Harald zur Hausen

ANNUAL REVIEWS

Annual Review of Virology

Cancers in Humans: A Lifelong Search for Contributions of Infectious Agents, Autobiographic Notes

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Annu. Rev. Virol. 2019. 6:1–28

The *Annual Review of Virology* is online at virology.annualreviews.org

https://doi.org/10.1146/annurev-virology-092818-015907

Keywords

Burkitt's lymphoma, nasopharyngeal cancer, EBV, cervical cancer, HPV, plasmid-derived human pathogens

Abstract

This review briefly covers periods of my early life; experiences during World War II; my school education; and my period as a medical student in Bonn, Hamburg, and Düsseldorf. Mainly emphasized is my scientific career after finishing my medical internship and periods as a postdoc at the Institute for Microbiology in Düsseldorf and the Virus Laboratories of the Children's Hospital of Philadelphia and as Senior Research Fellow at the Institute of Virology in Würzburg, Germany. Subsequent appointment as chairman of the newly established Institute of Virology, University of Erlangen-Nürnberg, in a similar position at the University of Freiburg, and then for 20 years as scientific director of the Deutsches Krebsforschungszentrum, Heidelberg, are discussed, covering the scientific developments during these periods. The emeritus period since 2003 was particularly exciting, leading to the discovery of autonomously replicating plasmids, derived from specific bacteria, and their link to common human cancers (colon, breast, and prostate).

THE FIRST YEARS OF LIFE

My existence, as well as the existence of my two brothers and my sister, was a consequence of the Russian October Revolution in 1917. This may sound strange, but it is easily explained. My father, Eduard zur Hausen, the youngest son of a farmer in the German city of Gelsenkirchen-Buer in Westphalia, finished his high school (*Gymnasium*) and started to study agriculture in Halle and Greifswald in Germany. The First World War in 1914 forced him to interrupt his studies and join the German army. Later, as a young officer, he had to suffer for almost four years in the siege and leaguer trenches in the region around Verdun, France.

At the end of the war in 1918 he could not resume his agricultural university training and joined the Baltische Landeswehr. This organization promised its members Baltic-Latvian citizenship and ownership of one-third of the land in the possession of great landowners, who were mainly of German origin. The Bolsheviks had entered the Baltic states and occupied Riga. The Landeswehr successfully expelled them (May 22, 1919) and, for a brief period, took over control of Riga. It must have been in the end of May or shortly thereafter that my father met his future wife, Melanie. She was 18 years old and had offered some secretarial help to the members of the Landeswehr, who were considered liberators after the previous months of terror. Her father was Latvian and owned a small factory for tools. He died of tuberculosis when Melanie was 12 years old. Her mother was partially of German and partially of Polish origin. After the death of my grandfather, my grandmother and her three daughters experienced financial problems, yet all the daughters had a good school education, mainly in German and Russian. In addition, my mother was musically talented and received further training as a pianist at the conservatory in Riga.

Obviously, my parents fell in love very quickly and decided, within a few weeks, to get married. When the German members of the Landeswehr had to leave, they married in 1919 in Mitau, at that time the capital of Courland. Their marriage lasted until the end of their lives (1959 for my father, 1977 for my mother) (**Figure 1**). Thus, without the circumstances and consequences following the Russian Revolution in 1917, this marriage and subsequently our family would not have been founded.

My parents' first child, Manfred, was born eight years later; the second one, Winfried, followed three years later; and then—again in three-year cycles—my sister, Eleonore, and then me in 1936 (**Figure 2**).

Figure 1

My parents, Melanie and Eduard zur Hausen, 1940.

Figure 2

My parents' four children: (*left to right*) Manfred, Eleonore, me, and Winfried.

WORLD WAR II AND THE POSTWAR PERIOD UNTIL 1950

My own memory goes back to 1939, when my father had to leave for the army. I noticed the deep depression of my mother and my brothers and sister at his departure.

In 1942 I entered elementary school in Gelsenkirchen-Resse. By the end of this year, we started to feel the consequences of the war: Bombing raids came closer and closer, since the area around Gelsenkirchen had a large number of coal mines and other industrial setups. As a consequence, all schools closed in the beginning of 1943, which was obviously bad for education but welcomed by many of the children. Except for three months in 1944 during which my Aunt Theodora (Dora), an unmarried elementary teacher, taught children at a school in Harle, a small village in Westphalia, with my sister and me as attendants, I had no school training until autumn of 1945.

Around 1942 I developed a deep interest in birds and animals. My mother and also some relatives and friends noted this; they all provided me with pictures and books of birds and animals. It must have been at the age of five that a friend of mine, Heinrich Schulte-Holthausen, and I were asked by some adults, "What are you going to do when you are grown up?" Our simultaneous and spontaneous answer came instantly: "*Naturforscher natürlich*" (natural scientists, of course). To some degree both of us held this up for life, although our paths separated later.

A couple of months before the war ended, near the end of 1944, my father took me to northern Germany, a region much less affected by continuous bombing raids. He had been dismissed from the army in the summer of 1944 when his battalion was surrounded by the advancing Russian army close to Voronezh in southern Russia. With great luck and aid from members of his battalion he escaped but suffered a serious heart attack during this time. Somehow, he managed to survive, eventually reaching a military hospital in Pirna, Saxonia. On July 20, 1944, the assassination

attempt on Hitler failed. One of the many consequences of this event was a lasting distrust of the Nazi regime in older officers of the army. The army discharged my father upon his release from the hospital, and he started to work in pesticide control in Oldenburg in northern Germany. In 1943, at the age of 16, my oldest brother had been drafted into the army. Somehow my father managed to bring Winfried and Eleonore to Cloppenburg, south of Oldenburg, to stay in private homes. In January 1945 he fetched me in Resse and took me to Langförden, a small village 50 km south of Oldenburg. My mother was going to join us a few weeks later. She was staying longer to care for our apartment.

The train tour starting from Recklingausen to northern Germany was a nightmare. There was a train collision as well as the constant fear of attacks by low-flying British fighter planes. More than 24 hours later, the next evening we reached the city of Osnabrück, where we had to stay overnight with a large number of other people in an extremely poorly ventilated bunker, continuously feeling close to suffocation. The next morning we walked for more than 20 km to a farm. This was probably the only time in my life when, at the age of eight, I slept while walking. After a couple of weeks, my father brought me to Langförden. Subsequently, my mother, Winfried, and Eleonore joined us there. Because Manfred had been drafted into the army, we did not see him again before the end of the summer of 1945.

After the war ended in May 1945, our family started plans to return to our home in Buer-Resse. We managed to do so during the summer of 1945, staying there with my mother while my father continued to work in northern Germany and tried to visit us as often as possible during an extremely difficult postwar period that was particularly aggravated by the lack of food. With the help of Aunt Dora, we managed to survive three very difficult years.

In the autumn of 1945 schools reopened. Thanks to my Aunt Dora, I was accepted into the fourth grade of the elementary school in Resse. At the age of nine my school background was extremely poor. It must have been in early 1946 that my aunt and my parents encouraged me to participate in a contest for entrance into *Gymnasium*, where out of 600 applicants about 160 were accepted. Somewhat miraculously, I found myself among the accepted ones. Mainly upon my father's advice I joined the humanistic branch, comprising nine years of ancient Latin lectures and later six years of classical ancient Greek. Much less emphasis was placed on natural sciences and mathematics. I learned German grammar from our Latin lectures and had initially great difficulties with mathematics. I passed the first year despite my certificate stating "serious reservations and concerns," probably because more than 50% of the pupils performed worse and had to leave the *Gymnasium*. At just 10 years of age in 1946, I was one of the youngest pupils; the oldest ones in the same class were 16 years old. After this difficult year I never had problems in school again.

In 1949 my father was promised an apartment in a newly built house in Vechta, a town close to Langförden. Our family moved to Vechta in May 1950, and I started in the fourth grade of the local *Gymnasium Antonianum*. During the following years I was not one of the top pupils, but I managed reasonably well, passing all grades and finishing school early in 1955 with a reasonably good certificate of having passed the *Abitur* (German university entrance qualification).

I continued to dream of a life as *Naturforscher* but remained undecided whether to study biology, chemistry, or medicine. As a young boy I had read biographies of Robert Koch and Louis Pasteur, as well as Sinclair Lewis's fascinating book *Arrowsmith*, and I came to the decision to study medicine, if possible, combined with biology.

Here I will say a few words about our family life: Both of my parents were very fond and proud of their children. My mother was like a clucking hen, most delighted when all her chicks were close by. This changed only when her sons brought their girlfriends and later their brides to the house. She was not satisfied with any of them, and that occasionally created some trouble.

Even her son-in-law, Rolf Ulken, a very friendly and calm engineer, sometimes suffered from her dissatisfaction. On the other hand, she was later devoted to her grandchildren, and they in turn all loved to be with her.

UNIVERSITY

Due to the difficult financial situation of my family—two older brothers studying at the same time—it became clear to me that I had to find my own resources to finance my university training. One year prior to finishing my *Abitur*, I asked my father if we could visit his sister (my Aunt Johanna) in Bad Godesberg to explore the possibility of my staying with her for my first semesters at the University of Bonn (only 7 km distant from her home). She was living there with her husband, Bernhard Kraneburg, a retired post office clerk, and their daughter, Cläre, an unmarried elementary school teacher in her fifties. I obviously made a good impression on them. Bernhard died a few months later; one or two days after the funeral my father arranged with his sister that I could stay there for the first semesters.

In late spring of 1955 I registered for medicine at the University of Bonn and attended a welcoming ceremony. The rector at that time, Professor Helferich, an organic chemist, welcomed more than 1,000 freshmen by handshake.

During my second semester I applied for a voluntary examination (*Fleiss-Prüfung*) in order to be eligible for a bursary. I passed this examination successfully and received a grant, which for the first time permitted me limited financial freedom. I finished my first medical examination (*Physicum*) after five semesters, again with good grades. This provided me with a very reasonable bursary, giving me the freedom of financial independence from my family.

After the first two semesters in Bonn I was granted permission to study biology in parallel. I realized, however, after trying to combine these two fields for four semesters, that I would not be able to finish my medical training within the regular period of 11 semesters. Therefore, I concentrated from then on exclusively on medicine.

My newly gained financial independence provided me with the opportunity to change universities. I went to Hamburg for two semesters, initially tempted by the existence of an Institute for Tropical Medicine. Disappointed, however, by the absence of prospects for involvement in interesting research projects, I changed universities once more and moved to the Medical Academy of Düsseldorf. There I finished my medical thesis as well as my final medical examination in the autumn of 1960.

During the last years of high school and during my university training I maintained a deep interest in questions surrounding the nature and biology of life: What is the biochemical and biophysical basis of life? Will we be able to reconstruct life ourselves? Questions fascinated me concerning evolution, human past (paleoanthropology), and the causes and evolution of human diseases. In my oral examination during my *Abitur* I knew every detail about the Neanderthals and their relationship to modern humans, obviously impressing my teachers, who knew very little about their brain volume, morphological characteristics, and by-then described geographical distribution.

During my medical training the poorly understood etiology of cancers and neurological and autoimmune diseases almost continuously kept my attention. In this period I heard of lysogeny, the uptake of bacteriophage DNA into bacterial hosts and their long-lasting presence and the occasional modification of host cell properties due to this persistence. It must have been during the last or second-to-last year of my studies when I developed the—at that time not completely new—idea that human cancers may have a somewhat related infectious origin. By early 1960, I was convinced to concentrate later on cancer research and, profoundly influenced by lysogenic bacteriophages, to put a focus on infections and cancer.

INTERNSHIP

By the end of the 1950s a large number of positions for medical interns became available. A two-year internship was required to receive the qualification to practice medicine. I decided at the age of 24 to receive this licensure. Being convinced at the same time to later follow up on research activities, I decided on the internship primarily based on a pleasant area and a good salary.

I started my first position in surgery in a hospital in Wimbern in Westphalia. Four months later I started in internal medicine after moving to Isny in southern Germany, an attractive little town close to the Alps. Retrospectively, in both hospitals the medical training was poor. Isny, however, offered beautiful nature and a pleasant life. I realized at this stage that I had the ability to easily communicate with patients. For this reason, I considered for the first time whether it would be worthwhile to practice clinical medicine. For gynecological training I went to my home city of Gelsenkirchen-Buer-Erle. With more than 1,000 deliveries each year, a head of this department close to retirement, and his head physician who fell ill for two months during my stay, this was the most labor-intensive and at the same time most satisfying period of my clinical phase.

Immediately thereafter I decided to start to work in my future research field, medical microbiology and virology. I obtained a position at the Institute of Medical Microbiology in Düsseldorf, the same place where I had previously worked for my medical thesis.

PROFESSIONAL CAREER IN DÜSSELDORF: FIRST ATTEMPTS TO MOVE INTO SCIENCE

Initially I started to work in the virus laboratory of this institute. The only activities there concerned the maintenance of primary African green monkey (AGM) kidney cell cultures for replication and propagation of poliomyelitis viruses, virus neutralization tests, and determination of complement-fixing antibodies. It took me less than four weeks to find this exceedingly boring and to long again for clinical activities. After a few failed attempts to find an interesting clinical position in pediatrics in Freiburg, Basel, and Bern, I became frustrated and decided to continue in microbiology.

This was another decisive point in my career. By then I had discovered that tissue culture studies could lead to very interesting results. I gave up all clinical plans and returned to my previous plans to stay in science.

In Düsseldorf I was completely left on my own. There were no stimulating discussions, but sufficient money was available. Whenever I approached the chairman of the institute, Professor Walter Kikuth, with a usually not-very-balanced proposal of my research, he listened for a while before he casually responded in a broad Baltic accent, "That sounds interesting; why don't you do it?" I received his permission to attend a two-week training course on cytogenetics in Münster and on bacteriophages in Köln (Cologne) at my own expense. Both of these courses opened my eyes to a new world of science. At the same time, the courses made it clear to me that I should not stay too long in the remarkably sterile atmosphere of this microbiology institute even though I had acquired reasonable experience in bacteriology and bacterial diagnostics. Nevertheless, I stayed for slightly more than three years.

In retrospect, I see this time in a different light: I could quietly develop my own ideas, mainly based on relatively careful literature studies, yet with the consequence of a poor publication record. I learned very little of existing trends in molecular biology but deepened my knowledge of infectious diseases, cancer, and neurological disorders. Another positive aspect was my increasing familiarity with microbiology.

By chance, I heard from one of the senior assistants that Professor Kikuth had given him a letter from Werner Henle in Philadelphia asking him for help in finding a young medically trained German with interests in virology. My colleague took the letter and disposed of it in a wastebasket. I instantly became very interested in this letter, since I had previously heard of Henle being a highly reputed German virologist who immigrated to the United States in 1936. His grandfather, Jacob Henle, was a famous anatomist at the University of Göttingen who discovered the Henle's loop in kidneys. During the Nazi terror regime, Jacob Henle's Jewish ancestry was the reason Werner could not find employment in Germany after finishing his medical training.

Immediately I asked my colleague in which waste bin he had disposed of the letter. When he told me, I rushed to the room, emptied the bin, and luckily found the crumpled letter. On the very same day, I wrote to Henle, informing him of my interest in this position and of my (still very limited) experience in virology and bacteriology. Shortly thereafter, I received a friendly letter from him saying that he and his wife, Gertrude (Brigitte), planned to visit Heidelberg during the forthcoming weeks and inviting me to meet them in the Europäischer Hof, a top hotel in Heidelberg (**Figure 3**).

I met both of them on a sunny summer day in this hotel. During lunch, Brigitte in particular told me about their research on Epstein-Barr virus (EBV). They had received cultured Burkitt's lymphoma cells from Tony Epstein in Britain in which he had seen herpesvirus-like particles electron-microscopically in a few individual cells. This story immediately interested me, although this was the first time I had ever heard of Epstein and the virus tentatively named after him. Apparently, my active interest pleased both of them, and we agreed that I would start in their laboratory in Philadelphia by January 1, 1966.

The contract arrived a few weeks later. It was everything but luxurious: We agreed on an annual salary of US$8,500, and Werner provided me with a preliminary refundable payment to cover airfare for my wife and my at that time two-month-old son, Jan Dirk.

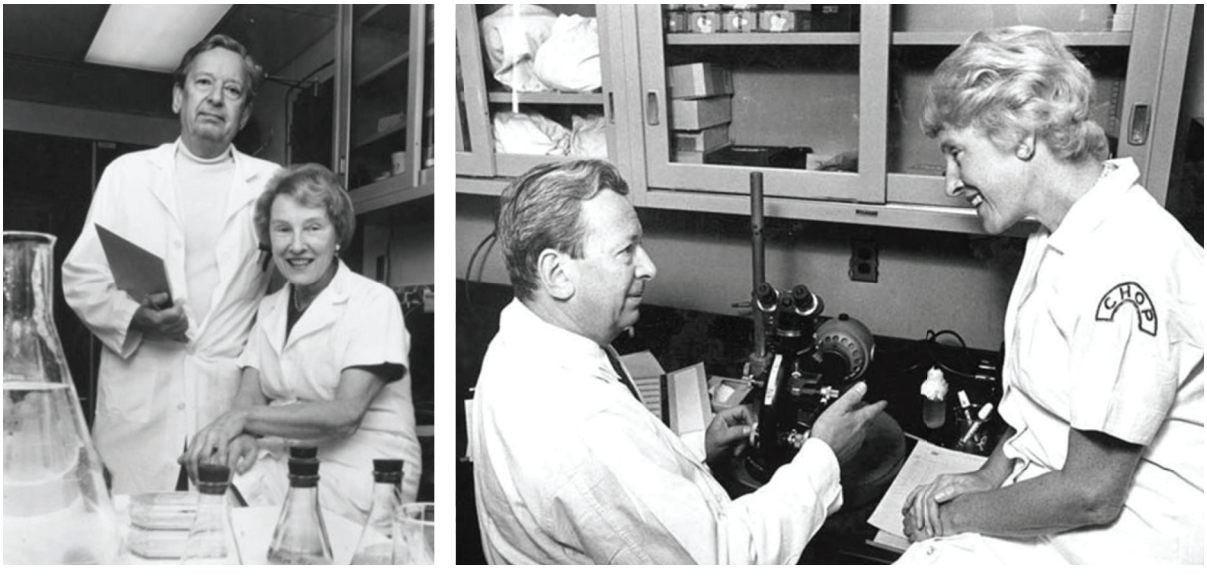

Figure 3

Werner and Gertrude (Brigitte) Henle in Philadelphia, approximately 1969 (with permission).

Thus, after a number of local arrangements and goodbye visits to relatives and friends, we decided that I would travel first by the end of December 1966 to Philadelphia to take care of making the proper arrangements for my young family before my wife and our son would follow four weeks later.

THREE YEARS IN PHILADELPHIA: THE HENLES, CYTOGENETICS, AND ADENOVIRUSES

When I arrived at the airport in Philadelphia on December 29, I quickly realized my insufficient knowledge of the English language. I had not noticed that the plane had arrived one hour earlier than expected. After leaving the customs area, I waited for about half an hour for the arrival of the Henles' secretary (Beverly Tompson) and former technician (Jeanie Knickerbocker) who were supposed to pick me up and bring me to the Drexelbrook Club in Upper Darby, where the Henles had booked a room for me. Because the two ladies did not arrive, I decided to undertake something on my own, went to a waiting taxi, and asked the driver to take me to Upper Darby. To my embarrassment, the taxi driver did not understand me. He asked some of the other drivers waiting there if any of them could understand me. Fortunately, one of them understood German and made some sense out of my broken English and directed the driver to the correct address.

It took me approximately two to three months before I was able to communicate reasonably well in English. Nevertheless, during the first few weeks I rented an apartment and became familiar with the Virus Laboratories of the Children's Hospital of Philadelphia, which was then surrounded by a poor neighborhood. The laboratories were surprisingly dark (no windows to the outside) but air conditioned. We could have our lunch in the laboratory, and—since Werner Henle and his wife were cigarette smokers—there were no smoking restrictions in the lab. Small cabinets for tissue culture work were available, but there were no laminar flow hoods.

Although both Henles urged me to join them soon in their immunofluorescent analyses of EBV expression in Burkitt's lymphoma cell lines, I tried to convince them that I needed to gain some background in molecular techniques of virology. After intensive discussions, I persuaded them to permit me to start to work with a reasonably well-studied virus (human adenovirus type 12) previously shown to be oncogenic upon inoculation into newborn rodents. I convinced them that this virus system would be perfectly suitable for my learning virus purification and nucleic acid extraction procedures and studying the effects of such infections on human chromosomes. In Düsseldorf I had already acquired some skills in chromosome spreading techniques and in the evaluation of chromosome structure. Werner Henle accepted my view and, to my joy, even bought an expensive Zeiss microscope equipped for phase contrast microscopy, immunofluorescence, and microphotography. Brigitte Henle was less convinced that this relatively inexperienced German postdoc needed expensive equipment but changed her mind later, when the first publishable data emerged approximately nine months after my arrival in Philadelphia (**Figure 4**).

This change in opinion was mediated by specific chromosome modifications I observed in several lymphoblastoid cell lines derived from patients with leukemia. Parts of chromosomes (mainly 1 or 16) did not condense. I demonstrated by ^{3}H-thymidine labeling that these noncondensed parts replicated their DNA during mitosis. Initially both Henles were somewhat skeptical about my finding and asked Peter Nowell (a well-known pathologist and cytogeneticist who became famous for the discovery of the Philadelphia chromosome) to confirm my findings. He did so and was obviously impressed. Nevertheless, Werner Henle remained cautious. When I asked him to join me as a coauthor of this publication, he refused, stating, "We are not in Germany, where the director always cosigns every paper." He corrected my English style, and after a few

Figure 4

In my laboratory in Philadelphia, 1967.

modifications the article was accepted by the *Journal of the National Cancer Institute*, my first publication in English (1) (**Figure 5**).

This first result greatly encouraged me, and I intensified my efforts in analyzing cytogenetic modifications of host cell chromosomes after viral infections. I concentrated on the analysis of human embryonic kidney cell chromosomes after infection with the oncogenic adenovirus type 12. A careful evaluation of mitotic chromosome spreads of these cells two or three days after infection revealed very frequent gaps and breaks in the long arm of human chromosome 1 and in the subcentromeric long arm of chromosome 17. I published these data in 1967 in the *Journal of Virology* (2), although I could not relate these modifications to functional aspects of the affected cells.

Another publication using radioactively labeled adenovirus type 12 DNA suggested the existence of hot spots for viral DNA integration into human cells (3). We later demonstrated that fragments of adenovirus DNA integrated into chromosomal DNA of nonpermissive hamster cells (4).

In 1973 and subsequently in 1988, Jim McDougall's group in Seattle reanalyzed this system, confirmed my findings, and mapped the affected region to 17q21-q22, close to the thymidine kinase gene in chromosome 17 (5, 6). In the following years, however, this remarkably specific modification of specific human chromosomes found little attention.

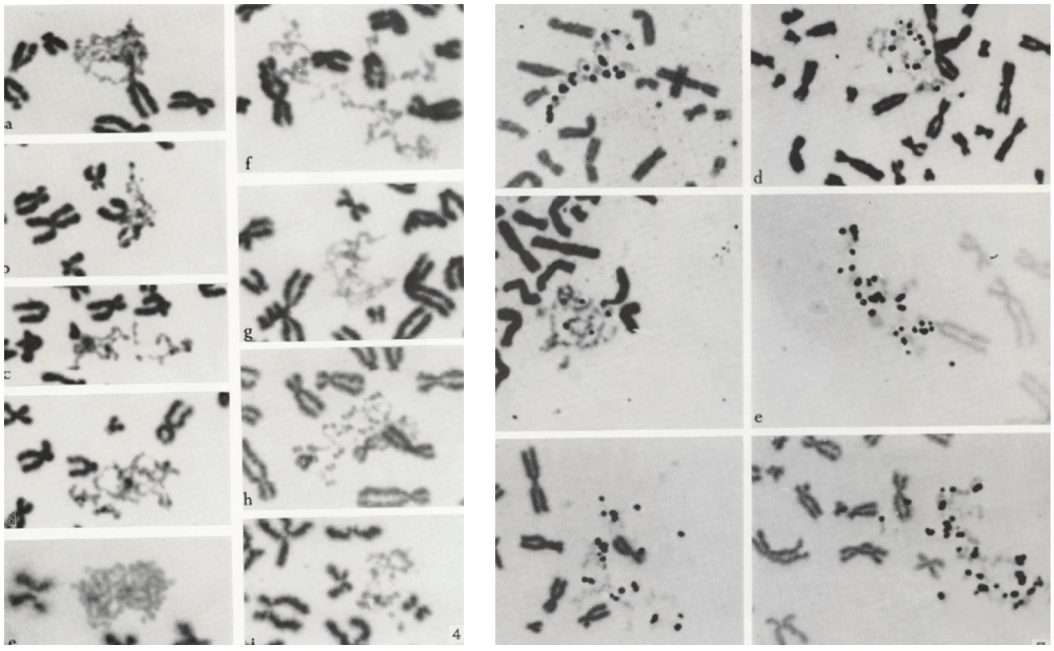

Figure 5

Incomplete chromosome condensation due to a delayed onset of DNA synthesis. Photos reproduced from Reference 1 with permission.

Hilary Koprowski, the former director of the Wistar Institute in Philadelphia, became interested in my adenovirus work. Around this time papers appeared revealing the reactivation of infectious simian virus 40 (SV40) polyomavirus from SV40-transformed cells (7). He encouraged me to try the same with adenovirus type 12–transformed Syrian hamster cells. Anticipating positive results, he prematurely prepared a full manuscript in which he omitted the results section. To his disappointment, I obtained exclusively negative data. In a study in collaboration with Frantisek Sokol of the Wistar Institute, we found an explanation for this failure: Pieces of adenovirus type 12 DNA were integrated into the transformed hamster cells without any evidence for integrated whole genomes (4). I published an additional study on adenovirus type 12 in 1969 that failed to find incorporation of host cell DNA into viral particles (8).

Although the Henles supported my adenovirus work, particularly because I emphasized that this would enable me in the future to do molecular studies on EBV, they cautiously urged me to start these studies as soon as possible. They had published their early studies on immunofluorescence in cultured Burkitt's lymphoma cells and correlated the high antibody titers measured by this procedure in sera from tumor patients with the presence of herpesvirus-like particles in these lines. This raised some doubts in the herpesvirus community, which questioned the specificity of this reaction. For these reasons, I initiated some experiments to stain these cells in suspension, plated them in a thin agarose layer on a slide, and examined individual positive cells by electron microscopy. A technician in Klaus Hummeler's lab was an expert in analyzing individual cells and proudly presented electron micrographs showing the particles in the isolated cells. The morphology was not absolutely beautiful, yet the particles were clearly visible (9) (**Figure 6**).

In addition to the adenovirus work, I was involved in cytogenetic studies on herpes simplex virus–induced chromosomal changes and on the chromosomal analysis of lymphoblastoid line, immortalized after EBV infection.

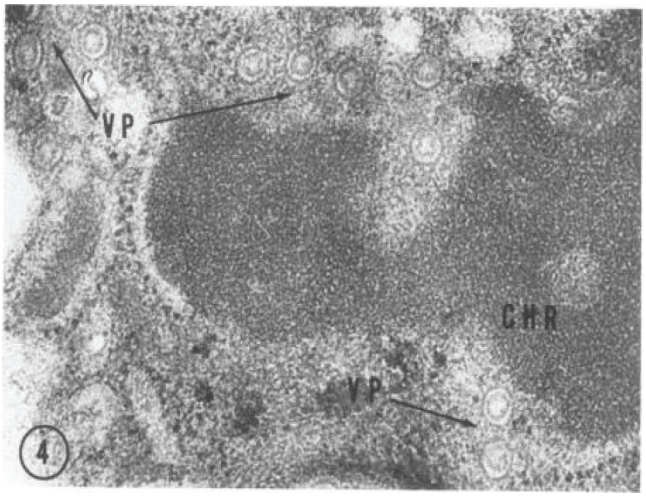

Figure 6

Isolation of individual immunofluorescent cells from the P3HR-1 line and electron microscopic detection of Epstein-Barr virus particles. Photo reproduced from Reference 9 with permission.

In the late summer of 1968, Eberhard Wecker, a well-known German virologist, visited the Henles and inquired about my work. He became very interested in these studies and offered me a senior assistant position at his newly created Virus Institute at the University of Würzburg in Germany. Since this offer also included the possibility for a habilitation (at that time a prerequisite for a subsequent appointment as professor), I gratefully accepted this offer and moved to Würzburg in March 1969.

WÜRZBURG, MARCH 1969 TO MAY 1972: FIRST STEPS IN MOLECULAR BIOLOGY OF SPECIFIC HUMAN CANCERS

Before I left Philadelphia, I said goodbye to Frantisek Sokol. We had become good friends during the previous two years when I collaborated with him at the Wistar Institute. He had emigrated from Slovakia more than 10 years earlier. As an experienced biochemist he also had an excellent background in the developing methodology of molecular biology. Although most of his colleagues at the Wistar were not very fond of him and considered him a bit arrogant, we became friends almost from the first day we met. He was of tremendous help to me, and we had discussions almost daily, frequently centering on my own research plans.

When he asked me what I was planning to do in Würzburg, my response was instant: I wanted to apply my newly acquired knowledge in molecular biology to the EBV system. He knew that the only available sources for this virus were suspension cell lines derived from Burkitt's lymphomas in which commonly a very small percentage of cells (usually less than 1–2%) produced the typical herpesvirus-type particles. However, the P3HR-1 cell line was a slightly higher producer—with up to 5% positive cells in immunofluorescence. When I told Sokol that I planned to use these cells for virus isolation and purification, and the subsequently extracted viral DNA for nucleic acid hybridization studies, he smiled and scoffed at me. He stated that by now several tumor virus systems existed (SV40-, polyoma-, my previously used oncogenic adenoviruses, and retroviruses) and asked why I wanted to spoil my career on a system with a very slim perspective.

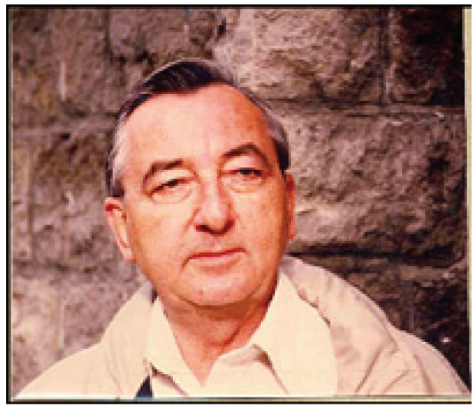

Figure 7

Eberhard Wecker, University of Würzburg, Institute of Virology. Photo courtesy of T. Hünig.

He did not convince me. I left with the firm conviction that every Burkitt's lymphoma cell would contain at least one EBV genome and that the low percentage of virus-producing cells in these lines represented the spontaneous reactivation of a latent EBV genome within these cells.

Eberhard Wecker in the—at that time still very small—Institute of Virology received me warmly, and he and his wife, Ilse, persuaded me to stay in their home for the first few days until I found an apartment not too far from the institute (**Figure 7**).

After a very brief period I hired a technician, Sharon, the wife of a US soldier who was stationed in Würzburg. Shortly thereafter, Heinrich Schulte-Holthausen, a chemist and my old childhood friend, joined the group. Some weeks later a PhD student, Hans Wolf, became part of the group as well.

After a couple of days the laboratory was sufficiently equipped to start initial experiments. The Henles in Philadelphia provided us with Burkitt's lymphoma lines and, with Wecker's aid, I soon obtained my first research grant and initiated work on EBV concentration and purification and viral DNA analysis. I cultivated P3HR-1 cells in the presence of ^{3}H-labeled thymidine, tried to concentrate the virus in a sucrose gradient, selected the fraction with the expected viral band, extracted the DNA, and subjected it to a cesium chloride density gradient. To my pleasant surprise, the very first gradient already revealed a radioactive band at a density of 1.718 g/cm^{2}. This result was repeatedly reproduced and was published in *Virology* (10).

The isolation of purified radioactive EBV DNA permitted us to answer the questions that had long been bothering me: Do nonvirus-producing Burkitt's lymphoma cells contain EBV DNA? Is this viral DNA detectable in primary biopsy material of Burkitt's lymphomas and nasopharyngeal carcinomas (NPCs), another EBV-linked cancer?

The first question was soon answered: Cells of the EBV-negative Burkitt's lymphoma line, Raji, indeed contained about six genome equivalents of EBV DNA per cell (11). Probably due to the low specific radioactivity used in these experiments, this figure represented an underestimate. This was later corrected by Nonoyama & Pagano (12), who basically confirmed our data but estimated the number of EBV genome equivalents per Raji cell three to four times higher than in our experiments. Nevertheless, our data represented the initial demonstration of a suspected tumor virus DNA in cells derived from a malignant human cancer.

For us the obvious next step was to analyze biopsies from Burkitt's lymphomas and NPCs: George Klein in Stockholm, Werner and Gertrude Henle in Philadelphia, and Peter Clifford

and Lars Santesson in Nairobi provided us with 10 different biopsies from Burkitt's lymphoma patients, an equal number of nasopharyngeal cancer biopsies, and a larger number of control cancers. Nucleic acid hybridization experiments demonstrated the existence of EBV DNA in these samples. Indeed, the calculated number of viral genome equivalents in the Burkitt's lymphomas ranged between 4 and 26 EBV genome equivalents per cell; for NPCs it varied between 1 and 19 (13). For the reasons mentioned above, it is likely that these calculations also represented underestimates.

I presented these results at the first meeting on herpesviruses and oncogenesis in Cambridge, United Kingdom, in 1974. It was considered a highlight of this meeting and was widely discussed. This resulted in a number of invitations to subsequent international and national meetings. In 1975 I received the Robert Koch Award for these publications.

When I had met Wecker in Philadelphia we had discussed the possibility for a habilitation after my arrival at Würzburg. At that time the habilitation was an important qualification for being appointed as a lecturer (*Privat-Dozent*) at German universities and for subsequent appointments as a professor. It commonly required a comprehensive thesis summarizing research results of the previous years of published research activities. Wecker told me that the Faculty of Medicine of the Würzburg University had recently changed the rules and would now allow also *kumulative Habilitationen*. This was supposed to represent a compilation of previously published scientific contributions. Wecker felt that my published work would fulfill these criteria. I should just write a short summary in German of my US papers (all written in English) and submit it to the faculty with his endorsement. So I did and submitted a 12-page summary. I did not realize that this was the first submission of a cumulative habilitation to this faculty, which created an outcry by several faculty members: "The publications are all in English; who knows whether one of the next *Habilitationen* will be in Chinese?" Finally, the faculty had to decide whether to accept this proposal. Although I was not personally involved in this debate, I subsequently learned from a faculty member that after a heated discussion my habilitation was accepted with a narrow majority of a single vote.

Toward the end of 1970 I received a phone call from Professor Adolf Windorfer, the chairman of Pediatrics at the University of Erlangen-Nürnberg in Bavaria, enquiring about my interest in applying for a position in clinical virology at this university. I quickly indicated my interest, and after a subsequent private discussion in Windorfer's home, I decided to apply for it.

Wecker in Würzburg discouraged me from accepting this position, if offered, because it would require focusing all my activities on diagnostic virology. In contrast to his view, I saw my big chance in setting up a new institute according to my own views. When in February 1972 the Medical Faculty in Erlangen voted in favor of my appointment as full professor for virology, I immediately accepted.

ERLANGEN 1972–1977: INSTITUTE OF CLINICAL VIROLOGY STUDIES ON EPSTEIN-BARR VIRUS IN NASOPHARYNGEAL CANCERS AND THE START OF RESEARCH ON PAPILLOMAVIRUSES IN CERVICAL CANCER

Two aspects were tremendously positive when I started to work in the institute in the basement of a building of the Children's Hospital of the University of Erlangen: First, Chancellor Köhler was extremely supportive in providing me with sufficient money for furniture and equipment for the laboratories and my own office. Second, Windorfer transferred two postdoc positions from the hospital to the new Virology Institute, enabling me to bring in Schulte-Holthausen and Wolf who had joined me in Würzburg. I hired a few new colleagues, including Georg Bornkamm and Bernhard Fleckenstein, who already had a good background in molecular biology and microbiology.

My first appointments, however, were two technicians, Marion Habermann and Gabriele Feulner, who later joined me for a few years when I moved to Freiburg. With two extremely helpful women responsible for cleaning glassware, Frau Buchner and Frau Schaufler, the team started to work in 1972. An initial grant provided by the Deutsche Forschungsgemeinschaft (DFG, German Research Organization) helped to buy an electron microscope, which bolstered our scientific reputation almost from the beginning.

My family moved to a small village 6 km north of Erlangen, Bräuningshof, surrounded by 2,000 m^3 of forest. Besides my two sons, Jan Dirk and Axel, a little dog, a Dalmatian named Assi, also became a member of the family.

Prior to leaving for Erlangen I had made the decision to start a new topic in our research. When Nonoyama & Pagano (12) confirmed our demonstration of EBV DNA in the virus-free Burkitt's lymphoma cell line, Raji, and in tumor biopsies, we became aware of the growing competition in the field of EBV molecular biology. Although we did not understand the reasons for the peculiar geographic patterns of Burkitt's lymphomas in children of Equatorial Africa and of NPCs in adults in southern China, I trusted that our molecular studies strongly supported serological data pointing to a role of EBV in these malignancies. Although I knew that we could continue—probably for a lifetime—to investigate the mechanism by which EBV could initiate malignant tumors, I was convinced that studies on the role of infections in more common human cancers would be more beneficial for human health.

Before changing to another topic, however, I decided to solve one other question hotly debated among the growing group of EBV virologists. The malignant cells of NPCs were invariably mixed and surrounded by intensive lymphatic infiltrates. At this time EBV was accepted as a B-lymphotropic agent. George Klein, a well-reputed immunologist at the Karolinska Institute, and his wife, Eva, vociferously postulated that the demonstration of EBV DNA in biopsies derived from this tumor originated from the infiltrating lymphatic cells.

Convinced that latent EBV genomes drove the malignant growth of NPC cells, I disagreed with Klein's interpretation and decided to clarify the existing situation. I had started in situ hybridizations of EBV-producing Burkitt's lymphoma lymphoblastoid tissue culture cells by applying a method developed by Perdue & Gall in 1968 (14). In my hands this method worked very well in cells producing EBV particles.

For these reasons, I asked Wolf to apply it on a number of frozen NPC biopsies from Klein and several colleagues in Kenya. Frozen sections were prepared in the Pathology Institute of Erlangen University. During the subsequent days I had to leave Erlangen for a few days. When I returned, I immediately asked Wolf whether those slides showed anything specific. He told me that the result was entirely negative, and he had thrown all of them into the garbage bin. Fortunately, the bin had not yet been emptied, and I recovered all the slides. A microscopic inspection revealed clearly labeled islands of cells, which in my opinion represented epithelial cancer cells. This interpretation was quickly confirmed by Volker Becker, the head of the Pathology Institute. We saw no labeling of the adjacent lymphatic cells (**Figure 8**).

When we published these data in August 1973 combined with immunofluorescence studies on EBV nuclear antigen in the labeled cells (15), the reaction in Stockholm was initially complete disbelief. In December 1974, however, Klein and several of his colleagues published a study describing the heterotransplantation of NPC cells into nude mice. This confirmed our in situ hybridization results, and he gave us fair credit to our earlier observations (16). However, this episode was largely the end of our specific engagement in EBV studies.

The relatively poorly studied human wart viruses were at the center of my interest. When I was still in Philadelphia, I came across a review by Rowson & Mahy (17) that described the relatively rare conversion of genital warts into squamous cell carcinomas. These reports fascinated me. In

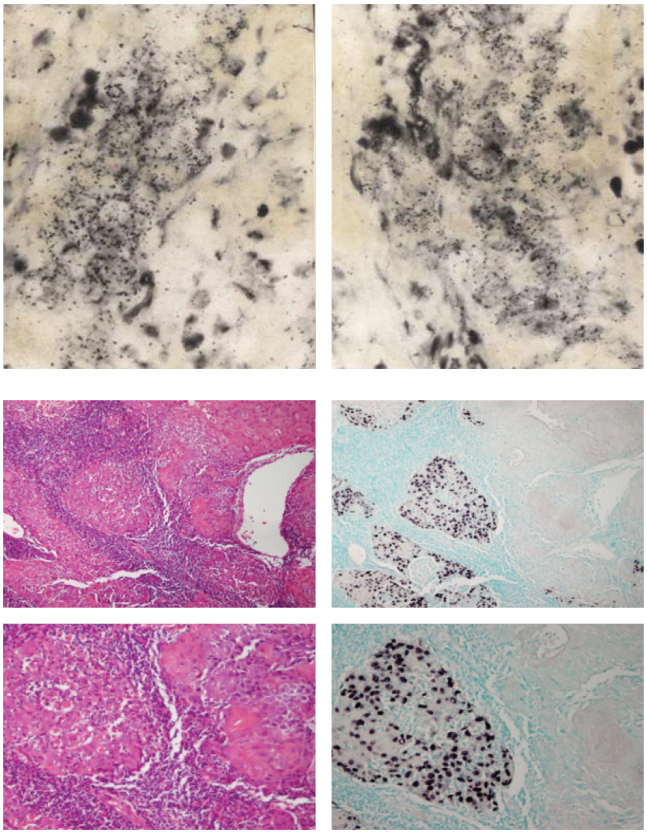

Figure 8

In situ hybridization of nasopharyngeal carcinoma sections. The upper two photos are from our laboratory and reproduced from Reference 15. The lower four photos are courtesy of Dr. K.W. Lo, Hong Kong.

1968, I decided to work on this topic. The hypothesis that came into my mind was a possible role of this agent in human cervical cancer. In 1846 an Italian physician, Rigoni-Stern, had postulated that cervical cancer could be linked to sexual contacts, but subsequent efforts to identify sexually transmitted agents responsible for this malignancy had failed. I was aware of Rous et al.'s studies during the 1930s of skin cancers linked to a rabbit papillomavirus infection (18) and of Olson et al.'s work in cattle documenting the induction of bladder cancer by bovine papillomaviruses (19). The new institute in Erlangen offered an excellent opportunity to investigate the possible role of human wart virus in cervical cancer.

Shortly after moving to Erlangen, I contacted the head of the University Dermatology Hospital, Professor Hornstein, and discussed with him the plans of a papillomavirus project. He suggested I contact his deputy, Wolfgang Meinhof. This proposal turned out to be very fortunate, since Meinhof was highly motivated. With the help of a young assistant, they instantly started to collect every wart that was surgically removed. Knowing from the literature that plantar warts produced high quantities of papillomavirus particles, I ground them with sea sand and subjected them to cesium density gradient centrifugation. Within a short period of time I obtained large quantities of highly purified papillomavirus particles. After DNA extraction and in vitro radioactive labeling of the DNA, I conducted the first nucleic acid hybridization experiments. Our initial

interest dealt with the question of whether the viral DNA obtained from plantar warts would hybridize to DNA from cervical cancer biopsies and to genital warts. No positive results were obtained. In contrast, plantar warts were highly positive, but warts from other locations on the trunk or arms revealed occasionally a faint reaction or were entirely negative. Since, electron microscopically, I saw typical papillomavirus particles in two or three extracts from genital warts, as well as in extracts from nonplantar warts, only one interpretation appeared to be plausible: Several types of human papillomaviruses (HPVs) must exist, either very distantly or not at all related to the isolated plantar wart virus.

In contrast to the group of Gérard Orth working at the Institute Pasteur, I avoided the pooling of different warts and analyzed them individually. Orth had initiated his work on papillomaviruses one or two years earlier, studying the rabbit papillomavirus, which, as mentioned before, caused skin cancers in domestic rabbits.

My avoidance of pooling different warts for virus extraction was based on somewhat vague evidence suggesting the existence of different types of HPVs: Almeida & Goffe, British scientists, had reported that sera from patients with genital warts agglutinated particles obtained from donor patients yet failed to react with papillomavirus particles from plantar warts (20).

I isolated and purified large quantities of plantar wart virus particles, extracted their DNA, and with my colleague Georg Bornkamm prepared radioactively labeled complementary RNA to DNA isolated from plantar wart virus. Hybridization experiments confirmed Almeida's suspicion: Clearly positive results were seen with most plantar wart hybridizations, some cutaneous warts were weakly positive, and genital warts and several cutaneous warts were entirely negative. We considered this as a first clear-cut hint that several different types of papillomaviruses must exist (21). Their number at present, 45 years later, totals more than 400 HPV types.

In spite of initial difficulties in isolating sufficient quantities of genital wart viruses, it became clear to us that genital wart viruses must be different from those in foot warts. Since a few reports had been published describing the malignant conversion of genital warts into squamous cell carcinomas (17), since 1969 I had considered the genital wart virus as a candidate causal agent for cervical cancer. We solved this question only after my transfer to Freiburg in 1979.

I received an invitation in December 1974 to a meeting on viruses in cervical cancer in Key Biscayne, Florida. Several publications had appeared previously claiming a role of human Herpes simplex virus type 2 (HSV-2) in the etiology of cervical cancer. After we found EBV DNA in Burkitt's lymphoma and in epithelial cancer cells of NPC, we felt confident that the same techniques should be applied to discover HSV-2 DNA in biopsies from cervical cancer (22). However, a large number of tests in our laboratory, mainly carried out by Schulte-Holthausen, failed to find any evidence for this DNA. I concluded that HSV-2 was an unlikely candidate for initiating cervical cancer and that the search for genital wart viruses in these tumors would be a much better option. I presented this at the Key Biscayne meeting. To my surprise, at the same meeting Bernard Roizman and Niza Frenkel from Chicago presented data of an HSV-2–positive cervical cancer containing 40% HSV-2 genome and HSV-2 transcripts in the tumor tissue. These results were apparently expected, obviously pleasing everyone in the auditorium. Subsequently, when I presented our negative results for HSV-2 DNA and discussed a higher likelihood of papillomaviruses linked to this cancer, I was intensively criticized that our methodology was not sensitive enough and therefore inappropriate. Although I did not hear of any follow-ups of the Roizman/Frenkel results, this discussion went on for a couple of years and only stopped when, in 1983 and 1984, we isolated the high-risk HPV types 16 and 18 directly from cervical cancer biopsies. Two years after the Florida meeting, a brief note I wrote shortly after this meeting was published in *Cancer Research* (23). An earlier review, however, had analyzed the HSV-2 data and emphasized genital papillomaviruses as better candidates (24).

During the period in Erlangen I continued with a number of additional studies, most of them directed toward the identification of novel viruses and their link to human diseases. These were unsuccessful, as were continued efforts to immortalize primary human cells by chemical or physical carcinogens. My colleague Ulrich Schneider established a few cell lines from leukemia patients. One of them, a T cell line, *Jurkat*, found global application and distribution, commonly not quoting the original publication by Schneider.

Based on a grant provided by the DFG, my colleague Volker Diehl and I spent two months at the Kenyatta Hospital in Nairobi collecting African cancer biopsies and genital warts. This was enabled by a Swedish radiotherapist, Bo Johannson, from the Karolinska Institute. HPV 18 was initially isolated from one of the cervical cancer biopsies collected there.

Shortly after starting to work in Erlangen I was contacted by members of the Medical Faculty to initiate and chair a special interinstitutional research program (Sonderforschungsbereich, SFB) titled *Methodenforschung zur Früherkennung des Krebses* (Research on Methods for Early Detection of Cancer). The initial application to the German Science Foundation had been made by one of the chairs of the Internal Medicine Hospital (Professor Demling) prior to my arrival to Erlangen but was put on hold for more than one year. I organized this jointly with a number of clinical and research groups of the university. Before I left Erlangen it had been successful in two rounds of external evaluations.

Erlangen and its university were milestones for my career. I reflect with gratitude on years of generous support by the president and chancellor of this university, the enthusiasm of my young team, and the thrill of organizing a new institute of virology. I also enjoyed teaching the then small groups of students in the developing field of tumor virology. Even though a few of my faculty colleagues disliked my engagement for research-oriented new professorships, we agreed that several of these appointments were excellent representatives of this university.

In 1976 the Faculty of Medicine in Freiburg offered me the professorship for virology and hygiene. I was tempted to accept this since I saw more opportunities for the expansion of research, space, and staff. I left Erlangen in 1977 with nostalgic feelings, uncertain whether I had made the right decision. This uncertainty was somewhat eased by the fact that several of my colleagues in Erlangen decided to join me in Freiburg. The points that tipped the balance in favor of Freiburg were the beauty of the city, the Black Forest, and the close vicinity to Alsace, France, and to Switzerland.

FREIBURG, 1977–1983

My time in Freiburg was, from the very beginning, somewhat complicated. I started my negotiations for the position in the Ministry for Cultural Affairs in Stuttgart. This meeting was unusually unfriendly and very different from the one I had in the corresponding Ministry in Munich when I negotiated my previous position in Erlangen. Nevertheless, we reached an agreement, and on March 1, 1977, I began my work in Freiburg. The Institute for Hygiene and Microbiology was made up of independent divisions of virology, bacteriology, immunology, and blood group serology. At the period of my arrival, the immunologist, Professor Vogt, acted as director. Two years later I was elected, followed in 1982 by the bacteriologist, Professor Bredt. This structure created some complications due to colleagues' fear that the virus division (with a full professorship as head) would predominate the others. This fear gradually disappeared. Another problem arose from the fact that the tenured academic staff members of the virus department were not equally productive and felt overwhelmed by the arrival, activities, and ambitions of the Erlangers who had joined me in the move to Freiburg. This required some patience but also some rearrangements and time.

More serious were problems with the university's management director (chancellor) and the Medical Faculty. The former chancellor considered ambitious scientists as a nuisance for the university administration and tried hard (but unsuccessfully) to block my early initiative to revive a special research program (in his words, "You are only disturbing our administration") that was supposed to be financed by the DFG and that up to then had been long forgotten. By persuading a number of colleagues, we revitalized it and in a subsequent external evaluation received the approval for funding.

Conflict with the Medical Faculty started soon after my election to committees for recruiting new professorships. I immediately noticed the preexisting arrangements, which did not necessarily follow quality standards. My opposition stigmatized me as a troublemaker and excluded me from subsequent committees.

I was on more cordial terms with some individual Freiburg Faculty colleagues. One of them was pathologist Walter Sandritter, and another was biochemist Helmut Holzer. Others were friendly and neutral, whereas still others observed our growing scientific reputation with recognizable distrust. In addition, many disliked my continued polemic remarks requesting a reform in the remuneration system of private compensation for full professors in medical faculties.

These were some of the negative issues accompanying the move to Freiburg. The research there, however, proved to be very positive. Well-funded by the DFG and SFB, we quickly expanded papillomavirus research activities, focusing on their suspected role in cervical cancer. Virtually within the first year Lutz Gissmann successfully isolated viral DNA from a genital wart, where the restriction enzyme analyses soon indicated that this DNA was different from all previous isolates (25). With the help of colleagues from the Biology Faculty, we learned molecular cloning procedures. Ethel-Michele de Villiers and Gissmann soon thereafter successfully cloned this genital wart virus DNA, which was labeled as HPV type 6 (26) (**Figure 9**). Upon using this DNA in nucleic acid hybridization tests, our first disappointment was evident: My initial hope that this virus may also be the cause of cervical cancer was not confirmed by the data. Nevertheless, besides DNA from genital warts, DNA samples from juvenile laryngeal papillomas (which I had collected over a number of years) were positive for hybridization. A DNA sample from one of them was closely related but not identical to HPV 6; it was cloned and labeled HPV 11 (27). By using this DNA in hybridization experiments with DNA from cervical cancer biopsies and carefully analyzing Southern blots, we noted that some of the lanes showed very faint bands, suggesting to me that they may contain distantly related but different DNAs of other HPV types. I selected two biopsies with relatively clearly visible faint bands and asked two of my students,

Figure 9

The papillomavirus crew in Freiburg: (*left to right*) Ethel-Michele de Villiers, Mathias Dürst, Michael Boshart, and Lutz Gissmann.

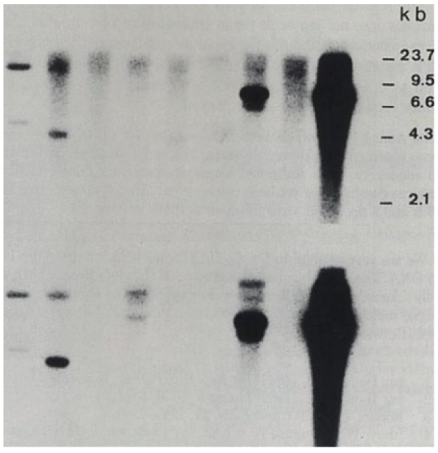

Figure 10

The first human papillomavirus 16 blot. Photo reproduced from Reference 28 with permission.

Matthias Dürst and Michael Boshart, to try to clone and characterize those sequences (**Figure 9**). Both were successful.

Dürst isolated and cloned the until-then-unknown HPV 16 DNA that was published in 1983 (28) (**Figure 10**); Boshart successfully cloned HPV 18 DNA (29). This was a breakthrough, since we quickly discovered that HPV 16 DNA was detectable in approximately 50% of cervical cancer biopsy DNA and HPV 18 in about 20%. We provided the clones to all laboratories who asked for them—with an obviously not-very-professional material transfer agreement. But even this unprofessional document was not honored by two of my coworkers and a couple of colleagues in other countries. Yet, our data were quickly confirmed. I felt some satisfaction in this situation because up to this moment, several colleagues had ridiculed our research, saying, "Everyone knows that warts and papillomaviruses are harmless."

The consequences of this discovery were far reaching: Previous assumptions that HSV-2 caused cervical cancer were fading away. A steadily increasing number of companies became interested in developing novel diagnostic tests for HPV infections and preventive vaccines. These vaccines became available globally more than 20 years after the identification of the agents. Initially the vaccines were recommended only for teenage girls, but after another 12 years, several countries began to recommend the vaccination of teenaged boys—in my opinion at least 10 years too late, since usually a higher number of sexual contacts of males in comparison to females of the same age predicts the former as the main transmitters of these most common sexual infections.

We continued to work on molecular aspects of these infections: Elisabeth Schwarz identified the HPV 16 E6 and E7 transcripts in cervical cancer cells (30). Their persistent presence in these cancers was a first hint to their role as viral oncogenes.

In the next years I aimed to find explanations for the following questions:

1. Can we define reasons for the long latency between primary infection, the slow development of precursor lesions, and the even slower progression to cancer (in cervical cancers, about 15 to 30 years)?
2. What regulates silent persistent infections?
3. Are additional factors involved in the eventual conversion to malignant growth?
4. What is the basis for the species specificity of some of these viruses?
5. Why does an immune response not eliminate such agents?

What intrigued me most were somatic cell hybridizations demonstrating that fusion of normal cells with malignant cells often resulted in nonmalignant hybrids. In some instances, even fusion of cells from two different malignant lines resulted in nonmalignant hybrids, whereas other hybrids maintained their malignant phenotype. The conclusion was obvious to me (and probably also to several other oncologists): Apparently the normal partner in these experiments contributed at least one gene that suppressed the malignant phenotype of the tumor-derived malignant partner. Because we had also observed the same effect in cell hybridization experiments of two HPV-DNA–containing cervical carcinoma cell lines (where we knew that the expression of viral E6/E7 genes was an essential determinant of malignant proliferation) (31), this phenomenon permitted only one explanation: The cancer cells contain at least one set of allelic genes, which are either mutated, silenced, or deleted. In normal cells, however, these genes suppress the function of viral oncogenes and keep these cells in a quasi-normal state. I had called this the cell-virus gene balance hypothesis of carcinogenesis or CIF concept (as cellular interference factor in normal cells suppressing viral oncogenic functions) in a publication many years earlier (32–34). It had dominated my thoughts for the following years. This led to the conclusion that (except for the rare inherited cancer risk mutations) no potentially tumorigenic infection is by itself sufficient for malignantly transforming human cells. The latency period between infection and initiation of tumor growth would depend on the number of modifications inactivating specific host cell genes (35).

The Freiburg period was also successful regarding a few other aspects. I noted cytopathogenic changes in lymphocytes cultivated from an African green monkey and found typical polyoma-type particles in the supernatant of this culture. The infection could be transferred to EBV-negative Burkitt's lymphoma cells that permitted further characterization of this virus. It was a novel polyomavirus type, labeled by us as B-lymphotropic polyomavirus (LPV). We subsequently cloned the DNA and sequenced the genome (36, 37). We demonstrated neutralizing antibodies against LPV in several human sera and suspected a similar infection in humans. A few years later several groups independently identified a closely related virus from humans, now labeled as human polyomavirus type 9.

Shortly thereafter, I isolated a novel human adeno-associated virus (AAV) from a papillomatous lesion later labeled AAV-5 (38). AAV-5 has subsequently been used by other groups as a gene vector system with particular affinity to cells of the respiratory tract.

Three other observations marked the Freiburg period. First, we discovered EBV reactivation by specific phorbol-esters (39). This reactivation proved to be very useful for the further molecular characterization of EBV.

The second observation resulted from my visit to the Weizmann Institute in Israel. I learned from Sarah Lavi that specific chemical carcinogens induced amplification of SV40 DNA in SV40-transformed Syrian hamster cells, apparently due to their mutagenic function. Previously I had persuaded one of my coworkers, Jörg Schlehofer, to test whether herpes simplex infections of various cells would lead to mutagenic results. The experiments did provide positive data (40). After returning from Israel, I asked Schlehofer to use Lavi's system but by infecting the same cells with herpes simplex virus. We showed that herpes simplex as well as a few other herpesviruses and adenovirus infections induced SV40 amplification in these cells (41). One practical consequence resulted from my suggestion to my coworker Regine Heilbronn to transfect human cytomegalovirus (CMV)–infected cells with the human polyomavirus JC (JCV). In immunosuppressed individuals, this widespread JC human infection could lead to an extremely serious disease in the brain. In tissue cultures JCV replicated only in human glia cell cultures, which were almost impossible to obtain. She showed that JC virus replicated exclusively in the CMV-infected human fibroblast cultures (42).

Third, we isolated a close relative of human EBV from cultivated AGM cells (43).

TWENTY YEARS AS SCIENTIFIC DIRECTOR OF THE DEUTSCHES KREBSFORSCHUNGSZENTRUM IN HEIDELBERG (1983–2003)

During the years in Erlangen and Freiburg I followed the developments of the German Cancer Research Center (Deutsches Krebsforschungszentrum, DKFZ) in Heidelberg with interest. I did not have any direct contacts to this center at the time, except for a temporary collaboration with the DKFZ biochemist Erich Hecker, whom I had asked for phorbol-esters to study the effect of these chemicals on Burkitt's lymphoma cells.

The DKFZ, which was founded by Heidelberg surgeon Karl-Heinrich Bauer in 1964, experienced a remarkably bad reputation in the German and international press during the subsequent 19 years. This was in part due to the overhasty appointment of several institute directors and low scientific published output, but it was in part also due to an apparent lack of clinical links and to the predominant research activities in murine models of cancer. "*Die Mäusedoktoren von Heidelberg*" (The Mouse-Doctors of Heidelberg) was one of the most frequently quoted headlines in the *Frankfurter Allgemeine Zeitung*, one of the major newspapers in Germany. Relatively generous external funding from the federal government and the state of Baden-Württemberg, internal quarreling, and a harsh international evaluation of the research quality had already resulted in the Federal Ministry for Science and Technology considering a complete shutdown of this center.

My personal thoughts were at that time that the cancer center could do much better by improving its research quality and internal structure and by devoting its activities to the direct analysis of human cancers. Since cancer interested me more than any other human disease, I played with several ideas on what needed to be changed.

In December 1982 I attended the sixtieth birthday celebration of Klaus Munk, the DKFZ virologist. The center was looking for a new scientific director, and I was curious to hear of the preliminary result. At this occasion I was approached by one of the division heads of the DKFZ Pathology Institute, Peter Bannasch, who expressed his surprise that I would not be interested in this position. This was news to me, too. Somebody had obviously been spreading this rumor.

Despite other potential candidates being approached earlier, the internal and external Council of the DKFZ voted in my favor. Subsequent negotiations with the Federal and State Science Ministries were successful, and by May 1, 1983, four months after my Heidelberg visit, I was appointed *Wissenschaftlicher Stiftungsvorstand* (scientific director) of the DKFZ, which had at that time more than 3,000 employees. An administrative director and I were jointly responsible for the development of this center for the next 20 years. The administrative director was Reinhard Grunwald for the first 12 years, followed by Josef Puchta for the last 8 years.

Restructuring the DKFZ's organization and research consumed a substantial part of my 20 years there. I consider these developments the most significant:

1. I established international evaluations of each of the eight individual institutes of the DKFZ in five-year cycles. Because we involved top scientists in the respective fields and mainly from abroad, these evaluations led to substantial consequences for several of the institute directors and individual divisions. This was repeated four or five times for each institute/research program and proved to be the most important step in reorganizing the center. Our model was quickly copied and adapted by other research organizations and several German university faculties.
2. Every two weeks I spent one day and subsequently one afternoon in one of the (at that time 48) divisions of the center, requesting a presentation of recent results by individual scientists, an overview of their financial and personal resources, and a presentation of future directives.

3. I blocked the extensive exploitation of PhD and other doctoral students (I noted that some of them had been working on their theses for six to seven years). I argued that most of the work should be finished within three years, but exceptions may be granted under certain circumstances (e.g., pregnancy, disease, or other unusual conditions).
4. Despite a modestly successful *Tumorzentrum Heidelberg-Mannheim*, to which the cancer center paid for a number of collaborative research activities, I realized the need for closer DKFZ-clinical interaction and established clinical cooperation units. These represented divisions of the DKFZ working as integrated parts in the surrounding university hospitals, with parallel laboratory facilities in the DKFZ. This had a difficult start, but after we established the first two units, it became more and more popular, resulting in many additional successors.
5. We reorganized the structure of the center: Instead of individual institutes, commonly representing one floor of the building, we created *Forschungsschwerpunkte*, floor-independent research programs. After approximately three decades the new structure seems to function well.
6. During my scientific directorship we added new buildings to the cancer center. Initially, we constructed a new communication center, a larger auditorium, and a convention hall, now almost continuously frequented by the staff and guests of the cancer center. Convinced of an important role of infections in human cancers, I proposed the construction of a new building for applied tumorvirology, which was completed in 1992.
7. After realizing that genome research was not effectively incorporated in any of the German nonuniversity research organizations and appreciating its importance for cancer research, I decided that we needed to do it ourselves. We established a number of efficient groups in genome research within our center and were later able to buy a new building for these activities.
8. Intrigued by the rapid global epidemic spread of acquired immune deficiency syndrome in the 1980s, I proposed to the Ministry of Science and Technology that it establish a bursary for young scientists to get two to three years of training abroad in laboratories working in this field. After this period they should return home and establish a laboratory in an institution of their choice. This proposal was approved, and we selected some very good candidates who found independent positions in the country after their return to Germany.
9. I invited the directors of Heidelberg University Hospitals to join in a discussion of creating what was later called a comprehensive cancer center. When I presented the concept of an interactive institution between the Medical Faculty of Heidelberg and the DKFZ, it received particularly energetic support from the head of the Hematology Division, Professor Anthony Ho, who had previously been engaged in comprehensive cancer centers in Canada and the United States. With his help we provided a concept booklet to the Federal Ministry of Research and Technology. It was approved in a local evaluation by foreign scientists with experience in comprehensive cancer centers and representatives of the respective Federal and State Ministries. An initial grant of €13,000,000 was provided to encourage other granting agencies (*Deutsche Krebshilfe*—German Cancer Aid) to provide further support.
10. A few other points proved to be helpful: One was the establishment of a Cancer Information Service for the public. Another was the prohibition of smoking in the center and the removal of existing cigarette and beer automats.

When I retired from my position as scientific director in 2003, I stated at a farewell party that I was leaving with good feelings, since the outlook for the DKFZ and the interactions of the

DKFZ with Heidelberg University were excellent. The official inauguration of the first German Comprehensive Cancer Center in Heidelberg took place a couple of years later.

During my 20 years as scientific director I tried hard not to be too absorbed by administrative and organizational activities. With time this became increasingly difficult. Nevertheless, throughout this period, I diligently attempted to keep two hours of every working day free for laboratory visits and discussions with the younger scientists. It was an enormous learning period for me in fields other than virology and microbiology.

Scientifically, many of the 155 publications that appeared during this time were related to work initiated in Erlangen or Freiburg or reviews that profited from my broader scope of the field. In several of them I acted as coauthor, since I initiated most of these papers and discussed them in the course of their experimental analyses. Several aspects, however, were novel and original.

One major change of direction with serious and important consequences resulted from a discussion with my wife, Ethel-Michele de Villiers, at the end of the 1990: Both of us felt that the papillomavirus field was sufficiently grazed and largely covered by commercial interests and industrial companies. It was time to start something new. A bit earlier we became aware of publications by Okamoto and his colleagues in Japan in 1997 describing a novel virus type (TT viruses) widely spread in human populations with some unusual characteristics (44). These investigators isolated single-stranded small circular DNA molecules from human blood without evidence for any disease association. Both of us were intrigued by these findings and quickly agreed that this may represent a fascinating research topic, in particular due to our experience with other ubiquitous viral infections initially thought to be without any straightforward pathogenicity for humans.

Ethel-Michele became quite enthusiastic about this proposal and initiated shortly thereafter experiments to isolate and clone some of these molecules. Close to 20 years later this shift had profound and far-reaching consequences, not directly related to the Okamoto isolates, but to a related research topic, which I discuss later.

THE EMERITUS PERIOD: 2003–2008

After I retired as scientific director of the DKFZ in 2003, Peter Lichter was appointed as an interim scientific director for one year until 2004 when Otmar Wiestler, a neuropathologist from Bonn, was appointed. Wiestler stayed in this position for approximately 11 years (until September 2015), when he was elected as president of the Helmholtz Society.

In 1999 I became editor-in-chief of the *International Journal of Cancer*, a position I held for 10 years. As editor-in-chief of this journal I moved from an asylum in Ethel-Michele's laboratory to a small office in the by now 12-year-old virus building of the cancer center. A few staff members at the center were amazed that I did not completely retire, but sooner or later they became accustomed to my presence. Shortly before my retirement, in 2001 I had published the book *Genom und Glaube* (*Genome and Religion*) for Springer-Verlag. Early during my retirement I finished the book *Infections Causing Human Cancer*, which was published by Wiley in 2006. Ethel-Michele and I jointly edited the volume *TT Viruses: The Still Elusive Human Pathogens* (45).

I used this period of time to write a few reviews, act as consultant for a few virus groups in our center, and continue some studies and considerations I had initiated when I took up my position in Heidelberg in 1983.

With great interest I followed the developments of HPV vaccines that had been licensed in many countries since 2006. I found the advertising of these vaccines quite disappointing for two reasons: First, it was instantly labeled as a cervical cancer vaccine. Knowing that the time elapsing between a high-risk infection and cervical cancer development commonly spans a period of 15 to 30 years, I did not understand the lack of emphasis on the prevention of essential precursor lesions

appearing one to three years after infection, and I questioned this policy in a number of public presentations.

A second aspect of advertising the vaccine appeared to me to be even more serious. The licensing agencies, as well as the producing companies, specifically propagated the vaccine for girls prior to reaching sexual maturity. None of them considered the importance of vaccinating boys of the same age group as well. Karin Michels and I published an article in *The Lancet* in 2009 emphasizing the need and reasons for vaccinating both genders (46). Initially this found little attention among those involved in the application of these vaccines. Eventually Australia and a very few other countries picked up this recommendation. In Germany it took 12 years after the first HPV vaccine license before a central commission in 2018 recommended vaccination of both genders.

THE NOBEL PRIZE IN PHYSIOLOGY OR MEDICINE IN 2008 AND SOME OF THE CONSEQUENCES

During the last part of 2006 and the first part of 2007 several of my international colleagues casually suggested to me in passing that the licensing of the HPV vaccine in the previous year would promote me to a top candidate for the Nobel Prize in Physiology or Medicine in 2007. I heard this with amusement, secretly hoping that this would become reality. When the Noble Prize for Medicine or Physiology was announced in October 2007 to Mario Capecchi, Martin J. Evans, and Oliver Smithies, I thought this was an excellent selection but experienced at the same time some disappointment that the predictions had not come true. Similar rumors had been spread even before 2006, obviously without serious substance. Consequently, I felt that my chances had passed, and I did not take notice of similar predictions in 2008. But when I received a phone call from Stockholm in my office on October 6 at 11 AM and listened to a voice with a Swedish accent, I instantly realized that this must be good news (**Figure 11**). The subsequent period was turbulent, hilarious, a bit exhausting, but fascinating, in part even more for my family and my friends than for me.

The scientific consequences had long-lasting and probably by far the most important effects. As part of a long tradition, I was asked to present a scientific lecture at the Karolinska Institute a few days before the award ceremony. Commonly, as expected by the auditorium, the new laureates presented reviews of their recognized research and provided an explanation of how they reached their results and what could be anticipated as future consequences. I decided not to stick to this tradition and to avoid a discussion on the papillomavirus work for which I was decorated. I selected the following theme: The search for infectious causes of human cancers: where and why? This was published about one year later (47). In this presentation I emphasized hematopoietic malignancies and colon and breast cancer, referred to inconsistencies in the commonly accepted red meat risk due to chemical carcinogens supposed to arise in the preparatory steps for consumption, and stated at the end, "It is tempting to speculate that a hitherto unidentified bovine infectious agent with pronounced thermostability, replication-incompetent for human cells and possibly structurally related to the polyomavirus family, may play a role in colorectal cancer, potentially also in lung cancers of nonsmokers and in breast cancer."

The preparation of this lecture and the accompanying discussions with Ethel-Michele, as well as the obvious surprise of my colaureates and several listeners in the auditorium, resulted in a decisive turning point in my late career. I returned to Heidelberg committed to pursuing an old idea suggesting an association between breast and colon cancer. This led to a long series of investigations, mostly in collaboration with Ethel-Michele, that led to the identification of what appears to be a novel type of infectious agent and human pathogen in dairy products. We believe that

Figure 11

The Nobel Prize Award Ceremony in Stockholm, December 2008: (*upper left*) with my wife, Ethel-Michele de Villiers; (*upper right*) with Ethel-Michele before the Nobel Lecture; (*lower left*) with my sons (*left to right*), Gerrit, Axel, and Jan Dirk; and (*lower right*) the Nobel Dinner with Crown Princess Victoria. Photos (*upper right*, *lower left*, and *lower right*) reproduced with permission from dpa.

these agents, which we have named infectious (bovine) plasmidom, are likely to be causal agents of colon and possibly breast cancer, as well as of other diseases of unknown etiology such as multiple sclerosis, Parkinson's disease, and Alzheimer disease. Because of space limitations, I refer the reader to the accompanying **Supplemental Appendix**, which includes an extensive summary of the exciting data we have obtained in support of these statements.

Supplemental Material >

CONCLUSION

I have had the unusual chance of a colorful and most interesting long life. I must express my gratitude to members of my family, friends, and colleagues who profoundly influenced my development, my personal life, and my career. First, I am grateful to my parents, Melanie and Eduard zur Hausen, who during very difficult times took great care of my two brothers, my sister, and me. My aunts Dora and Johanna and my cousin Cläre Kraneburg provided enormous help and support in my early years. I am deeply indebted to my former academic teachers; Werner and Gertrude Henle; Eberhard Wecker; and a large team of my former and present colleagues, coworkers, and

friends in Düsseldorf, Philadelphia, Würzburg, Erlangen, Freiburg, and Heidelberg. I also greatly appreciate the help of the DKFZ *Stiftungsvorstand* (Board of Directors), who permitted me to continue my work after retirement in 2003. I trust they recognize that the subsequent period has been productive.

A very special note of gratitude goes to my three sons, Jan Dirk, Axel, and Gerrit, who tolerated the frequent absence of their father. They managed their own successful careers.

Nobody else, however, influenced my personal life and my scientific career more than my wife, Ethel-Michele de Villiers. She has repeatedly stated, mockingly, that we two split our activities: She does the work, and I do the talking. Indeed, a large proportion of experimental data obtained during several decades as well as a number of excellent ideas are hers. Looking at her work and her intellectual input and proposals, frequently underestimated by several of her colleagues, I see she has a point in saying this. Thank you, Ethel-Michele, for tolerating me, clearly a passionate and addicted scientist (not very dissimilar from you).

DISCLOSURE STATEMENT

The author is not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

The intensive help in preparing this manuscript by Ethel-Michele de Villiers and Lisa Braun is gratefully appreciated.

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