

**Berton Zbar M.D.**  
**Memoir of a Physician-Scientist**  
**National Cancer Institute 1965-2005**

*The National Institutes of Health*

By a remarkable stroke of luck, I learned that working at the National Institutes of Health (NIH) would fulfill my military obligation. I had been draft deferred while in medical school, internship and residency and it was time to repay my obligation. Dr. Joel Buxbaum, a neighbor and co-worker in Salt Lake City, informed me of the opportunities available in the US Public Health Service. President Kennedy had been assassinated in 1963 and U.S. involvement in Vietnam had increased under President Lyndon Johnson. The idea of going to Vietnam to fight in the war terrified me. I was afraid of being maimed, wounded or killed. Like many young physicians, I went to the National Cancer Institute (NCI, NIH) to avoid going to Vietnam to participate in the Vietnam War. We were sometimes referred to as *yellow berets*. I never dreamed that I would spend 40 years at the NIH.

The National Institutes of Health (NIH) is a federal government institution dedicated to improving the health of the American people. The organizing concept is of separate divisions or “institutes” each focusing on a specific health problem: for example, cancer, neurologic disease, and arthritis. The genius of the designers of the institution can be seen in the structure of the Clinical Center, the research hospital that is the emotional core of the NIH. Here research laboratories are located adjacent to the hospital wards to ensure that the focus of the research effort is on the patient.

The NIH provides incredible power for the study of single human diseases. Physician scientists have the freedom to bring people affected with specific rare illnesses to Bethesda for comprehensive evaluation and study. The cost of transportation of patients to Bethesda and the cost of medical care and research tests is born by the government. This feature of bringing patients from the United States and Canada, and in some instances other countries to the NIH is supplemented by the ability to make trips to visit patients in their communities (field trips). For 20 years, I used the ability to visit patients in their communities (house calls) to study patients/families affected with rare inherited forms of kidney cancer.

The NIH is an ivory tower where scientists are privileged to work as long as they dedicate themselves to improving public health, and work effectively. 19<sup>th</sup> century musicians were free to compose music as they choose; the only requirement was that once a year, the musicians provided their compositions for their sponsors (princes) to enjoy. Biomedical scientists who work at the NIH have that kind of remarkable freedom; I was free to do whatever research I wanted. My sponsor was the NCI Site Visit Committee who came every four years to review my work.

*Have a healthy disregard for the impossible*

I spent 40 years working at the NIH and ended up as a Laboratory Chief. How do you summarize a research career? What are the highlights of this long personal and scientific journey? The central philosophy that I evolved is reflected in the quotation: “Have a healthy disregard for the impossible” (Larry Page, Google, CEO). This philosophy was based on the idea that research investigators at the NIH should use the security of a government position to solve difficult problems of human disease.

I never cared about money. My salary was enough for my needs. I didn’t care about prestige. I could have been a professor in a medical school. My focus was on analyzing and solving biomedical problems and in taking part in the advancement of cancer research. Sometimes I felt like Sisyphus, continually being defeated. But I was engaged in a struggle with an adversary of considerable strength. I put everything I had into the struggle. And in those rare moments, when a goal was achieved, my joy was intense.

### **Start of research career: Herbert J. Rapp, Tibor Borsos and Peter Medawar**

I’ve described the fact that though I had many teachers, I received little or no mentoring in my educational experience. That changed when I met Tibor Borsos and Herbert J. Rapp. They came from Manfred Mayer’s immunochemistry laboratory at Johns Hopkins to the National Cancer Institute to start a program to improve cancer diagnosis. Tibor Borsos was an authority on the first component of complement. Herbert J. Rapp was an expert on the mathematical basis of complement action, the one hit theory of immune hemolysis. I worked with Herbert J. Rapp from 1966 until his death from depression and suicide. I worked with Tibor Borsos until his retirement from the NCI in 1988.

These men trained me in the discipline of science: both the techniques of science, and the importance of rigorous thinking. There was accuracy in measurement, in pipetting, and the importance of controls in science. The laboratory aphorisms were “Never trust anyone, even yourself.” “Never trust anyone who is sloppy in their grammar.” “When you think you have made a discovery, you need to be particularly careful. Because now you have a vested interest in your observations and you will have a tendency not to perform all the controls.” These principles guided me in my work for 40 years. In some of our work that involved DNA sequencing, I had two laboratory technicians read the same sequences to ensure that the readings were correct.

The experiments that we described in our publications had to be reproducible. At a time when articles are appearing about the lack of reproducibility in some areas of science, in our laboratory, reproducibility of experimental observations was the bedrock of our work. If work that we published was not reproducible, we would consider that a major embarrassment. There was a sense in which work in the laboratory was monastic. We were disciplined, dedicated and single minded. Getting it right was the major value.

### **Peter Medawar : *The Art of the Soluble***

Beside Herbert J. Rapp and Tibor Borsos, the other scientist whose philosophy had a major impact on me was Peter Medawar. Peter Medawar was an English physician who systematically studied tissue transplantation and was awarded the Nobel Prize in medicine for his work. He was

also a philosopher of science. In an essay that I found particularly important he wrote: “Like other amateurs, Koestler finds it difficult to understand why scientists seem so often to shirk the study of really fundamental or challenging problems. With Robert Graves he regrets the absence of ‘intense research’ upon variations in the – ah – ‘emotive potentials of the sense modalities’. He wonders why ‘the genetics of behavior’ should still be ‘uncharted territory’ and asks whether this may not be because the framework of Neo-Darwinism is too rickety to support an inquiry. The real reason is so much simpler: the problem is very, very difficult. Goodness knows how it is to be got at. It may be outflanked or it may yield to attrition, but probably not to a direct assault. No scientist is admired for failing in the attempt to solve problems that lie beyond his competence. The most he can hope for is the kindly contempt earned by the Utopian politician. If politics is the art of the possible, research is surely the art of the soluble. Both are immensely practical-minded affairs.” This quotation sat under glass on my desk for years.

### **Tibor Borsos and Herbert J. Rapp**

Tibor Borsos is articulate, a genuine “know it all”. Born in Hungary he came to the United States after World War II with his family. Tibor is very smart. He helped me become a successful scientist. He was a good friend and colleague. You could find Tibor in the laboratory standing next to a fish aquarium containing thermometers to measure water bath temperature shaking a rack of test tubes containing sheep red blood cells.

Herbert Rapp was a short, chubby man. He was bald, before baldness became fashionable. While Tibor was mercurial, Herb was more deliberate and even-tempered. You could find Herb at his desk writing and thinking. He was intense. He looked a little like Buddha. There was a sign on his office door giving permission for those in search of critical thinking to enter. Another sign on the door said *In vivo veritas*. There was a cartoon taken from Punch next to his desk. It showed the lonely inventor, sitting and working and then making a useful observation or invention. Then, all the inventor’s peers jumped on his wagon that eventually collapsed, leaving the inventor alone once again. I think that Herb saw himself as that lonely inventor.

Herb was a father figure for me. He loved music. He was very stubborn. He became convinced that animal studies dictated what kinds of cancer treatment should be administered in people, *In vivo veritas*. He was also convinced that cancer would turn out to be one disease not 200 diseases. He demoted me when I returned from Utah in 1976. Before he became severely depressed, he was convinced that all cancers could be effectively treated by intralesional injection of the bacteria, BCG. Nothing would dissuade him from this conviction. Knowing the cure for all human cancer was an occupational hazard of aging cancer research scientists.

Tibor had several conversations with me that changed my life. He told me that I had self-destructive tendencies. That I behaved in a way that negated my ability to reach my goals. I found this conversation particularly helpful. In another conversation Tibor recommended that I stop mourning over separation from Michael Gene Hatch. He cautioned me not to spread myself too thin, and of the dangers of unfocused work. Tibor helped me get the position as laboratory chief in discussion with Dr. Alan Rabson. He suggested an experiment that was to prove very useful in my work.

When our laboratory was located in Building 37, we had lunch together in Building 36 every day. We sat at the same table for lunch. There was Herb and Tibor and the assorted laboratory research fellows. As in the Auburn Building, conversation on work related subjects was discouraged. Herb and Tibor took pride in punning in two or three languages. I was often depressed by lunch conversations because Herb and Tibor knew so much more than I did.

### **BCG and Bladder Cancer**

Herbert J. Rapp had developed a mathematical theory of complement action. But now that he had moved from the Johns Hopkins School of Medicine, he changed his field of research to the detection of immunologic changes in cancer. His staff used carcinogen feeding to rabbits and then inbred guinea pigs to produce hepatomas and then studied these cancers for the presence of new antigens. When I arrived in the laboratory, Winthrop (Hall) Churchill had started to work with transplantable versions of the guinea pig tumors. I worked also with Terry Wepsic, a pathologist, and Barry Kronman, a surgeon. Our major accomplishment was the demonstration that under certain exacting conditions manipulation of the animal's immune system could lead to cure of a transplantable tumor. This was the heyday of immune therapy with bacteria called BCG *Bacillus Calmette–Guérin*. Herbert J. Rapp deserves the credit for leading our laboratory's efforts that led to successful immunotherapy in people with early stages of bladder cancer.

In those early years at the NCI I struggled to define myself in research work. The struggle to accomplish something was painful. I had tremendous anxiety trying to make a scientific contribution. I felt like I was struggling in the dark, like Jacob's wrestling with a man/angel. In this struggle, I held on to work with guinea pigs too long. I was afraid to leave the research work that I knew. In some respects, my behavior was similar to my father's fear of leaving the laundry business. One problem with leaving the guinea pig research work is that I didn't have any specialized research training. What I learned from Rapp and Borsos was the discipline of science, not any particular branch of science.

My research career began in the Auburn Building, an NIH rental building on Auburn Avenue in Bethesda. This was the first of many locations where I worked at the NIH. Animals were kept in the basement of the Auburn Building where Eliza Saunders, a research technician, sustained a severe rabbit bite. Laboratories were on the first floor. Tibor Borsos and Harvey Colten enjoyed listening to Gustav Mahler's First Symphony. This symphony, played at full volume, could be heard daily. The *Frere Jacque* march from the symphony is engraved in my memory. Hal Churchill and I worked further down the corridor. Around noon, there would be an enthusiastic shout from Tibor who was doing an experiment "they're lysing!" "They're lysing!" His research work involved sheep red blood cells and the way proteins interacted to cause the blood cells to lyse. When the fluid in the test tube turned red, he knew that the experiment had worked.

Tibor took great pride in his work and in the way he worked. He could put his feet up in the afternoon. His experiments took several hours to complete. The work that I enjoyed took years to complete and required patient recruitment on an international scale. I remember working together with Winthrop Churchill with test tubes in a large water bath. I shook the test tubes to mix the contents so hard that they fell out of the test tube rack and the experiment was ruined. Although Herb was first and foremost a complement biologist and immune-chemist, he decided

to use his knowledge to detect unique characteristics in malignant animal cancers. This project in one form or another was to occupy me for many years.

### **Why didn't I pursue a career related to complement research?**

I didn't pursue a career related to complement research (Tibor's field) because Hal Churchill and I were not in the complement part of the laboratory. We were in the applied biology portion of the laboratory rather than the basic research part. Both Harvey Colten and Mike Frank, physicians who were in the complement part of the laboratory, pursued careers related to complement research and its clinical interface. They took their knowledge of complement assays and applied it to human beings, Harvey Colten with complement synthesis and Mike Frank with hereditary angioneurotic edema. What they did was to apply the techniques that they learned in the laboratory to people. A good approach for research minded physicians.

### **The career that I pursued**

I did not follow in Harvey Colten, Mike Frank, Herbert Rapp or Tibor Borsos' footsteps. In a way, what I did was harder. I took the discipline of science, the discipline of a wet research laboratory and applied the discipline to an entirely new field – human cancer genetics. I made substantial contributions to this field which is why Bob Wiltrot nominated me and Marston Linehan for the Lasker prize several times. The approach that Herb Rapp and Tibor Borsos used for cancer study was primitive, the search for antigens in chemically induced guinea pig tumors. They studied transplantable tumors for much too long. They had little understanding of biology or medicine. Herb was so insistent that his way, his approach was the correct one. The work that I accomplished was certainly much more relevant to human cancer biology, diagnosis and treatment than both Tibor Borsos' work and the work of HJ Rapp.

### **Suicide of HJ Rapp**

Herb Rapp developed severe, apparently intractable diabetes and depression. He had adult onset diabetes and his neuropathy impaired his ability to play the piano. He was treated as an inpatient at the Washington Psychiatry Center. But eventually he ended his life by hanging himself in his garage. He might have benefited from electric shock therapy.

### **The Turning Point**

Around 1985 we were informed that our laboratory space was needed by other investigators. This was a very traumatic time. Administrators suggested that we join Jeffrey Schlom's laboratory. Another alternative was that we move to Frederick, to the National Cancer Institute-Frederick Research Facility. I spoke to other investigators about joining their laboratories: Dr. Steve Rosenberg and Dr. Anthony Fauci but I could not find a good fit.

### **Genetic studies of human cancer 1985-2005**

In 1985, I felt that I was about to lose my job. My research was stuck; I kept repeating the same type of experiment. I knew that I had to do something different, but I was frightened to change. I

began a series of experiments that were to occupy the rest of my career. Through a series of experiments with guinea pig tumors I observed that the genetic material of the tumors, the DNA, was unstable; there was a tendency to lose genetic material: I wondered whether loss of genetic material occurred in human tumors.

I had an epiphany while at a Steam Boat Springs Meeting when I heard a talk by Brenda Gallie, a Canadian geneticist, on retinoblastoma (a malignant eye tumor of children), and was introduced to the work of Alfred Knudson. I realized that the approach that was being used to study retinoblastoma could be adapted for use in studies of human lung cancer and human kidney cancer. I could test for the deletion or rearrangement of DNA in these human tumors. I established collaborations to do this work with John Minna, Medical Oncologist, then at the National Cancer Institute and with Marston Linehan, Urologic Oncologist, also at the National Cancer Institute.

At this time, I was studying to take the Boards in Internal Medicine, and Medical Oncology Boards. The Boards advised applicants to be familiar with recently published papers in the field. This requirement led me to read papers on chromosomal changes in a family with kidney cancer, and chromosomal changes in lung cancer. Both these manuscripts that I read while preparing for Internal Medicine Boards proved critical in the work that I was to do. These reports suggested that alterations on human chromosome 3p were critical in the pathogenesis of these tumors.

The bottleneck in beginning both sets of experiments was the obtaining tumor samples. I developed working relationships with the key members of Dr. Minna's and Dr. Linehan's laboratory to facilitate transfer of tumor samples to my laboratory. The individuals involved were Emile Trahan, and Robert Worrell in Dr. Linehan's laboratory and Dr. Bruce Johnson in Dr. Minna's laboratory.

These experiments had a number of pitfalls. In one set of experiments with kidney cancers, I used a certain restriction enzyme to "cut" the DNA into smaller fragments. When I looked at the results, the answer was just what I was looking for except that each tumor sample gave the identical result – that result was too perfect. I consulted Bert Vogelstein, a Cancer Geneticist at Johns Hopkins, and he told me that I was dealing with a subtle artifact. The tumor DNA and the normal DNA had different susceptibility to being cut by the enzyme. I consulted Bert Vogelstein from time to time for advice. Cathy Talmadge, an outstanding technician, brought knowledge of molecular biology techniques to the laboratory. Dr. Michael I. Lerman brought a broad knowledge of molecular biology into the laboratory.

The individuals who participated in the original studies of kidney and lung carcinomas were: Hiltrud Brauch, Cathy Talmadge, Raymond White, Jean Marc Lalouel, Marston Linehan, and John Minna.

### **Kidney cancer has a consistent genetic defect**

Jacqueline Whang-Pang and John Minna reported that small cell lung carcinomas had a consistent deletion of chromosomal material on the short of chromosome 3 (3p). I wanted to try to confirm this observation using polymorphic DNA markers; I wanted to work with human

materials. By chance, I learned that another investigator had already begun similar work. So, in pursuit of original work, I looked for another project closely related to the work on small cell lung carcinoma. Remembering the chromosome 3;8 translocation family, and the work of Gyula Kovacs, I selected kidney cancer as a second human cancer to analyze because existing work suggested that chromosome 3 was altered in kidney cancer. These research projects had the advantage that the same analytic tools could be used for both projects. Within a year, we found that both small cell lung carcinoma and common renal carcinomas had a consistent loss of DNA sequences on the short arm of chromosome 3.

The observation that both small cell lung carcinoma and common kidney cancer had a loss of DNA sequences on chromosome 3p (so-called loss of heterozygosity) was of great importance because it suggested that there was a gene(s) located on chromosome 3p that was important in the pathogenesis of kidney and lung cancer. These areas of chromosome 3p loss might correspond to the “second hit” of Knudson’s “two hit theory”. The “first hit” would be a somatic mutation of a gene located in the region corresponding to the area of chromosome 3p loss. When we suggested in a paper in *Nature* in 1987 that there were kidney cancer gene(s) located on chromosome 3p, we did so with considerable trepidation. It was an audacious statement, but one supported by our data.

### **The chromosome 3;8 translocation family**

In 1979, a family with a constitutional chromosomal translocation involving chromosome 3p and chromosome 8q with increased susceptibility to kidney cancer was reported in the *New England Journal of Medicine*. Family members that inherited the chromosome 3;8 translocation had a greatly increased risk of developing kidney cancer; family members who did not inherit the chromosomal translocation had a risk equivalent to that of the general population. How did this chromosome 3; chromosome 8 translocation lead to the development of kidney cancer? Did the translocation disrupt and inactivate a critical gene and thus lead to kidney cancer? Did the translocation juxtapose two ordinarily separate genes and produce an oncogene? Working with Fred Li, Laura Schmidt and Marston Linehan, we began our studies of this remarkable family. In Boston, we interviewed family members and collected blood samples. Returning to our laboratory at the National Cancer Institute-Frederick, we extracted DNA from the blood samples and began our analyses. We demonstrated that the portion of the inherited chromosome 3p translocated to the long arm of chromosome 8 was deleted in the renal tumors that developed in family members. Furthermore, many of the renal tumors had individually specific, somatic mutations in the von Hippel-Lindau disease gene.

These results suggested that renal tumors in this family developed as a result of a three step process: 1) the inheritance of the chromosome 3p, chromosome 8q translocation; 2) the deletion of the portion of chromosome 3p translocated to chromosome 8q; 3) the somatic mutation of the remaining, single copy of the von Hippel-Lindau disease gene. This was another validation of the Knudson two-hit theory of cancer development with the added feature of an inherited constitutional translocation rather than an inherited mutation in a cancer-causing gene.

One of the puzzling results of studies of this family was that not all tumors from affected members of the chromosome 3p chromosome 8q family had somatic VHL mutations in their

renal tumors. With the discovery of three additional renal carcinoma genes (PBRM1, SETD2, and BAP1) located on chromosome 3p, the likely explanation for those renal tumors is that there are somatic mutations in PBRM1, BAP1 or SETD2. Renal tumors in the setting of inherited chromosome 3p translocations can develop with a loss of the distal portion of chromosome 3p plus a somatic mutation of either VHL, PBRM1, SETD2 or BAP1.

### **Identifying kidney cancer genes: nationwide canvassing for kidney cancer families**

Once the tumor studies were completed, Michael I. Lerman suggested that we try to identify the genes that were responsible for kidney or lung cancer. After debating the pros and cons for several months, we selected kidney cancer as our target. Kidney cancer definitely “ran” in families, while lung cancer did not. Having an inherited susceptibility to kidney cancer was a characteristic that we wanted to exploit. We wanted to identify genes that were fundamentally responsible for the development of kidney cancer. For a small laboratory, with little experience in human genetics, this was a bold and almost foolhardy undertaking. We felt that with the available techniques and Knudson’s conceptual framework, we ought to be able to solve the problem and to identify kidney cancer gene(s). Thus began a 6-year forced march to identify a kidney cancer gene that was ultimately successful in 1993. When we began, the bottleneck was no tumor samples to analyze. We had already faced and solved that problem. Now, the bottleneck was finding families with multiple members affected with kidney cancer. We received samples from our first kidney cancer family from Bert Vogelstein. Then, I initiated the first of many nationwide recruitment efforts for families with multiple members affected with kidney cancer. The objective was to find multigenerational families with multiple living members affected with kidney cancer or related tumors. I began a letter writing campaign to physicians who were likely to encounter families with multiple members with kidney cancer. I obtained the mailing lists from national medical organizations of urologists, nephrologists, pathologists, and dermatologists and with the assistance of the Publication Department of the National Cancer Institute-Frederick Cancer Research Facility mailed out tens of thousands of recruitment letters. Family referrals then came in to my laboratory or to Marston Linehan at the Urologic Oncology Branch.

In some respects the recruitment of families/patients with inherited cancer resembled the canvassing that I did for my father in his laundry business. What I did for my father involved the distribution of flyers advertising the Stafford Hudson Laundry business in Forest Hills, Queens, New York. What I did in patient recruitment involved the mailing of tens of thousands of letters to physician specialists across the country. I once joked that I had send a letter to every physician in the United States at least once.

Once a family contacted us, I spoke to a family member to enlist their cooperation in this cancer research project. Usually, there was no difficulty in enlisting the cooperation of the family in research that affected their specific rare illness. Families were pleased that someone with expert knowledge in their health problem were interested in helping them. Members of families with inherited cancer were often too busy to come to the National Institutes of Health for evaluation and donation of blood samples. This led to a very active program of field trips to visit members of families with multiple members with kidney cancer located in the United States and Canada. Initially I went to my NCI administrator, John Barone, and requested funds for travel to visit

families affected with kidney cancer. John Barone spoke to Dr. Alan Rabson, then Director of Cancer Biology and Diagnosis, and I was told the following day that I had a budget of \$10,000 for travel to visit cancer families.

Thus began the most enjoyable part of my working career. In talks about our work, I had a slide that stated: "National Cancer Institute. We make house calls." I wanted to emphasize the point, that the NCI, a large government institution had relevance in people's daily lives. That the large government institution reached out and came into their homes to help them with their health problems. I visited families affected with different types of inherited kidney cancer. I made the early trips alone; on later trips I was accompanied by a dermatologist (Jorge Toro), a genetic counselor (Lindsay Middleton), and an administrator. Field trips were the result of the intensive recruitment program to identify families with inherited kidney cancer.

Each disease had its own targeted mailing program. For example, in the studies of the Birt-Hogg-Dube syndrome, a dermatologic disorder associated with kidney cancer, I sent letters to all members of a national dermatologic society repeatedly for several years. Some 40 to 50,000 recruitment letters were mailed to identify families affected with this inherited dermatologic disorder. In addition I was able to locate the original Canadian family described by Drs. Birt, Hogg and Dube in their landmark publication. An anecdote related by a member of the first von Hippel-Lindau disease family we recruited is illustrative. I telephoned a family member who lived in Louisiana, explained who I was, and what our research program was. She expressed interest in our research program. I asked her whether I could come visit her and her family the following week. She was surprised by the alacrity of the response. These field trips, very intense experiences, are engraved in my memory.

### **Field Trips**

We criss-crossed the United States and Canada visiting families with inherited kidney cancer. There always was a sense of excitement coming into a new town or city to visit a family with inherited kidney cancer. Would we be able to locate the family? Would there be a good turnout of family members? Would the family welcome us? Would we be able to find our lodging for the night? What was the town like? We visited Louisiana, Hawaii, Washington State, New York State, Missouri, Wisconsin, Minnesota, Michigan, Maine, New Hampshire, Utah, We visited South Carolina, Ontario, Saskatchewan and Nova Scotia. Families were members of all classes; no sector of society was spared. We visited small towns in Pennsylvania, Canada, and Louisiana. We interviewed family members and collected blood samples in kitchens, living rooms, churches and synagogues. We attended many family reunions and one Bar Mitzvah.

### **Von Hippel-Lindau disease**

The first form of inherited kidney cancer that we studied was von Hippel-Lindau disease. This is a remarkable disease that is associated with a predisposition to develop tumors in multiple organs: eyes, central nervous system, adrenal glands, kidneys, and testicles. At first, I was reluctant to study this inherited disease. I was looking for examples of "pure" inherited kidney cancer, that is, an inherited predisposition to develop just kidney cancer and no tumors of other organs. But finding such families was exceedingly difficult. Because of the greater availability of

families with von Hippel-Lindau disease, we began our studies of kidney cancer predisposition with families affected with von Hippel-Lindau disease.

### **Learning Linkage analysis, and pulsed field gel electrophoresis**

When Michael Lerman and I decided to clone either the small cell lung cancer gene or the kidney cancer gene, there were two critical decisions to be made: which cancer gene to pursue and which approach to take. We decided to clone the kidney cancer gene because we knew kidney cancer families existed. We decided to use linkage analysis rather than cloning the chromosome 3; chromosome 8 translocation breakpoint, because we were convinced that linkage analysis, although a long, complex process, would yield an unequivocal result. We did not have confidence that identifying the chromosome 3p; chromosome 8q translocation breakpoint would yield a kidney cancer gene. Successful cloning of a kidney cancer gene by linkage analysis required: a large panel of families affected with kidney cancer, a series of polymorphic probes for chromosome 3p, and knowledge and experience in linkage analysis. We used repeated nationwide mailings to obtain a critical mass of kidney cancer families. Michael Lerman and his group isolated DNA fragments from chromosome 3p that detected inherited variations in DNA size. Mastering Linkage analysis was accomplished with the assistance of Sherri Bale, Jean Marc Lalouel, and the staff of the National Cancer Institute – Frederick Cancer Research Center Computer Center. Linkage analysis was not in use at the NIH at the time our work on the von Hippel-Lindau gene was performed.

Eventually, we were able to localize the von Hippel-Lindau gene to a region on chromosome 3 with polymorphic DNA markers located on either side of the gene, and to identify one DNA markers that did not recombine the disease gene.

To actually identify the von Hippel-Lindau gene, we needed to switch from genetic analysis to physical mapping. Yusuke Nakamura in his work on Familial Adenomatous Polyposis had encountered a similar problem in his work to identify the Familial Adenomatous Polyposis gene. Nakamura used the strategy of identifying overlapping germ line deletions in the gene region to identify FAP. Reasoning that germ line deletions might occur in the region of the VHL gene, we set out to collect more than 100 families affected with VHL. This was necessary because we anticipated that the frequency of germ line deletions in the region of VHL would be low; a large number of families would be necessary to find rare germ line deletion events. We studied the DNA from 117 families and identified 3 families with germ line deletions in the VHL region.

The technique of pulsed field gel electrophoresis was used to identify germ line deletions and to map the VHL gene region. This was an awkward, difficult technique that was mastered with the help of Helen Hunt. At one point in this work, all of our laboratory benches were covered with pulsed field gel electrophoresis set-ups.

As indicated below, Masa Yao, found the anomalous DNA fragment that indicated the first germ line deletion in VHL. Farida Latif found the critical cosmid in the region of nested deletions that contained the previously unidentified gene responsible for von Hippel-Lindau disease.

### **Eureka moment**

Some inherited illnesses are caused by a physical loss of a segment of genetic material, as though a piece of the DNA had been cut out with a pair of scissors. If VHL disease was caused by this scissor effect, it would simplify our attempt to find the VHL gene. Based on other hereditary illnesses, it was likely that physical loss of genetic material would occur infrequently. So, we collected a large number of additional families with VHL during a second national recruitment of families affected with this illness.. Eventually, Dr. Masa Yao, a Research Fellow and Urologist from Yokohama, Japan, saw an unexpected black horizontal stripe on a piece of X ray film. We held the film up to the window. Masa Yao had discovered a von Hippel-Lindau disease family with physical loss of genetic material. It was a Eureka moment.

### **The von Hippel-Lindau gene is a gatekeeper for sporadic renal cell carcinoma**

Alfred Knudson suggested an insightful theory of the pathogenesis of human cancer. Reduced to basics, the theory suggested that sporadic and inherited forms of cancer were caused by inactivation of the same gene. Knudson's analytical work was done on retinoblastoma, an eye tumor found in children, but the implication was that these observations applied to other forms of human cancer. Once we identified the von Hippel-Lindau gene, we turned to studies of non-inherited renal cancer and were able to demonstrate that the VHL gene was inactivated in virtually all cases of sporadic renal carcinoma.

### **Characteristics of the laboratory research work:**

The laboratory research work required great attention to detail because misidentification of a blood sample would compromise the entire analytic process. I collected the blood samples personally because of my concern that the samples be identified accurately. But there were many steps between collection of a blood sample and the final sample of DNA. These steps were performed by two or three technicians. In our studies of the disease that we named hereditary papillary renal carcinoma, we processed blood samples from a large family affected with the illness. I became suspicious that the technician processing the blood samples was making mistakes in sample identification. When the family came to the NIH for clinical evaluation, I had DNA extracted from these new blood samples and compared the results with DNA extracted by the technician. The DNA results should have matched if they were collected on two separate occasions from the same person. The DNA results did not match indicating that sample identification errors had been made in the handling of the DNA samples. To correct this problem, I made a second field trip to the family with hereditary papillary renal carcinoma, recollected blood samples, and reprocessed the blood samples for DNA. This second field trip gave us DNA samples that we could trust and led to the identification of the gene responsible for hereditary papillary renal carcinoma.

This work benefitted from a stable working environment, research freedom, and a dedication to understand the genetic basis of kidney cancer. There was a critical mass of physician scientists, a bank of families with inherited kidney cancer, and a bank of some 10,000 DNA samples collected from family members and from patients with kidney cancer. Each time an inherited kidney cancer gene was discovered we could return to the family bank to determine whether this

specific gene was responsible for disease in any of the families in the family bank. This process was invaluable because not all families had all the typical clinical manifestations of the disease. One of the families that we studied was remarkable in that three of five siblings had died with multiple, bilateral renal carcinomas. Although the family was evaluated and treated at leading medical research centers, no explanation for the development of renal carcinoma in three of five siblings was provided. Marston Linehan and I made a field trip to this family to obtain medical information and blood samples for DNA. We collected medical records and histologic slides from family members affected with the illness. Histologic slides were reviewed at the NCI by Dr. Maria Merino. The next generation of family members were evaluated clinically at the NCI by Marston Linehan and his group. Occult renal neoplasms were sought by scans supervised by Peter Choyke. The illness was not caused by mutations in the kidney cancer genes that we had already identified: VHL, and MET. Fourteen years after the initial field trip, we were able to determine that the illness in this family was caused by a recently identified gene, known as FH, and that the family was affected with the disease hereditary leiomyoma renal cell carcinoma.

Fourteen years after the initial field trip, I made a second field trip to members of this family. Because we had identified the novel gene responsible for the disorder, we were able to determine which family members carried the mutated gene and which family members were at risk of developing the disorder. The experience with this family illustrates the importance of maintaining the bank of kidney cancer families and the bank of DNA samples from members of the families.

### **How we came to study kidney cancer**

I have often been asked how we came to study kidney cancer. It was chance. I had read a paper in Nature by Bert Vogelstein that described the loss of genetic material from human bladder cancer. I thought that the place to begin studies of human cancer was with a previously studied cancer. I went to see Dr. Marston Linehan, Chief, Urologic Oncologist, NCI and asked him whether he had samples of bladder cancer. Marston Linehan said that he did not have samples of human bladder cancer but that he had many samples of human kidney cancer. Remembering the paper that I had read for the Medical Oncology Boards about a family with a chromosome 3 translocation and increased susceptibility to kidney cancer, and other papers that described chromosome 3 loss in kidney cancer, I decided to make use of what human cancer materials were available. In a way this choice illustrates how efficient the National Cancer Institute was in studying human cancer. Steve Rosenberg, Chief of Surgery at the NCI, was doing innovative studies of immunotherapy in patients with kidney cancer. For his studies, Dr. Rosenberg required that the kidneys containing the kidney cancers be removed. Dr. Linehan and his group removed the kidney cancers and stored the normal and malignant tissues in his frozen tissue bank. When I came along, I was able to use these normal and tumor samples for sophisticated genetic analysis.

### **Characteristics of Inherited Kidney Cancer**

In patients who develop kidney cancer without an inherited predisposition, a single tumor develops in one of the kidneys. The situation in patients with an inherited predisposition is dramatically different. Both kidneys are filled with multiple kidney tumors. Photographs of kidneys from patients with inherited kidney cancer show grape-like tumors varying in size from

barely visible pinheads to tumors several centimeters in diameter. These dramatic differences in tumor number between inherited and non inherited kidney cancer reflect the underlying pathogenesis of these kidney tumor types.

### **Is there another kidney cancer gene? Hereditary papillary renal carcinoma**

Having identified the VHL gene the burning question was whether there were other kidney cancer genes? Was the VHL gene responsible for all susceptibility to kidney cancer or were there other genes that could increase susceptibility to this malignancy. This question reminded me of old discussion of people who could be divided into the “lumpers” and the “splitters”. My tendency was to be a lumper.

This issue was decided when Dr. Linehan was referred a large family with multiple members from South Carolina. The referring urologist said to Dr. Linehan, that the family did not look like they had von Hippel-Lindau disease. When this family came to the NIH, and when the histologic slides were examined under the microscope, a discovery was made. Each and every renal tumor from members of this family had a distinctive appearance under the microscope. The tumors had a papillary growth pattern, a growth pattern that was different from the growth pattern of renal tumors associated with von Hippel-Lindau disease. The two different histologic appearances produced a problem. Pathologists in the United States classified both the type of renal cancer associated with von Hippel-Lindau disease and the type of renal cancer found in the new family as renal cell carcinoma. No distinction was made between the two forms of renal carcinoma. Dr. Linehan and I visited the Urologic pathologist at the Walter Reed Army Institute and this opinion was reinforced.

But the biology was convincing because each and every tumor in members of this family had the same appearance under the microscope. It was as though the histologic appearance of tumors in this family had been stamped with a die. So, we pursued this point and searched for families with a predisposition to develop kidney cancer with this particular type of appearance under the microscope. In fact, several families had been described in the old medical literature, from Sweden, and Australia, that remarked on a peculiar pathology found in all renal tumors of these families.

Eventually, this work led us to the discovery of a second renal carcinoma gene, the MET proto-oncogene. By a curious coincidence, my supervisor at the time, was Dr. George Vande Woude, a discover of the MET gene. Dr. Vande Woude had sought a role of this gene in human cancer. We had found it.

### **Discovery that germ line mutations in the MET proto-oncogene cause hereditary papillary renal carcinoma type 1**

The small number of affected families we identified and the primitive state of the genetic map when the work was performed complicated our analyses of hereditary papillary renal carcinoma type 1. Rather than making use of the genetic and physical mapping techniques that we had used

to clone the von Hippel-Lindau disease gene, we used a combination of linkage analysis and the candidate gene approach to determine that the MET proto-oncogene was responsible for hereditary papillary renal carcinoma type 1. Using linkage analysis we determined that the gene responsible for hereditary papillary renal carcinoma was located on the long arm of chromosome 7. The MET proto-oncogene was one of the few genes known to reside in this location. We decided to focus our effort on the MET proto-oncogene because it was located in the region of interest and because MET was known to be a receptor tyrosine kinase. Mutations in RET, another receptor tyrosine kinase, had been shown to be responsible for Multiple Endocrine Neoplasia type 2A, another inherited cancer syndrome. The similarities in tumor biology between RET and Multiple Endocrine Neoplasia type 2A and MET and hereditary papillary renal carcinoma type 1 provided a strong reason to pursue mutation analysis of the MET proto-oncogene.

When Laura Schmidt tested for germ line alterations in MET in affected members of a large hereditary papillary renal carcinoma by single strand conformation polymorphism analysis, she detected a band shift that was suggestive of an inherited mutation but the band shift was not consistent. In one experiment we detected a band shift, but could not confirm the result in a second experiment. In the laboratory, we referred to this change as “the disappearing” mutation. Eventually, Laura Schmidt found that a DNA polymorphism in the binding sequence that was amplified by the polymerase chain reaction could cause variations in the amplified DNA. Once the primers used for the polymerase chain reaction were redesigned to avoid this polymorphism, we were able to consistently detect germ line mutations in the MET proto-oncogene in affected members of hereditary papillary renal carcinoma type 1 families.

### **The Birt-Hogg-Dubé Syndrome**

Dr. Gladys Glenn, a medical oncologist and internist, was the first National Cancer Institute internist to evaluate members of renal carcinoma families. Her history and physical examinations were particularly detailed. Dr. Glenn noticed that some of the members of renal cancer families that she examined had skin lesions and brought this information to the attention of Dr. Linehan. This led Dr. Linehan to establish a collaboration with Dr. Maria Turner, Dermatologist, National Cancer Institute to examine the skin of consecutive patients from the Urologic Oncology Clinic. Dr. Jorge Toro was the Dermatology Fellow who performed many of the skin examinations. This collaboration led to the discovery that some members of renal cancer families had the cutaneous lesions of a rare inherited skin disorder that had been originally described in a Canadian family by Drs. Birt-Hogg-Dubé. These unusual observations could be explained in several ways. These families could be affected by two distinct inherited diseases, one associated with a predisposition to develop skin alterations, and a second disease associated with a predisposition to develop kidney tumors. A second possible explanation was that the families has an inherited predisposition to develop skin alterations and that the kidney tumors had occurred by chance. Finally, the families could have a single inherited illness associated with a predisposition to develop both skin lesions as well as kidney tumors. There was one analytical approach that would provide an explanation for these observations and also provide a way to identify the associated gene. We decided to recruit families who had an inherited predisposition to the skin alterations characteristic of the Birt-Hogg-Dubé syndrome. We would then compare the frequency of kidney tumors in family members with characteristic skin lesions (affected) to

family members without the characteristic skin lesions (unaffected). We found that the presence of the characteristic skin lesions increased the risk of developing a kidney tumor by factor of 9. Another clinical feature of families with the Birt-Hogg-Dubé syndrome was an apparent susceptibility to spontaneous collapse of the lung (pneumothorax). Using the analytical approach described above, we found a 50-fold increase in lung collapse in family members with skin lesions.

Our methods of genetic analysis changed during our work on the Birt-Hogg-Dubé syndrome. We used micro-satellite markers instead of restriction fragment length polymorphisms and performed a whole genome scan that localized the minimal critical region to be a region on chromosome 17p. Using recombination mapping, the minimal critical region was limited to 700 kilobases. Michael Nickerson developed a high throughput DNA sequencing procedure to determine the DNA sequences of all exons in the minimal critical interval in a panel of affected individuals from different families affected with the kidney syndrome. He sequenced about 150 exons and detected frameshift mutations in a previously unidentified gene in eight of nine tested families.

### **Discovery that germ line mutations in a previously unknown gene cause the Birt-Hogg-Dubé syndrome**

The key strategic decision in our work to identify the gene responsible for the Birt-Hogg-Dubé syndrome was what clinical feature to use for research analyses. Although our central focus was kidney cancer, there were a number of problems involved in using kidney cancer as the target. First, when the work was performed it was not certain that kidney cancer was a manifestation of the Birt-Hogg-Dubé syndrome. Second, even if kidney cancer was a manifestation of this inherited disorder, kidney cancer in Birt-Hogg-Dubé patients occurred infrequently. So, it would be difficult to bring together a critical mass of affected patients for linkage analysis. For these reasons, we decided to recruit patients on the basis on the characteristic cutaneous lesions that defined the Birt-Hogg-Dubé syndrome. Patients were recruited through nationwide mailings to dermatologists. We included photographs of typical cutaneous lesions as well as photographs of the histologic appearance of the lesions. Some 40,000 to 50,000 recruitment letters to dermatologists were mailed over a several years period. The recruitment of the original family described by Drs. Birt, Hogg and Dubé was particularly useful in linkage analysis since there were 26 affected individuals in this family.

Our methods of genetic analysis changed. We used microsatellite markers instead of restriction fragment length polymorphisms and performed a whole genome scan that localized the minimal critical region to be a region on chromosome 17p. Using recombination mapping, the minimal critical region was limited to 700 kilobases. Michael Nickerson using high throughput DNA sequencing to determine the DNA sequence of all exons in the minimal critical interval in a panel of affected individuals from different families affected with the Dubé syndrome. He sequenced about 150 exons and detected frameshift mutations in a previously unidentified gene in eight of nine tested Dubé families.

### **Hereditary Leiomyoma Renal Cell Carcinoma**

In the collaboration between Dr. Maria Turner and Dr. Linehan, yet another group of families was identified with yet another skin condition and an apparent susceptibility to renal carcinoma. Affected members of these families had multiple smooth muscle tumors of the skin, smooth muscle tumors of the uterus (fibromas), and yet another histologic type of kidney cancer.

Families with these hallmarks were recruited and collected by Dr. Jorge Toro and myself. Several mini dermatology clinics were held by Dr. Toro and myself in different cities where members of families with the Dubé syndrome and hereditary leiomyoma renal cell carcinoma were studied and blood samples collected. The gene responsible for HLRCC was identified by another group and confirmed by the NCI group. Dr. Linehan and his group found that the renal cancers associated with HLRCC are particularly aggressive, and require surgical treatment tailored to this biologic behavior.

### **Do the kidney cancer genes produce distinct diseases? Histology of renal tumors**

The studies of inherited kidney carcinomas have demonstrated a close correlation between histologic appearance of the inherited kidney carcinoma and the predisposing gene. Thus, germ line (inherited) mutations of the VHL gene predispose to clear cell renal carcinoma; germ line mutations of the MET proto-oncogene predispose to type 1 papillary renal carcinoma; germ line mutations of the FH gene predispose to type 2 papillary renal carcinoma; and germ line mutations of the BHD gene predispose to several different forms of renal histology. While the VHL gene is the gatekeeper for non inherited clear cell renal carcinoma, the other kidney cancer genes (MET, BHD, FH) do not appear to be gatekeepers for the corresponding non inherited forms of the disease.

### **Biochemistry**

The identification of the kidney cancer genes has led to an explosion in biochemical studies to define the functions of the proteins encoded by these genes. These studies in turn have suggested drugs that are proving useful in the treatment of patients with metastatic kidney cancer.

### **Accomplishments**

We were able to identify the genes that were responsible for 3 distinct forms of inherited kidney cancer, to characterize a 4<sup>th</sup> distinct form of inherited kidney cancer, and to establish the field of kidney cancer genetics.

### **Credit for Discoveries**

Credit refers to the recognition by the community of being the scientist responsible for a discovery. Allocation of credit is difficult when scientists collaborate. In the discovery of genes involved in the pathogenesis of human kidney cancer, my laboratory was responsible for the experimental work that led to the identification of the genes responsible for three distinct forms of inherited kidney cancer. This work was a mammoth undertaking and could not have been accomplished without the nationwide recruitment effort, sample collection, and genetic analysis that occurred in my laboratory

## **Kidney cancer genes: from one to many**

When we began our work in 1985, the idea that there was a gene(s) that produced an inherited predisposition to kidney cancer was audacious. But a systematic approach led to the identification of the von Hippel-Lindau disease gene, the gene that plays a gatekeeper role in the pathogenesis of common kidney cancer. Once that discovery was made, the pressing question was: whether there was other genes that also conferred an inherited predisposition to kidney cancer? The clinical and genetic analysis of additional families referred to National Cancer Institute Urologic Oncology provided a clear cut answer and led to the recognition that the MET proto-oncogene and another gene, the Dubé gene (Folliculin) could cause inherited kidney cancer. With further study, there are now 12 genes identified that can lead to inherited predispositions to renal cancer. Ongoing research suggests that additional genes that confer inherited susceptibility remain to be found. And that there are surprising discoveries ahead.

## **The Leukemia Service of the Medicine Branch**

When I started work on the Leukemia Service of the Medicine Branch. Ed Henderson was in charge of the Leukemia Service and Seymour Perry was in charge of the Medicine Branch. My responsibilities were the care of children and adults with leukemia. Combination chemotherapy had just been developed by Emil Frei and Jay Freireich, who had left the NCI to go to MD Anderson Hospital, Texas. Pioneering work had been performed by Howard Skipper in mice and we were applying the principles that Skipper had developed to children with leukemia. We were told that Emil Freirich had said that no patient was to die from leukemia; they might die from the toxic effects of combination chemotherapy, but not from the disease.

The head nurse on 2 East, the ward where the children with leukemia were housed, was June McCullah, a red haired, thin, firebrand of a nurse who was very helpful. I also did some clinical work on the adult leukemia service. Acronyms for the chemotherapy programs: MOPP. Other fellows thought up a name for a mythical chemotherapy program called MAIM. We started the intravenous drips, did the spinal taps, and monitored the white blood counts and the platelet counts.

## **Howard Hughes and Carl Newman**

The two patients at the National Cancer Institute that made the greatest impression on me were Howard Hughes and Carl Newman. I remember Howard Hughes because of a fatal mistake that I made in his treatment and I remember Carl Newman because of the dramatic nature of his treatment and the remarkable clinical results.

Howard Hughes was a thin, fair-haired, 8-10 year old boy with acute leukemia admitted to 2East at the National Cancer Institute. He had a markedly elevated white blood cell count. I needed to lower his leukemic cells rapidly to prevent brain damage, but I failed to take the necessary precautions. In my orders I forgot to request administration of allopurinol, a drug used to prevent uric acid nephropathy. Because of this omission, Howard Hughes developed renal failure. He

was transferred to Walter Reed Army Hospital for dialysis but died. I felt guilty. Computer generated lists would have prevented this tragedy. I had no one to talk to about this mistake.

Carl Newman had acute myelogenous leukemia. He was a teen-ager at the time I treated him. Part of the treatment protocol involved intrathecal administration of chemotherapy drugs. This treatment was required to kill leukemia cells in the central nervous system. Carl would not cooperate for the procedure. There was an enormous struggle while I performed a spinal tap. We needed several people to hold Carl in position; I passed the needle into the spinal canal and administered the chemotherapy drugs. Carl went on to a long-term cure of his leukemia. He is currently in his 50s. His sister and I live in the same community. The second year of the two-year fellowship was a research year. I choose an NCI scientist to work with. I began with Wendall Rosse, an expert with an interest in platelets and paroxysmal nocturnal hematuria (PNH), but he left for a faculty appointment at Duke University, I then started work with Jerome Block but that didn't work out. I met Tibor Borsos and Herbert J. Rapp and we clicked.

## **Laboratory Chief 1988 to 2005**

### **HIV/AIDS**

As Laboratory Chief, I was confronted with some difficult administrative problems. One of our laboratory technicians became seriously ill. For a period of time, it was unclear what the cause of her health problem was. Eventually, the employee informed a few members of the laboratory that she was infected with the HIV-AIDS virus. Before she informed all the members of the Laboratory, there was a period of time when some members of the Laboratory knew the diagnosis while other members of the laboratory did not. Our nursing staff informed us that we were not permitted as a matter of policy to inform other lab members. When her diagnosis became known to the entire laboratory staff, some laboratory employees became uncomfortable knowing that they were working in close proximity to someone infected with HIV-AIDS. They were uncomfortable using the telephone that the infected employee had used. This problem was ultimately resolved when the employee became too sick to continue working.

### **Sexual harassment**

A male African American laboratory technician was accused by a female Caucasian high school student of sexual harassment. She felt uncomfortable with some of the remarks that the technician had made to her. This was a sensitive situation because it was difficult to determine the truth of the matter. I brought the matter to Teri Cecil, my Administrator, who met with me and the laboratory technician to discuss the situation. I also discussed the matter with other women in the laboratory. This situation was resolved when the laboratory technician avoided speaking with the student and the student returned to high school.

### **Laboratory spying**

Our laboratory group discovered a previously unidentified disease gene that caused kidney cancer. We wanted to know what the normal function of this disease gene was. Because of the difficulty of the problem I assigned two research fellows to work independently on this problem.

One of the research fellows began to go through the research notebooks of the other research fellow without his permission or knowledge. The research fellow whose work was being studied without his permission suspected spying and set up a video camera to record what happened at his desk after hours. Then the research fellow brought the evidence of spying to Dr. Laura Schmidt and to me. This led to a discussion with the research fellow, Bob Wiltrout, Director, NCI-FCRF and the director of NCI Fellow Training

### **International studies of kidney cancer**

Our work on kidney cancer involved the participation of a large number of individuals at the National Cancer Institute, and investigators throughout the world. One reviewer stated that that our work had established the field of genetics of kidney cancer. The identification of genes responsible for different forms of inherited kidney cancer was a stimulus to patient identification throughout the world. The DNA tests that we developed prompted investigators throughout the world to search for families with these rare inherited forms of kidney cancer, to identify the mutations responsible for their diseases, and to bring these patients the benefits of modern treatment.

Similarly, the demonstration that mutations in one of these genes is primarily responsible for the development of non-inherited kidney cancer provided a sound foundation for effective drug therapy of metastatic disease.

### **Problem Solving**

If I were to select the characteristic of work that I particularly enjoy, it would be solving problems. Using logic and analysis to solve problems is a particular pleasure. It doesn't seem to matter what the problem is. I enjoy the challenge and the process of problem solving. I like to think that I can solve most any problem if given the resources. Medical research is a form of problem solving. The challenge in medical research is to select the correct problem to try to solve. The research problem needs to be important and needs to be solvable. This is how I worked in the research laboratory. I selected a single problem, and threw all my resources at it. When the problem was solved, I went to the next problem to be solved. Great energy was expended in selecting the next problem to approach in the research laboratory.

### **A research physician**

When asked "what do you do?", my reply is that "I am a research physician." I make use of my training and experience as a physician and oncologist, as well as my training as a scientist, to solve problems of medical importance. I'm most secure analyzing clinical problems with modern genetic tools. My most recent work on bilateral, multifocal papillary renal carcinoma type 1 has that quality. I want to understand and explain disease. I'm attracted to the elegance the beauty of linkage analysis, the incisiveness of genetics. My career at the interface of familial cancer and modern genetics was a perfect match for my abilities and interests. Research freedom provided by the NIH enabled me to pursue this interest with a small staff.

## **Retirement**

I retired from the National Cancer Institute after 40 years of service in 2005. I remained in retirement for 9 years until I resumed work, part time, in the National Cancer Institute Urologic Oncology Branch. When I come to work in the morning and walk toward the South entrance of the Clinical Center, I am filled with wonder at this marvelous institution where I work.

## **The Urologic Oncology Branch**

I have worked together with Dr. Marston Linehan, Head, Urologic Oncology Branch since 1985. This has been a remarkably productive collaboration. Marston Linehan has been responsible for patient management; I was responsible for genetic analysis. Together we created the field of renal cancer genetics. Our collaboration has been fruitful in terms of gene discovery, patient diagnosis, biochemistry, and patient treatment.

## **The Jewish High Holidays and BCG**

I spent one afternoon on the Jewish High Holidays in our guinea pig room in Building 37 on the NIH campus. I should have been fasting. Maybe I was. I certainly should not have been working. What I was doing was injecting solutions of a living attenuated bacteria (BCG) into guinea pig tumors. My hand slipped, the syringe fell and I injected my thumb with the solution of BCG. Not good. The adverse consequences were a badly swollen thumb, a change in my immune status, but otherwise I was OK.

## **Edgar Ribí**

Edgar Ribí was one of our collaborators in the work on BCG and cancer. Edgar Ribí was born in Switzerland, a glider pilot, a candidate for the Swiss Olympic team, an electron microscopy expert, with a physical chemists approach to biology. He worked at the Rocky Mountain Laboratory in Hamilton, Montana. Edgar had developed a preparation of BCG cell walls attached to minute droplets of oil as a candidate non living vaccine for the prevention of tuberculosis. Herbert Rapp and Edgar got together and decided to test whether the “Ribí vaccine” would be effective in the treatment of experimental tumors in guinea pigs. I did the laboratory work and, sure enough, the non-living vaccine that Edgar had developed proved to be safe and effective in guinea pigs.

Edgar was larger than life. He loved to fly and he loved the mountains. Once he flew Suzi, Adam and I to the Moose Creek Wilderness. We hiked to Cathedral Woods. Tragically, Edgar died in a plane crash coming home from Spokane. One of his favorite expressions was “Howdie!” While walking from the Rocky Mountain Laboratory to my motel one evening I was stopped by a policeman who did not recognize me. I think I had a beard at that time.

*End*