diagnosed as rabies and their brains were only studied if they had bitten a human; therefore, spongiform encephalopathy may be underdiagnosed.[34]

General neuropathology

During the 1960s and 1970s, Gabriele Zu Rhein became an outstanding figure in the study of diseases with only a few reported cases. She participated in

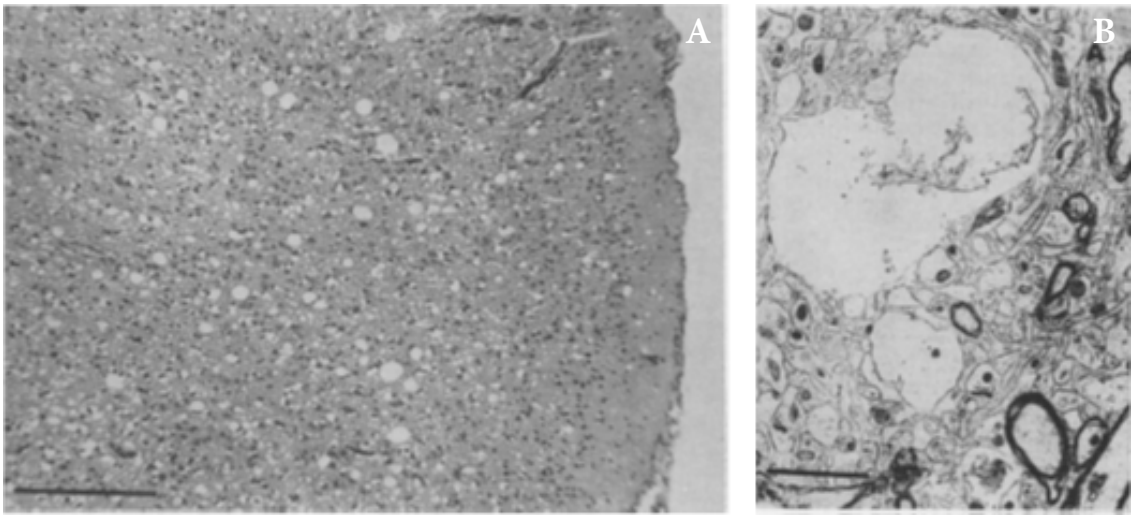

Figure 6. Spongiform polioencephalopathy of the cerebral cortex of a squirrel monkey inoculated with transmissible mink encephalopathy agent. A) Light microscope images (bar equals ¼ mm). B) Electron micrograph. Oblong vacuole with membranous content surrounded by cell processes including myelinated axons (bar equals 2 μm).[38]

multidisciplinary groups, in which she was responsible for neuropathological studies using electron microscopy.

In the study of severely mentally disabled patients from the Central Wisconsin Colony were diagnosed according to their clinical, aetiological, and pathogenic findings. In this context, neuropathological findings were particularly relevant in the diagnosis of syndromes with multiple congenital abnormalities and impairment of the functions of the central nervous system. There was a small group of patients who were difficult to classify, generally presenting disability from birth or soon after, with a mixture of central nervous system symptoms. Neuropathological study was essential for diagnosis and genetic counselling of these patients, as many cases were familial, presumably due to recessive inheritance.[40]

Between 1957 and 1964, Gabriele Zu Rhein systematically studied the presence of axonal dystrophy in the gracile nucleus of the medulla oblongata in a significant number of autopsy studies. She discovered that this alteration appeared around the fourth decade of life and increased with age as a sign of ageing. It was absent in paediatric populations, although brain atrophy secondary to influenza, congenital microcephaly, and other disorders were frequently observed in a small group of children with phenylketonuria.[41]

In 1969, Opitz and Zu Rhein's study group coined the term Zellweger syndrome, also designated cerebrohepatorenal syndrome by McKusick, referring to a peroxisomal disorder first described in 1964, which manifested as multiple developmental defects associated with a psychomotor disability and epilepsy. It displayed an autosomal recessive inheritance pattern.[42,43]

That same year, several cases of lissencephaly were reported. They were classified as syndrome type I, in which the cerebral cortex presents four layers, associated with severe psychomotor disability and the typical epilepsy described in Miller-Dieker syndrome. This description was based on an initial report from 1963 and another from 1969 by Zu Rhein's group.[44,45]

In 1973, they published the cases of male patients from two families with X-linked aqueductal stenosis; the second family presented thalamic and cortical malformations.[46]

In 1977, they described two families with cerebral dysgenesis syndrome and microcephaly, together with severe

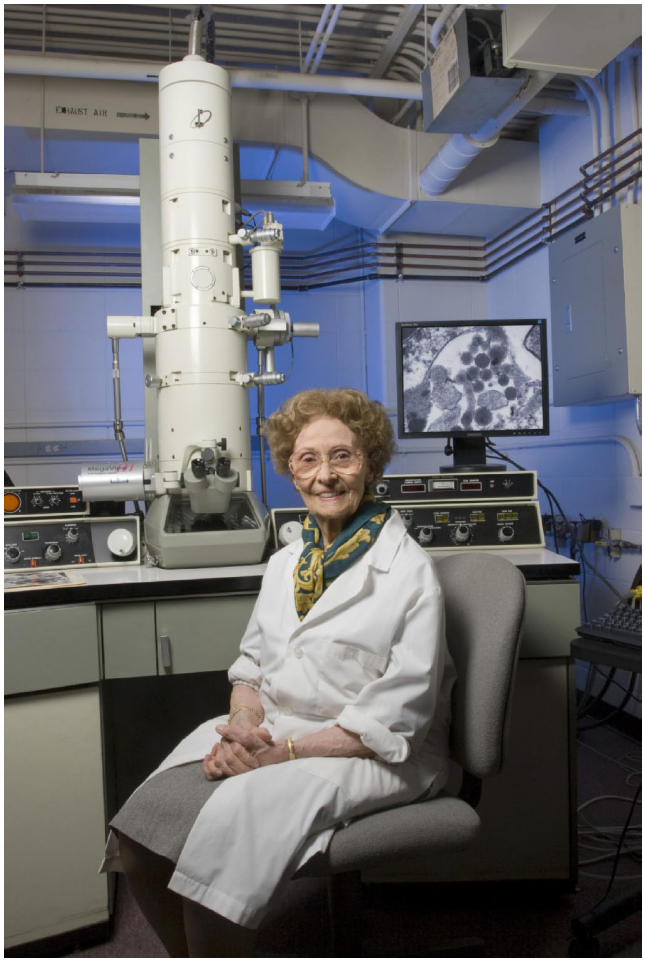

Figure 7. Gabriele Zu Rhein in 2006 with the electron microscope. Pleomorphic bodies suggestive of *Mycoplasma pneumoniae* are shown in the screen.[1]

intellectual disability, spasticity, jaundice, and respiratory infections. In many cases, myelin was practically absent in the white matter and areas of spinal cord.[40]

They also published articles on familial haemophagocytic lymphohistiocytosis: a preliminary article in 1976 reported brain and ocular manifestations, and another more extensive study in 1985 included electron microscopy studies. This syndrome includes diffuse proliferation of lymphocytes and cytologically benign histiocytes in the reticulo-endothelial system and the central nervous system, with erythrocytes phagocytised by histiocytes.[47,48]

Another outstanding chapter addresses lysosomal diseases with metabolic defects and accumulation of substances. In 1960, they described the neuropathological study of the brain biopsy of a girl with congenital familial spongy idiocy (van Bogaert-Bertrand syndrome or Canavan disease), which led to the spongy degeneration of the brain. This was the first ever case of the disease to be diagnosed by brain biopsy.[49]

In 1972 and 1975, they reported familial cases of GM1 gangliosidosis type 2 (or juvenile GM1 gangliosidosis) showing a β-galactosidase deficit with accumulation of substances in the central nervous system and organs, causing progressive psychomotor impairment and somatic disorders of fatal outcome.[50,51]

In 1973, they reported four cases of mucolipidosis type II or inclusion-cell disease, with their neuropathological findings. This disease involves a lysosomal defect with deficiency of several acid hydrolases and accumulation of glycolipids and mucopolysaccharides, which progresses with severe psychomotor disability, short stature, and multiple dysostosis, with patients dying in early childhood.[52]

Regarding viral diseases, by 1960 they had already presented three cases of acute necrotising encephalitis with neuropathological studies, as mentioned above.[5] In September 1967, Gabriele Zu Rhein and Sam Chou actively participated in a conference on measles and subacute sclerosing panencephalitis, held in Bethesda (Maryland), during which they exhaustively described the ultrastructural pathology of this brain disease from biopsy tissue of an affected boy.[53] In 1971, she participated in the description of ophthalmoscopic and neuropathological findings of bilateral cytomegalovirus retinitis in a patient after kidney transplantation.[54]

Regarding the pathology associated with the Epstein-Barr virus, a report was published in 1967 on a case of Burkitt lymphoma with predominantly abdominal symptoms, with an electron microscopy study of the pericardium and meninges performed by Zu Rhein.[55] In 1979, they reported a case of lymphomatoid granulomatosis in a 38-year-old woman, with a pathological study of necrotising vasculitis performed by Gabriele Zu Rhein.[56]

In 1990, they presented a case of a 7-year-old boy with epilepsia partialis continua and Rasmussen encephalitis, including a neuropathological study of a surgical specimen

taken during a partial left hemispherectomy, showing the characteristic changes of chronic encephalitis.[57]

Veterinary neuropathology

The vast range of subjects addressed by Gabriele Zu Rhein also includes veterinary diseases. For instance, in 1970, she participated in the study of meningeal and cerebral infection in a deer infected by the meningeal worm *Pneumostrongylus tennis*, a common parasite in the species.[58] In 1977, she also reported a case of Uhl anomaly in a mink. This is a rare cardiac malformation characterised by hypoplasia of the right atrium and ventricular myocardium. This congenital defect was identified in humans in 1952.[59]

History

Gabriele Zu Rhein collaborated with her colleague Igor Klatzo to write a biography of the neuropathologists Cécile and Oskar Vogt published in 2002.[60]

The culmination of a career: dementia associated with Mycoplasma pneumoniae

After a lapse of several decades after the PML project, Gabriele Zu Rhein faced a new challenge in the study of the pathology of the central nervous system. In 1990, Gary Ludwig of Neenah (Wisconsin) consulted her about a case of possible PML, which was not PML but something new. The patient presented disseminated foci of demyelination and capillary abnormalities, such as focal compression of endothelial nuclei that compromised the vessel lumen. Using the electron microscope, they observed intracytoplasmic inclusions with focal accumulation of endothelial nuclei (Figure 7). Zu Rhein had no qualms consulting other specialists about her hypothesis that inclusion particles were *Mycoplasma pneumoniae*. Years later, one of these specialists, James Powers of the University of Rochester, sent her two more cases to confirm the diagnosis by electron microscopy. In total, they studied the cases of three immunocompetent adults with subacute or chronic neurological syndrome of fatal course with cognitive and motor impairment, which they presented in a poster at the 2007 annual meeting of the AANP in Washington, receiving an award, and reported in an article published together with James Powers, Shyh-Ching Lo, and Christine Hulette.[1,61]

In 2011, a letter to the Editor of the *Journal of Neuropathology and Experimental Neurology*, signed together with Powers,[62] mentioned two more cases of this entity, one reported by José Ferreira, from Quebec,[63] presenting identical lesions in the brain, and another by Roy Rhodes et al.,[64] from New Brunswick, in which the brain biopsy revealed the presence of bacteria without cell walls inside capillary endothelial cells. Immunostaining yielded positive results for *Mycoplasma pneumoniae* in the case reported by Rhodes, and subsequently in her own cases and the case reported by Ferreira. They considered this to be a new disease of the central nervous system.[1]

If Zu Rhein's discoveries on PML represented the peak of her career, her discovery of encephalopathy associated with *Mycoplasma* infection, when she was nearly 90 years old, was the culmination of her professional life, after more than 50 years as neuropathologist.[65]

The end of a long life

Gabriele spent her last year at Oakwood Village Tabor Oaks Assisted Living in Madison and died on 23 February 2016 at Oakwood Village University Woods. She was survived by her brother Hans, her nephew, and his children. Her remains rest in Munich.[66]

Conclusions

Gabriele Zu Rhein had special qualities that contributed to her professional and scientific success. Among other terms, she may be defined as tenacious, methodical, and with a tendency to interdisciplinary work, in which she was in charge of the neuropathological aspects of the studies thanks to her mastery of electron microscopy. She never had qualms sharing her findings, and when doubts arose, she consulted with other neuropathologists, who also consulted her. She was also able to assert herself as a woman in a men's world, with the added difficulties that her status as a foreigner may cause.

Her career extended for over five decades, and she preserved her judgement and an admirable intellectual lucidity until the end of her life. She made her last publications when she was 92 years old.

Her three greatest achievements were her studies on PML and JC virus, TME, and dementia associated with *Mycoplasma pneumoniae*, but she was also an outstanding general pathologist in the study of congenital diseases and several viral infections of the nervous system, other than PML.

Conflicts of interest

The author has no conflicts of interest to declare.

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