Robert J. Huebner, M.D.:

A Virologist's Odyssey

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Acknowledgments

A chance introduction to Dr. Victoria A. Harden, Historian, of the National Institutes of Health, resulted in the genesis of this manuscript after a prolonged incubation period. At a meeting of the Washington Society for the History of Medicine in 1995, I heard Dr. Harden making authoritative comments about Rocky Mountain Spotted Fever. I introduced myself to Dr. Harden as someone who had worked in the Virus and Rickettsial Laboratory of NIH with Dr. Robert J. Huebner from 1948 to 1952. Dr. Harden, who has been trying to "build NIH history brick-by-brick" became interested immediately, indicated that she had no information about the virus laboratory at that particular time period, and would appreciate my personal memoirs for the years when I was associated with Bob Huebner. He was an NIH research icon about whose work she wanted more information. So, three years later, I submitted a short autobiographical memoir about the time that I had spent at NIH, along with several other anecdotal laboratory adventures with which I was involved. Dr. Harden seemed to be pleased with the material that I had turned in, and she asked what I was planning to do for an encore. I hedged a reply, but I began contemplating whether I could perhaps make some further contribution to documenting medical history. In 1998 when I saw Dr. Harden at another History of Medicine Society meeting, I mentioned that I might be interested in writing a biography of Huebner whose virological exploits, I had come to realize, were largely unknown or forgotten by my contemporary infectious disease colleagues. Dr. Harden

encouraged me to start examining relevant sources of information and to keep her informed about what I was able to learn and document.

I began my quest by meeting with Harriet Huebner, not yet a widow, and describing to her the project that I was undertaking. Harriet was extremely supportive of this endeavor. She gave me a copy of Huebner's extensive bibliography, his curriculum vitae, and a list of his awards and honors. I also started interviewing some of Huebner's remaining associates at the Laboratory of Infectious Diseases of NIH, and I was able to obtain the names of various colleagues who were associated with Huebner at the National Cancer Institute. During theses initial explorations, Bob Huebner passed away in late August 1998. I attended the memorial service at his farm on September 5, 1998, had an opportunity to meet other colleagues and was able to renew acquaintance with others of his family members whom I had not seen for a long time.

I began to collect selected reprints of the Huebner writings through the National Library of Medicine to supplement the ones I had retained during my time at NIH. By early spring 1999 I had collected considerable source material. I happened to encounter Dr. Harden by chance in March or April 1999. When I informed her about what I had been doing, her reaction was that since I "had accomplished so much," I "should be made official." She arranged for me to be associated initially with the Office of NIH History through a modest contract and then later officially as a volunteer. I was then able to use the resources of the History Office to facilitate the task of research and writing.

Harriet Huebner, who had been Bob's administrative assistant and second wife, provided rich insight about his life and personality during the course of many frequent, pleasant interviews during the writing of this manuscript. At the time of his retirement,

Harriet and Bob did not retain any official work-related correspondence. Harriet described details of Bob's professional and personal life, his personal thoughts, his philosophy of scientific research, and their close collaboration at work and at home. She also provided access to other family members who acted as custodians of family history. Bob's sister, Catherine M. Huebner of Cincinnati, Ohio, provided many details of Bob's early life along with notes, newspaper clippings and photographs. My first information-seeking trip to the Huebner farm in Ijamsville, Maryland yielded a cornucopia of information including several large scrapbooks containing news clippings, other publicity items and a collection of testimonials written by many guests on the occasion of Bob's retirement. The wives, Berdi and Harriet, had carefully compiled this information over many years. My hostesses on the occasion of my visit to the farm were Susie (Roberta Sue Huebner) Creamer and Ginny (Virginia Rose) Huebner. Susie provided an extensive overview of the farm, family life, and the Huebner cattle breeding enterprise. Other Huebner siblings whom I interviewed on various occasions were Betty (Elizabeth Jean Pfeiffer), Danny (Richard Daniel), and Jim (Robert James). They each provided their own perspective on a Huebner upbringing. Jim regaled me with many humorous anecdotes about his father. I was Kay (Frances Kay) Huebner's guest at a symposium she organized on Alzheimer's disease at the Jefferson School of Medicine (Philadelphia) in honor of her father. I had an opportunity there to meet some of Bob's former associates at the National Cancer Institute. On that same occasion I had a wonderful encounter with Dr. Murray B. Gardner of the University of California at Davis. He was a major Huebner associate and participant in the Virus Cancer Program. He later provided extensive

literature about his role in the program in California, and we had several extended pleasant telephone conversations about his interactions with Bob.

Current and former members of the Laboratory of Infectious Diseases, NIAID, helped fill the information gaps of the years from 1952, when I left NIH, until Bob transferred to the National Cancer Institute in 1968. The major informants were Drs. Bob (Robert M.) Chanock, Al (Albert Z.) Kapikian, and Jan (Janet W.) Hartley. They each contributed documents and personal recollections of their research and collaboration with Huebner. Chanock provided extensive information of his own biography, history of the Laboratory, continuing humorous anecdotal information about Bob Huebner, and he provided access to the annual reports of the Laboratory of Infectious Diseases for the mid-to late 1960s. I had several interviews with the ailing Bob (Robert H.) Parrott, who despite his fragile health, enthusiastically described his research association with Huebner. I had an opportunity to meet with Dr. Leon Rosen on one of his infrequent visits to the LID from his home near Paris, France (arranged by Bob Chanock). He described his important participation in the Junior Village studies. Dr. John L. Sever described his collaboration with Bob Huebner on the production and testing of multiviral antigens and antibodies and studies of rubella. Dr. Alexis I. Shelokov of LID, though not working directly with Huebner, was the source of much interesting anecdotal information over the course of writing this manuscript. Dr. James A. Rose, who was on temporary assignment from NCI to Wally Rowe's unit, had ample opportunity to study interactions between Rowe and Huebner and also to observe some of Huebner's idiosyncrasies.

The person who provided me the most valuable insight into Bob Huebner's involvement with the National Cancer Institute was Dr. Carl G. Baker, former Director of

NCI from 1969 to 1972. I had several extensive meetings with Dr. Baker who generously lent me some of his personal papers and books. I also had access in the files of the NIH History Office to Dr. Baker's meticulous writings relating to the Virus Cancer Program. The first set of writings was a series of oral interviews which he conducted in 1995 with persons who were involved prominently in the Virus Cancer Program. The second written source was a detailed description of the administrative history of the Virus Cancer Program from its inception in the late 1950s until 1972. These two major efforts by Dr. Baker provided much of the material dealing with Huebner's cancer activities. Another source for the cancer chapters was a series of interviews with NCI personnel that was part of the National Cancer Institute Oral History Project. Ms. Gretchen Case of History Associates, Inc. of Rockville, Maryland conducted these interviews in 1997. Review of the Annual Activities of the National Cancer Institute in the NIH Library also helped document the scope of Huebner's activities.

I want to thank Dr. Thomas A. Waldmann, Chief, Metabolism Branch, National Cancer Institute for the introduction in 1998 to Dr. Alan Rabson, Associate Director, National Cancer Institute. Dr. Rabson graciously provided access to former and current members of the National Cancer Institute who had worked with Huebner. I had opportunities to interview some of these persons who interacted with Bob Huebner while he was associated with cancer investigation. Dr. John B. Moloney of NCI, discoverer of the mouse retroviruses that bear his name, and former Director of the Virus Cancer Program, provided details of his relationship with Bob Huebner during the course of several interviews. He also gave me a copy of the Zinder Report. Dr. James Duff, who worked closely with Huebner at NCI in an administrative capacity, offered information

during several interviews about Bob's intensive recruiting and collaborative activities with the extensive network of project managers in the Virus Cancer Program. Dr. Jack Gruber provided additional administrative details about other aspects of the Virus Cancer Program. Drs. Raymond V. Gilden, Padman S. Sarma, and Johng S. Rhim, all active associates of Bob Huebner, described their participation in research projects and supplied some of their reprints written with Huebner. Dr. Robert E. Stevenson, active administratively in the Virus Cancer Program for many years, during an extensive interview, described many aspects of Huebner's unusual intellectual traits. Dr. Stevenson also provided access to his own personal papers deposited in the Library of the University of Maryland, Baltimore Campus. These papers documented some of the origins and early activities of the Virus Cancer Program. Dr. Gary J. Kelloff of NCI, during the course of several interviews provided a vivid account of collaboration as a young associate with Bob Huebner. Special thanks are due Dr. Douglas R. Lowy of NCI for his critical review of the oncogene chapters.

Dr. John Parascandola, Historian of the USPHS, provided the Commissioned Corps Personnel Records of Drs. Huebner, Bell and Rowe.

Throughout the writing of the manuscript, Dr. Victoria Harden provided encouragement and preliminary editorial advice. The NIH History Offices subsidized the critical editorial review performed under the auspices of History Associates, Inc. of Rockville, Maryland. The editor assigned to the task was Nancy K. Berlage, Ph.D. She performed in an outstanding fashion, allowing no word, phrase, statement or opinion of mine to go unchallenged. She literally wore out the redlining feature of her laptop computer on every chapter submitted to her in order to produce a coherent manuscript.

Drs. Harden and Berlage were the muses who inspired and enabled the completion of the manuscript. Other History Office personnel provided valuable help in the final stages of the manuscript preparation, including Ms. Marilyn Berman who retyped some of my threadbare notes, and, especially, Ms. Brooke Fox, the Office Archivist, who kept meticulous track of bits and pieces of the manuscript on the hard drive of her computer. She cheerfully retrieved these expeditiously on short notice.

Many thanks are also due to my wife, Jean, who provided encouragement—and lunch—and who patiently bore alone the long hours I could have spent with her during my "retirement" while I was writing the manuscript.

Introduction

On Saturday September 6, 1998, friends, family, neighbors and former colleagues gathered at the home on Hidden Hills Farm, Ijamsville, Frederick County, Maryland, to celebrate the life of Dr. Robert J. Huebner. He had passed away on August 26, 1998, after enduring for 20 years the physical and intellectual indignities of progressive dementia caused by Alzheimer's disease. The memorial service occurred on a bright, sunny day near the farmhouse located on an elevated part of the farm overlooking the verdant Maryland countryside, thus providing a pleasant setting for what otherwise might have been a depressing afternoon. His family recalled with fondness many pleasant memories. Colleagues described in detail his expansive personality, his abundance of ideas for investigational research, the originality of his thought processes and his talent as a raconteur. Neighbors and associates in the cattle breeding business described the originality of his methods in farming, and his ability to breed consistently prize-winning Angus beef cattle. On display in the living room of his home were his many diplomas, awards and medals. After lunch, an ancient videotape was played depicting his earliest scientific triumph and the affectionate bonding with his colleagues with whom he was associated in this achievement. The memorial service concluded with the scattering of his ashes in the little stream (Bush Creek) that ran through the lower south meadow of the farm. This stream is a tributary of the Monocacy River, and the ashes eventually made their way to the Potomac River, then on to Chesapeake Bay from where they reached the

Atlantic Ocean. This final ceremony concluded the group's tribute to one of the outstanding pioneers in virology of the 20th century.

I first became acquainted with Bob Huebner's career in 1947 when I was a senior medical student at Boston University. I had become interested in infectious diseases after exposure to three stimulating faculty members: Dr. Alice B. Marston, the Professor of Bacteriology; Dr. Louis Weinstein, Lecturer in Infectious Diseases and Chief of Service at the Haynes Memorial Hospital, the Infectious Disease Service of Boston University; and Dr. Chester S. Keefer, Professor and Chairman of the Department of Medicine. In 1947 I was impressed and fascinated by Bob Huebner's unraveling of the mystery of Kew Gardens Spotted Fever, a.k.a. rickettsialpox that he seemed to resolve with speed and efficiency. I also became aware later of his work on the clinical and epidemiological aspects of the second outbreak of Q fever at the National Institute of Health. During my internship in 1947 at the Evans Memorial, the Medical Service of the Massachusetts Memorial Hospitals (later University Hospital), one of my residents, Dr. William L. Hewitt, knowing of my interest in infectious diseases, suggested that I might find it advantageous for my career to spend some time doing laboratory study at the then Division of Infectious Diseases of the National Institute of Health in Bethesda, Maryland. Dr. Hewitt, who was assisting Dr. Keefer in an evaluation of the first patients treated with streptomycin, had spent several years at the Division of Infectious Diseases and was a Surgeon (equivalent to a Major in the Army) in the Commissioned Officers Corps of the United States Public Health Service. Bill Hewitt was well acquainted with Dr. Charles Armstrong, Chief of the Division of Infectious Diseases at NIH, and he offered to arrange

an interview for me with Dr. Armstrong at NIH. Dr. Armstrong at that time enjoyed a national and international reputation as an outstanding virologist.

I met Dr. Armstrong for an interview in January 1948. He was exceedingly pleasant and cordial, and he suggested that I might like to meet some of the members of the Division to see with whom I might enjoy working. I was introduced to an impressive array of famous research microbiologists. During our tour of Building 7, a young officer in regulation uniform breezed into an office on the third floor. Dr. Armstrong introduced me to Bob Huebner. My initial impression was that Bob appeared to be a warm, open friendly person full of exuberant energy. When Dr. Armstrong explained that the purpose of my visit was to survey the laboratory as a place for future infectious disease experience, Bob said, "Great! Welcome aboard," and he breezed out of the room. Bob was then in the midst of one of his frequent return visits from Southern California where he was actively engaged in his field studies of Q fever. At the end of my interview, Dr. Armstrong asked me what goal I had in mind for coming to the laboratory and whether I had someone with whom I wanted to work. I told him that I had no specific research projects in mind but that I would like to learn laboratory methods in virology and to become acquainted in working with rickettsial techniques as well as following any leads that might develop while working in the laboratory. I also indicated that I would enjoy working with Bob Huebner. These answers apparently met with Dr. Armstrong's approval. We shook hands, and he told me to expect the confirmation of my appointment in June 1948 along with instructions for reporting to the laboratory in August. Dr. Hewitt must have given an enthusiastic recommendation about me to Dr. Armstrong.

On arrival in Bethesda on August 1, 1948, I was perturbed to discover that Bob Huebner was still in California, no one was certain when he would return, and Bob's laboratory personnel had not been expecting me or warned that I was coming. I spent the next several anxious months getting vaccinated with all the currently available rickettsial vaccines, including the one for Q fever, before I was allowed within the laboratory working space proper. Once I could enter the working area I was accepted warmly as a member of the laboratory. I was generously taken under the wings of Bob Huebner's key personnel including Betty Ransom, the Chief Bacteriologist, Chick Turner, Head of the Serology Unit, and Charlie Knauff, the Head Animal Technologist. They instructed me in the laboratory techniques then in use in the Laboratory. In October or November 1948 Bob returned briefly from California and outlined a project for me related to Q fever. He returned permanently from California in early 1949.

I was Bob's very first professional medical or doctoral associate in the laboratory from August 1, 1948, to September 1, 1952. I had planned originally to spend 2 years in the laboratory prior to my going on to the completion of my clinical training in internal medicine and infectious diseases. The intervention of the Korean War in 1950, with my military status temporarily "frozen" in the Public Health Service, necessitated postponement of these plans so that I spent several years more in the laboratory than I had anticipated. Every thing works out for the best. With the additional time spent in the laboratory I was able to complete the Coxsackie virus projects that Bob Huebner and I had started in the summer of 1949. These four years in association with Bob provided the most rewarding and enjoyable experiences of my years in medicine. I was given an opportunity to participate in meaningful research activities with an outstanding

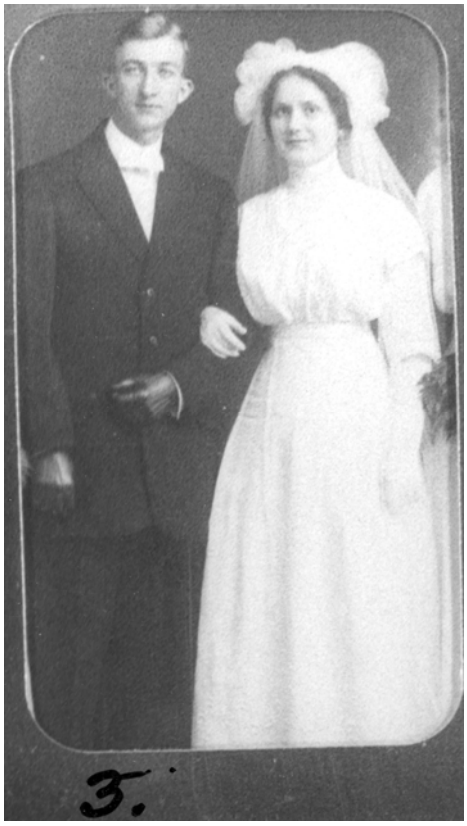
practitioner of virological research science. The more important benefit was getting to know a remarkable, generous and kind human being. My relationship to Bob was a little unusual inasmuch as I was the initial experiment in his having a close associate at hand in the laboratory to assist him in the beginning of his ever expanding penetration into the search for unexplored and undiscovered viral agents. He was unfailingly kind to me, and there were never any angry words between us. He was always very hospitable to me and later to my family, a trait that he exhibited increasingly as his circle of associates and scientific peers expanded. He freely expressed his confidence in me by suggesting that I make full-time research my career. He encouraged my pursuits of leads in the laboratory that interested me, and he was instrumental in letting me embark on a project with my own small unit. I was somewhat annoyed initially at a later date when he requested that I suspend this activity temporarily in order to take over the management of the laboratory aspects of the Texas pleurodynia study when it became apparent that the laboratory personnel were having problems with the study. In retrospect, this was the finest compliment that Bob could have paid me.

In later years, after his retirement and declining health when I had occasion to mention my early association with Bob to my contemporaries in the field of infectious diseases, they would invariably confess to me that they had no inkling of who Bob was or of what he had done. I found it intolerable that Bob's work should be forgotten so quickly in view of his magnificent accomplishments in advancing the world's knowledge of virology. I welcomed the opportunity, when it became available, to reacquaint the scientific and medical communities as well as the general public with the scope of his

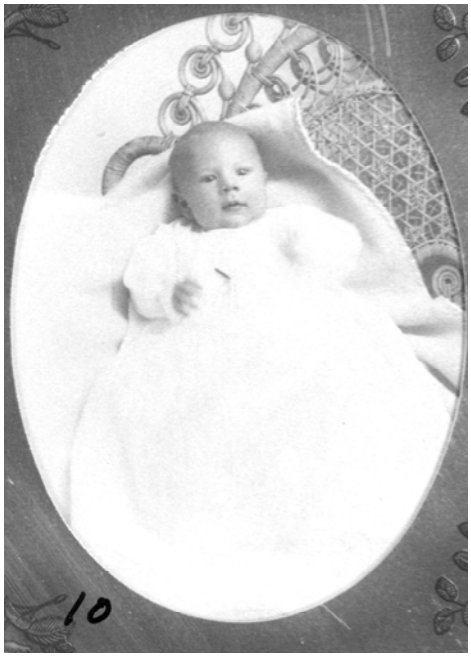
achievements as a scientist and as a great human being. This undertaking is my tribute to the memory of Bob Huebner.

Chapter 1

The Early Years



1912. Wedding picture of Bob Huebner's parents, Joseph and Wilhelmina (Brickner) Huebner. (Office of NIH History files, contributed by Catherine Huebner).



1914. Bob Huebner's baby picture. (Office of NIH History files, contributed by Catherine Huebner).

Robert Joseph Huebner was born February 23, 1914, in Cheviot, Ohio, a middle-class suburb on the west side of Cincinnati. He was the first child of Joseph Frederick and Philomena Margaret Brickner Huebner. Philomena Huebner was born November 24, 1890 in St. Leon, Indiana and passed away April 19, 1956. Joseph F. Huebner, Sr. was born October 16, 1888 and died December 8, 1946. Bob's parents were first-generation United States citizens and staunch Roman Catholics. Their forebears came from southern Bavaria and the area near Breslau, now called Wroclaw, in the part of Germany ceded back to Poland after World War II. Joseph and Philomena had a large family—nine children; this demographic characteristic was not unusual among Catholic German and Polish Americans in the late nineteenth century, but was becoming increasingly less common in non-rural middle class communities. The Huebners spent their married lives

in Cheviot; their children were born and grew up there. The children came in rapid succession: the oldest (after Bob), Beatrice Margaret Huebner Haesl, was born June 24, 1915; Mary Cecilia Huebner Mettman, was born next on July 3, 1917; the second son, Joseph Frederick Huebner, Jr., arrived March 15, 1919, (deceased November 19, 1998); the third, Richard Harold Huebner, on February 7, 1921, (deceased January 2, 1998); and the fourth son, John Walter Huebner, on October 18, 1923. Another girl came next, Catherine Maria Huebner (the family historian), born October 17, 1926; then a boy, William Henry Huebner, on July 5, 1929; and finally the youngest, Margaret Estelle Huebner Pohlman, was born June 15, 1933. (Nineteen ninety-eight would be a sad year for the Huebner family: three of the sons, including Bob, passed away during the year.)

Bob's early childhood, while unremarkable, was a happy time of play and recreation with his many siblings and the numerous neighborhood children. He attended St. Martin's, the parish elementary parochial school, and later graduated from Elder High School, a well known Catholic school on the west side of Cincinnati. Like other boys his age, young Huebner was interested in sports. He loved to listen to the Cincinnati Reds games on the radio, and he was a competent tennis player. He also appreciated music, listening to opera on the radio Saturday afternoons while reading or studying. He took violin lessons for a while, but never learned to play well.

C. 1914. Front row, left to right: Mother, Father (holding Bob), Aunt Margaret. Back row, left to right: Aunt Catherine and Alice, a friend of the family. (Office of NIH History files, contributed by Catherine Huebner).

Bob's interest in science and medicine was foretold at an early age. Bob's younger brother Bill later recalled an interesting anecdote: "I somehow felt that I was one of your first patients, prior to your receiving your degree in medicine. I vividly remember when I was chasing Father Metzartz's dog, named Spot. You caught me, picked me up, put me on your shoulder and carried me back to the house. At the age of five I was so impressed having a big brother that cared. Unfortunately you dropped me on my face cutting my lip. Instead of letting a doctor sew it up, you put it back together without stitches" (2).

Bob's extended family included two maternal maiden aunts—Aunt Margaret and Aunt Catherine Brickner. They were extremely fond of Bob and his siblings and their house was a second home to all of them. From the time that Bob was a baby, he was a favorite of his Aunt Catherine. In 1956, she went to live on the farm that Bob bought when he was at the National Institutes of Health and helped take care of his children until she died there in 1970 at age 93. Aunt Catherine, having no family of her own, spent the last 25 years of her life with Bob's family. According to Harriet Huebner (4), Bob's second wife, she was not an easy woman, was sharp-tongued and very puritanical, but generous of spirit and completely devoted to Bob's nine children. Aunt Margaret was also an important influence (3). She and Bob's father spent many hours with Bob reading, walking, fishing and attending concerts. She married later in life and died shortly thereafter. Bob never stopped mourning her.

1928. Huebner family and friends. Front, left to right: Joe, Marian Jim Warden (friend), Jack, Dick, and Bob (holding up the wheelbarrow). Back row, left to right: Ben Huebner and Ruth Fox (cousin). (Office of NIH History files, contributed by Catherine Huebner).

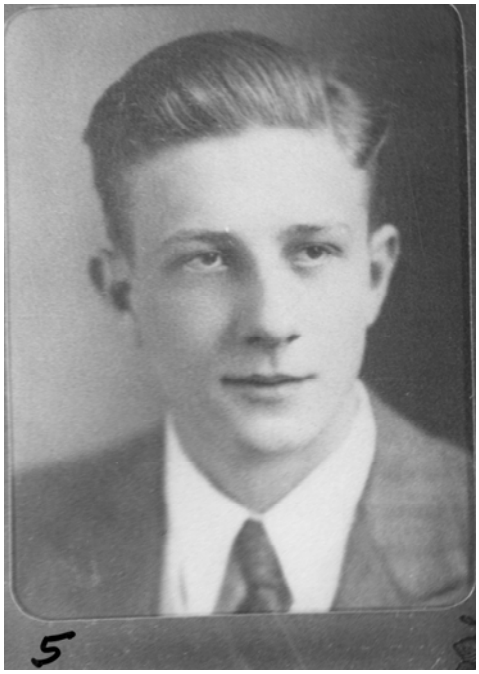
Bob's adolescence and early adult years were influenced by the societal and economic upheavals of the "Great Depression." Like many other families, the Huebners suffered through hard times. Joseph Huebner had attempted to improve his family's finances by buying into and operating a movie theater in Cheviot, but in the depressed economic climate, it was difficult to make such an investment succeed. The business failed. He apparently invested heavily in Paramount sound equipment in 1928 and went bankrupt the year "when Al Jolson sang "Mammy" for Warner's (from *The Jazz Singer* in 1927)" (5). Bob's Uncle John, who owned the other movie theater in the area, apparently invested more wisely, surviving the stock market crash of 1929 and the

subsequent national economic downturn. Unfortunately, Joseph was not so lucky. After holding down a series of jobs, he went back to his former trade as a tailor making men's vests. According to his second wife Harriet (6), Bob "adored his father, a sensitive, poetic man who, nevertheless, (or perhaps because of his gentle nature) was not too successful in his business ventures." Joseph died young at age 58 of heart disease. Unlike her husband, Bob's mother was a strong, assertive woman who held the family together through pragmatism and hard work. Philomena was "less loved than feared" but "capable of immense strength and courage." Harriet observed that Bob "was an interesting mix of both parents—highly sensitive and intuitive, full of warmth and humor but very aggressive in his approach to science, never losing sight of practicality" (6).

With the change in the family's financial fortunes, Bob realized in 1929 at age 15 that money was a major problem that could impact heavily on the quality of life. Financial matters would continue to plague him for many years, even after he became an internationally renowned scientist. As a teenager, he attacked his financial problems with the same energy and verve that would later characterize his adult workstyle. He took a job in a local drug store and worked an astounding 80 hours a week to help pay his high school and college tuitions. Later, when Bob's mother traveled to Washington, D.C., in 1949 to see him honored with the Washington Academy of Science Award for his work on rickettsialpox and Q fever, the local Cheviot newspaper reminisced about those early years of hard work (7): "Many Western Hills residents will remember the distinguished young scientist pictured here as the affable youngster who not so many years ago was waiting on them at Tilly's pharmacy (now Bernen's) at Harrison and Glenmore Avenues. Since those days which served as his introduction to the magic of medicine, Bob Huebner

has traveled far both physically and in the unexplored realms of medical research." Cheviot's ode to an accomplished former resident of the area highlighted some of Huebner's enduring characteristics: his strong work ethic, sociability and intellectual tenacity.

Bob graduated from Elder High School in Cincinnati in 1932. He enrolled as an irregular student in Xavier University, Cincinnati, where he went intermittently for four years. His education was varied, minimally geared toward science, and he majored in economics and English literature. Unsure of his future plans, he fulfilled the requirements for entrance to law school. The devotion to Catholicism Bob had been steeped in at home continued. He found time to attend religious retreats with some of his friends who entered the seminary, one of whom became a priest. Given his commitment to religious devotions, he may have considered at one time going into the priesthood. His occupational goals changed, however, and he decided to go to neither seminary nor law school. Instead, he chose medical school. As he had little scientific background, he enrolled in the University of Cincinnati in order to complete the required pre-medical school courses. After a year and a half, he had fulfilled the prerequisites, so he withdrew before getting an academic degree and applied to various medical schools. Despite that Bob's high school and college performance was that of an average student—no doubt, the heavy workload took its toll—the Saint Louis University School of Medicine accepted him for matriculation in September 1938. Huebner made his farewells and headed off to St. Louis. For the first time he moved out of the family home on 3723 St. Martin Place, Cheviot, Ohio, leaving behind the security of his childhood and the comfort of familiar surroundings.



1932. Bob's graduation picture from Elder High School. (Office of NIH History files, contributed by Catherine Huebner).

Entrance to medical school is often an emotional and cultural shock, but Bob was probably fortunate in his acceptance at Saint Louis University. The school, a private university under Catholic and Jesuit auspices, traces its history to the foundation of the Saint Louis Academy in 1818. The Society of Jesus took over direction of the school in 1827. The small Jesuit college received its charter as Saint Louis University in 1832, becoming the first university established west of the Mississippi River. The School of Medicine was established in 1836 as the Medical Department of the University and had the distinction of awarding the first M.D. degree granted west of the Mississippi River in 1839. A period of turbulence ensued in the 1840's and 1850's leading to the separation of the medical school from the university and its establishment as a proprietary enterprise. In 1903 the entity, then known as Marion Sims-Beaumont College of Medicine, was

incorporated back into the University when funds were made available for its purchase. The college's decision to merge with the university was reinforced by the recommendations of the Council of Medical Education and Hospitals of the American Medical Association, which insisted on university affiliations for all schools of medicine that wished to become accredited.

Thus, in September 1938, Bob Huebner entered the mainstream of American medical education. By the 1930s and 1940s many medical schools accredited by the American Medical Association had instituted systematic curriculums subject to approval by the AMA. The first one and one-half years required studying the so-called basic sciences including gross and microscopic human anatomy, anatomy of the nervous system, physiology, biologic chemistry, pharmacology and pathology. In the second half of the second year, students were introduced to patients and learned about taking histories, how to perform a physical examination and make a diagnosis. They also learned about patient care from lectures in general medicine and surgery. In the third and fourth years students gradually assumed greater responsibilities for patients; they attended, under supervision, specialty clinics and served as clinical clerks on the medical and surgical hospital services while still attending scheduled lectures. Some medical schools required their students to take and pass part I of National Board Examinations in order to be promoted to the third year of school and then to pass part II in order to graduate. Most students would then go on to take part III after the year of internship, which satisfied the requirements for licensure in most of the United States.

There were still some quaint teaching practices in the 1930's and 1940's. Students learned to compound their own medications in pharmacology, and continued learning to

write medication prescriptions in Latin. How different from today's methods! Currently, commercially manufactured, expensive pills come out of bottles and creams, and ointments are squeezed out of tubes. Now, students and physicians are required to know how the medications work in the body, cause uncomfortable or serious reactions, and how they may react adversely with other medications given simultaneously. Every physician today depends on antibiotics, which did not become available until sulfonamides appeared in 1939. Over the years, up to the present, astounding advances have occurred in medical and surgical therapy, diagnostic imaging modalities, understanding of disease processes, immunology and molecular biology that were not available when Bob started medical school. However, in Huebner's day and, probably still true today, the schools taught students that history and physical examination accounted for 80-85% of the successful effort in making a diagnosis whereas laboratory tests accounted for only 15-20% of the effort. When Bob started medical school, physicians had the training to make many successful or brilliant diagnoses, but they had extremely limited therapeutic tools.

Medical schools during the depression years of the 1930's offered meager financial help for students without affluent parents. Scholarships were limited and available to only a few students, those who were in the direst financial straits. This situation would change in the years during and after World War II when the United States Government financed medical school tuition through the Naval V-12 Program, the Army Specialized Training Program (ASTP) and the "GI Bill" sponsored by the Veterans Administration. Now, most medical schools have substantial endowments, scholarships and other financial resources to help students cope with the astronomical tuitions and

living expenses associated with modern medical education; however, the average student or his/her family usually accumulates substantial debt during the course of medical school attendance.

Medical school opened up a whole new world of opportunity for Bob Huebner, although the old financial troubles continued to be troublesome. Fortunately, young Huebner was able to borrow money from his paternal uncle, John—the successful theater operator—whom he paid back gradually with interest over many years (1,8). He also received encouragement and modest financial help from his favorite maternal aunts, Margaret and Catherine. Yet, despite the generous help forthcoming from his kin, he found that he needed additional money to help pay his expenses. Apparently, the school administrators had little empathy for poorer students who found it difficult to make ends meet and disapproved of their employment outside of class. He was suspended from medical school on three separate occasions for working part-time in violation of school regulations (9). Nevertheless, he was reinstated each time because of his good grades. One job was as a "bouncer" in a St. Louis brothel (9). He was able to get more respectable employment as a washer of glassware in the laboratory of the Nobel Laureate, Dr. Edward Doisy (9). He was also put to work in the latter's laboratory extracting estrogen from the urine of pregnant mares. Bob remembered how difficult it was to hold down a job, pay the bills, and keep on top of his studies. "I had no choice," he later recounted, but "they always let me back in." Bob's financial resources were always stretched so tight that he often found it difficult to find enough money to pay for food, so he "always took a job that included food." At one point, though, he "was down to 125 pounds," a skeletal weight for someone almost six feet tall. Food became a major

obsession with him at that time (9), and enterprising medical student that he was, he did his best to combine his scientific interests with his physical demands. Bob utilized his newly acquired medical learning to help remedy his chronic hunger. He made a serious study of products that would provide the best nutritional value for the least cost. He found that *Grape Nuts* fulfilled this criterion, and, for a long period, this was the mainstay of his diet (9)!

Despite the financial and academic pressures of medical school, Bob let romance enter his life. This occurred in the person of Grace Berdine (Berdi) Hoffman, a young, attractive, vivacious student nurse who was in training at St. Mary's Hospital, an affiliate of the medical school. Born in Regan, Nebraska, on October 29, 1916, Birdi grew up on a wheat farm in Nebraska, and then moved to St. Louis for nurse's training. Bob lost no time in courting her. Berdi was a Methodist, but adopted Bob's faith when they wed. They were married October 29, 1939, at the beginning of Bob's sophomore year in medical school. One year later, almost to the day, the young couple welcomed their first child, Elizabeth Jean (Betty) (born October 30, 1940). Since Berdi had to work to support the family, Bob took Betty to Cheviot to live with his family for about one year. According to Bob's sister, Catherine (1), the family spoiled Betty outrageously. Berdi came to Cincinnati later, worked as a nurse at Good Samaritan Hospital, and she and Betty lived with the two maiden aunts. Frances Kay (Kay) was born February 20, 1942.

Bob's life was extremely hectic at this time with medical school and growing family obligations. Remarkably, he excelled scholastically and was elected to the AOA (Alpha Omega Alpha) Honorary Medical School Society when he was in his junior year.

He graduated in the top five out of a class of 100—no shabby achievement. Bob received his medical degree from St. Louis University in June 1942.

World War II had intruded into Bob Huebner's life shortly after he entered medical school. The War started September 30, 1939, when Hitler's Germany invaded Poland. During the early 1940's, after the defeat and the evacuation of the British Expeditionary Force from Dunkirk, France, the United States became involved increasingly in the Lend–Lease program to Great Britain. This increasing involvement and the inevitability of the United States' eventual belligerent status led to the institution of the peacetime draft through the Selective Service System. Physicians were subject to the draft. Bob registered with the S.S. Official Draft Board # 29, Hamilton County, Cincinnati, Ohio. His number was Order No. 309. On October 14, 1941, Bob Huebner filled out an application for appointment to the United States Public Health Service (10). The Public Health Service had become a component of the Armed Forces of the United States after the Japanese bombed Pearl Harbor on December 7, 1941. The PHS had a mandate to provide wartime medical service to the Coast Guard. Bob received a commission in the reserve corps of the United States Public Health Service at the lowest rank compatible with his level of training.

September 1942. Bob Huebner and his brothers. The brothers were called home on emergency leave from the Armed Forces when their father had a serious heart attack. Their father survived to see his sons home safely after World War II. From left to right: Joseph F. Huebner (1919-1998); Richard H. Huebner (1920-1998); Robert J. Huebner (1914-1998). (Office of NIH History files, contributed by Catherine Huebner).

When Bob graduated from medical school in June 1942, he moved his growing family to Seattle, Washington, where he spent a year's rotating internship in the United States Marine Hospital from July 1, 1942 to June 30, 1943 (10). His family continued to grow, and Geraldine Adele (Gerry) was born April 20, 1943. He was commissioned in the U.S. Public Health Service Reserve on July 1, 1943 as an assistant surgeon (equivalent to an Army first-lieutenant). After spending nearly a month as a Ward Surgeon at the Marine Hospital he was assigned to the United States Coast Guard base in Ketchikan, Alaska. He then went on sea duty as Ship's Doctor aboard the USS Hemlock

CGC (Coast Guard Cutter) from August 11, 1943 to February 12, 1944 (10). This 6-month service became the source of a number of Huebner anecdotes—he was a great raconteur—including the transport of a load of prostitutes to Seattle (11). Another anecdote recalled that routine sea patrol was usually drab and dreary while on board the USS Hemlock in the frigid waters off the coast of Alaska and the Aleutian Islands. The skipper of the cutter frequently experienced the need to medicate himself with the medicinal ethyl alcohol in the ship's infirmary. Bob, who was an inquisitive and quick study in all his activities, occasionally had to assist with navigational duties and to direct the helmsman at the ship's wheel (12). This unsolicited apprenticeship as a skipper continued until he was transferred on February 12, 1944, to the base infirmary in Ketchikan, (10) where he remained until his transfer to the U.S. Public Health Service Dispensary in Washington, D.C., on July 1, 1944.

While he was on shore duty he decided to apply for a commission in the Regular Commissioned Corps of the U.S. Public Health Service. From its inception until shortly after the end of World War II, the Regular Corps required interviews and a very comprehensive written examination. In prior years, appointments had been sought eagerly; competition was still keen among the applicants and acceptance was highly selective. The corps was attractive to many young medical men especially during the Depression years, as it offered financial security, as well as opportunity for professional advancement. A commission was no easy achievement. Bob was the only applicant in the Alaskan Theatre at that time, and special accommodations had to be made in order for him to take the examination. On the day of his examination he was locked in a back ward

of the onshore infirmary, and completed the test under the watchful supervision of one of his superior officers, Dr. Tom Carlson (10). Needless to say, he passed.

As part of the appointment process, Dr. Wendall A. Preston, Surgeon, USPHS, District Coast Guard Medical Office, 13 Naval District Alaskan Sector, provided an assessment of Bob's performance in the PHS to date. Preston wrote: "On his own initiative this officer has partaken in the medical and surgical work at the USCG Base Infirmary at Ketchikan in addition to his own work aboard the Hemlock. He was away from Ketchikan approximately one-half the time so that he is fairly well known. He is quite adaptable and would seem to be especially useful in any particular service work where dealing with patients in an efficient way is quite desirable. He is quite interested in psychiatry and would be especially valuable in psychiatry if he were given special training. Also interested in orthopedic surgery" (10).

Bob received his commission. He was transferred from Alaska to the U.S. Public Health Dispensary in Washington, D.C. and moved his family to Fairlington, Virginia and after November 1944 to 3121 McComas Avenue, Kensington, Maryland. In its infinite wisdom, given the information about Bob's possible aptitudes as described by Preston, the wartime personnel office assigned him to the Ear, Nose and Throat Clinic. Bob was not thrilled with this assignment.

The lack of formal training in research and the nature of his pedestrian assignments to date in the Public Health Service do not appear to have provided a promising professional background for Bob Huebner in view of his many future scientific accomplishments in microbiological research. In the standard account of his arrival at NIH, Huebner took action to change his somewhat unsatisfactory situation shortly.

Restless with his assignment to the USPHS Dispensary, he needed an activity that would challenge him intellectually. During the fall of 1944, Bob attended a gathering of the Commissioned Corps officers and was introduced to Dr. Charles Armstrong, then Chief of the Division (later Laboratory) of Infectious Diseases of the National Institute of Health. This Division was the direct descendant of the original USPHS Laboratory of Hygiene established in 1887 at the Marine Hospital on Staten Island. Known simply as the "Hygienic Laboratory," the Laboratory had several locations in Washington, D.C., before finally moving in 1940 out to the campus of the National Institute of Health in Bethesda, Maryland. Dr. Armstrong at that time was a nationally and internationally renowned investigator for his work on botulism, St. Louis encephalitis, lymphocytic choriomeningitis and poliomyelitis. During the course of their conversation, Bob hinted that he might like an opportunity to work in a research laboratory. Dr. Armstrong, an astute observer and a discriminating judge of character, apparently sensed potential in the young but inexperienced officer. He invited Bob out to visit the infectious disease laboratory in Bethesda, Maryland, housed then in Building 5. After a pleasant conversation and further critical evaluation, Dr. Armstrong recruited Bob as a member of his Division in November 1944.

While this account of how Bob Huebner arrived at NIH is probably the one that is closest to the truth, there are several anecdotal versions of how Bob finally arrived at the National Institute of Health. Dr. Norman H. Topping, in his autobiography *Recollections*, (in the chapter about the National Institutes of Health) (11) claimed that he recruited out of the PHS clinic three promising young officers to work with him in his rickettsial disease unit at NIH. They included Drs. Richard G. Henderson (who later died of

laboratory-acquired scrub typhus), Charles C. Shepard, and Bob Huebner. Many years later, Bob offered another version that he related to a boisterous social gathering at the Commissioned Officers Club on Old Georgetown Road near the NIH. (12): While he was working in the ground level Ear, Nose and Throat Clinic of the USPHS Dispensary, the clinic received a call from the 5th floor office of the Surgeon-General, Dr. Thomas Parran (who concentrated the attention of the wartime public on the hazards, prevalence and effective treatment of sexually transmitted diseases). The Surgeon-General had a sore throat. He ordered that one of dispensary physicians should administer an injection of penicillin to him in his office (a poor decision under any circumstance because of the potential for serious allergic reactions without the availability of resuscitation equipment). The dispensary had received one of the earliest repository forms of penicillin that was designed to retard the release and to prolong the effective duration of the penicillin dose. The medium for the administration of the penicillin was a form of beeswax in oil that was semi-solid and required heating so that the penicillin could be propelled through the needle. Bob was selected to handle this assignment. Bob prepared for the injection by heating the penicillin-beeswax mixture over a Bunsen burner. He then rushed up five flights of stairs to the Surgeon-General's office. He injected the needle into Parran's buttock, but the mixture had solidified and would not come out of the needle. The Surgeon-General ordered Bob to prepare another syringe and to try again. Bob repeated this procedure two more times and was unsuccessful in getting the penicillin into Dr. Parran. After the third uncomfortable attempt, the exasperated Surgeon-General said, "Huebner! You better plan a career in research because you sure as hell don't know how to take care of patients." This experience might have precipitated

Bob's desire to leave medical practice behind and head to NIH for a career in research. The story, however, should be taken with a grain of salt. Bob was an entertaining raconteur, and he was not above embellishing reality, especially when under the mild influence of intoxicating beverages. The repository penicillin mixture was probably not generally available until 1946 or 1947, several years after the alleged incident was said to have occurred; yet the story is not completely implausible, as the Armed Forces certainly could have had access to penicillin well before the public did.

No matter which version is most credible, undoubtedly Bob's research career began very quietly. Some twenty years later he recalled the unceremonious way in which he began his work at NIH in a letter of condolence sent to Miss Mary Emma Armstrong (13) following the death of her father. Of his first day at NIH he wrote:

"I arrived without prior scientific training except what one acquires willy-nilly at medical school. I went directly to Dr. Armstrong's office. He greeted me kindly but in a courtly, great man's fashion, took me across the hall and said, 'Here is your office and laboratory'. It was perfectly empty, and he left me there without one word of further instruction. However disconcerting this may have seemed at the time, it was the best thing that could have happened to me. I was left entirely to my own devices. Within a few days I had stolen a chair to sit in (that I still have 24 years later), acquired a desk and some 'loose' laboratory equipment.

"One year later your Dad and I had breakfast at NIH. His only question about my work was, 'How do you like working here'? I said, 'Fine.' He said, 'Well, that's fine, I guess you want to stay on.'

"When I finally got underway in my laboratory work he was a Gibraltar of support, something I very much needed in my studies of rickettsialpox in New York and Q fever in California."

Despite the seeming casualness of their first days together, Huebner would become very close to Dr. Armstrong. The relationship that developed between the brilliant mentor and the apt student would come to resemble that between father and son. In Dr. Armstrong's division, Huebner had found a place where he could shine, where his aptitude for research could be nurtured and his innovativeness abound. Bob Huebner was on his way. His family was settled, and he had a stable income; but perhaps most importantly his considerable intellectual powers now had an outlet. It would not be long before he was asked to draw on his research prowess to solve a puzzle that would help curb a public health nuisance: rickettsialpox.

1) Written, oral and family history and photographs from Catherine Huebner, Robert J. Huebner's sister on July 3, 1999.
2) Letter from William H. Huebner, brother, September 19, 1982
3) Harriet Huebner—personal communication
4) Harriet Huebner—ibid.
5) Armstrong, R., "Will This Man Conquer Cancer," *The Saturday Evening Post*, August 24, 1968, p. 78.
6) Harriet Huebner—letter to Dr. Peter H. Raven, Home Secretary, National Academy of Sciences, May 16, 1989.
7) News item—Local newspaper, Cheviot, Ohio, March 11, 1949 in R. Huebner's personal papers.
8) Susie Huebner (Roberta Sue Creamer)—Interview, September 17, 1998.
9) Robert J. Huebner—Personal communication.
10) United States Public Health Service Commissioned Officer Corps—Service record.
11) Topping, Norman H., Autobiography, with Cohn, Gordon. *Recollections*. Chapter—At the NIH. Pp. 51-134. University of Southern California, Los Angeles, 1990.
12) Robert M. Chanock—Personal communication.
13) Lent by Miss Mary Emma Armstrong—Letter sent by Robert J. Huebner on July 14, 1967.

Chapter 2

Rickettsialpox

As a newcomer to the laboratory, Bob Huebner was assigned to an established research group that could ease him into the investigative practices used by his new colleagues and the research problems on which they were engaged. In this case, it was the unit working on rickettsia and their relationship to Q fever, a newly-recognized disease that was not yet well-understood. The group had begun these studies in the late 1930s, but had been interrupted by the outbreak of World War II.

"Rickettsiae" is a generic name applied to a group of gram negative viruses that exhibit some characteristics of bacteria. They are obligate, intracellular parasites, i.e., organisms that can only exist as parasites. They differ from most bacteria in that they require living cells for growth, but unlike viruses, they are retained by the Berkefeld (bacterial) filter. They are causative agents of many diseases and are usually transmitted by arthropods (lice, fleas, ticks, mites) that serve as vectors. Serious diseases caused by rickettsias include epidemic typhus (louse borne), endemic typhus (flea borne), Rocky Mountain Spotted Fever (tick borne), scrub typhus (mite borne), and a group of spotted fevers primarily found in the Mediterranean and eastern Asia areas characterized by an initial lesion followed by a generalized rash, the more recently described Ehrlichioses (granulocytic and monocytic types) and Q fever. Rickettsialpox, the disease that Huebner would work on, is a member of the spotted fever group.

Huebner spent his first year and half with the lab working on the rickettsia—Q fever studies. These investigations provided an excellent introduction to the world of

research at NIH, and they resulted in Huebner's first publications. Little did he know then how directly relevant these investigations would prove for the path-breaking success he stumbled into during his second year at the laboratory, when he was called upon—by luck of the draw—to solve the problem of a new "mystery" illness afflicting inhabitants of Kew Gardens, in the Borough of Queens, New York City. For his first on-site public health assignment, he was be able to draw on the expertise he gained through the rickettsiae work to unravel the mystery of Kew Gardens Spotted Fever, or, as it came to be known, "rickettsialpox." By the end of 1946, Bob Huebner would catapult to the attention of the general and scientific community with a breakthrough in public health that was made in record time (1,2,3).

In February, 1946, numerous residents of the Kew Gardens housing development came down with a strange illness that was characterized first by a high fever, then by a small pimple-like lesion that broke down leaving a scab, followed by swollen regional lymph glands and the subsequent development of a generalized rash similar to but distinct from typical chickenpox. The physicians attending these patients did not recognize this constellation of signs and symptoms. As new cases appeared, they speculated that they were confronting a previously unrecognized infection. They informed the local health department of their fears and suspicions, but were initially rebuffed. Various local newspapers, however, caught wind of a story, and they published several alarmist articles describing the strange illness infecting the residents of Kew Gardens. When the number of patients approached 100, the New York City Health Department finally decided to investigate the outbreak. In July 1946, after the

Department's own efforts were deemed inconclusive, officials sent a request for assistance to NIH. (4).

In the meantime, the media reports of the mysterious illness fired the imagination of Charles Pomerantz, a former manufacturer of ladies' coats, who had became a pest exterminator and a self-taught authority on ticks and mites. Despite his lack of a formal science education, Pomerantz came up with an ingenious, but medically plausible explanation. He reasoned from the various news stories that biting insects might be vectors, "the carriers" of the disease. Since ticks were not prevalent in the epidemic locale, he focused his attention on the possibility that mites and their hosts, house mice, might be the carriers of some microbe causing the illness. On his own initiative, Pomerantz inspected the exteriors of the buildings where the illnesses were occurring, but he found nothing to justify his suspicions. Rather than give up, the intrepid amateur scientist phoned Dr. Benjamin Shankman (5), the physician who saw the first cases, explained his theory, and asked for permission to examine the interior premises of the apartments. Despite the unusualness of his request, Pomerantz gained authorization and proceeded to conduct his own investigation. He descended into the basements of the houses and peered closely at the areas such as the little cracks in the walls and the tops of the incinerator doors where mites were apt to congregate. Scooping up mites engorged with blood meals, he gathered 45 specimens and traveled all the way to Washington, D.C., to take his find to an entomologist in the Division of Insect Identification of the U.S. Department of Agriculture. A few days later the mites were identified as members of a rare species called *Allodermanyssus sanguineus*, first discovered and classified in Egypt in 1913. Although researchers had in the past suspected that these mites were

potential transmitters of disease, they had never been able to definitively show this. (It has been suggested that these mites were brought inadvertently into the United States in the luggage of Russian immigrants after World War II.) Now, Pomerantz was offering the link that might very well show this to be the case.

When the request to investigate the outbreak had first arrived at the NIH in July, Bob Huebner, as the only commissioned officer in the laboratory available to respond, was given the assignment. At that time, he held the rank of senior assistant surgeon in the United States Public Health Service (USPHS), the equivalent of a Navy lieutenant or an Army captain. Ordinarily a senior officer with more extensive research experience would have investigated an epidemic of this proportion involving an unknown pathogen, but it so happened that all the senior officers in the Division of Infectious Diseases were taking their first extended leaves since the end of World War II. Someone, however, had to keep the lab running, and Bob, lacking seniority, had remained behind. Besides, as a junior officer with a growing family, he had been unable to afford an extended vacation on his modest salary. As it turned out, instead of a few weeks holiday, he got the opportunity of a lifetime to distinguish himself as a research scientist.

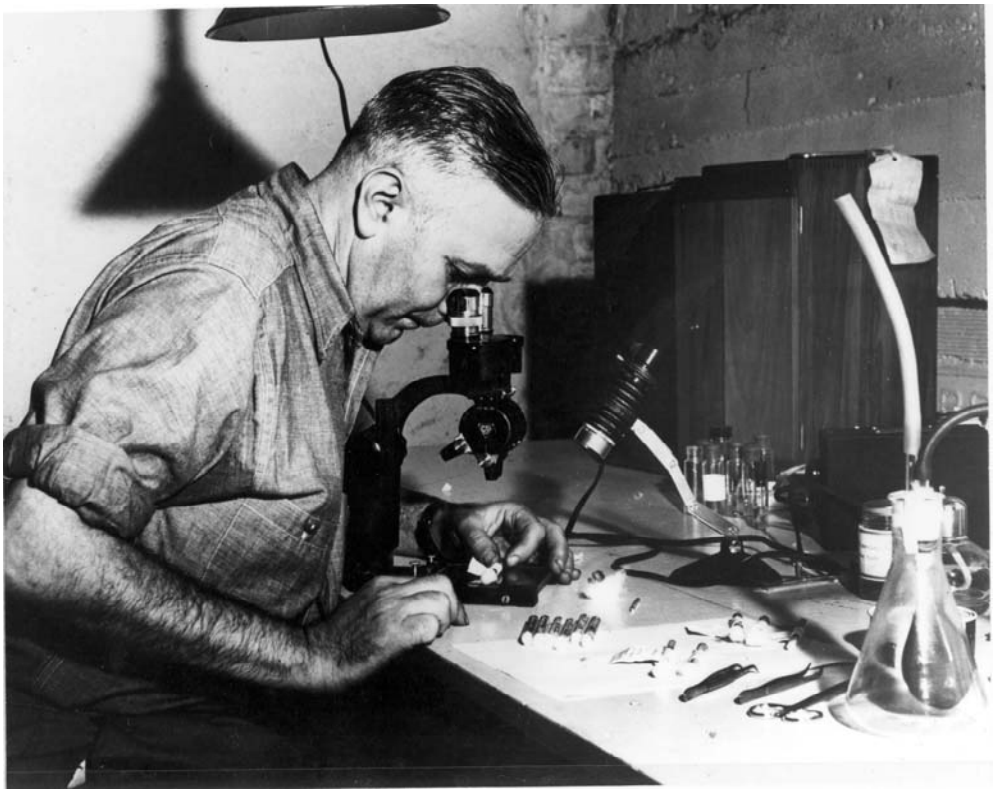
Upon arriving in New York, Huebner followed the accepted protocol of the United States Public Health Service and immediately established good working relationships with New York City Health Department officials and those physicians whose patients were to be included in the investigation. He started by examining many of the patients and then obtaining blood and tissue samples from them for injection into laboratory animals to try and isolate an infectious agent. With the help of the Health Department, he was able to establish laboratory facilities locally to do some of the

processing, but the most critical specimens were sent back to Bethesda. Huebner took blood from some of the patients and injected it into animals almost as soon it was collected, often right there on the spot. Bob had brought jars of mice with him from Bethesda to be used for this purpose. This was one of the valuable lessons I learned from Bob about increasing the likelihood of success in isolating potential infectious agents during an active field epidemiological investigation: collect and process specimens as expeditiously as possible, and in the field, if feasible.

After collecting all the blood specimens that he needed, Huebner traveled back to NIH where he could use the lab's facilities to perform any tests that might be needed. He monitored the mice specimens closely, looking for any changes that might give a clue to the mechanics of the disease. One weekend, shortly following the inoculation of mice with blood from the patients he first examined, Bob checked the jars that contained some of the mice injected with the blood of MK, a 22-year old female patient. He noticed that all the mice had died overnight except for one mouse that appeared to be moribund. This mouse conveniently expired while Bob was inspecting the jar (6). He immediately harvested internal organs (liver and spleen) and passed a suspension of the organs to several more mice. They became ill several days later. He was then able to pass material from these mice to guinea pigs and to the yolk sacs of embryonated chicken eggs. In doing so, he succeeded in establishing the serial passage of an organism that had the cultural and morphological characteristics of a rickettsia. These characteristics included lack of growth on the usual culture media for bacteria, failure to pass a bacterial filter, growth in the yolk sac of the chick embryos and visibility under the microscope with typical rickettsia-staining dyes, and characteristic scrotal inflammation in the guinea pigs.

The isolation of the germ from the patients constituted the first leg of the tripod in solving the nature of the illness.

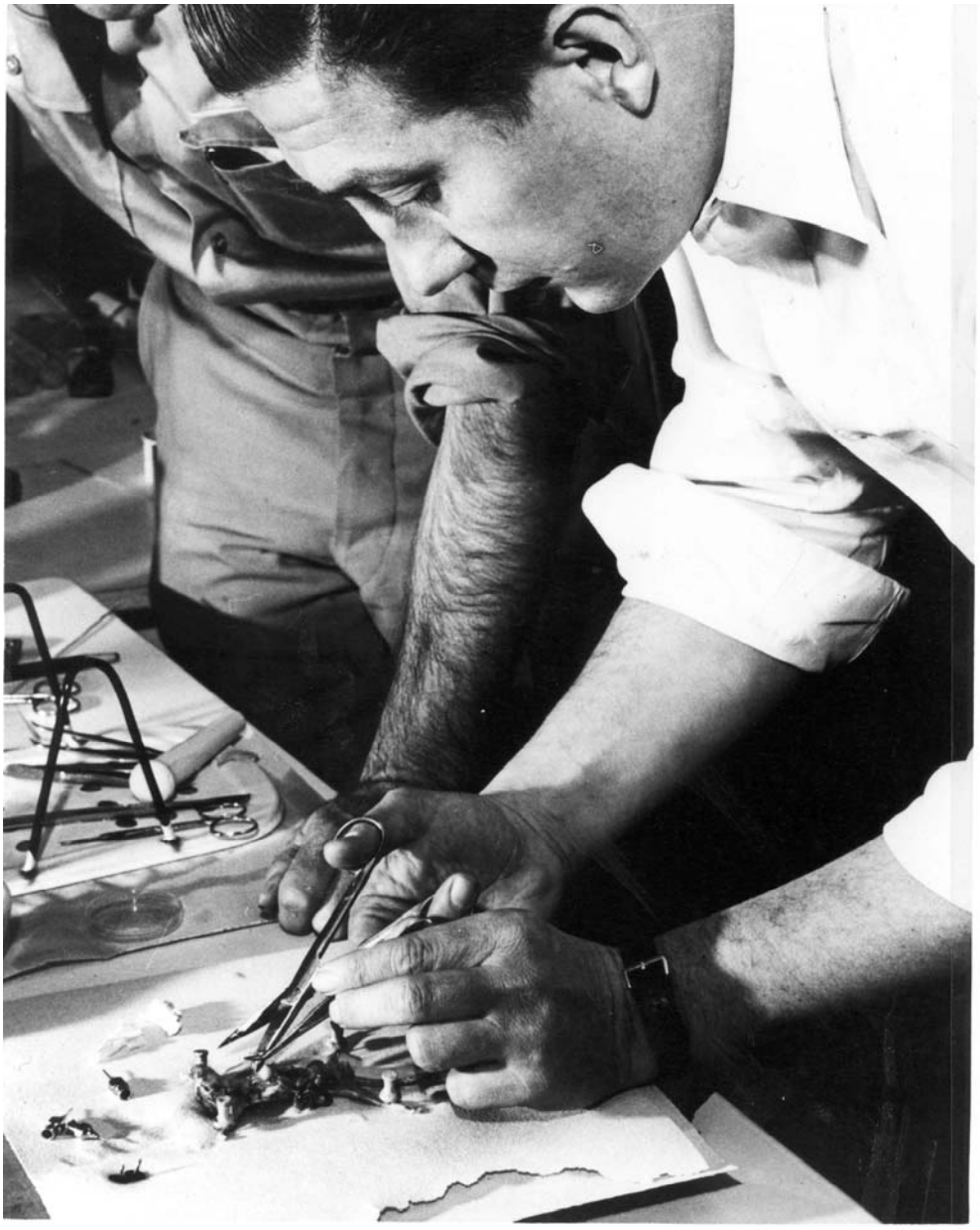
Now that he had potentially found the causative agent, the next step was to find out how the illness spread. Bob Huebner was aware of the extensive mouse infestation in the apartment project, but he did not yet suspect or know how the illness was transmitted to the patients from the mice. In mid-August 1946, he received a phone call from the entomologist at the Department of Agriculture whom Pomerantz had contacted, informing him of Charlie's discovery of the mites. The next day, August 16, 1946, Huebner called Pomerantz, asking if Charlie would like to gather mites for him. Pomerantz was astonished to receive the call. After consulting with Pomerantz, Bob came to the conclusion that the investigation should focus on determining if the mites and mice played a role in transmitting the disease. The next morning, Charlie met Bob at the Health Department Field Laboratory and deposited a closed vial of freshly collected mites on the desk in front of him. This prompted the rapid exodus of several personnel out of the laboratory. Charlie spent the next seven weeks in the basements of the development collecting mites with an occasional break to trap mice. Some of the inhabitants of the buildings where the infestations were occurring remarked to Bob that sometimes they had the visual impression that "the walls had movement." Bob, Charlie and other researchers noted that sometimes the corridor walls had so many mites on them that the walls, indeed, seemed to be moving. On one occasion Charlie peeled back some of the wallpaper, and found the underlying wall teeming with mites! (6)



Summer 1946. Charles Pomerantz at the microscope. (Office of NIH History files).

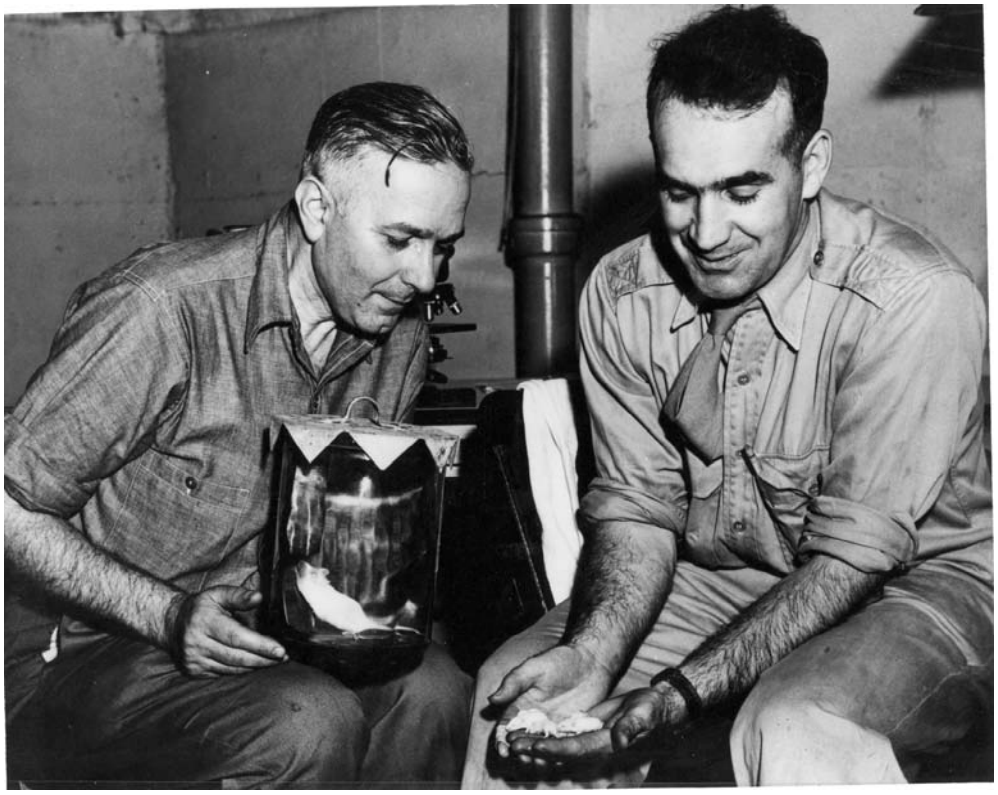
Now that the focus was on determining the connection between the human infections and the mites and mice infestations, Bob determined that they needed to procure as many samples of mites and mice as possible to see if they carried the same organisms. To process so many specimens, they needed to establish another field laboratory in one of the basements of the housing development. Huebner wanted a PHS professional entomologist or parasitologist to provide expertise in the conduct of the field laboratory. He requested help from Dr. Leon Jacobs, a parasitologist with the Division of Tropical Medicine at NIH. Dr Jacobs identified a few mites for Bob, but he was caught up in his own research work in Bethesda and was not anxious to go to New York for additional fieldwork. Jacobs had just returned from Army service and wanted to progress

with his cultivation of *Entamoeba histolylica*, work that he had already deferred for 3½ years (7).



Summer, 1946. Dr. Robert Huebner dissecting a mouse in the USPHS field laboratory in the Kew Gardens apartment complex. (Office of NIH History files).

Bob was persistent in seeking additional help. He requested the services of Drs. Cornelius B. Philip, Glen Kohls or William L. Jellison from the Rocky Mountain Laboratory of the USPHS. Dr. Jellison was available. Dr. Jacobs acquainted Jellison with the scope of the investigation, describing the particular mites his groups had identified. Dr. Jellison went to New York on September 10, 1946. He had been working intensively for three weeks with Bob and Charlie collecting and processing large numbers of mites when he developed a typical attack of rickettsialpox. He was unaware of having been bitten until he observed the initial lesion 7 days before the onset of fever. Bill Jellison recalled Bob's reaction to the news that he had been infected: "Dr. Huebner showed up one morning at our temporary laboratory in Queens and said, 'Our mission is accomplished.' I was able to reply, 'Mine is too.' I had a temperature of 102, a rocky feeling and a primary eschar on the left arm. He took me over to the Marine Hospital. Soon after they put me to bed they came in and set mouse traps around my room. They did not want their local mice, which apparently were quite common, to become infected with rickettsialpox."(8) Although the investigators were not yet completely certain about the routes of infection, they felt it more prudent to exercise whatever precautions they could to prevent the spread of infection.



Summer 1946. Charles Pomerantz (left) and William Jellison (right) inspecting mice. (Office of NIH History files).

Huebner now isolated the causative organism of rickettsialpox from mites in a series of experiments using pools of engorged mites that had fed on normal mice. He also obtained positive isolations in white mice, guinea pigs and the yolk sacs of embryonated eggs. He compared these isolates immunologically with the organism isolated from patient MK (and also sister EK) and found them to be identical. Bob Huebner proposed the name *Rickettsia akari* for the organism. Akari is the Greek term for mite (2). In isolating the organisms from mites and showing them to be identical with the organisms isolated from the patients, Huebner had built the second leg of the tripod.

Summer 1946. Charles Pomerantz (left) and William Jellison (right) collecting mites from the tops of incinerator openings. (Office of NIH History files).

Summer 1946. Incinerators at Kew Gardens. (Office of NIH History files).

Huebner was getting close to solving the final piece of the puzzle. The third leg of the rickettsialpox tripod was the isolation of *R. akari* from the house mouse. Pooled tissue from mice trapped in the housing development was injected initially into mice and guinea pigs; this produced a highly lethal disease in both species. Surprisingly, this disease was identified not as rickettsialpox but immunologically as the virus lymphocytic choriomeningitis. Heubner found that the virus existed as an enzootic infection (an

infection affecting animals in a specific area) among the mouse colonies in the Kew Gardens housing developments.

Bob Huebner then enlisted the expertise of his Bethesda colleague Dr. Charles Armstrong, who had discovered the lymphocytic choriomengitis virus in 1934 and described its behavior in animals and man. Dr. Armstrong worked with Huebner to eliminate the virus as a factor in the investigations. Basically, their logic was to produce antibodies in mice so that they would not become ill with the virus; then, when these mice did become ill and die, the researchers would know it was from something other than this particular virus - hopefully the rickettsiae. Dr. Armstrong immunized a group of mice by subcutaneous injection with a sublethal dose of lymphocytic choriomeningitis in order to protect them from infection with the virus present in the Kew Gardens mice. One month later, he pooled suspensions from a new group of mice trapped at the housing development (the rickettsialpox focus) and injected the suspensions into some of the immunized mice; they became ill 9 to 10 days later. In turn, pooled tissues from the ill mice produced illness when passed in mice and guinea pigs. Both subpassages produced the external and pathological signs of rickettsialpox.

Huebner recovered *R. akari* from the tissues of guinea pigs and mice and found that these isolates were identical immunologically to those isolated from human blood and mites (3). In this way, Huebner demonstrated the cycle of infection with the agent of rickettsialpox. Mice harboring the infection had blood-sucking ectoparasites that bit the humans who were accidental hosts. The mice were the reservoir of infection. The mites feeding on mice acquired the organisms after a blood meal. The mites in turn fed on humans who became accidentally infected hosts.

Elimination of rodent infestation was the obvious public health strategy for controlling the spread of infection. Later, tetracycline and chloramphenicol were found to be effective antibiotics for treating clinical rickettsialpox. For several years after Huebner's discovery, the incidence of cases in New York City remained at approximately the same level then began to decline gradually until the illness slowly disappeared from the area. Sporadic cases were reported elsewhere in the United States, and in other countries geographically distant, such as the Soviet Union. At present, the disease incidence is insignificant.

The entire investigation from the beginning, starting with Bob Huebner's involvement, was completed in a little over 7 months. Dr. Jellison submitted this fact to the *Guinness Book of World Records* in 1980 as an example of the fastest solution to this type of vector-borne infectious disease problem (8). It was a major accomplishment, and it demonstrated Bob Huebner's intuitive grasp of field epidemiology as well as the firm foundation of his scientific methods. All aspects of the laboratory studies were controlled with meticulous care.

In recognition of his achievement with rickettsialpox, Bob received several prizes including the Bailey K. Ashford Award sponsored by the American Society of Tropical Medicine in 1949. This award was given annually to a young investigator under age 35 who has made a major contribution to the field of microbiology. This award also carried a stipend of $1000 provided by the Eli Lilly Company that Bob Huebner used as part of a down payment towards the purchase of the family farm in Frederick County, Maryland in 1951.

Perhaps just as important for his future success, Huebner established a pattern of work relations that would serve him in good stead. On this very first assignment, Bob Huebner demonstrated those qualities that were the hallmark of all his research projects. These included unusual energy, critical thinking, resourcefulness and his ability to work and to cooperate with those individuals who could be helpful and contribute to the joint common effort. He also demonstrated his abilities to work cooperatively with scientific colleagues outside his field of expertise such as Dr. Charles Armstrong, Dr. William Jellison and to seek appropriate expertise when and where he could find it, even if it came from such an unlikely source as exterminator-turned-medical-sleuth, Charles Pomerantz. By cultivating this type of cooperation, he built relationships that resulted in lifelong feelings of mutual affection, respect and admiration. That was also exactly what he would do during his next investigative foray into Q fever in California.

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6) Robert J. Huebner—personal communication.
7) Leon Jacobs—Letter to Robert J. Huebner September 7, 1982 prior to retirement.
8) William L. Jellison—Letter to Retirement Committee for Robert J. Huebner October 3, 1982.

Chapter 3

Q Fever in Southern California

Bob Huebner's next major investigative project was the study and elucidation of the Q fever endemic concentrated in Los Angeles County. Huebner researched in the area intermittently for about 2 1/2 years while he and his colleagues completed a series of meticulous laboratory and epidemiological studies that helped clarify many of the unanswered questions about the nature and spread of Q fever in humans and animals. His findings pointed the way to viable public health measures that could be implemented to curb an infection in humans that caused significant morbidity and occasional mortality.

Dr. E.H. Derrick, Director of the Laboratory of Microbiology and Pathology, Queensland Health Department, Brisbane, Australia, first recognized Q fever as a "new fever entity" in 1937 as a result of his study of an outbreak of a febrile disease among abattoir workers in Brisbane, Australia in 1935 (1). He named the disease "Q fever" (Q for query) and published detailed clinical records of 9 cases. Most of the cases, Derrick found, occurred in meat workers and dairy farmers. The fever lasted variably from 1 to 4 weeks. The most prominent symptom was headache, often severe, persistent and frequently the first complaint. Derrick thought that Q fever bore some resemblance to the typhus rickettsia group of illnesses. It was not, however, accompanied by a rash, and blood studies did not show the positive "Weil-Felix" immunological response characteristic of the typhus group. Fortunately, he was able to isolate the causative organism from human blood and urine specimens. Suspecting a rickettsia, he used guinea pigs for original isolation and subsequent serial passage. For definitive diagnosis, Derrick

sent infective guinea pig liver to Dr. F. MacFarlane Burnet and Mavis Freeman, virologists with the Walter and Eliza Hall Institute, Melbourne, Australia. They confirmed Derrick's suspicions that the organism was a rickettsia and reported their findings simultaneously in the same journal (2). In 1939, Derrick named the organism *Rickettsia burneti* after Dr. Burnet who was responsible for identifying the organism.

The USPHS involvement with Q fever began almost simultaneously around 1938 when investigators at the Rocky Mountain Laboratory in Hamilton, Montana, began to record observations on the prevalence of a fever-causing entity in the area. Gordon E. Davis and Herald R. Cox isolated the organism of Q fever from *Dermacentor andersoni* ("wood") ticks in western Montana in 1938 (3). They identified the organism as a rickettsia and named it *Rickettsia diaporica* because of its ability to pass through Berkefeld W filters that retained the other rickettsias causing typhus and spotted fevers. Dr. Rolla E. Dyer, Chief of NIH's Division of Infectious Diseases and a specialist in typhus, was also working on rickettsias. After visiting the Rocky Mountain Laboratory where he first encountered the organism, Dyer became ill with a febrile disease like the one described by Derrick in Australia. His blood showed cross immunity with the Australian strain. Fortunately, Dyer did not die from his illness, and in 1939, he was able to show that *Rickettsia diaporica* and *Rickettsia burneti* were indistinguishable from each other (3a). The year before, Herald Cox (4) and Emden J. Bell had cultivated the organism in chick embryo yolk sacs and produced a vaccine that was successful in laboratory animals. Cox (5) continued the work and by 1941 had sufficiently improved cultivation methods so as to make vaccine production possible. Ida Bengston (6)

developed a complement—fixation test, further establishing the immunologic relationship of the American and Australian agents.

The investigators in Building 5 at the NIH initiated additional studies on the nature of the Q fever organism in the early 1940's. Unfortunately, but characteristically, the first outbreak of laboratory-acquired illness with Q fever (in 1940) affected many of the personnel. Drs. Rolla Dyer, Norman Topping and Ida Bengston (7) studied the outbreak intensively and successfully isolated and identified the Q fever organism from the hapless victims. One laboratory worker, Mr. Asa Marcey, died. Although the outbreak exacted a heavy toll, it provided Drs. J. W. Hornibrook and K. R. Nelson the opportunity to describe, for the first time, atypical pneumonia as a major clinical feature of the disease (8). Based on this experience, Drs. Ralph D. Lillie, Thomas L. Perrin and Charles Armstrong were able to describe the pathology in animals (9).

The NIH Division (Laboratory) of Infectious Diseases suspended its study of Q fever after the laboratory outbreak in order to concentrate on more pressing problems related to the health of troops and theatre area populations during World War II. Q fever, however, became a problem, occurring as occasional explosive outbreaks in troops serving in Italy and some regions of the Balkans (11). The Army Epidemiological Board investigated these outbreaks. The Board reached the conclusion that the outbreaks represented "point source" (locally-occurring) infections related to the stationing and bivouacking of troops in locations where they were exposed to local livestock, usually either sheep or goats (12). Characteristically, attempts by the Army investigators to work in their laboratories with the isolated agents resulted in outbreaks of infection among their laboratory personnel (13,14).

After the War ended, NIH scientists resumed their studies of rickettsia and Q Fever. It was this investigation that Bob Huebner joined when he arrived at the Laboratory of Infectious Diseases. Huebner and his colleagues Norman H. Topping and Charles C. Shepard began an investigation of the immunologic characteristics of six strains acquired from different sources of *Coxiella burnetti*, the causative agent of Q fever (32A). While this work was in progress another outbreak, the second, of Q fever occurred in Building 5 between December 17, 1945, and May 30, 1946, with an explosive incidence of 18 cases between February 6 and February 11, 1946. Bob reported on the epidemiological features of the outbreak and correlated the increased incidence of cases with the intensity of antigen preparation that resulted in the aerosolization of highly infectious rickettsia-laden chick embryo yolk sacs (32B). Bob also participated in the clinical evaluation of 45 of the 47 patients who were hospitalized at the USPHS Hospital in Baltimore, Maryland in 1946 (8). The resulting publications were the first that listed Bob as an author.

The USPHS was called upon for the first time by local health officials to investigate two naturally occurring, sharp outbreaks that were reported within a short period of each other in the United States. The first appeared in Amarillo, Texas, in March 1946 (15) and the second in Chicago, Illinois, in August 1946 (16). Norman Topping and Charles Shepard, from the LID, both investigated the Amarillo outbreak, and Dr. Shepard alone investigated the Chicago outbreak. It turned out that the two outbreaks were alike in many respects; both arose from occupational exposure of susceptible persons to livestock either being slaughtered or moving to slaughter. Attack rates were high, occurring in over 50 per cent of exposed persons.

Topping's and Shepard's studies of the two outbreaks showed that infected cattle in Amarillo and infected calves and sheep in Chicago had been the sources of human infection, and that transmission to slaughterhouse workers had come from the infected tissues and body fluids either by direct contact or by means of droplets of spattered fluids. The researchers considered the potential roles of tick as vectors based on the work done at the Rocky Mountain Laboratory. The researchers found, however, that ticks were seen very rarely on animals in either of these locations, and, in Chicago, cases did not tend to be associated with people working with hides (where ticks would have appeared), but were concentrated instead in persons handling viscera. They completed serological studies of blood by the complement fixation method and were able to diagnose the infective role of Q fever in the outbreaks.

Although these studies somewhat clarified the *means* by which the humans had become infected, the *manner* of infection of the animals was not obvious. More difficult still, it was impossible, for several reasons, to gain a clear picture of what source provided the natural reservoir of the disease in the United States. For one, the infectious source had long been removed when the above outbreaks occurred, and the epidemiological investigations were necessarily retrospective. Moreover, the apparent lack of an insect vector in the two American outbreaks and their explosive and isolated nature seemed to be different from the original picture of Q fever first described by Derrick in Queensland, Australia, in the 1930's.

In the spring of 1947, an endemic area of Q fever infection was discovered in Los Angeles County that appeared to provide suitable material for investigation of the natural reservoir problem, and it resulted in the dispatch of Dr. Shepard and Bob Huebner to that

area (17). The NIH response came as a result of communications from Frank W. Young, M.D., of Artesia, California (18,19). Dr. Young, a family physician, had seen several ailing patients with similar symptoms, but had been unable to make a definite diagnosis. Based on his clinical analysis, Young suspected Q fever. It was impressive that Young thought of Q Fever, given the state of knowledge at that time. He sent to NIH a number of blood specimens from patients ill with atypical pneumonia. As Young had surmised, many of the specimens turned out to be positive for Q fever, as indicated by the complement-fixation test. Thanks to Young's foresight, NIH was alerted to the presence of Q fever in this region.

The cases were occurring in the milk shed area of Los Angeles County (17), which encompassed a zone of flat land 10-30 miles southeast of the city of Los Angeles. In order to understand the nature of the epidemic, it is important to be aware of the nature and the practices of the dairy industry at that time as well as the geography, climate and demographics of the region. This dairy area was one of the most concentrated in the world. There was little or no pasture available (hence, the coining by Bob Huebner of the phrase "unpasturized cows"). Nearly all the feed was brought in from other places, often many miles away. Because of the high cost of feed and lands, almost all the cows were imported from areas where pasture was available. They were usually bought as 3-year old animals or older, so-called "second calf heifers" The cows were concentrated in pens or corrals at most dairies. The considerable manure that accumulated in the pens was removed by scraping with a bulldozer to the center of the pens where it was loaded into trucks and hauled away to fertilizer plants. The dairies themselves consisted of the pens,

an open milking barn, a closed feed barn and piles of baled hay protected on top against rain (17).

Although most of the population in the area was concentrated in towns of a few thousand inhabitants, many of the residences were quite close to the dairies, and occasionally dairies were located right in town. There were over 400 dairies found in this region, and few people could travel far from their homes without passing one or more of them. Now, of course, the dairies have gone the way of the citrus groves and have made way for the suburbanization of Los Angeles County.

On arrival in California, Drs. Shepard and Huebner sought the cooperation of the local physicians in the search for cases of old or newly occurring Q fever. As was standard procedure, they enlisted the help of the Los Angeles County Health Department. The cooperation of the dairy owners, often suspicious of strangers who might interfere with the milk production, was paramount for the success of the investigation. Initially, federal assistance was welcomed eagerly, especially when it was discovered that one of the health officers was a recent Q fever patient. Later, as the epidemic picture unfolded, some owners became paranoid and actively hostile, especially after the investigators implicated unpasteurized milk as the primary source of Q fever infection.

In the months of April, May and June 1947 Shepard and Huebner found 17 serologically proven cases of Q fever. They first isolated C. Burneti from blood specimens drawn from four patients, and found a diagnostic rise in complement fixing antibodies in three of them. In order to confirm a diagnosis, the workers sent the blood samples—handling them with extreme care—by airmail to NIH where they were studied by the usual cultural and immunological methods and tested for Q fever. The serum

complement-fixation test was relatively insensitive but highly specific for chronologically recent and moderately remote infection with *C. burneti*; it was the routine serological test in use for Q fever. The presence of Q fever was confirmed.

While Huebner and Shepard now had a clear understanding of what disease entity they were dealing with, there were still some missing pieces in the puzzle. What was the common link in all of these cases and how was the disease being spread? In order to determine the answers to these questions, the two scientists focused on the epidemiology of the clinical cases. The striking feature was their peculiar relationship to the dairies. Huebner and Shepard found that in all but two of the case histories, patients had visited dairies or lived near them; yet, none had been actually employed by a dairy. Most of them had not come within 10 to 20 feet of a cow, meaning that their contact with cattle was much more remote than that of dairy workers (17).

Shepard and Huebner determined that the next logical step was to study dairy workers in the area. Complement-fixation tests on 20 dairy workers showed that 10 were positive for Q fever in varying degrees of dilution. Shepard and Huebner theorized that some of the dairy workers may have been infected many years previously and had lost serological evidence of infection since antibodies may decline over time. As a control on their results, they compared serums submitted for routine pre-marital tests for syphilis in Los Angeles County, representing the general population, with similar serums from the District of Columbia. Five serums from Los Angeles showed a positive titer for *C. burneti* antibodies, but none from the District of Columbia gave a positive reading. All of this evidence pointed to extensive exposure not only among dairy workers, but also among Los Angeles County residents as a whole.

The next phase of the study was the examination of blood from dairy cows. One hundred and thirty bloods from 9 different Los Angeles County dairies were tested, and 21 were found to be positive for antibodies to *C. burneti*, an overall incidence of 16.2 per cent with varying incidences among the dairies tested. These bloods were controlled against sera previously drawn from dairy cattle in Texas and Maryland, none of which showed positive tests for Q fever.

Huebner and Shepard now had enough data to present their preliminary conclusions in a report that they published in the *American Journal of Public Health* in 1948. In the article, they described how Q fever had been occurring in an apparently endemic manner in the milk-shed area of Los Angeles County. Most of the cases lived near or visited dairies. Serological studies with a test of high specificity showed that many people who did not give histories of clinical attacks of Q fever showed complement-fixing antibodies for Q fever. Half of the dairy workers and people living near dairies showed specific antibodies. Of 130 sera of cows in the area, 21 showed antibodies, some in high titer possibly indicating recent infection (17). These findings suggested to Shepard and Huebner that a problem of major public health significance for people and for the dairy industry existed in Los Angeles County; furthermore, they demonstrated the need for additional massive epidemiological and laboratory studies to define and elucidate the causes for the existence of the problem (17). The ease with which he and Shepard were able to find large numbers of people sick with an uncomfortable and occasionally fatal disease, the evidence from blood studies of current and former morbidity, and the suspicions that cattle were the reservoir of infection all

added up to a major public health threat. Huebner was convinced that further study was necessary. The NIH and the local authorities agreed and approved additional research.

At this point, Bob Huebner took over the major responsibility for the conduct and directions of the study. Charles Shepard's career interests lay elsewhere, away from Q fever and epidemiology, and he shifted to other research work. Over the next several years, Bob Huebner demonstrated his extraordinary organizational skills and his ability to assimilate and master enormous amounts of data. By mutual agreement he enlisted the cooperation of the California State Department of Health. In order to have adequate controls on his surveys of the milk industry and population studies, he brought into the project the personnel at the Rocky Mountain Laboratory at Hamilton, Montana. The facility there processed about a third of the specimens, and the Bethesda laboratory processed from one-third to one-half. Much of the work was handled locally at a facility called the "Q Fever Laboratory," which was established in the endemic area at Downey (Hondo), California as a cooperative undertaking of the NIH, the California State Department of Health and the Los Angeles County Health Department. This "facility" was a rather unimpressive shed-like structure where, despite its appearance, excellent work was done.

The expanded Q fever studies resulted in a flurry of activities and a series of carefully researched reports entitled "Q Fever Studies in Southern California" that were either written or co-authored by Bob Huebner. (20-27). These reports, along with other manuscripts and reviews, would greatly enhance the understanding of Q fever in a natural setting.

June 1950. Q fever laboratory in Los Angeles County, California. (Office of NIH History files, contributed by E.A. Beeman. Author's 1949 Chevrolet Impala is on the right).

The first important finding in this expanded phase of activity was the recovery of *C. burneti* from raw milk (20). This demonstrated that an animal product might provide a likely source for human and animal infection with Q fever. The preliminary epidemiological data pointed suspiciously to five dairies as source points. Rickettsial organisms identified by all available criteria as *C. burneti* were recovered with ease by each of the three laboratories from 40 out of 50 specimens of raw milk collected from 5 widely separated dairies located in Los Angeles County. These dairies had variable numbers of cows and workers with complement-fixing antibodies for Q fever and association with known cases of illness. As it turned out, control materials such as cattle blood and urine, and feces from sick calves did not yield *C. burneti* when injected into experimental animals. Neither did material from various insect species. The failure to recover *C. burneti* from these sources, as well as the absence of a demonstrable illness in

infected animals, suggested to Huebner that he should consider another source for infection. In the absence of concurrent severe infection in the cow, Huebner theorized that a local infection of the udder (often indicated by mastitis) might explain how *C. burneti* came to be shed in raw milk. He had put this hypothesis to the test by purchasing (at an outrageous price) and autopsying a cow that was shedding organisms into her milk. He found that the prevalence of *C. burneti* in the udder was limited: he isolated the organism from only four quadrants of the udder and the associated lymph nodes, but from no other organs (21). Ultimately, he determined, *C. burneti* was not associated with mastitis of the udder or with the diminution in either the quantity or the quality of the milk. While these findings helped Huebner eliminate some possibilities, key questions about how the illness was spread remained unanswered.

Faced with a Q fever problem in Los Angeles County of significant magnitude, the authorities involved decided to expand the scope of the epidemiological studies even further in order to derive additional hypotheses about the possible sources of infection and modes of transmission. Under the direction of the state epidemiologist, Huebner embarked on an initial pilot study, with the help of the county health department, and of Dr. Joseph A. Bell, the ranking epidemiologist at NIH, (22). This was Bob Huebner's first cooperative venture with Dr. Bell. The two men subsequently enjoyed a fruitful and productive association in many future joint investigations. In the pilot study, the epidemiologists were able to gather information on 300 cases through exhaustive efforts. After analyzing their findings, they devised three general hypotheses about the conditions that were conducive to the spread of Q fever in the endemic area: occupation in the dairy or livestock industry; residence in close proximity to a dairy or livestock yard; and the

household use of raw milk. The researchers arranged for future studies to specifically determine the worth of each of these hypotheses, resulting in a report on an astounding 10,000 persons (24). As it would later turn out, not one of these possible modes of spread accounted for more than one-half of the cases.

The milk industry in Los Angeles County was also unusual in having a large number of "certified dairies" that sold unpasteurized milk. These dairies were inspected and their products certified to be free of the usual bacterial pathogens found in milk, such as campylobacter, salmonella, E. coli O157:H7, yersinia, listeria, tuberculosis, brucellosis, cryptosporidia, or staphylococcal enterotoxin (30). However, prior to Huebner's Q fever work in California, no one had thought of looking for *C. burneti* in raw milk. There were valid—though perhaps not in retrospect particularly good—reasons why no one had considered this before: while these other common bacteria could be found by routine bacterial isolation methods, *C. burneti* could not. It did not grow on the usual media for growing bacteria, and it needed living cells in order to propagate. The usual dairy was not equipped to handle this technology. Moreover, despite the obvious hazards of drinking unpasteurized milk, the dairies had extensive lists of very satisfied clients who preferred the taste of milk unadulterated by beneficial heat.

Influenced by the preliminary study that indicated that 32 per cent of the cases studied used raw milk and by the shedding of *C. burneti* in the raw milk of dairy cows, the researchers advanced to the next obvious step: determining the effects of pasteurization on the survival of *C. burneti* in naturally-infected milk (23). In preliminary studies at Bethesda, (that were later expanded) (31), Huebner found that the resistance of *C. burneti* to heat was greater than that of other rickettsias. Surprisingly, it even exceeded

that of most common bacterial pathogens. This observation suggested that pasteurized milk and products prepared from pasteurized milk must be investigated as potential sources of Q fever infection. In a series of five controlled experiments, Huebner investigated the effects of two methods of pasteurization on the survival of *C. burneti* in naturally infected milk, (1) the holding vat method and, (2) high temperature-short time (HTST) pasteurization. He used four separate rigidly observed and controlled tests to examine the efficiency of each method. These experiments were performed in two large milk-processing plants under the supervision of milk inspectors from city, county and state health departments. At each plant, they used 400 to 600 gallons of pooled milk from two dairies known to contain *C. burneti*. After suitable sterilization and preparation of the pasteurizing equipment, they tested the milk in aliquots by each of the two methods of pasteurization, utilizing the conventional time and temperature parameters for the pasteurization of milk. The temperature in the HTST experiment was 160.5F for 15 seconds, and the temperature for the holding vat experiments was 143F for 30 minutes. All temperatures were controlled very carefully.

At the end of the experiments, the milk samples were processed at the local Q fever laboratory and the Bethesda laboratory. At both locations, milk specimens were injected into guinea pigs. Special chemical (phosphatase) tests indicated that adequate pasteurization had been accomplished in all samples. At both laboratories, however, the end results showed that all the milk pasteurized by the HTST method were negative in producing infection in the guinea pigs as manifested by the lack of development of complement-fixing antibodies. On the other hand, in the milk pasteurized by the vat holding method, one of four batches showed evidence of infection in both laboratories.

As a result of this finding Bob Huebner felt that further tests of vat-pasteurized milk were needed. He purchased 32 quarts of vat-pasteurized milk off the shelves of Los Angeles markets, and he injected standard amounts into guinea pigs at the Q fever laboratory. Three specimens of whole milk and one cream specimen produced serological evidence of infection in at least one of two guinea pigs. Two of the whole milk specimens and the cream specimen were adequately pasteurized according to the phosphatase test. Bob Huebner found these results disturbing because studies in his laboratory in Bethesda showed that the lethal heat end-point for *C. burneti* sealed in vials of saline or milk was 60C or 140F which was only slightly below the vat holding temperature of 143F. Yet, one could not be sure that during vat pasteurization all portions of the milk were heated uniformly. He thought that more reliable pasteurization methods had to be employed in order to eliminate all *C. burneti* even though the epidemiological studies did not appear to incriminate pasteurized milk as a cause of Q fever.

Results of the initial epidemiological investigation of the first 300 cases of Q fever prompted a more extensive study involving 10,000 persons in the Los Angeles County area and a control group of 2000 persons in other locations in order to test the hypotheses developed in the initial study regarding the possible methods of infection. The authors of the study were Drs. Joseph Bell, Bob Huebner and the state epidemiologist, M. Dorothy Beck (24). Their conclusions were as follows:

1. The complement-fixation test used in the study was shown to be highly specific for recent infection with *C. burneti*. The test used was of relatively low sensitivity but high specificity, and the positive results represented only part of the total past infections that had occurred.

2. Q fever had been occurring endemically in the Los Angeles area for several years, and it was highly probable that more than 50,000 people had been infected in the preceding years. It appeared that a sizable proportion of these infections caused many persons to have an acute illness with fever for 2 or more days' duration with diagnoses of influenza, atypical pneumonia or similar designations.

3. The most frequent and by far the most important sources of human infection were local dairy cows, their young calves and some of their raw products, particularly raw milk and hides.

4. The persons most apt to have been infected were those who had used raw milk in their households, those whose residence had been located near a dairy or livestock yard, those who had worked in industries handling live or recently-killed local dairy cows and young calves, e.g., employees in dairies, in meat packing plants, fat rendering plants and those employees in creameries and hide plants handling the raw products.

5. Among each of these employee groups, the incidence of positive complement-fixation tests for Q fever was highest in those who also used raw milk.

In their report, Drs. Bell, Beck and Huebner drew several conclusions about the epidemiological findings: the evidence suggested that local dairy cows and their raw products, particularly milk were the most frequent source of human infection and that these infections had caused many persons to have an illness not heretofore recognized as Q fever. In spite of these important observations with regard to the incidence and possible sources for the spread of Q fever, an additional epidemiological finding was soon to be discovered that helped to explain the intense infectious potential of the dairies: that the parturient placentas and the birth membranes of infected cows were highly contagious

and capable under the proper conditions of generating aerosols heavily laden with *C. burneti* that could be dispersed into the vicinity of the dairies (25). This finding occurred following the appearance of Dr. Lauri Luoto at the Q fever laboratory in California (25). Dr. Luoto was a veterinarian who gave up private practice, obtained the degree of Master of Public Health at Johns Hopkins University, and then joined the USPHS. After spending several restless months in Bob Huebner's laboratory in Bethesda, he was finally sent to California where he could indulge his primary interest in studying the diseases of large domestic animals. During the course of his rounds of the dairies, where the cows were actively shedding organisms in their milk, he noted that the placentas and birth membranes from cows that had recently delivered calves remained lying about the corrals for many hours. The disposal of these specimens was haphazard. He decided to examine the placentas of these animals for infection with Q fever.

Dr. Luoto initiated the study of the tissues from serologically positive cows taken from infected herds and in which parturition was imminent. Placentas were collected from the uterus and vagina, from the floor of calving stalls and from the grounds of corrals following normal parturition. Clean portions of tissue were excised at random and were kept frozen till ready for processing.

Dr. Luoto then made an astounding discovery that helped explain a major factor in producing the intense contamination of the environment of the dairy with Q fever. The placental tissues of 33 serologically positive cows were tested and 13 (39 per cent) were found to contain *C. burneti*. Attempts to demonstrate *C. burneti* in 4 serologically negative cows that recently gave birth were unsuccessful. Titrations were performed with suspensions of placental tissue. Some tissues diluted as high as 1 to 100,000,000 were

infectious for guinea pigs. From these findings it was evident that placentas of some infected cows represented rich sources of C. burneti. Luoto and Huebner postulated that the intensity of environmental dairy contamination would appear to depend upon the frequency of parturition and the number of infected cows in the dairy herds. In the Los Angeles County milk shed, great numbers of cows were concentrated on many dairies; parturitions were frequent and placentas were allowed to remain on the grounds of open corrals. The dry climate in this area, together with the known ability of *C. burneti* to survive desiccation, suggested that infected placentas could provide sporadic but excellent opportunities for dissemination of viable organisms into the environments of both humans and animals. Furthermore, contamination of the hides of newborn calves by heavily infected placental membranes could occur, and this could be an important factor in the genesis of Q fever infection among abattoir and hide plant workers. As further evidence of air-borne spread, Dr. Edwin Lennette and co-workers later isolated *C. burneti* from sheep and goat pens in northern California and thus demonstrated contamination of the atmospheric environment. (28).

In the years since these findings, observers have recognized that other animal species can be infected with *C. burneti* and can shed organisms in parturient placentas. Several interesting reports appeared in 1988 describing the outbreak of “Poker Player’s Pneumonia” in a group of men in Nova Scotia, Canada. The men apparently were all exposed simultaneously to a cat infected with Q fever that was giving birth to a litter of kittens while the men were enjoying their weekly poker game (29).

In an effort to test possible control measures for the Q fever problem, Bob Huebner and Lauri Luoto with John Winn of the nascent Communicable Disease Center

of Atlanta, Georgia (then assigned to the California State Department of Health Viral and Rickettsial Laboratory, Berkeley, California) decided to initiate a pilot controlled vaccination study using a vaccine prepared at the Rocky Mountain Laboratory (26). They immunized one-half of a group of serologically negative cows before the cattle joined three herds known to harbor Q fever. They demonstrated the high susceptibility of uninfected cows to Q fever and the occurrence of suitable exposure to the disease by the fact that up to 50 percent of the cows constituting the control group showed evidence of infection. They believed the vaccine could have engendered some resistance to infection since 3 times as many non-vaccinated cows gave evidence of infection, and 5 times as many non-vaccinated cows as vaccinated cows shed *C. burneti* in their milk during the 6 to 9 month observation period. This study suggested the possibility that vaccine might be used to control Q fever in dairy cattle and the need for further research and field studies on this phase of the problem. Although the vaccine demonstrated partial control of infection, the magnitude of the effort at that time to eliminate Q fever by vaccination was fraught with extreme difficulty to say nothing about the resistance of the dairy industry to the economic disruption resulting from a massive vaccination effort.

While Bob Huebner was involved in all the above activities, he commuted back to Bethesda periodically to review laboratory operations related to the California studies and to outline work for his key personnel, primarily, his chief bacteriologist, Miss Sara Elizabeth (Betty) Ransom, and the supervisor of the serology unit, Mr. Horace C. (Chick) Turner. On one of his whirlwind return trips in October 1948 he also found a new laboratory associate recently out of his medical internship (the author—Edward A. Beeman).

After outlining a California-related project for me to start, he was soon off again to California. This project encompassed the immunological comparison of 9 strains of *C. burneti* (including the original 6 strains studied in 1946 by Topping, Shepard and Huebner (32) and 3 newly isolated strains from California) to determine whether the California strains would be suitable for antigen use in the complement-fixation test.

To make Q fever antigen was a lengthy process. First, seed material was injected into the yolk sacs of chick embryos. The eggs were candled daily. When the embryos appeared moribund, the yolk sacs were harvested, stained with special Machiavello or Giemsa stains to assess the amount of rickettsial growth, pooled, then ground in a Waring blender under a protective hood. The emulsion was shaken with an equal amount of ether and allowed to stand and separate. The aqueous portion was then drawn off in a separation funnel and the residual ether was vacuumed off the aqueous portion. This latter material was then subjected to differential ultra-centrifugation to obtain a button of dead rickettsial organisms at the bottom of the centrifuge tube. This button was re-suspended in an appropriate liquid medium; it was the antigen material for use in the complement-fixation test. Live antigen preparations for injection into guinea pigs for antibody production were made without the use of ether, which is lethal to the rickettsias. Betty Ransom and I became involved in the feverish grinding of egg yolk sacs for Q fever antigen and antibody preparation.

These activities set up the conditions conducive to the third laboratory outbreak of Q fever at NIH. This outbreak was not supposed to have happened because we were theoretically in a bio-safe facility, Building 7, which had been recently constructed and occupied in 1947. Fortunately, the outbreak was limited involving only 8 non-immunized

NIH personnel who had wandered into the rickettsial unit and one visiting foreign scientist. The outbreak was unique, however, because the landlord and the landlady of one of the unit's personnel also developed Q fever without ever having visited the laboratory. I chronicled the details of the outbreak with my first publication, which was hidden in the *Public Health Reports* (33). However, due notice was taken of this paper, and I was listed as one of the last entries in that "Book of Virtues" entitled, *The United Public Health Service, 1798-1950* (33a). I was given credit for having made the great epidemiological observation that infection with *C. burneti* could be transmitted by fomites. In 1949, a re-enforcing observation, the one usually quoted in the medical literature, was made by Dr. John Oliphant of the USPHS. He reported an outbreak of Q fever among laundry workers who laundered garments of personnel from a laboratory (probably the Rocky Mountain Laboratory) that was working with Q fever (34).

The immunological comparison of the 9 strains studied by the author mirrored the results of the original study of the 6 strains by Topping, Huebner and Shepard (32) and remained in the laboratory as unpublished data (35). The early diagnostic and serological studies showed great differences in the ability of antigens made from different strains of *C. burneti* to fix complement in the presence of sera from convalescent patients or to fix antibody in the blood of guinea pigs infected with different strains of *C. burneti*. The results reported in the NIH strain comparison studies probably represented manifestations of the phenomenon in *C. burneti*, described at a later date, now known as "phase variation." Newly isolated *C. burneti* strains from animals and ticks are characteristically in phase I, i.e., they will react in the complement fixation test only in late convalescent sera. After a variable number of passages in embryonated chicken eggs, phase I strains

are converted to phase II strains, i.e., they will react with early as well as late convalescent sera from guinea pigs. Any phase II strain can be converted to phase I by passage through a susceptible animal. A single passage through a guinea pig can suffice (36,37). It was likely that the antigens used for complement fixation testing in the California studies were derived from strains in Phase II.

Betty Ransom (31), in 1949 was performing a series of studies on the resistance of *C. burneti* to chemical and physical agents including the lethal thermal end-point of *C. burneti* suspended in milk in order to simulate the conditions of commercial pasteurization. In this group of experiments, she found that the organisms survived temperatures as high as 63C (143F) when suspended in milk, sealed in vials and submerged in water baths for 30—40 minutes. This was actually the same temperature as the vat holding temperature that was used for the commercial pasteurization of milk in California at that time.

In 1948, after the experience of the Army Epidemiological Board studies of Q fever in World War II and during the Q fever studies in California, Dr. Cornelius B. Philip of the Rocky Mountain Laboratory proposed removing the Q fever agent from the genus *Rickettsia* and renaming it *Coxiella burneti*, the prototype of a new genus *Coxiella* (10). The organism was named after its co-discoverers Dr. Herald R. Cox and Sir F. MacFarlane Burnet. The organism warranted separation by virtue of important differences between it and other members of the genus, *Rickettsia*. *C. burneti* is filterable, very resistant to physical and chemical agents, does not elicit agglutinins against proteusX strains which are responsible for the Weil- Felix reaction (in other rickettsias), does not produce the cutaneous rash associated with other rickettsial diseases of man and

does not elicit an acute “toxic reaction” in experimental animals as do other members of the genus Rickettsia. It differs from other pathogenic rickettsias in that it does not require an arthropod vector to maintain itself successfully in nature.

In the spring of 1949, Bob Huebner returned permanently to Bethesda from southern California. He apparently had completed his role in the field studies in Los Angeles County. His only other connection with Q fever in that area was the preparation of an exhibit on the epidemiology of Q fever presented at the annual convention of the American Medical Association in San Francisco in 1950 and later at the convention of the American Veterinary Medical Association. At the AMA convention on June 30, 1950, Bob Huebner and Joe Bell (27) presented their summary paper “Studies in Southern California in a Symposium on Q Fever” before the section on Preventive and Industrial Medicine and Public Health. They discussed the results of their findings, and they concluded from their epizootiological and environmental studies that dairy cows and their raw products presented a tremendous reservoir of *C. burneti* in the Los Angeles area. Furthermore, they stated that since Los Angeles County was a major livestock center and contained one of the largest and most concentrated dairy industries in the world, this situation made it probable that Q fever would remain a public health problem in that area until effective control measures were developed. They also concluded that, although universal pasteurization of milk would help prevent some infections, and the vaccination of occupationally exposed groups might afford protection to those groups, it appeared that truly effective control of the disease in man would have to wait the development of measures for control of infection in the animal sources of human disease. Fortunately the passage of time, real estate development and natural demographic

changes have helped to moderate the public health problem represented by Q fever in Los Angeles County.

I was able to talk recently with the state epidemiologist on call for the health department in Sacramento (38). He indicated that Q fever as of August 3, 1999, is non-existent in the Los Angeles area. Sporadic cases have been reported elsewhere in the northern part of the state associated with some dairies. Certified dairies have had problems with other bacterial pathogens. An outbreak occurred a few years ago when some animal rights activists kidnapped a herd of pregnant goats from the campus of the University of California at Berkeley. When the goats gave birth, the activists who adopted the kids developed Q fever. A few years ago it was possible that an occasional transient visitor might become infected in California with Q. fever and then bring the incubating infection back to his home area, but today that seems unlikely (39). Nevertheless, it is still required that an outbreak of Q fever be reported to the appropriate public health authorities.

I did not know in the spring of 1949 (and it was only many years later that I learned) that Bob Huebner had been "politely invited" to leave California (43). Although Huebner and Bell had delivered the message again publicly about prevention of Q fever through animal control at the AMA Convention in 1950, they had been sending the message locally while they were conducting their studies actively in the late 1940s. The "invitation" probably came from the state and local public health authorities who succumbed to pressure applied by politically active groups in the livestock and dairy industries, most notably the owners of certified milk dairies who were angry that the blame for this "massive" public health problem was laid on them. They also envisioned

the threat to their economic health that might occur with any curtailing of their industrial activity.

A reflection of the mental and emotional attitude of the people in the milk and livestock industries is described in a letter written by Dr. William Jellison who was an active participant in the Q fever studies (40): “After several months work in the Los Angeles area word got around that there was something suspect in the milk supply and inquiries were made to our laboratory.

“There was an early agreement with the city and county health departments that they would handle all local publicity. Finally, an invitation was issued to the press for a meeting and the facts, as then known, were presented with the caution that this was a sensitive situation. They could do as they wished about the publicity. Not a single paper violated this confidence.”

Jellison continued, describing another meeting called early in the NIH investigative phase to inform health officials and representatives of the dairy industry about the nature of the problem. The investigators faced a largely hostile and paranoid audience. A physician associated with the dairy industry asked to speak, stated that he had never heard of Q fever and believed that the entire investigation was a hoax to embarrass the dairy industry. Dr. R. R. Parker, a respected senior investigator from the Rocky Mountain Laboratory who had been invited to the meeting, then proceeded to skewer the know-nothing critic with the established experimental and published evidence that confirmed the presence of Q fever in Los Angeles County. The explanation had a temporary calming effect on the troubled dairymen.

Jellison described another meeting to which he was invited when Bob Huebner was unable to attend. This was the Annual Meeting of the California Veterinary Medical Association in San Luis Obispo held during the later years of the Q fever studies. Jellison was astonished to discover that, despite the ongoing investigation and the communications to the medical and state health communities for the previous few years, the Veterinary Association seemed to be blissfully unaware of and had no official opinion on the Q fever problem in Southern California. Jellison enthusiastically castigated the veterinarians for taking such an ostrich-like, head –in-the-sand attitude to a serious health problem involving creatures for which they had some responsibility.

In this letter, Jellison recorded some of the forces that were least sympathetic to the efforts of Bob Huebner and his conscientious colleagues to illuminate and find solutions to a major health menace in Los Angeles County.

June 1950. Q fever field laboratory in Los Angeles County, California. (Office of NIH History files, contributed by E.A. Beeman).

In these Q fever studies, Bob Huebner participated actively in a “hands on” fashion. In his own words he “milked and bled over 1000 cows and was butted over the fence more than once” (41). In the enthusiasm and energy in pursuing his projects, Bob demonstrated a trait that became a nightmare for some of the administrative people who were responsible for his activities in the field and later in the laboratory. He frequently ignored the bureaucratic niceties for which he was responsible. In his work in California he often entered contractual agreements with various suppliers without informing the administrative people back in Bethesda. However, in view of the usual success in most of his projects, these imperfections were overlooked with minimal reprimand.

In summary, Bob Huebner demonstrated unequivocally the role of infected dairy cattle as a reservoir for Q fever spread among humans in an unusual endemic area. The vehicles for infection included raw milk, other raw products and birth tissues of infected animals that resulted in a constant prevalence of uncomfortable illness in a population living in close proximity to dairies. His studies provided information for public health control measures. In view of his success in unraveling the complexities of rickettsialpox and Q fever, Dr. Charles Armstrong, who had retired recently, and Dr. Karl Habel, the new Laboratory chief, firmly believed that Bob Huebner could follow his own research interests rather than serving at the pleasure and the demands put upon the USPHS. In the Commissioned Officer’s Efficiency and Progress report covering the period from May 1946 to May 1949 (42) Dr. Habel wrote: “Dr. Huebner has been one of our outstanding research men and in a short period of time has made several major contributions in the field of infectious diseases. Dr. Huebner has worked out the complete picture of a new infectious disease (Rickettsialpox) including the etiology, reservoir and vector. The past

two years has brought forth a great fund of information concerning Q fever in cattle and humans in southern California, and this represents the best work to date in this disease. It is recommended that this officer be permitted to continue to expand his very productive activities in the field of infectious diseases at NIH."

After our discussion about his immediate future plans, Bob expressed the desire to study and to look for new viruses in community outbreaks. He also wanted to try to unravel the relation of virus involvement in various clinical respiratory syndromes. However, for the projects envisioned, the research tools and the new laboratory animal hosts were not yet available. Fortunately, the right tools and techniques appeared shortly thereafter; one of the biggest breakthroughs was the use of the suckling mouse as a host. Following in short order came the development, expansion and improvement of tissue culture methods for the growth and isolation of previously unknown viruses. Over the course of the next decade Bob Huebner and his associates would isolate "70 new viruses," classify them, and define the clinical syndromes with which they were associated. I had the good fortune to be able to participate with Bob in some of his early forays into this new viral activity.

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Chapter 4

Coxsackie Viruses—Herpangina

The next few years represented a transition period in the orientation of Bob Huebner's research activities. Following the completion of his California Q fever studies, Bob felt that he could make no further significant contributions to the field of rickettsial diseases. Instead, his attention was drawn to the myriad of undefined fever illnesses loosely attributed to "viruses." The lay and medical public described many of these illnesses as the "common cold," "flu," or "grippe." Except for the influenza virus itself, most of the possible agents for these illnesses had not yet been identified.

During his studies of rickettsialpox in New York City and Q fever in California, Huebner was fulfilling the needs of the Division of Infectious Diseases, which in turn was responding to requests for help in solving specific infectious disease problems submitted to the United States Public Health Service. Charles Armstrong, when he was Chief of the Laboratory from 1942 to 1948, recognized Bob's initiative, brilliance and assumption of responsibility in finding solutions to the problems that were presented to him. Dr. Armstrong had given strong support and encouragement to Bob during the rickettsial and Q fever studies. In recognition of Bob's solid ability and accomplishments, Dr. Armstrong, as well as the new Chief, Dr. Karl Habel, felt that it was appropriate at this time for Bob to embark on research endeavors of his own choosing (1). On the occasion of Dr. Armstrong's death, Huebner's wrote in his condolence letter to Mary Emma Armstrong, the former Chief's daughter, of the impact this research veteran had had on his career:(2): "His support characteristically was wholly unexpressed—yet

complete and unequivocal. I never had any doubts about him, and his complete confidence in me made it impossible for me to succumb to any doubts that I had to take on large responsibilities in the field."

Huebner assumed that leadership admirably. In 1949 and 1950, he led a team effort in the isolation of members of a relatively new group of viral agents from the Coxsackie group and established them as the specific cause of a previously described but largely forgotten acute febrile childhood illness—herpangina. Each member of the team involved in these studies had specific responsibilities, but Bob Huebner provided the overall direction. He was acutely aware at all times about the various aspects of the study and any new significant developments. He also provided a philosophic focus for the joint endeavor; he had keen insight into the importance of the study and kept all participants aware of the study goals. He was adamant about maintaining strict controls on all aspects of the study with emphasis on the work in the laboratory as well as in the field. The experiences in 1949 and 1950 would help establish protocols for the future investigations of the many new viruses and illnesses that were to be discovered in the next few years (1).

I was aware in the spring of 1949 that Bob seemed to be casting about for ideas to help keep the both of us busy. Bob kept returning to the concept that he would like to study acute outbreaks of febrile illness in small or defined communities and to identify the causative agents responsible. He thought in terms of continuing surveillance in communities for ill-defined fevers and the collection of specimens to test for unknown new viruses. Bob speculated that many upper respiratory illnesses, with or without fevers, and with loose diagnostic identification, constituted a vast wastebasket of many diverse

viruses. Clinical symptoms had only been loosely connected to these illnesses without viral confirmation. He hoped that he could provide some enlightenment in this area by finding the viral or other causes. There were, however, some major obstacles to this approach: our own inexperience in viral research methods, the difficulty in finding suitable communities and appropriate outbreaks; and the limited range of laboratory hosts available for doing studies of this nature. The laboratory techniques described previously for rickettsial agent research were of limited use for the envisioned future viral projects, and the usual laboratory animal hosts (mice, rats, monkeys, egg embryos) were too cumbersome for the large-scale surveillance activities that Bob envisioned. In 1949, tissue culture techniques, still in the developmental stage at NIH (3) and elsewhere, were not yet in use in our newly named Laboratory of Infectious Diseases. (The Division became the Laboratory when the National Microbiological Institute became part of the National Institutes of Health in 1948 (4,5).) Over the next few years, starting in 1953, the availability of reliable tissue culture methods to the Laboratory would enable Bob and his colleagues to make rapid strides in discovering new viral agents of respiratory illnesses.

In this interim period between specific research projects, Bob and I became aware of reports circulating in the scientific literature and at professional meetings of a new group of viruses, the Coxsackie viruses, that had been discovered by employing an infrequently used laboratory host—the suckling mouse. In 1947, Dr. Gilbert Dalldorf and associates (6) had isolated the first virus of this group from the feces of two patients ill with a paralytic disease during an outbreak of poliomyelitis in Coxsackie, a community in upstate New York. (The name Coxsackie is derived from the Native American Mohawk dialect and was said to mean Hoot Owl Place. Much later when I was doing an infectious

disease consultation on a full-blooded member of the Mohawk Nation he confirmed to me that this was the correct interpretation. I have not had an occasion to get a second opinion (1).) After discovery of the initial strain of Coxsackie virus, Dalldorf reported similar viruses of the same and different antigenic types from 28 of 433 fecal specimens collected during poliomyelitis outbreaks in New York and Delaware (7). Dr. Joseph L. Melnick of Yale Medical School (8), his coworkers and other investigators had described additional isolations of similar viruses from New England, Delaware, North Carolina, Ohio, Pennsylvania, Texas, Alabama, Georgia and Louisiana (9). Many isolations were demonstrated in feces; a few were made from throat washings and blood and tissues taken at autopsy. Additional isolations were made from sewage and flies collected in several areas. Coxsackie viruses had shown a seasonal prevalence in the summer and early autumn months when poliomyelitis was also prevalent, and occasionally both viruses had been found simultaneously in the same or pooled fecal specimens (13).

By spring 1949, scientists had gained enough information to better define the new virus. The term “Coxsackie” was used to designate viral agents that produced myositis (muscle degeneration), loss of muscle function and death in suckling mice less than 2 weeks of age. They had also characterized the small size of their physical structure (10-20 millimicrons) and the lack of inactivation by ether due to the absence of ether-soluble components in their structure; the poliomyelitis virus also had this latter characteristic and it would later be shown that the so-called ECHOviruses did too. Scientists also demonstrated that the viruses were quite hardy; they were resistant to the then-common antibiotics penicillin and streptomycin, and they remained viable in various diluents under a variety of unfavorable conditions. In addition, scientists had found that the agents

had exceedingly narrow host ranges in the laboratory. Several strains produced mild febrile illness in cynomolgus monkeys (a type of macaque widely used in biomedical research) and chimpanzees with the shedding of virus from the throat and in feces but no lesions of muscles or central nervous system (7). Despite the limitations in cultivating the virus, one laboratory reported a strain grown using the new tissue culture techniques, and we would later adapt a strain to grow in chick embryos (10). Suckling mice, however, particularly after Dalldorf's work, became the laboratory hosts of choice for growing Coxsackie viruses.

After his initial discovery of the Coxsackie virus, Dalldorf went on to classify his isolates in mice on the basis of the signs of illness and the tissue changes produced. The typing of strains immunologically by their antigenic composition was important to identify the specific types with the illnesses that they cause, to establish the identity of the strain and to allow comparison with similar strains isolated in different laboratories. Dalldorf classified his isolates into two groups, A and B. Group A produced extensive myositis (muscle inflammation) but no cerebral lesions in suckling mice; group B produced less extensive myositis but also produced severe encephalopathic (brain) symptoms ending in cystic degeneration of large areas of the brain. Later, it would be found that group B infection produced necrosis of the interscapular fat pads and visceral lesions, especially pancreatitis, and that some of the strains that caused severe pancreatitis were lethal for adult mice. In 1949 Dalldorf classified his group A viruses into three immunologic strains labeled types 1,2 and 3, but at that point had not found any type-specific differentiation then among group B. Shortly afterwards, Melnick and his colleagues developed data indicating that at least 7 immunologically distinct types

existed in group A with many isolations remaining to be classified (11). There are at present about 23 types in group A and 6 types in group B.

Before 1949, all studies of the occurrence of Coxsackie viruses in man were almost exclusively limited to reported “outbreaks” or to “cases” of certain types of illness. Researchers had isolated Coxsackie viruses based on the presence of clinical syndromes resembling paralytic poliomyelitis, aseptic meningitis, “summer grippe,” pleurodynia, influenza-like illnesses and fevers of undetermined origin. The Coxsackie finding was almost accidental: scientists had made these isolations while looking primarily for poliomyelitis in the summer and fall months when it was prevalent. The researchers found specific elevations of serum antibodies for Coxsackie viruses in patients who had some of the above illnesses by means of neutralization and complement fixation tests (9).

Infection of laboratory workers had also been reported (although the published data would not come out until 1950) (12). Acute febrile illnesses presenting thoracic and abdominal pain, suggesting epidemic pleurodynia, were found to be associated with the presence of laboratory strains in throat washing and stools. The illnesses were followed by elevation of specific antibody in serums taken during convalescence. Eventually it would be shown that the laboratory strain causing these isolated illnesses was a group B Coxsackie virus that could cause epidemic pleurodynia.

Bob Huebner and I noted this unfolding area of research with great interest (1). Bob’s unerring epidemiological sense immediately observed the flaw in the various investigations that assigned etiologic causation of these undefined clinical entities to the isolation of the Coxsackie viruses under uncontrolled conditions. In the studies reported,

cases, in which virus isolations occurred, had no relationship to or contact with other cases that were shedding virus. In addition, researchers had made no attempt to determine the prevalence of virus in persons who were not ill.

In the late spring of 1949, Dr. Joe Bell invited Bob and me as his guests to the annual meeting of the American Epidemiological Society in Pittsburgh, Pennsylvania where several papers were being presented about Coxsackie viruses. While there, we had an opportunity to meet Drs. Dalldorf and Melnick and to discuss their presentations and previous work on Coxsackie viruses. (We also, incidentally, met Dr. Jonas Salk who was just beginning his work with poliomyelitis vaccine). Bob succeeded in irritating Dr. Dalldorf, and especially, Dr. Melnick about the way they were assigning etiological significance for the isolation of Coxsackie virus to poliomyelitis and other poorly defined summer illnesses without adequate controls for their isolates.

After the Pittsburgh meeting, Bob remarked that the season was soon approaching when the Coxsackie viruses and poliomyelitis were usually prevalent. His intuition and prescience prompted him to speculate that this might be a good time to anticipate the appearance of viruses if they should occur in nearby communities. On our return to Bethesda, Bob started talking to many local community physicians and NIH people asking them to be on the alert and to report to him any cluster of cases of the same or similar illnesses. Fortunately, we soon became the beneficiaries of provident serendipity. In late August and early September of 1949, a fortuitous local outbreak of febrile illness occurred in Parkwood, a small suburban community close to the NIH, involving 8 persons in 5 nearly adjacent households (14). These patients developed successively similar acute febrile illnesses. The laboratory isolated a Coxsackie virus, Dalldorf's group

A type 2, uniformly from their stools. We were alerted to the outbreak by several investigators at the NIH who used to meet regularly with the physicians in the Building 7 second floor conference room as part of a daily luncheon group. These men had family members, primarily children, who had become ill.

Among the patients the clinical features of this illness were similar and non-specific. The signs and symptoms, lasting an average of 3 days, included fever, headache, muscle pains, occasional stiff neck, sore throat, nausea, and occasional vomiting. One of the men who had alerted us to the outbreak also became ill. He was the father of one of the sick children. His symptoms, though similar to the other patients, simulated a meningeal type of illness and were severe enough that he had to be hospitalized at the Public Health Service Hospital in Baltimore under the care of Drs. Charles G. Spicknall and Luther L. Terry (the future Surgeon-General). Since he was exposed to his sick child, his physicians collected specimens from him to be tested for infectious agents. The laboratory repeatedly isolated Coxsackie group A type 2 from this patient's stool, and he developed a significant rise in antibodies for the infecting virus (14).

As a result of this sequence of events, Bob initiated a survey of the entire community (80 households) in the middle of September 1949 in order to determine the actual presence of Coxsackie virus and to act as a control for the cases involved in the outbreak. This was the type of disease event that Bob had been hoping to study if it were to occur locally. Bob immediately enlisted the help of Dr. Joe Bell to provide epidemiological expertise. Assisting him in the epidemiological investigation was Dr. Roger M. Cole, one of my medical school classmates. Dr. Cole had arrived recently at the Laboratory after spending a year as a post-doctoral Public Health Service Fellow at the

Haynes Memorial Hospital in Boston training under the supervision of the eminent Dr. Louis Weinstein, who pioneered the study of infectious diseases. Since we were dealing with a private civilian population, the research group solicited the cooperation of local community health officers. These officers were Dr. Robert H. Riley, of the Maryland State Division of Public Health and Dr. V. L. Ellicott, Montgomery County Health Officer. The group also contacted practicing physicians in Montgomery County, Maryland and obtained permission to collect stool specimens and blood samples from their patients. Miss Irma Parr, a Public Health Nurse, also assisted the Laboratory and collected fecal specimens and obtained personal and illness data during the community survey.

Dr. Charles Armstrong, the former Laboratory Chief (1942-1948), provided initial advice and guidance to the project as well as specific help with studies on the laboratory primates that were used to test for poliomyelitis. He also astutely observed a difference in the appearance of suckling mice infected with the group A type 1 strain compared with mice infected with the other group A strains isolated during the course of the initial and subsequent studies. These differences were confirmed by distinctive microscopic tissue changes consisting of focal rather than diffuse muscle destruction (1). This was an important observation for our laboratory. It enabled us to differentiate clinically the appearance of mice infected with the type 1 strain from other group A Coxsackie strains.

Over the next few weeks (after the initial outbreak), we collected 373 stools from 296 of the 308 persons residing in 80 of the 84 households in the area. In the 5 households involved in the outbreak, all 8 persons previously ill and positive for virus in the stool were still shedding virus in the stool during the survey, and 3 additional

household contacts, not previously tested, were also positive. A total of 276 persons were tested in the remaining 75 households: 5 persons, only one of whom was ill in August or September, in 5 scattered households, were found to harbor Dalldorf's group A type 2 virus. The majority of isolations occurred in persons under the age of 15 years. Based on virus isolation and homologous positive neutralizing antibody presence, we demonstrated that within the 5 involved households, the virus tended to be associated with the occurrence of a febrile illness during the months of August or September.

The survey also found that two immunologic types of virus other than type 2 were in the Parkwood community; these were Dalldorf's group A type 1 (discussed above) and an unclassified type designated initially as NIH-233 (later "H-2" before getting its official classification as a group A type). Another unclassified type (NIH-248, later " H-1") not present in Parkwood was found in 3 persons in a nearby area. It was interesting that both Dalldorf's type 1 and NIH-248 were from groups of adjacent households. These isolations had interesting implications. They again suggested person-to-person contact, and they provided us with our first indication of the presence and ubiquity of multiple types of these viruses in the community in addition to the strain causing the initial illnesses.

In order to determine the length of time that these viruses might persist in the feces of infected persons and to detect the possible incidence of persons who might develop a carrier state for the virus, the survey team sampled the community again for virus isolation. In December 1949, a resurvey in Parkwood of all persons who had positive stools and a random sample of persons with negative stools disclosed type 2 virus in the stool of 1 person 76 days after the original isolation. No other virus of any

other type was isolated at this time. Intervening tests of positive persons, however, showed the persistence of type 2 virus in their feces for periods ranging from 9 to 47 days. In the resurvey period, 158 persons were tested also for neutralizing antibodies against Dalldorf's group a type 1 virus as well as type 2. All persons living in the 5 households involved in the original outbreak associated with type 2 virus possessed antibodies against that virus; in addition, only two persons neutralized type 1. These results provided further support for the etiological association of Dalldorf's group A type 2 with the illness observed.

On the other hand, our studies brought into question the association between Coxsackie virus and poliomyelitis that Melnick and Dalldorf had postulated. Out of 60 isolations taken from 31 persons we found four immunologically distinct types of group A Coxsackie virus. However, in these 31 persons, we did not associate our isolations with a serious illness or any illness justifying the diagnosis of meningitis or of poliomyelitis in the absence of an epidemic of the latter disease. Our surveillance methods, relying on trained observers and laboratory isolation techniques, would have enabled us to detect these clinical entities if they had been present. These findings substantiated Bob Huebner's insistence on adequate controls in field studies and cast doubt on the causative association of poliomyelitis-like illnesses in uncontrolled studies that reported the isolation of Coxsackie viruses.

The evidence and the data established during the investigation of the original outbreak and the subsequent community survey provided clear evidence that the illness observed was caused by the virus isolated from the cases. Our group was at first unable to give a name to the illness, except to designate it as a non-descript, febrile "summer

illness." We would not recognize until the following year, after another outbreak in the same community, the critical diagnostic physical sign of the illness.

It took almost a year to process all the material collected in the laboratory and to prepare a manuscript for presentation (14). Bob Huebner gave me the opportunity to present the paper (14) before the Section on Pediatrics at the Ninety-ninth Annual Session of the American Medical Association in San Francisco in June 1950. I also had the responsibility of monitoring Bob's "Q Fever in Southern California" exhibit at the convention. This meeting also coincided with the outbreak of the Korean "conflict" (1). The war resulted in my being "frozen" in the Public Health Service for an additional two years, but it enabled me to continue participating in research during that time with Bob Huebner.

After Bob Huebner and I returned from San Francisco in mid-July 1950, we became involved in an additional small epidemic in Parkwood similar in its presentation to the one that occurred one year previously. This new epidemic would ultimately enable us to establish definitively the Coxsackie A viral etiology of a specific infectious illness, herpangina. The symptoms of the illness had been described many years previously, but its origins had remained obscure, generally unknown and unrecognized by most physicians. Our study described herpangina more precisely and clearly established its link to a specific Coxsackie virus (15).

The cluster of cases involving six children in nearly adjacent households in Parkwood prompted the institution of another community survey similar to the one conducted the year before in 1949. Miss Ruth Emily Anderson and Mrs. Irma Parr Powell, Public Health nurses, collected fecal specimens and obtained personal and illness

data during the community survey. Coincident with this most recent outbreak, Bob decided to expand surveillance for the illness we had observed beyond Parkwood and into the Washington, D.C. area. For this purpose he initiated a cooperative association between NIH and the resources of The Children's Hospital of the District of Columbia. This association would last for many years and involve many joint cooperative studies. He also enlisted the cooperation of practicing physicians associated with Children's Hospital including Dr. Montgomery Blair, Superintendent, and staff members Drs. Frederick G. Burke, Sidney Ross and William F. Burdick. He also included Drs. Lewis K. Sweet and Leroy Hoeck of Gallinger Memorial Hospital (now D.C. General Hospital). Dr. Robert H. Parrott, Chief Resident at Children's Hospital (later Clinical Associate at NIH, and then Chief of Pediatrics and of the Children's Hospital Research Foundation) also became involved in the study and was instrumental in finding many cases that appeared in the hospital out patient department.

The index (or initial) illness of the 1950 outbreak in Parkwood occurred July 16, 1950, in the 5-year-old daughter of Dr. Birdsall N. Carle. Dr. Carle during this period was in charge of the Brucellosis Unit in Building 7. His daughter's clinical symptoms resembled those seen in the patients the previous year; however, Dr. Carle noted the critical physical sign that would come to define this illness and those illnesses that occurred in subsequent patients. His daughter complained of a sore throat, and Dr. Carle observed a number of small ulcers that appeared on the anterior fauces of the tonsils and on the soft palate. Since the lesions and all other evidence of illness disappeared on July 18 without antibiotic treatment, little more attention was given to the matter. However, during the successive 15 days, 5 similar illnesses appeared among children living in three

nearly adjacent households. In each instance, the illness was characterized by sudden fever of short duration and a sore throat. During the course of the illness, each child developed small punched-out ulcers with grayish bases and surrounding red areolas on the anterior pillars of the tonsils. Several patients also had lesions on the soft and hard palate, and one child had them on the tongue. The lesions were neither very painful nor attended with swelling. Once Coxsackie A viruses of the same immunologic type were isolated from all the patients, Bob Huebner, myself and others involved in the study realized that this was the same illness that had occurred in 1949. Our "summer illness" was really herpangina. However, none of the observers in 1949 and 1950 had had experience with this particular constellation of signs and symptoms.

With the insights gained from the 1949 Coxsackie group A isolations and the experience with the new 1950 outbreak of similar isolations, Bob Huebner speculated that this syndrome might have been reported previously. As a former student and current alumnus of the Saint Louis University School of Medicine, he had been impressed with the extensive clinical experience of his pediatrics professor, Dr. J. Zahorsky. Dr. Zahorsky came from a Hungarian ethnic background. His name translates "From the Hills," which is also the title of his autobiography. Dr. Zahorsky was a co-author of a text with T.S. Zahorsky entitled *Synopsis of Pediatrics*, published in 1934 by the C.V. Mosby Company in St. Louis. The text was still in print several editions later when I attended medical school from 1943 to1947. Bob Huebner theorized that with Dr. Zahorsky's vast pediatric experience, the latter had probably seen and described the disease we were seeing. Bob, fortunately, had saved his copy of Dr. Zahorsky's small text. During the period of our investigation Bob had started living on his farm in Frederick County,

Maryland. At this time the indoor plumbing in the farmhouse was temporarily inoperative, and the family was using the newly constructed outhouse. On an occasion when Bob had to use the outhouse, and as one who was not inclined to waste time, Bob used the opportunity to peruse the Zahorsky text. To his delight, jumping out at him from the pages (17), he found a section under the title "Herpangina" that described precisely the illnesses that we had been observing. Considering the source from which we had made all of our isolations of herpangina viruses, it seemed fitting that Bob should make his discovery in an outhouse. Dr. Zahorsky had first reported the illness in the *Southern Medical Journal* in 1920 (16), and he had first used the term "herpangina" to describe it in the *Archives of Pediatrics* in 1924 (17). In 1947, Dorland's Medical Dictionary (18) also used the term as the descriptive name of a specific infectious disease. Reports of other outbreaks of febrile sore throats with blister-like lesions had appeared before our study, but they usually attributed the etiology to herpes virus infection or described them as examples of aphthous stomatitis (9). From the descriptions in these reports, it was difficult to determine if the illnesses represented herpangina or not.

We spent the remainder of the summer of 1950 and the fall months doing the community survey that mirrored the one performed in 1949. Our laboratory isolated virus from all the cases and from some of the household contacts. We also demonstrated serological evidence of infection in all the cases. The agent isolated in Parkwood in 1950 we labeled "H-3." At the same time that we were studying these cases, physicians associated with Children's Hospital were collecting through the outpatient department stool and blood samples from children suspected of having herpangina. In addition to the patients in Parkwood, Coxsackie group A viruses were isolated from 26 of 31 patients in

the Washington area. As a result of these studies, 3 additional group A strains were recovered. Two of them H-1 and H-2 (NIH-248 and NIH-233) had been isolated in the 1949 Parkwood survey. Feces from a patient in the Washington area yielded a new immunological strain labeled "H-4." Thus, excluding the original Coxsackie group A type 1 strain, we associated 6 serological (immunological) strains with the specific childhood disease now recognized as herpangina and established them as the etiological agents. (15).

During these studies, Bob Huebner gave me the primary responsibility for directing the laboratory support staff (19). Many of the methods for working with the suckling mice and a new group of viral agents involved the techniques of classical virology with which we both had to become familiar. This involved, primarily, learning to work with the tissues of the suckling mice from which we made reagents such as live antigen suspensions for producing antibodies in adult mice. We also used the reagents in the virus-neutralization tests employed for serological testing Despite the innovativeness of these techniques, the work still was labor intensive and, in light of future advances, somewhat crude, lacking in the elegance of modern DNA technology and molecular virology. Nevertheless, we were able to develop accurate, reproducible and well-controlled methods to get the necessary data. I was responsible for making the necessary reagents such as viral suspensions, immune murine sera and antigens for use primarily in the neutralization and complement fixation tests. In addition, I made all the viral isolations, supervised the testing of human sera for antibodies and did the immunological typing that resulted in the recognition of four previously unrecognized group A Coxsackie virus serotypes.

In order to determine the accuracy and specificity of testing for human antibodies against group A Coxsackie virus, Bob and I evaluated the serological methods using neutralization and complement-fixation tests to demonstrate type-specific antibody responses to recent and past infection with these agents (20). We found the neutralization test to be accurate and type-specific whereas the complement-fixation test was more group than type-specific. This contrasted with Bob's previous experience with Q fever; in that case, the complement-fixation test was a highly specific indicator of recent and past infection (21). Therefore, we used the virus-neutralization test exclusively to test for human antibodies and to determine the strength of antibody production in adult mice and then used the adult mouse serums for type-specific antibody testing.

In addition to these studies, Bob had another independent, but herpangina-related, study underway, which was supervised by Dr. Angela Briefs, (22) a visiting scientist and recent immigrant from Germany. They also cooperated with Drs. Joel Warren and Sidney S. Breese, Jr., of the Department of Virus and Rickettsial Diseases, Army Medical Service Graduate School (WRAMC). Huebner and Briefs determined the physical properties of two group A Coxsackie (herpangina) viruses propagated in eggs and mice by using ultra-centrifugation and electron microscopy. They used Dalldorf's group A type 2 that had also been adapted to the chick embryo from mice (10) and the H-3 type propagated in mice only. They found the size and sedimentation constant of the type 2 strain from the mouse and egg tissues were the same. For both the type 2 and the H-3 strains the sedimentation constant of the component presumed to be the virus was on average 150S. The particle size determined from electron micrographs was approximately 37mμ. These characteristics were similar to other apparent herpangina

strains, but not with strains such as group A type 1 and others that were later classified as belonging to group B (23). The findings described by this study were integral to defining the physical properties of the virus; this information was essential for identifying the virus and in comparing it with similar agents of the same physical attributes in other laboratories.

Other studies in our laboratory shed light on the epidemiology of the group A Coxsackie viruses. My own work in the lab helped confirmed the prevalence and ubiquity of these agents during the epidemic period. One of the herpangina patients from Children's Hospital provided a stool that simultaneously harbored two group A viruses, both H-1 and H-2 (24). At the time of the second mouse passage from stool when the virus was being typed immunologically, I observed that the mice survived initially when neutralized with H-1 serum but then succumbed. When suspensions made from these late surviving mice were injected into a new litter, this litter was protected from infection by H-2 serum. The experiment was repeated using second passage virus suspension in two litters of mice but neutralizing one litter with H-1 serum and one with H-2 serum. These mice all died and virus was recovered corresponding to the serum with which the mice were not protected. Other mice were injected with virus and both H-1 and H-2 serum. These mice all survived.

In order to exclude accidental contamination of the original stool specimen in the laboratory, a portion of the original stool which had remained frozen was thawed, a fresh suspension made, and the entire process was repeated with identical results. At this point, I asked Dr. Bob Parrott to get a blood sample from the patient who was convalescing. The blood taken when the patient was first seen was tested simultaneously with the

convalescent sample by the neutralization test in mice against the standard H-1 and H-2 virus suspensions. Both the acute and convalescent blood samples showed a significant rise in antibody titer against the H-1 virus and only a slight insignificant rise against the already high titer of H-2 virus. We interpreted the data to mean that the patient was ill with H-1 virus when he was seen initially and had previously become infected with H-2 virus from which he was recovering by the time the second sample was taken. This seemed likely, given that the clinic physicians did not obtain from the parents a history of a similar illness within one to two months prior to the presenting episode. This simultaneous dual infection with herpangina strains alerted us to the possibility that we might encounter other situations in the future with the same or similar organisms and this indeed happened. It also reinforced our concept of the widespread distribution of the Coxsackie viruses.

This dual infection episode also revealed a facet of Bob Huebner's character that I found unusual, pleasing and very atypical of many heads of research groups. When I asked him in which order he would like to be listed in the manuscript describing the dual infections in relation to herpangina epidemiology (24)(1), he replied, "Ed, the idea, the observations, the investigation and the manuscript preparations were all the results of your effort. I was not involved. Do not include me on the paper." I was frankly amazed! There are very few chiefs of laboratories who do not insist that their names be included on all publications coming out of their laboratories. Bob apparently was impressed with the work because he quoted the findings repeatedly in subsequent papers. Bob was very generous with me and future associates in sharing credit for the joint common achievements of research activities.

We received another epidemiological clue to the behavior of the viruses and dual infections by the experience of receiving from another laboratory a reference "type" strain with which to compare some unknown isolations recovered in our laboratory (1). This outside virus was supposed to be a prototype of group A type 4. When we started working with this outside virus, and, having had the experience of working with material containing dual infections, we soon discovered that this "reference" strain contained two viruses, type 4 and another group A strain. When Bob and I asked the investigator the origin of the virus, he replied that the strain had been isolated from mixed sewage. This provided more evidence of the widespread ubiquity of these agents during the months in which they were prevalent.

While the laboratory was churning out data on virus isolations, immunological typing and the results of neutralization tests in human sera, Drs. Roger M. Cole and Joe Bell were correlating the data epidemiologically with the herpangina illness information and the behavior of the group A Coxsackie viruses provided over the past two years (25). In a carefully documented analysis of their observations on the epidemiological aspects of the group A viruses, they provided the following numerical data of the surveys and conclusions of the studies: Over a 14 month period (August 25, 1949 through October 31, 1950) 2,670 fecal specimens representing 1,232 persons tested once or more had been tested for the presence of Coxsackie viruses. Smaller numbers of throat swabs, blood clots, spinal fluids, urine, and autopsy specimens were also tested. Specimens were obtained from persons ill with various diseases both in and out of hospitals and from periodic routine stool surveys of communities in the metropolitan area of Washington, D.C. The results of the testing were as follows (25):

1. One hundred and fifty-eight fecal specimens, 24 throat swabs and 13 rectal swabs were positive for group A Coxsackie viruses of 7 different immunologic types. No group B or ungrouped viruses were isolated, nor were viruses isolated from other types of specimens. By determining that only a group A Coxsackie virus was present, the researchers could reason that there a relationship between this particular virus and the clinical illness that had become manifest in the community.

2. Ninety-nine persons were positive once or more by feces; 5 of these persons were positive again in a second year for a different type of virus and 2 persons were tested and positive by throat swab only—making a grand total of 106 persons positive for group A Coxsackie viruses of all types and by all specimens tested indicating widespread prevalence in the community of these agents.

3. Most persons were tested as a result of periodic routine stool surveys to serve as negative controls following the isolation of group a Coxsackie virus from ill patients in the community.

4. The researchers examined the data looking for correlations between the prevalence of virus with various social and environmental factors. They found that sex and race were not associated significantly with the distribution of these viruses. In contrast, age, exposure, season and relation to a specific illness were important attributes of group A Coxsackie virus distribution. Virus occurred in significantly greater numbers among:

a) Persons under 9 years of age; b) Persons exposed in the household to a previous positive person; c) Persons tested during August and September;

d) Persons who were cases or contacts of herpangina. There was no valid evidence from the NIH group's work or from the literature that these viruses were significantly associated with poliomyelitis or with other disease. This is emphasized because the early investigators made the original isolation of Coxsackie viruses from patients with paralytic disease, presumably poliomyelitis, and the early reports emphasized the finding of Coxsackie viruses among patients with suspected poliomyelitis.

5. Group A Coxsackie viruses of different immunologic type may occur: a) Successively in different years in the same person and apparently cause herpangina each time. b) Nearly simultaneously in siblings with herpangina. c) Simultaneously in a single stool of a child ill with herpangina.

6. Group A Coxsackie viruses might be found in the stools of an individual for as long as 76 days indicating the potential in isolated instances for infectivity to other people for a prolonged period. They usually persisted for less than a month suggesting rapid clearance of virus from the stool in most cases.

During the period of study many of the epidemiological features of herpangina and group A Coxsackie viruses were observed; they included:

a) Epidemics of herpangina occurred in two successive years in a single community.

b) There were multiple cases within families.

c) Herpangina tended to break out in late summer and early fall.

d) It was predominant in children under four years of age.

e) The prevalence of herpangina was not affected by sex or race.

f) The mode of spread was from person to person probably by the fecal-oral route.

g) The incubation period ranged from 1 to 10 days and most frequently 4 days.

h) Contact and convalescent carriers of the causative viruses occurred.

i) The etiology of herpangina is apparently multiple immunologic types of group A Coxsackie viruses.

An important factor in explaining the spread of group A Coxsackie viruses in the groups under observation was provided by another extensive study performed in the Parkwood community by testing for neutralizing antibodies in the blood samples obtained in two successive years, 1949 and 1950 (26). Blood was studied for neutralizing antibodies against 6 virus strains. The summary and conclusions were as follows: Infection with group A Coxsackie viruses resulted in the development of type specific neutralizing antibodies. This was therefore the basis for employment of the neutralization test in the serological survey described in the study. The neutralization tests resulted in the following conclusions:

1. Sex is unimportant in influencing the possession of neutralizing antibodies. The influence of race could not be determined in the all white population studied, and the blood specimens were collected on insufficient occasions to determine the influence of season.

2. The number of persons having antibodies increased with age. A larger percentage of adults than children had antibodies against each virus type, and adults possessed antibodies against a greater number of virus types than children

did. These observations plus the demonstration that in households where virus was isolated those persons with pre-existing antibodies did not become infected therefore offered an explanation for the age distribution of herpangina as well as that of the occurrence of virus.

3. The presence in many adults of antibodies to most of the virus types in this study indicated that these viruses were ubiquitous and caused infection frequently in man.

4. The neutralizing antibodies persisted for a year and probably longer with essentially unchanged titer; therefore, an individual titer cannot be used as the sole criterion for the diagnosis of an acute or recent group A Coxsackie virus infection. Acute and convalescent serum specimens in association with illness and the simultaneous isolation of virus is much stronger evidence of current or recent infection.

5. Effective exposure of susceptible individuals to infected persons, especially within households, plus serologic evidence of susceptibility, determined the acquisition of infection. Thus, a herpangina virus introduced into an area of the community with a high percentage of susceptible individuals spread within families and only among families having frequent contact with each other and produced infection only in those persons who did not possess neutralizing antibodies. Although other persons in households somewhat removed geographically within the community were equally susceptible as judged by their lack of antibodies, virus was never recovered from them, and they did not develop

antibodies following the 1950 outbreak of herpangina; the best explanation of this was their lack of effective contact with known infected persons.

6. Occasional individuals (all adults) without demonstrable antibodies at the levels tested, who lived in virus-infected household, did not become infected as measured by excretion of virus and did not develop antibodies. Possible explanations include immunity from a remote infection many years ago with loss of antibodies or other unknown reasons.

7. This study (26) of the prevalence of neutralizing antibodies in a community provided substantiating evidence for the hypotheses proposed by Drs. Cole and Bell that age and effective exposure particularly within households to infected individuals largely governed the spread of group A Coxsackie virus infections in man.

The above epidemiological observations and the conclusions reached on the basis of the neutralizing antibody studies provide a detailed summary of the knowledge and insights that we acquired about the etiology and spread of group A Coxsackie viruses as exemplified by the disease herpangina. These observations were probably applicable to most other members of the enterovirus (27,28) group of viruses. Later, poliomyelitis, Coxsackie, and ECHO viruses were categorized together because of their presence in the alimentary tract, small size, resistance to ether and RNA chemical composition. They have been given numerical designations and are also termed “picorna,” i.e., small, RNA viruses. With the passage of time, many more group A strains up to a total of about 23 have been discovered, and other clinical syndromes have been recognized. The physical

signs in the throat characteristic of herpangina have also been observed in some of the higher numerical enterovirus strains. However, herpangina still remains a distinct infectious disease of childhood (28).

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Chapter 5

Coxsackie Viruses—Epidemic Pleurodynia

Following the completion of the laboratory phase of the herpangina studies and the subsequent preparation of the relevant manuscripts, Bob Huebner gave me an opportunity to start my own independent study of a subject that I found fascinating (1). During the preceding two years, I had become intrigued with the age susceptibility of suckling mice to the lethal effects of the Coxsackie viruses. Group A Coxsackie viruses cause flaccid paralysis and muscle destruction in suckling mice up to the age of 6 or 7 days. There is a variable interim period when the mice can still become infected, as noted by the development of neutralizing antibodies, but the mice show no objective signs of illness. Thereafter, the virus does not appear to infect the young growing mice. Inasmuch as skeletal muscle is the primary tissue affected by group A Coxsackie viruses, I hypothesized that testing the muscle of variously aged mice for biochemical or immunological changes might provide some clues to explain the age susceptibility of mice to infection with group A Coxsackie viruses.

Dr. Karl Habel, at Bob Huebner's suggestion, put me in charge of a new unit called the Laboratory of Basic Virology; this allowed me the facilities and staff I needed to get started. I began my so-called "basic studies" with a comparison of respiratory enzymes of the muscles of infected and non-infected suckling mice utilizing a Warburg (water) bath with its attendant glass hardware (which I calibrated). Several laboratory biochemists, including Drs. Dean Woods and Herman DuBuy, aided me in these studies. I imagine that "basic studies" (the techniques commonly used to initiate a virological

investigation) at the present time would probably involve the use of molecular techniques, primarily the various methods used in working with and manipulating DNA. These methods were only in their gestational stage at the time I began my early investigations. I had started the initial experiments on the respiratory enzymes when, in the summer of 1951, I became involved again in another collaborative effort with Bob Huebner. This activity occupied my time until I left NIH in September 1952, and I did not get to pursue or complete my independent study.

Bob asked me to help with the laboratory phase of the investigation into the etiology of an outbreak of epidemic pleurodynia that was occurring in the vicinity of Bonham, Fannin County, Texas (2). In the summer of 1951, the Texas health authorities provided Bob the opportunity to assist in their investigation. The outbreak in northeastern Texas was widespread, the local medical community had little prior experience with this illness, and the health officers were anxious to determine the cause and receive recommendations for effective control measures to limit the spread of the disease. This was also the period before the Centers for Disease Control (then the Communicable Disease Center) had the capacity to investigate disease outbreaks, and such requests for help still came to the National Institutes of Health.

By the time we began our investigation into the Texas outbreaks, clinical reports had been compiled which described the disease. We also had access to laboratory reports indicating that group B Coxsackie had been found in single or isolated cases of illness. Based on this information, Bob realized the Texas situation presented exactly the type of community that he was interested in studying. The outbreak provided a unique opportunity to perform laboratory studies of this disease simultaneously as it occurred in

the general population. As with the herpangina outbreak, here again the investigative group studied large geographical areas and included large groups of persons who had no signs of the disease.

As he had done in his earlier public health investigations, Bob again called upon the epidemiological expertise of Dr. Joe Bell, asking him to assist at the site of the epidemic in Texas. Bell had recently acquired a young Public Health Service Officer in his section, Dr. Paul M. Beigelman, who had been assigned to temporary active duty with the outbreak of the Korean War. Dr. Beigelman did a major portion of the "grunt" work in Texas, collecting specimens of stools for virus isolation and blood specimens for antibody testing. Huebner also worked closely with Dr. Joe A. Risser of Bonham, the physician who had alerted the Texas health authorities to the outbreak when many of his patients became ill. Dr. Risser was a talented family physician and general practitioner. Bob Huebner and Joe Risser immediately developed an affinity for each other, and Bob was very impressed by Risser's professional competence. Joe Risser became one of many in the legions of Bob Huebner's fans.

Huebner gained the opportunity to study the outbreak when Dr. J.V. Lyons, Director of Laboratories, Texas State Department of Health and Dr. George W. Cox, State Health Officer, Austin, Texas, brought the outbreak to the attention of NIH Laboratory of Infectious Diseases. While Joe Risser had been the first to recognize the clinical entity, other local physicians had also reported the outbreak. Representatives of the Texas State Health Department (primarily Dr. James A. Strong) and the NIH Laboratory of Infectious Diseases with the assistance of practicing physicians in the epidemic area (Drs. J.A. Risser, J.M. Donaldson, L.C. Biggers, L. Morgan and E.C.

Williams from Bonham, Texas) carried out the collaborative studies. An additional epidemiological study, in addition to the one around Bonham, embraced the area around the nearby small community of Telephone, Texas, that included 700 persons, approximately 65 of who gave clinical evidence of concurrent illness or histories suggestive of recent attacks of pleurodynia.

While this disease had been recognized for many years both in its epidemic and sporadic manifestations (3), many physicians were still unfamiliar with the illness, even in 1951. Epidemic pleurodynia (also labeled epidemic myalgia, Bornholm disease, epidemic muscular rheumatism, acute benign dry pleurisy, epidemic pleuritic pain, Bamle disease and "devil's grip") is an acute, specific, febrile, infectious disease of limited duration, usually occurring in children and young adults. The disease is characterized by a) abrupt onset of severe paroxysmal pain in the regions of the attachments of the diaphragms, i.e., the lower chest and upper abdomen; b) aggravation of the pain by movement and respiration; c) periods of remission and exacerbation of the pain and fever; and d) frequent concomitant occurrence of severe headache, anorexia and malaise. The disease usually occurs as sharply localized, explosive epidemics during the summer and fall months, although endemic areas have been reported. When occurring as sporadic cases or at the beginning of an epidemic, pleurodynia may be confused with appendicitis, biliary colic, acute cholecystitis, pancreatitis, perforated peptic ulcer, acute coronary syndromes, pneumonia, pleurisy, pulmonary infarction and occasionally the pre-eruptive phase of herpes zoster. These latter illnesses, however, may be distinguished by the usual clinical and laboratory methods, and they usually do not occur as epidemics during the summer or early fall. Cases of pleurodynia seen during the course of an

epidemic should not offer much diagnostic difficulty; nevertheless, cases seen only sporadically frequently provide opportunities for "diagnostic romance." By 1951, many astute observers had written excellent reviews of epidemic pleurodynia. Daae and Homan first described the disease in 1872 in Norway (4). Finsen had observed the disease initially in 1852 in Iceland, but he did not publish his observations until 1874 (5). In 1888 Dabney described the first outbreak of the illness in he United States (6). In 1933, Sylvest published his classical monograph describing his observation of patients on the island of Bornholm, Denmark, off the coast of Sweden, (hence "Bornholm Disease") and reviewing the reports of European epidemics up to that time (7). Harder (8) reviewed the American literature up to 1935, and Scadding (9) brought the subject up to date in 1946. Finn, Weller and Morgan (10) reviewed an extensive outbreak involving patients at the Boston City Hospital in 1947. Dr. Herbert Morgan of the Thorndike Memorial Laboratory of the Boston City Hospital, during his concomitant study of the laboratory aspects of the outbreak, came close to discovering the etiology of epidemic pleurodynia; he might have done so if he had used 1-day old instead of 3-day old suckling mice to inoculate them with the test material from the patients.

Some researchers had postulated a viral etiology for pleurodynia for many years. In 1949, Curnen, Shaw and Melnick (11) described isolations of their Connecticut 5 strain (later classified as Coxsackie group B type 1) from patients with a diagnosis of "non-paralytic poliomyelitis." A year later, they reported illness in a laboratory worker with a disease resembling pleurodynia (12). The worker had had chest pain with fever and had developed antibodies against the group B type 1 virus. Weller, Enders, Buckingham and Finn (13) went back to the material saved from the 1947 Boston

outbreak and isolated 2 viruses (group B type 1). Lazarus (14) in Seattle, Washington, isolated group B type 3 strains from some patients with pleurodynia. These individual cases suggested group B Coxsackie viruses as possible etiologies for pleurodynia.

When Huebner began to investigate the outbreak in Texas, he relied on the methods developed in the study of the herpangina outbreaks. He immediately began to collect stool samples and blood serums and they soon deluged Bob's laboratory. Because he had standardized laboratory methods previously during his work with the group A Coxsackie strains (15), Bob felt that the viral isolations could be handled in a routine fashion by the technical personnel without professional supervision. This assumption proved incorrect as it became apparent that the laboratory personnel needed some guidance, confronted as they were with confusing, unexpected and uncharacteristic isolation results. One day during this period, Bob came into the small laboratory where I was working, and asked if I could temporarily defer my own experiments and assume the laboratory supervision of the pleurodynia project. Even though he was still "my Boss," Bob phrased his request in the most gracious manner. I readily agreed to this request albeit with the realization and some disappointment that I would not be able to continue or finish my own experimental study. A brief inspection of the staff's methods soon indicated the source of the laboratory technicians' problems. The laboratory was using one-day old mice instead of two to three-day old suckling mice because published reports indicated that the group B Coxsackie viruses established infection with greater ease in the youngest mice rather than in mice of two to three days of age. The few days' difference in age resulted in noticeably larger mice in which signs of illness could be recognized more easily. The laboratory technicians were unaccustomed to working with the younger

animals. They had also not recognized that the first four isolations from stool specimens yielded the unique signs of illness in mice that characterized infection with Coxsackie group A type 1 (described in the previous chapter). Mice infected with this type showed the characteristic spastic postures and attitudes in contrast with the other group A herpangina types where the infected mice demonstrated a complete flaccid paralysis. Using the techniques that I had used previously to separate multiple herpangina viruses in the same stool specimen, (17), it was possible to show that 2 of the patients were also harboring a group B Coxsackie virus—type 3. Because of the slow development of the manifestations of infection with the B virus, the staff had not recognized its presence initially. Just as the staff had not been able to recognize the symptoms of infection with Coxsackie group A, type 1 viruses, they also had trouble recognizing the illness caused by the group B viruses. In primary and early passages, the viruses propagated slowly and irregularly, producing almost none of the signs in the suckling mice characteristic of the group A strains. Ataxia, tremors, spasticity and weakness occurring between the third and twelfth post-injection day were the only signs prior to death, and these manifestations were difficult to distinguish from frequent undetermined causes of infant mouse mortality. However, in subsequent passages more regular production of ataxia and tremors at expected intervals, followed by 100 per cent mortality, established clearly the presence of an agent. We established these isolations subsequently in each instance by reciprocal cross neutralization tests. These isolates were similar to or identical with the Coxsackie B type 3 strains sent to our laboratory by Drs. Dalldorf (18), Melnick (18) and Lazarus (18) for comparison with their strains.

The results of the initial epidemiological analysis showed conclusive isolation and serological evidence to support the association of group B Coxsackie virus type 3 with the cases of epidemic pleurodynia. While Huebner's group did not publish all of the epidemiological data for this study or describe it in great detail, the researchers provided enough information for the reader to get a sense of the basic epidemiological findings (19). Typical cases of epidemic pleurodynia with clinical features as described in the previous paragraphs and by others (6,8,9,10,14) occurred in the vicinity of Bonham from late June to September 1951. The pattern of the outbreak was similar to those described in rural areas by Sylvest (7) and Pickles (20,21). Multiple cases occurred in certain families, as many as 3 or 4 persons becoming ill almost simultaneously or in rapid succession with intervals of three to five days between cases.

The 22 cases described in the published report (2) were selected from among patients referred by practicing physicians in Fannin County, Texas, and some of the nearby areas. The physicians included Dr. J.M. Donaldson, the Fannin County Health Officer, and Drs. L. Morgan, J.L. Stevens and E.C. Williams from the Medical and Surgical Clinic in Bonham, Texas. The patients included only those who were examined by the observers during an illness characterized by fever and the sudden onset of acute pain in the chest aggravated by breathing and on whom the observers obtained stool and blood specimens during illness.

Stool specimens from 18 of the 22 patients studied yielded Coxsackie viruses. The first agents to be recognized and typed by immunologic methods were strains of Dalldorf's group A type 1 as described above. The 4 patients who harbored group A type 1 included 2 patients who also harbored group B type 3. Stool specimens from 16 patients

including the 2 already noted yielded the B-3 virus. Thus, 2 patients had only group A type 1 virus in their stools. One of those patients was later thought not to have pleurodynia, although she lived in a household with 2 siblings who had typical illness with B 3 in their stools.

Serological studies yielded confirmation of infection, but also some puzzling results. In 14 of 22 patients serum specimens were collected close to the onset of illness as well as during convalescence. They were examined by neutralization and complement-fixation techniques for antibodies against B 3; they were also examined for antibodies against A 1 and A 4 as controls. Seven persons, from whom B 3 was isolated, demonstrated a rise in neutralizing antibodies. One patient had high levels of B 3 antibodies in both serums. Five patients showed no evidence of antibodies against B 3 but in 4 of these patients no virus had been isolated from the stool. For some unknown reason one patient with illness and repeated positive stool isolations showed no evidence of viral antibodies. There was little correlation between the results of the neutralization and the complement-fixation methods as noted previously for the group A viruses (22). Bob was able to definitely establish a group B Coxsackie virus in a naturally occurring epidemic as the etiological agent for epidemic pleurodynia.

Persons involved in the Texas “adventure” later had differing recollections of the time spent in that “unique” rural environment (16). Dr. Paul Beigelman, the NIH officer attached to Joe Bell’s epidemiology section, had some fond memories about his field experiences in Texas. (Dr. Beigelman, after finishing his Armed Forces assignment at NIH, went to the Peter Bent Brigham Hospital in Boston to finish his residency training

and fellowship in endocrinology. He later joined the faculty of the University of Southern California School of Medicine in the Diabetes Section.) He wrote (23):

"Dear Bob,

I am sorry to be unable to attend the celebration in your honor. Certainly, my contacts with you in the early 1950's were, and remain, memorable. Your dynamism, energy, originality and productivity were, by then, legendary. You may have forgotten by now, but you proposed a viral etiology of diabetes mellitus [based on the production of pancreatitis in adult mice by Coxsackie B viruses and the frequent appearance of insulin dependant diabetes in man after an apparent viral infection –EAB]. Some of us scoffed, but time (about two decades) proved you right, and us wrong. (As usual!)

"Do you remember our pleurodynia project in Telephone, Texas? Every day for a month the temperature topped 100F. Our subjects thought it hilariously redundant to send their frozen feces to Washington, D.C. We collected blood in the cotton fields during harvest time. It was 1951 by the calendar, but life was in the early 1900's. The women did the work, chewed snuff, wore sunbonnets and went barefoot (this was pre-hippy America). The men played dominoes and 'set.' In one area populated by a 'holy roller' sect, our encroachments, as research scientists, physicians and government agents, albeit we came with smiles and charm, elicited a rather negative reaction. We were told to exit immediately, and never to return on pain of encountering hostile gunfire. As I recall, we never did respond to that challenge.

"However, our daily contacts were with some of the kindest, most pleasant, hospitable and decent people in my memory. Nobody bothered to lock their doors (also they were poor sharecroppers); they were so honest that a rather battered old sponge I had left behind was mailed to me in Washington."

Bob Huebner had a slightly different memory of what he called the "mysterious epidemic in Telephone, Texas." Quoted in a *Saturday Evening Post* article (24), Bob recalled the tiny town and the time he had spent there: "That is down in Sam Rayburn country and Telephone's main claim to fame was that it had no telephones. [Rayburn (1882-1961) was a prominent Texan Democratic Congressman and former Speaker of the House of Representatives between 1949-1953 and 1955-1961.] I was pretty sure when I got there that the disease was the devil's grip, epidemic pleurodynia, that everybody was catching from using the same water dipper at the country store. But I needed stool specimens to pin it down, and the folks didn't go for that idea at all. Finally, I started paying them fifty cents a specimen. This old fellow told me that was the damnedest thing he had ever heard. He said he knew Washington was full of it, but that was the first he had heard they had started buying it" (24). Bob apparently learned over time to employ any number of enterprising maneuvers to accomplish his goals out in the field.

Bob was not finished investigating outbreaks that reinforced his studies of the etiology of pleurodynia (25). In 1954, the West Virginia Health Department requested that Bob assist them in the investigation of an epidemic of chest pain, possibly pleurodynia, which involved some coal miners in Beckley, West Virginia. Dr. Horace W. (Buddy) Bernton (25) had an opportunity to observe Bob during this study and to describe Bob's working habits under the conditions of field epidemiology. Dr. Bernton

was at NIH from 1954 to 1956 fulfilling his military obligation in the Public Health Service. He was a Clinical Associate at the new Laboratory of Clinical Investigation in NIAID. He was assigned to Bob's laboratory until the Clinical Center was able to receive patients. Bob took Buddy Bernton to the epidemic locale to assist him with the study. Buddy found that working with Bob was a tiring, hectic, frenetic, but an ultimately enjoyable experience. Buddy, at that time, was a husky, vigorous physician 15 years younger than Bob. Buddy had difficulty keeping up with an active, rapidly moving Bob Huebner. The team worked from early morning till late at night, examining patients and gathering clinical specimens for testing, including feces, urine, throat swabs, blood and various materials from environmental sources. In the evening after the day's activities, Buddy would drop into bed exhausted at the motel and would fall asleep immediately. When the alarm went off early the next morning, Bob was up already, fully dressed, writing up the previous day's notes and outlining the schedule for the coming day.

After a careful analysis of the clinical and laboratory results, Bob concluded that the chest pain simulating pleurodynia was not infectious in origin. Instead it was caused by the miners working in a cramped position trying to extract coal from a narrow seam deep in the mine with the result that many of them developed chest pain from muscular strain. This last episode ended Bob's active involvement with epidemic pleurodynia.

The pleurodynia investigations had several important outcomes. They allowed Bob to continue vindicating his research philosophy, namely, that in undertakings of this nature: there was no substitute for intensively controlled observations on the occurrence of prevalent infectious agents in the human population, any more than there could be substitutes for controlled experiments in the laboratory; and the evidence in 1951

appeared to support the hypothesis that epidemic pleurodynia was caused by one or more types of group B Coxsackie viruses. Relying on that philosophy, he was able to demonstrate the specific etiological role for group B Coxsackie viruses in causing epidemic pleurodynia. In doing so, he laid the groundwork for continuing understanding of the disease. In subsequent years, researchers would continue to investigate pleurodynia and develop new and important findings. In 1956, Kibrick and Benirschke further expanded knowledge of the clinical spectrum of these agents when they isolated the Coxsackie group B type 3 virus from newborn infants who died with acute aseptic myocarditis and meningo-encephalitis (26). Since this discovery, group B Coxsackie viruses have been recognized as one of the causes for acute viral myocarditis in adults.

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Chapter 6

Exploring Respiratory Viruses and Adenoviruses

Following the studies of the Coxsackie viruses, Bob progressed to another phase of his research career that lasted about six years, from 1952 to 1958. In 1949, prior to starting the Coxsackie virus investigations, Bob had discussed with me his interest in studying respiratory illnesses with the intention of determining possible viral causes for the vast mass of undifferentiated or unknown respiratory illness etiologies. With his initial foray into respiratory virus research, Bob and his associates made a major new discovery. They isolated and described several strains of a previously unknown respiratory virus genus and designated them as the Adenoviruses, a name reflecting the origin of the tissue from which they were isolated. Bob and his associates also described a new clinical entity, caused by a specific strain of adenovirus (type 3), that they called pharyngoconjunctival fever, again reflecting the anatomical sites involved in the illness. The discovery of the adenoviruses opened the period of Bob's research career during which he made the explosive discovery of many previously unknown viruses. During that time, feverish activity in the Laboratory of Infectious Diseases established the nature of the new viruses, their biological and physical characteristics, their relationship to disease, their epidemiological behavior, their behavior in human volunteers and the effects of immunizations for certain of these new agents. Bob was aided in these activities by a number of important and fortunate factors. He had available new laboratory tools in the form of improved culture techniques that vastly improved researchers' capability to

isolate and grow viruses; he had a ready source of reagents; he had access to advanced physical techniques such as electron microscopy, protein electrophoresis, and ultra-centrifugation; and he now could use the early applications of DNA technology. Most importantly, he gathered about himself a group of talented, brilliant, loyal, industrious associates and collaborators without whom much of the work would not have been accomplished. He was also able to maintain his previous associations with the local hospitals and their physicians who provided him with clinical material in the form of patients to study and specimens for laboratory examination. For a while, he also had an association with the Department of Microbiology, School of Hygiene and Public Health of Johns Hopkins University, in the person of Dr. Thomas G. Ward who participated in the isolation and early volunteer studies related to the adenoviruses. The opening in 1953 of the Laboratory of Clinical Investigation, part of the new NIH Clinical Center where patients with illnesses under study could be hospitalized and precise clinical observations made, also aided Bob's endeavors. The facility also served as the site for the controlled study of human volunteers who participated in investigations of specific infectious illnesses. In 1955, Huebner and "Dr. Joe Bell had the foresight and imagination to establish a longitudinal surveillance of the microbial experience of infants and young children in a welfare nursery (Junior Village of Washington, D.C.) during intervals of health as well as disease. This study yielded a veritable cornucopia of new viruses and epidemiological insights" (1). The end of Bob's participation in the Junior Village studies capped the period of his involvement with the respiratory viruses.

Adenoviruses

Back in September 1952, I had completed my tour of duty at NIH (2). I departed in order to resume and finish my clinical training as a Fellow in Internal Medicine at the Mayo Clinic in Rochester, Minnesota. My leaving left a void in Bob Huebner's laboratory that needed to be filled. My replacement was Dr. Wally Rowe, who went on to develop a brilliant national and international reputation in virological research. Wally came with previous laboratory experience gained at the National Naval Research Institute, which was associated with the National Naval Medical Center in Bethesda, Maryland. While there, he had studied extensively over several years the pathogenesis and immunology of lymphocytic choriomeningitis virus infection in mice (3). He arrived at Bob's laboratory in Building 7 on August 1, 1952. Wally spent the first month becoming oriented to the laboratory, the personnel and the ongoing activities. He was quiet, unassuming and congenial. I immediately felt warmly toward him when he congratulated me on the techniques and the quality of the laboratory studies in which I had participated while working with the Coxsackie viruses (2). His intellectual talents were widely recognized throughout his professional career until his untimely death from colon cancer in 1984.

New tissue culture techniques were one of the laboratory developments that aided Bob Huebner's anticipated research efforts. Even by 1952, only a single investigator at the Laboratory of Infectious Diseases was using a reliable tissue culture system, although the researchers at the National Cancer Institute had relied on tissue culture methods since their introduction a number of years previously. At the NCI, Dr. Wilton Earle had been researching tissue culture methods for many years. Dr. Earle was a widely recognized expert and pioneer in the field of tissue culture used for cancer research (4). His

techniques, involving the use of large, rather complicated glass apparatus for the propagation of bits of cancer tissue bathed in a nutrient solution, did not lend themselves well to use in virus isolation and cultivation. Also at NIH, Dr. Harry Eagle, in the early 1950s, had devised the minimal essential medium of chemicals, nutrients and vitamins that goes by his name for use in tissue culture work and suitable for virus cultivation (5). When Huebner first began his adenovirus work in 1952, the only investigator at the Laboratory of Infectious Diseases who had established a tissue culture system for use in research investigations was Dr. Alexis Shelokov (6), then working with Dr. Karl Habel, the Chief of LID.

Alex Shelokov, Roger Cole (my medical school classmate and collaborator on the herpangina studies) and I were fellow interns in Boston during 1947-1948. Alex spent an additional 2 years training as a resident in medicine with Dr. Louis Weinstein at the Haynes Memorial Hospital, the infectious disease unit of Boston University. He came to the NIH-LID in July 1950 and began to work with Dr. Karl Habel, Chief of LID and a highly respected virologist. In 1951, Dr. Habel assigned Alex temporarily to the Harvard laboratory (Children's Hospital, Boston) of the Nobel Laureates Drs. John Enders, Thomas H. Weller, and Frederick C. Robbins to learn current tissue culture techniques for viral cultivation (6). Enders, Weller, and Robbins won the Nobel Prize in 1954 for developing tissue culture methods for growing poliomyelitis virus in human embryonic and other tissues, thus eliminating the need to use the nervous tissue of living rodents and non-human primates. In addition to their fortuitous discovery on the cultivation of poliomyelitis, they were the pioneers in developing the roller tube technique and employing a monolayer of cells derived from many sources, in addition to humans, to

serve as a substrate for virus growth. A number of methods are available to disperse cells in a single layer over the surface of the tubes; this helps in examining the tubes to look for virus growth evidenced by cytopathic changes. Tubes containing specific cell lines became available commercially, but initially investigators prepared their own.

On his return from Harvard in 1951, Alex set up the first tissue culture system in LID for the cultivation of viruses that he had learned at the Ender's laboratory at Harvard. The system employed the roller tube method, namely, roller tubes in a rotating drum with the appropriate nutrient mediums to nourish the tissue cells. It also relied on various cell lines for general use in virus isolation and cultivation. When Bob Huebner and Wally Rowe decided to look for new respiratory disease agents in 1952, they apparently felt that their search would be expedited by using this relatively new method.

In their first attempts at virus isolation, Huebner and Rowe used tubes in which they placed tissue fragments (explants) taken from of human adenoids and obtained during the winter and spring of 1952-1953 from operations on young children. Dr. E. Clarence Rice (Pathologist at Children's Hospital, Washington, D.C., and its Research Foundation, and the Surgical Department of the United States Naval Hospital, Bethesda, Maryland) supplied the adenoids. During the course of examining the growth of the adenoid tissue in the culture tubes, Rowe, Huebner and colleagues encountered a characteristic degeneration which could be serially transmitted to other culture tubes, and which they identified subsequently as a new viral agent, the adenovirus (7). I asked Dr. Janet Hartley who worked on these new agents with Drs. Huebner and Rowe why they used adenoids in the initial studies for virus isolation (8). She offered two possible explanations. The first explanation was an application of "Sutton's Law." Willie Sutton

was a notorious bank robber. When he was apprehended and asked why he robbed banks, he responded, “Because that’s where the money is.” It seemed logical that the location of the adenoids in the nasopharynx, functioning as a lymphoid protective filter organ against infectious invaders, might be a potential source of viruses. The second explanation was that Bob Huebner and Wally Rowe might have been looking for a new epithelial cell line to use in tissue cultures, hoping to provide a suitable substrate for respiratory virus growth. Whatever the explanation, the isolation of the adenoviruses from adenoids is a tribute to the intuitive genius of Bob Huebner. In the immediate absence of a localized outbreak of respiratory illness that he could study, Bob turned to another method, and used a tissue that might provide a potential source for the elusive and unknown respiratory viruses that he was seeking.

During the first week of culture, most of the adenoid explants showed normal growth of epithelium with a few areas of fibroblastic (fibre-like connective tissue) outgrowth (7). After 8 - 28 days of culture 62 % of adenoids observed for this period demonstrated the characteristic cytopathic (destructive changes to cells) change with complete destruction of the epithelium. Wally noted the cytopathogenic changes involving the epithelial outgrowths of the adenoidal tissue. He was not sure what to do with this observation, and he sought the expertise and experience of Alex Shelokov. Alex thought that what Wally was seeing denoted “characteristic degeneration” of tissue explants after prolonged incubation, and he suggested that Wally discard the “degenerated” material. Instead, relying on his keen investigative sense, Wally Rowe transferred the fluid from the tubes showing changes into tubes containing other cell lines (HeLa cells, human embryonic tissue and fresh adenoid cultures not showing

degenerative changes). He found that he was able to establish an agent in successive cultures and maintain it in several cell lines. Shortly afterward, Wally showed Alex other tubes with the same changes. Wally then told Alex that on a "hunch" he had transferred the supernatant fluids from the first tubes to the second tubes. Alex congratulated Wally for having isolated an "agent" and for not taking the advice of an "expert" (6). Dr. Alex Shelokov (6) later remarked, when I discussed this with him recently, that the average observer looking at these changes in the cultures that had not been seeded with a known infectious inoculum, would have attributed the changes to spontaneous degeneration of the tissue rather than to a viral agent and would have discarded the tubes.

The original publication (7) describing the isolation of the adenoviruses appeared in 1953. Over the course of the next several years, the intensive activity at the laboratory resulted in the publication of additional studies describing the biological properties of the group and the isolation of many individual adenoviruses classified into 6 immunologic types (9). Bob's group also delineated a specific illness associated with one of the types (10,11) and engaged in an epidemiological survey associating that immunologic type with the illness seen in a Northern Virginia community (12).

The viruses over time were found to have the following properties that helped distinguish them as a new and distinct group: a) they produced unique cytopathogenic changes in human explant cultures (epithelium of adenoids and tonsils), in HeLa cells derived from a human carcinoma strain with acid production, and in monkey kidney cells and rabbit trachea cells; many of the early isolations were made from adenoid tissue and typed by immunological means; later isolations came from throat swabs, tears and feces of sick patients; b) they produced no pathologic changes for the usual laboratory animals

(rabbits, guinea pigs, rats, mice, hamsters, suckling rodents and chick embryos), a feature that helped characterize the group; c) they resisted the lethal effect of ether; d) they were heat labile (56C for 30 minutes); e) they passed through bacterial filters; f) they were resistant to antibiotics; g) they had type specific neutralization antibody generating properties; h) they produced group-reactive complement-fixing antigens and antibodies (not type specific); i) they produced a non-infective soluble antigen in tissue culture fluids with infectivity residing in the cells after differential centrifugation. These properties became the established criteria for classifying a viral isolation as a member of the adenovirus group. Studies by later authors showed the viral genome to consist of DNA and the virion (virus particle) to possess distinctive electron microscopic structure and physico-chemical properties, thus adding to the list of criteria (9,13).

The tissues and clinical specimens for these studies were collected in part by Dr. Jean Lockhart, Children's Hospital, Washington, D.C.; Dr. E. Clarence Rice, Pathologist, Children's Hospital, Washington, D.C.; Dr. Theodore Winship, Pathologist, Garfield Hospital (later incorporated into the Washington Hospital Center); Dr. Daniel L. Weiss, Director of Laboratories, D.C. General Hospital, Washington, D.C.; Dr. Leroy Hoeck, Director of Pediatrics, D.C. General Hospital, Washington, D.C.; Cdr. John R. Seal and the personnel of the Naval Medical Research Unit No. 4, Great Lakes Naval Training Station, Great Lakes, Illinois; and the personnel of the Ear, Nose and Throat Clinic, U.S. Naval Hospital Bethesda, Maryland. Bob Huebner maintained an association with Children's Hospital of Washington, D.C., and other local institutions that kept sending him fecal specimens, fecal swabs and throat swabs in 1951 and 1952 after the completion

of the Coxsackie virus studies. The laboratory stored these specimens until they could be processed appropriately.

A summary manuscript written by Huebner and his associates appeared in the *New England Journal of Medicine* in 1954 (11). It described the isolation of these new respiratory system viruses in tissue culture from adenoid and tonsil tissue removed at the time of surgery and from nasopharyngeal and conjunctival secretions and feces of persons with respiratory illnesses. The authors designated these new viruses initially as "adenoidal-pharyngeal-conjunctival" agents indicating the important anatomic sites in which they were found. Two years later, one of the committees on viral taxonomy suggested the term "adenovirus" be used to describe the group, playing on the term "adenoid," the anatomical site that was so important to its discovery (14).

Bob Huebner and his associates segregated one hundred and forty-three isolated strains into 6 immunologic types on the basis of cross neutralization tests in tissue culture. They speculated early in the studies that additional immunologic types probably existed (9). They were right, and there are now currently more than 30 immunologic types; many of the higher numerical types are associated only rarely with an assortment of illnesses while the lower numerical types are associated primarily with respiratory and ocular syndromes.

The first 6 immunologic types found were thought to cause frequent infections in children. Serologic surveys in the Washington, D.C. area indicated that 50 % of infants six months to one year of age had been infected with at least one type. Based on this information, it was projected that by the age of 15, the average person would have had infections with several types. Most persons studied had antibodies to 4 or more types by

age 34, and a few had antibodies against all 6 types recognized by these studies. These findings are reminiscent of results of antibody studies found earlier during the herpangina investigation which found increasing antibodies with advancing age. That may have prompted researchers to look for similar characteristics in the study of human adenovirus antibodies.

The amount and specific kinds of illnesses produced by these infections were the subject of various investigations in the early 1950s. Early into the work, it became apparent that there was convincing evidence that types 3 (9,10,11) and 4 caused specific respiratory illnesses. At the same time that Huebner's group was discovering the new viruses, Maurice Hilleman, of the Walter Reed Army Institute of Research, and J. H. Werner were studying similar viruses (Hilleman and Werner's RI-67 (15)). They produced respiratory illnesses from Type 4 in military personnel, primarily new recruits. They described these as "acute respiratory disease" and "primary atypical pneumonia" (now called atypical pneumonia produced by adenovirus). Hilleman and Werner were only slightly behind Bob Huebner in discovering adenoviruses.

Huebner and his colleagues isolated these viruses from persons undergoing tonsillectomy and adenoidectomy by growing tissues taken from adenoids and tonsils in tissue culture (43 strains classified in 5 immunologic types had been obtained from these sources) (11). This suggested that a possible role in persistent chronic disease of tonsils and adenoids should be investigated (the indications for tonsillectomy and adenoidectomy are still being debated). Possibly of more importance was the demonstration of viruses in tissues containing specific antibody against them by the simple expedient of growing infected tissues for prolonged periods. Huebner speculated

that this method of “unmasking” viruses represented a highly sensitive and essentially new technique for isolating viruses (11). Bob Huebner found this technique particularly helpful. During the course of the investigations, he found that older methods of taking swab cultures, directly from the surface at the time of receipt in the laboratory of the eventual virus-yielding tonsils and adenoids, were negative when inoculated into tissue culture; whereas the new method using tissue explants resulted in positive isolations.

In addition to the 1954 summary publication, Huebner later published another manuscript (9) which indicated that types 1, 2, 5, and 6 had been isolated almost exclusively from the spontaneously degenerating tissue cultures of human adenoids and tonsils; one or more of these viruses were present in 57.4 % of children under age 15 years whose tissues had been cultured. For this study, Huebner had found that growing the tissue in culture was a much more sensitive technique for isolating the viruses from tonsils and adenoids of well children than were the tests of tissue suspensions or of throat swabs taken at the time of tonsillectomy as noted above.

Bob Huebner and associates made single isolations of types 1 and 5 from nasopharyngeal secretions of persons with acute pharyngitis. Type 3 was isolated almost exclusively from nasopharyngeal and conjunctival secretions and anal excretions, the majority of isolations having been obtained from persons with acute febrile pharyngitis and conjunctivitis. It was identical to the agent isolated by Neva and Enders (16) from a child with roseola infantum (exanthem subitum). Type 4, the prototype of which was the RI-67 strain found by Hilleman and Werner (15), had been isolated 6 times from Naval recruits with acute respiratory infections and once from spontaneously degenerating tissue cultures of an adult adenoid. It seemed that the availability of reliable tissue culture

methods was stimulating the interest of other investigative groups in the emerging opportunities for the study of respiratory viruses including the adenoviruses that Bob Huebner was studying so intensively. The identification of the Neva and Hilleman strains with serological types isolated in Bob's Huebner's laboratory helped lend credibility to the validity of his observations.

Studies in volunteers, including vaccine studies, were proceeding simultaneously (17,18), initially at various correctional facilities and later at the NIH Clinical Center. All 6 types were administered intranasally to human volunteers. Both nasopharyngeal secretions and tissue culture fluids containing active virus were used as inoculums. Few objective manifestations of illness appeared in the volunteers, and those that did appear only rarely exceeded similar clinical manifestations observed in controls. However, most of the volunteers presented evidence of active infection as indicated by sharp rises in the levels of complement-fixing and neutralization antibodies and by the appearance of virus in nasopharyngeal secretions taken three to six days after virus administration. Failure to produce overt illness was explained speculatively by the presence of pre-existing antibodies in the serums of a majority of volunteers but many volunteers gave no measurable evidence of pre-existing serum antibodies.

Besides furnishing precise evidence concerning the immunologic specificities of the different adenoviruses, these studies allowed Huebner to draw summary conclusions about adenovirus infection: the data showed that an infectious process resulted from intranasal administration of either infected human secretions or infected tissue culture fluids, and that infections with the viruses were induced frequently without the production of overt illnesses. (More detailed information about the volunteer trials

contrasting the various respiratory viruses is contained in the chapter on Volunteer and Vaccine Studies.)

Pharyngoconjunctival Fever

During the course of the intensive studies of the adenoviruses, Bob Huebner was able to mobilize the facilities, personnel and resources of his own unit in the Laboratory of Infectious Diseases along with the LID Epidemiological Unit of Dr. Joseph Bell (who had collaborated with him on previous studies), Dr. Thomas G. Ward of Johns Hopkins School of Medicine and the newly opened Clinical Center of the National Institutes of Health. By combining the clinical (1), epidemiological (2), and laboratory (3) methods used at these different institutions, Huebner was able to rapidly refine knowledge about the adenoviruses. The first illness that the LID associated with the virus was the entity that Huebner's group labeled Pharyngoconjunctival Fever named after the fever and acute inflammation of the nose, throat and ocular conjunctiva associated with it. The group was able to study this illness in several outbreaks and determine its relationship to adenovirus 3. These studies largely defined the clinical spectrum and prevalence of adenovirus respiratory infection in the United States.

The study of pharyngoconjunctival fever began on February 11, 1954, when Dr. Robert H. Parrott admitted a two-year old girl with acute febrile rhinitis, pharyngitis and conjunctivitis to the Infectious and Parasitic Disease Service of the Clinical Center at NIH. The girl was to be a subject in the clinical and etiological study of acute respiratory illnesses, part of Bob Huebner's ongoing study of adenoviruses and their relationship to undefined respiratory illnesses. Dr. Parrott had been associated previously with the studies of herpangina (see the chapter on Coxsackie Viruses). He came to NIH in July

1952, sponsored by Bob Huebner, after completing his pediatric residency at Children's Hospital, Washington, D.C. He was recruited primarily to serve in the new Clinical Center. While he was waiting for the Clinical Center to receive the first patients, he worked in the laboratory from 1952 to 1954 with Bob Huebner and Wally Rowe. He helped with the clinical observation of outpatients and the acquisition of clinical specimens for adenovirus study in the laboratory. He was one of the co-authors on the original publication describing the isolation of the adenoviruses from adenoids (7).

Bob Parrott, in his capacity as a liaison person between NIH and DC Children's Hospital, used to spend one day a week at that hospital's out patient department looking for suitable patients with acute respiratory illnesses as candidates for clinical investigation. This two-year old did not fit the usual mold because she had acute conjunctivitis in addition to fever and naso-pharyngeal inflammation. When he first encountered this patient, he used all his powers of persuasion to convince the parents that their child would be making a major contribution to the advancement of medical knowledge by being admitted for study and treatment at the newly opened Clinical Center at NIH (19). With their approval, Dr. Parrott arranged to have the girl transferred to NIH, and on February 11,1954 she was admitted to the Infectious and Parasitic Disease Service of the Clinical Center. Her admission to NIH was fortuitous, setting off a chain of events that would ultimately lead to the identification of a new clinical entity caused by an adenovirus.

At the time of her admission and physical examination by Dr. Parrott, the girl coughed in his face. Six days later he developed mild rhinitis, pharyngitis and unilateral conjunctivitis, the exact same signs and symptoms evidenced by the patient. Eight days

after the admission of the of the two-year old, the pediatric nurse who attended her and a seven-year old girl who occupied the other bed in her hospital room also showed manifestations of febrile pharyngitis. With accelerating frequency during the next week, similar cases of fever with rhinitis and pharyngitis occurred in an ambulatory adult study patient and in two ambulatory pediatric patients. Another pediatrician had rhino-pharyngitis without fever thirteen days after the admission of the first patient. Sterile cotton swabs were taken of the posterior pharynx and conjunctiva of all patients and tested for adenoviruses since these were the agents that Huebner's group was then studying. Paired acute (or pre-illness) and convalescent blood serums were collected for complement fixing and neutralization testing for adenovirus antibody. Multiple throat cultures for bacteria were also taken on all patients during the acute illnesses, primarily to rule out streptococcal or other bacterial infections as the cause of the illnesses (10).

Clinical observations in these 8 patients demonstrated illnesses that had the appearance of a distinct nosologic entity with somewhat variable features. Signs and symptoms in descending order of frequency showed pharyngitis, rhinitis, fever for 5 to 6 days, cervical lymphadenopathy and conjunctival inflammation. Three patients had transient liver enlargement accompanied by tenderness in two. The illnesses showed no apparent response to antibiotics. Throat cultures were negative for pathogenic bacteria (10).

Adenovirus type 3 was isolated from 6 of the 8 patients, either from the pharynx or conjunctiva. Seven of the eight patients showed development of neutralizing antibodies against type 3 except for one patient who had an elevated level in the only blood tested (Blood for antibody testing had not been drawn prior to onset of illness).

Complement-fixing antibodies were more variable because these antibodies demonstrated adenovirus group specificity rather than the characteristic type specificity of the neutralizing antibodies. The study thus suggested that adenovirus type 3 was the cause of pharyngoconjunctival fever (10).

In light of these initial results, Dr. Joe Bell, Bob Huebner's constant collaborator, and his epidemiological team undertook the study of several local community outbreaks of pharyngoconjunctival fever. Joe Bell and Bob Huebner had teamed up successfully in studying Q fever in California, herpangina in Maryland, and epidemic pleurodynia in Texas. The opportunity presented itself again to engage in another study and combine epidemiological surveillance with laboratory support to establish the etiology of a specific illness. With the ensuing investigations they were able to establish adenovirus type 3 firmly as the etiology of the disease and to describe its epidemiological behavior in the community (12). Historically, several observers had recognized this entity clinically without knowing its etiology. In 1943 Derrick (20) had described 3 cases of conjunctivitis associated with fever that he thought were probable cases of inclusion conjunctivitis (swimming pool-bath conjunctivitis). The clinical symptoms he described were similar to the three cases observed in the NIH study. In 1953, T.A. Cockburn, an ophthalmologist, (21) described an epidemic in Greeley, Colorado, of a febrile illness commonly associated with conjunctivitis and sore throat that he called "Greeley Disease." Eight paired acute illness and convalescent phase serum specimens from Greeley patients were obtained by the LID. (These were requested by Huebner's laboratory and received through the courtesy of Dr. Morris Schaeffer, Medical Director, Communicable Disease Center, Department of Health, Education and Welfare,

Montgomery, Alabama, and Dr. F.S. Cheever, Professor of Microbiology, Graduate School of Public Health, University of Pittsburgh.) They showed a specific neutralizing antibody response to adenovirus type 3 following recognition by LID of the NIH outbreak of pharyngoconjunctival fever in February 1954. Within a few months on July 23,1954, pediatrician Dr. Frederick G. Burke of Georgetown University School of Medicine, advised the group that he was seeing a number of such cases in Alexandria, Virginia, many of them children who had been attending the Burgundy Farm Summer Day Camp. (Dr. Burke had previously worked with Huebner in the herpangina study.) The researchers followed the trail to the camp where Dr. Thistle McKee, the consulting pediatrician, indicated that many children had been absent with a febrile illness associated with conjunctivitis. The Fairfax County (Virginia) Health Department represented by Drs. Harold Kennedy and Thomas F. McGough (Alexandria) and the camp authorities represented by Dr. Robert Burnham and Miss Constance Bell gladly welcomed Dr. Bell's team's proposal for investigation of the camp outbreak. This represented another example of the welcome and fruitful collaboration between community public officials and NIH at the epidemic locale that had become par for the course for investigations related to Bob Huebner's community studies.

The population studied was divided into groups of illness-related persons and the control persons. The epidemiological study groups were: 1) the Burgundy Farm Summer Camp campers, counselors, maintenance workers and administrative personnel; 2) household members of Burgundy Camp campers; 3) Broyhill area residents; 4) Hollin Hill area residents (the latter two are residential areas in Northern Virginia where campers lived); and 5) sporadic cases. Nine hundred and seventy persons were observed,

(comparable in number to the persons observed in the Texas pleurodynia outbreak). Of these, there were 300 cases of illness from which there were 80 isolations of adenovirus type 3. An additional 59 isolations were made from household contacts and community residents. The standard epidemiological methods employed during the study were similar to those employed in previous community studies. They included case finding, history of illness, examination by physicians where possible, collecting material for laboratory study, e.g. throat swabs for viruses and bacteria, fecal swabs or specimens, acute illness and convalescent phase blood specimens for antibody tests and the collection of similar materials from family and neighborhood contacts of patients and the control population. Following the completion of the laboratory tests and the compilation of the population data, the research group then correlated the virus isolations with the clinical illnesses and compared the results in the control groups who had no evidence of infection or illness.

From this carefully studied outbreak, Joe Bell and associates were able to further conclude (12) that adenovirus type 3: 1) invaded tissues of infected persons; 2) was present in a lesion (throat or conjunctiva) regardless of its presence in other tissues; 3) was not present in well persons, even those who were intimate contacts of a person with the illness; 4) was present almost exclusively during the acute illness and was rarely found before onset or after recovery; 5) was associated with a particular clinical syndrome and not with other common illnesses; and 6) was found in similar illnesses in three distinct outbreaks and in sporadic cases widely distributed throughout the study area. The signs and symptoms previously described were present in varying proportion among the cases studied. Until further studies could be done, it was estimated that one-

fourth to two-fifths of the cases would not be clinically recognizable if seen alone and not in association with typical cases (12).

In addition to providing insight into the specific clinical symptoms associated with pharyngoconjunctival fever caused by adenovirus type 3, this study offered a better understanding of the public health implications and epidemiological patterns of the illness. The researchers concluded further that the disease occurred in localized epidemics and in sporadic form, in all ages but predominantly in children, and in both sexes. The study observations suggested that: 1) infected human beings were a common source of infection; 2) infection generally produced illness (always in this epidemic experience); 3) healthy carriers were not an important source of infection; 4) the disease was highly infectious for young contacts; 5) older persons were more likely than children to be immune and had presumably developed immunity from a previous attack; 6) contaminated swimming pools were a suspected but unproven source of infection; 7) the incubation period was probably 5 or 6 days; and 8)that the period of communicability, as indicated by the presence of virus, decreases from 100% during the first few days of illness to practically zero after the ninth day of illness. Although the disease had been recognized as a clinical entity since Derrick's work in 1943, its prevalence was not known (20). Now, with this new significant study, researchers and the public community understood its high rate of incidence and the populations it was most likely to affect. While no studies were carried out during this investigation on prevention or treatment, the authors gained the clinical impression that various antibiotics would not influence the course of the disease.

Based on this data, Joe Bell and his associates concluded that the clinical, etiological, and epidemiological data had differentiated one disease entity from the poorly defined mass of undifferentiated respiratory illnesses generally known as the common cold, catarrhal fever, non-streptococcal sore throat or acute respiratory disease. Bob Huebner was thus on his way to achieving his goal of further delineation of specific entities from among the mass of nondescript respiratory illnesses. (12).

In roughly the same time frame, Dr. Ernest Jawetz and his colleagues at the University of California at San Francisco were able to identify another clinical entity caused by a different adenovirus type. An investigator at NIH-LID in the late 1940s, Dr. Jawetz had become interested in adenoviruses and the illnesses produced by them. He and his staff were able to isolate adenovirus type 8 from a similar but distinct ophthalmologic infection called epidemic kerato-conjunctivitis. This eye infection tended to be associated with swimming pools and was also common among shipyard workers in Japan. It was characterized by inflammatory changes in the cornea as well as the conjunctiva and by the swelling of the pre-auricular lymph nodes. Dr. Ernest Jawetz (22) isolated adenovirus type 8 from patients using local swimming pools and from United States shipyard workers. The susceptibility of these two disparate population groups was not apparent.

After the discovery in 1954 of pharygoconjunctival fever, ongoing etiological, isolation and serological studies involving the adenoviruses and other respiratory viral agents proliferated. A major investigative program (to be described in the next chapter) would take place at the District of Columbia orphanage, Junior Village, contributing to the etiological significance of adenoviruses. By the end of 1957 a clear picture of the role

of adenoviruses in the causation of human illness had begun to evolve. In addition, other distinguishing features of illness were uncovered (23). By this time, 18 immunologic types had been established, some of which were associated with varying degrees of frequency in illness and infection.

Some of these investigations were carried out in cooperation with investigators at military installations and added significantly to the base of information. Huebner's group published in 1957 some generalized conclusions about adenovirus and illness: 1) Acute respiratory disease (ARD) including pneumonia without cold-agglutinins was highly prevalent among military recruits, occurred in epidemic form and was associated with types 4 and 7, less frequently with type 3; 2) Pharyngoconjunctival fever (PCF) occurred primarily in the general population, occurred in epidemic form, was associated with type 3, less frequently with type 7. Further random observations strongly related summer outbreaks to swimming pools. Other features observed occasionally were pneumonitis in winter and superficial keratitis in summer; 3) Non-bacterial pharyngitis occurred chiefly in infants and young children (most common of the adenovirus infections), was associated with type 3, and less commonly with types 1, 2 and 5. It resembled PCF but without conjunctivitis. Its sequelae included otitis media and pneumonitis in infancy; 4) Non-bacterial conjunctivitis occurred in all age groups, primarily older persons, usually in endemic form, associated primarily with type 3. Older persons tended to have less fever, children tended to have more fever; 5) Epidemic kerato-conjunctivitis had a low prevalence in the United States with a higher rate in Japan, was associated primarily with type 8 (later found with other types), usually occurred in epidemic form in the United States chiefly as an industrial problem (23).

Additional serological studies in various age groups indicated a high prevalence of infections in infants and young children with a lower incidence, as people grow older. Extrapolation to the total population of the United States suggested that adenovirus infection accounted for a substantial amount of respiratory and ocular infection (23).

The path breaking studies conducted by Bob Huebner, his laboratory associates and his collaborators in epidemiology largely defined the clinical spectrum and prevalence of adenovirus infection in the United States. With these studies, he worked toward his goal of defining and differentiating the various respiratory illnesses. He had begun this process by focusing on the clinical characteristics of adenoviruses and finding their various etiologies. Over the course of this process, he refined his methodology and implemented the community-based epidemiological approach, an approach that allowed him to gain a broader understanding of the public health context of adenovirus-produced illnesses. He had developed the fundamentals of the community-based epidemiological approach during community surveys for Coxsackie viruses in 1949-1952. This approach reached its fruition with the pharyngoconjunctival fever community survey. At the same time this important study marked a sea change; it represented one of the last occasions when personnel of the Laboratory of Infectious Diseases of the National Institutes of Health investigated a local outbreak of illness. The nascent Communicable Disease Center (now the Center for Disease Control—CDC) was just beginning in the mid-1950's to investigate clusters of infectious diseases using their Epidemic Intelligence Service (EIS) officers. Huebner would further elaborate his community approach with the Junior Village studies, but these investigations were initialized by Huebner and not at the behest of the US Public Health Service. In collaboration with Joe Bell, Huebner would design a

new approach to the search for additional viral agents by instituting longitudinal survey techniques in the imaginative Junior Village studies. . The type of studies he proposed to follow the adenovirus investigations was the kind that CDC either did not have the capacity or was not equipped to handle at that time. The continuing professional progression manifested by Bob Huebner is indicative of the uniqueness, innovativeness and insightfulness that he brought to the continuing investigation of respiratory viruses.

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Chapter 7

The Junior Village Era

Nearing the completion of the of the major components of the adenovirus studies, Bob Huebner shifted to another potential method of correlating isolated viral agents to the illnesses that they might be causing. In 1955 Dr. Huebner implemented a broader, far-reaching study turning his attention to other respiratory infections. These ubiquitous infections were an extremely important public health problem in the United States, detrimental in their prevalence, morbidity and economic costs. With the aid of Dr. Joe Bell, his long-time collaborator in research, Huebner had the insight to implement an innovative program that charted the longitudinal microbial experience of a nursery group of children in a District of Columbia orphanage over several years during periods of illness as well as good health. Bob selected this group for study because he thought that it represented the population class most likely to be afflicted with many infectious diseases and thus provide the best opportunity to isolate and identify the causal agents both known and unknown. The program was unique in conception and was executed expeditiously by the personnel of the Laboratory of Infectious Diseases under Bob's direction. The ultimate purpose of the study was to provide information hopefully leading to better diagnosis, treatment and public health measures for control.

Bob's impressive continuing research success and growing scientific reputation led to recognition of his intellectual talents and to appropriate promotion within NIH. In 1956 Bob Huebner became Chief of the Laboratory of Infectious Diseases (LID), a position he would hold until 1967. He succeeded the previous Chiefs, Dr. Charles

Armstrong (1942—1948), Dr. Karl Habel (1948—1954), and Dr. Dorland J. Davis (1954—1956) (1). During his tenure as chief, Dr. Huebner relied on a philosophy of research and a set of research principles that served the LID and its associates well over the course of several decades. Huebner's approach emphasized a basic commitment to the fundamentals of public health work: isolating the agents that caused disease by following a logical progression of research steps. While Huebner's philosophy did not ignore or denigrate the new technologies that were coming into play in virology research, he did think that these technologies had their proper place in research. That is, the new technologies should not drive the research or define the research problem—a development becoming evident in other research specialties—but should aid in the solution of the already well-delineated objective. It was this sort of attention to synergy between public health goals, technology and research that enabled Huebner and his associates to have the sort of success they did. Some fifty years later, in a tribute to Huebner's scientific acumen, Dr. Robert Chanock who succeeded Bob Huebner as Chief of LID in 1968 (up to the present), described how these philosophical principles had shaped the positive course of LID and gave a large part of the credit to Huebner (2): "During the past 54 years studies performed by LID scientists have been driven by long-term goals directly related to the issues of health and not by technological advances or changing trends of what was considered fashionable in research. It is remarkable that the previous chiefs of LID resisted the siren call to shift to a reductionist approach when confronted with the dazzling and unending progression of new opportunities for biological insights made possible by technological advances. Despite many temptations to limit study to smaller and smaller models of infection, the research goals of LID have

remained the same, namely: 1) delineation of the etiology, pathogenesis and epidemiology of medically important virus diseases and 2) development of means for their control. This credo for conduct of research in LID was first articulated by Armstrong and Huebner over 50 years ago, and it continues to serve as our compass. An important subtext of this credo is that LID scientists are allowed and encouraged to pursue an infectious disease problem from beginning to end. This means that they must also master most or all of the approaches and technologies required for successful pursuit of such a broad objective." The holistic research focus did not preclude, however, the incorporation of technological advances where beneficial. Rather, Dr. Chanock further noted, the LID was flexible and had readjusted some of its specific approaches in order to accommodate, and make the best use of, technological developments in the areas of immunology, biochemistry, electron microscopy, and molecular biology.

Under Huebner as well as the various other chiefs, the LID tended to follow a policy of function following training: that is, researchers were given tasks that conformed to the extent of their previous training and experience. For example, when Huebner first arrived at LID, he had little laboratory experience. He gained this by working with an established and experienced team learning the rudiments of and the laboratory tools for working with rickettsias, before he moved on to semi-independent, then independent, research projects involving more complex problems. On the other hand, Wally Rowe who had two years experience working at the Naval Medical Research Laboratory, initially under the tutelage of a mentor and then on his own, did not have to go through the same exercise in training. Instead, right away, he assumed the responsibility of setting up the tissue culture system for the Huebner virus unit and monitoring it for evidence of virus

growth. While the chiefs of LID followed the principle of having their staffs learn the basics of laboratory research procedures before moving on to complex tasks, such as those using advanced technology, they also were supportive of continuing education so the researchers could take advantage of appropriate educational opportunities. The Chiefs encouraged and arranged for further continuing education and training in areas of learning that would enhance the professional skills of the LID scientists in working on specific problems oriented to laboratory programs. For example, Dr. Joseph Bell, after he had been at the LID predecessor laboratory for a while, went on to receive epidemiological training and advanced degrees at the Johns Hopkins University School of Public Health; Chief Karl Habel sent Dr. Alexis Shelokov to Dr. John Enders' laboratory at Harvard to learn the technique of tissue culture by the roller tube method which turned out to be an important factor in isolating the adenoviruses back at LID. In addition, investigators were encouraged to take the evening graduate courses given at NIH by the Foundation for the Advanced Education in the Sciences (FAES), and the Laboratory paid for these courses. The chiefs knew what they were doing: this open-minded approach to the continuing pursuit of knowledge was not only beneficial to the research, but it also enhanced the scientists' professional credentials, improved their research skills overall and built ties with research communities.

Huebner, himself, benefited by incorporating technological advances into his own research without making them the primary driving forces. One can trace the progression of his research from studies of relatively little technical complexity to those that relied upon more complex technology. Bob Huebner, Wally Rowe, Joe Bell and their associates pursued the goals outlined in Chanock's review as their studies progressed

using clinical, virological, immunologic and epidemiological approaches. Their basic clinical approach included investigations of community outbreaks, epidemiological surveys, serological surveys for specific infectious agents, initial cross-sectional hospital-based studies (expanded later by Dr. Robert Parrott of the Children's Hospital National Medical Center, Washington, D.C.), the study of experimental infection in healthy adult volunteers, the elucidation of modes of the spread of infections and the development of vaccines where feasible against specific infectious agents. After completing less complex but accurate and controlled observations of the behavior between viruses and their hosts, Huebner's group progressed to more complex studies of viral agents at the biochemical and molecular levels.

The Junior Village era began with the foresight and imagination of Bob Huebner and Joe Bell. The two researchers provided a major impetus to the study of naturally occurring infectious illness with their longitudinal surveillance of defined populations during periods of health as well as disease. They initiated this major undertaking with a survey of the microbial experience of infants and young children in an orphanage and welfare nursery—Junior Village of Washington, D.C.—when the children were well and when they were sick. The study was unique in the length and intensity of the clinical observations by physicians and nurses, the numbers of laboratory specimens examined, the discovery of new viruses and new serological types of previously recognized viruses and the observations of the patients while they were well. This prolonged study established in 1955 provided a treasure trove of viruses and other microorganisms. With due apologies to Robert Louis Stevenson, Junior Village was a veritable "Child's Garden of Viruses" and bacteria.

Intense activity characterized the first few years of the study, with intermittent and diminishing activity toward the final years in the 1960's. An extensive range of publications was produced over the span of a little over a decade, documenting the isolation of numerous new viruses. The most comprehensive and detailed account of the study was published early on, covering the first three years (1955-1958). This classic manuscript by Dr. Joe Bell and his collaborators (3), *Illness and Microbial Experience of Nursery Children at Junior Village,* provides, from the epidemiologist's point of view, the best overall and definitive description of, the methods the researchers used and their findings. From this publication, the reader can gain a good understanding of the work and what it meant to be involved in the study, during its initial and most active stage. Joe Bell's collaborators on the manuscript included Bob Huebner, Drs. Leon Rosen, Wally Rowe, Roger Cole, Francis M. Mastroda (pediatrician), Thomas M. Floyd, Bob Chanock and Nurse Ruth Anderson Shvedoff. The authors acknowledged with thanks and appreciation the help of the many additional professional, technical, clinical and administrative personnel who assisted in this initial comprehensive study. These individuals not only provided invaluable contributions from the beginning, through the first years of the study, but also for an additional 5 to 6 years beyond the period covered by this comprehensive publication. The principal investigators worked harmoniously with the institution's nurses, members of the District of Columbia Welfare Department and the medical and supervisory staffs at Junior Village. (The following in brackets is to be put in a footnote.) [They were especially grateful to Drs. James H. Johnson, A. Martin Lerner and Janet W. Hartley and to nurses Isabel Child and Christine Cummings for professional assistance; those providing technical and statistical assistance included Mrs. Joan Austin,

Mrs. Lotta Chi, R.B. Clark, HMC, Mr. Lee Cline, Mrs. Ermine Compton, Mr. John D. Estes, Mrs. Janet Hovis, Mr. Norman Ikari, Mr. Walter James, Mr. Jerome Kern. Mr. William Lane, Mr. Richard Lynt, Miss Dorothy Moore, Mrs. Barbara Neal, Miss Edythe Rose, Mrs. Shirley Shifflett, Mr. Horace C. Turner, Mr. Richard Whitt, Mrs. Doris Wong and Mrs. Myra Wormald. The authors also appreciated the cooperation extended by the District of Columbia Welfare Department that provided access and particularly the medical and supervisory staffs at Junior Village under the direction of Dr. Jack Kleh and Mr. William Stone]

Bell and his co-authors designed this massive undertaking to try and improve knowledge about the still undifferentiated acute respiratory diseases, including the common cold and acute fevers that had no recognizable signs in the nose, throat or lungs. They gathered statistics documenting how persistent and dangerous a major public health problem these infections were. According to various contemporary studies done in the years prior to the Junior Village Study, (3), such illnesses had been occurring on an average of 2—6 times per person per year in certain locales (3). An U.S. National Health Survey taken around the same period showed that acute respiratory diseases were a major cause of disabling illness (3). It was clear to Bell, Huebner and their colleagues that a new innovative study was required in order to garner additional information that might help reduce the impact that these illnesses had on public health and, ultimately, the economy.

In the few years prior to the onset of the study, newly developed laboratory methods (e.g., the use of the suckling mouse, tissue culture techniques, with their menu of various cell lines, and new culture media) had enabled identification of many hitherto

unrecognized viruses that were potential causes of diseases. Relying on these methods, Huebner and Bell approached the problem of prevalent acute respiratory infections by initiating in 1955 the comprehensive, long term, clinical, epidemiological and laboratory study of acute infections and illnesses as they occurred naturally in nursery babies at Junior Village. The study objectives were: a) to observe the natural occurrence of acute illnesses and infections as they spread throughout this limited group of normal children, b) to find new infectious agents and to determine which agents were causing acute illness and c) to develop methods for disease control.

The question of informed consent in pediatric populations in institutions has been a sensitive one in the past. In order to escape problems engendered by the lack of appropriate informed consent, the investigators made every effort in this study to explain its purposes and procedures to the appropriate, responsible persons in the District of Columbia Welfare Department. The children were wards of the D.C. Court system and after being apprised of the researchers' intent, the Court allowed the studies to proceed (9). Given the legal status of the orphans, the investigators followed the proper and most expeditious approach in allowing themselves to start their study. Definite advantages also accrued to the subjects of the study. In exchange for submission to regular physical examinations and provision of specimens for laboratory evaluation, the investigators made arrangements for full time medical care for the infants and toddlers including careful periodic observations during times of illness, therapeutic intervention, such as antibiotics when appropriate, and approved pediatric immunizations. The study time probably represented the period in their lives when the children received the best and most sympathetic medical care.

The Institution

Junior Village provided an ideal location and institution for the study. It was maintained by the District of Columbia's Department of Public Welfare to provide domiciliary and other care for children who had no parents or guardians or whose family situations were incompatible with appropriate child-care. Other institutions, not Junior Village, housed children who had been deemed mentally retarded or deficient, children who were ill and needed convalescent care and those determined to be "juvenile delinquents." Unlike those children, Junior Village children were considered "normal" except that they represented that portion of the general population of Washington, D.C. with unstable or tragic family situations that precipitated homeless children. Children were discharged to parents, guardians or foster homes as soon as conditions warranted, but some spent most of their childhood at Junior Village.

When the study began in 1955, Junior Village cared for some 250 children, approximately 50 of whom resided in the nursery that constituted the study group. In February 1956, the nursery group moved from the western part of the city to better facilities in the southern part, and the older children moved in the fall. At both the old and the new locations the institution was situated on a large tract of land and utilized city water, sewage and other public services. The living arrangements constituted a more or less independent local community since an elementary school, recreation, sleeping, dining and church facilities were maintained on the premises. Although older children had the opportunity to associate with other children outside the institution while attending high school and summer camps, the nursery children had few direct contacts with outside children.

The Study Group

Drs. Bell, Huebner and associates selected the Junior Village nursery group for study because it was subject to a continual turnover and was housed in somewhat crowded facilities that were conveniently available for intensive clinical and laboratory observations. They wanted to observe a group of normal babies at the time of their first infection with commonly occurring microbial agents, and, for economy of time and effort, it was desirable that the members of the group have a high incidence of such infections. The nursery children (6 to 35 months of age) who constituted the study group lived and ate apart at both locations (the old and the new). At the old location, the nursery children had resided in the administration building but in the new location they resided in a separate two-story brick building. In general, crib infants and creepers slept in one dormitory and toddlers and runabouts in another, both rooms were joined by an open hallway, and playrooms, toilets, wash rooms, kitchen and dining facilities were common to both groups. Each dormitory covered 700 square feet and contained 26 cribs together with other nursery furnishings. They were so crowded that almost all cribs touched each other side-to-side or end-to-end. Frequent overpopulation necessitated that two children often slept together in the same crib. Despite the overcrowding, the researchers and their public health counterparts felt that, overall, the sanitation was good, and that toilet, bathing, playing and eating facilities were adequate

The D.C. Welfare Department was entirely responsible for routine custodial and medical care. A chief counselor and four assistants bathed and fed the nursery children, prepared their formulas, assisted in other food preparation, changed diapers and did laundry until May 1957 when a laundry service became available. Volunteer workers and

older children occasionally assisted in the care of the nursery group. These homeless children had a serious need for tender loving care that was important in the maintenance of nutrition and emotional stability. However, the counselors had large numbers of babies assigned to them, and they had neither the time nor the resources to provide the attention and love these children desired. According to several of the physicians (3) who attended the children, the young residents of Junior Village, while making plaintive noises, would hold on tightly to the medical attendants hoping to seek attention and affection.

The counselors kept records that included daily notes on signs of illness such as fever, anorexia, diarrhea, insomnia, and irritability. In the early part of the study, a Welfare Department physician visited 1 to 3 times weekly and when called by the chief counselor who was a registered nurse. Beginning in September 1956, one of the authors, a pediatrician (Francis M. Mastrota), was employed part time by the Welfare Department for daily medical care of the nursery group. At the old location, ill children had been maintained in their domicile, as there were no hospital facilities available on the premises. At the new location, an infirmary was maintained in a separate building on the grounds. At both locations, children with serious illnesses were sent to D.C. General Hospital or to the NIH Clinical Center for medical care or for special study.

Dr. Bell calculated the occupancy rate and the demographics of the nursery group with great accuracy. A total of 587 children, some with re-admissions, resided in the nursery group during the 156-week study period. The mean population per week was 52.7 children; the median was 53, with some variation when some weeks had more or some less than 50 children. The population tended to be largest in the winter and spring months—months when illnesses proliferated and thus good for tracking. The 479

children had 8224 child-weeks of residence in the nursery during the study period. The mean duration of residence per child was 17.2 weeks; the median was 12 weeks and 25% of the children remained in residence for less than 5 weeks and 25% stayed for more than 23 weeks. At the time of admission 48% of the children were female, 79% were African-American and the age distribution was fairly uniform by months of age: 21% were 6 to 11 months of age and 43% and 36% respectively were 1 and 2 years of age. The ethnic distribution of the nursery group at the time closely followed the ethnic distribution of children in the District of Columbia. In 1958, the Department of Education reported that 78% of District of Columbia school children were African-American. The mean duration of residence for white males was 6-8 weeks, white females 16.5 weeks, African-American males 17.1 weeks, and African-American females 20,9 weeks. This tendency for white babies and male babies to have shorter periods of residency at Junior Village influenced the proportionate distribution of the number of child-weeks observed so that an average of 85% were African-Americans and 55% were female. The age of the study group was also an important epidemiological factor. However, the researchers resorted to estimating the ages of about one-quarter of the children because birth records were incomplete. Occasionally the children were slightly younger or older than the 6 to 35 month age limit for the nursery group.

In brief, the study group consisted of some 50 babies living together as an epidemiological unit in such close association with one another that there was abundant opportunity for spread of infection. There was also ample opportunity for the introduction of infection from outside the institution through the continual flow of newly admitted children, visitors, counselors, volunteer workers, attendants and study personnel. Within

the institution the older children could also introduce infection when they visited the outside dispensary which was adjacent to the nursery (but maintained separately) in both the old and new locations. The older children were used occasionally to help care for nursery children and when they were hospitalized simultaneously with ill nursery children in the infirmary.

Clinical Procedures

Through the courtesy and active cooperation of the Welfare Department, the Laboratory of Infectious Diseases study was superimposed on the custodial and medical care functioning of the institution. The Welfare Department's medical observations of the children were utilized by the study, and study personnel supplemented these observations with routine clinical, epidemiological and laboratory examinations. After the study was well underway, unexpectedly high rates of virus infection were found making it necessary to increase the intensity of the clinical and laboratory observations. These changes were accomplished gradually during the first half of the three-year study so that the intensity of the observations was fairly constant throughout the last half. Clinical observations of the children initially, performed three times a week, gradually increased to daily examinations. The study staff was gradually increased so that by September 17, 1956, a full time pediatrician, a registered nurse, 3 nurses aides, and 2 part time nurses maintained daily observations on all children both ill and well. They made an effort to describe anything that looked like a departure from normal health and to denote the time of occurrence.

The health monitoring was extensive so that the researchers could be alerted to an outbreak of infection. One way of doing this was to monitor changes in the children's

temperature, a reliable sign of infection, rather than to rely solely on non-specific signs in nose, throat or lungs. During the early days of the study rectal temperatures were taken only on children with definite or suspected illness, but by 1956, temperatures were taken on all children in order not to miss any episodes of infection that would otherwise escape detection. The study also began to use the new stainless steel probes, which were much more accurate and sturdier than the standardized clinical mercury thermometers made of glass. Of course, special care was taken to clean and chemically treat thermometers to prevent the spread of infection, and cultures from the thermometers were taken during epidemics, as well as more routinely, in order to insure that that there had been no viral or bacterial contamination. In as much as clinical thermometers had been recognized in the past as a source of contamination, this sort of quality control was an absolutely crucial key to the success and reputation of an etiological study of this nature.

The staff provided diphtheria, tetanus and pertussis (DPT) vaccine routinely to all the children in 1956, and, by 1957, they were administering poliomyelitis virus vaccine (the new killed Salk vaccine). At the time, the Academy of Pediatrics Committee on Immunization Practices routinely recommended these vaccinations. The children might not otherwise have received the vaccines had they not been at Junior Village. A controlled trial tested the effects of oral benzathine penicillin on the prophylaxis of streptococcal sore throat. The study group (staff) performed controlled studies with two adenoviral vaccines, inactivated measles vaccine and a Coxsackie B pentavalent vaccine with unpublished results. The adenoviral and Coxsackie vaccines had been developed as the fruits of Huebner and Bell's research with these agents.

Specimen Collection

In order to capture as many different agents as possible from several body sites, the researchers engaged in a massive collection of specimens in order to try to correlate the isolation of a pathogen with the illness that it might be causing. This was a very elaborate undertaking, and it is informative for the reader to have a sense of the incredibly detailed record keeping and scheduling necessary to make the study successful. Without careful and highly controlled procedures, the efforts would have failed from the beginning. Instead the researchers gained from the successful implementation of the clinical and laboratory procedures a large mass of data that enabled description of illnesses and infections according to age, sex, race distribution and their temporal interrelationships.

The staff collected throat and anal swabs from all children, ill and well, once a week (Wednesday) and at the time of onset of all definite and suspected illnesses. They looked for viruses and bacterial pathogens and tried to determine when the onset of infection occurred and with what infecting organism. In 1957, specimens were also collected with a frequency that would allow researchers to determine how soon a child would begin shedding a microbial agent after admission to an infected environment.

The specimen collection did not stop there—in a few special studies, throat and anal specimens were collected daily through the course of an illness and occasionally specimens were collected from the lesions of conjunctiva, ears, nose, lips and skin. Blood specimens were collected routinely at the time of admission, 6 weeks after admission, each 3 months thereafter and at the time of discharge if the children had not

been “bled” recently. This schedule was adjusted throughout as the needs of the various special studies changed.

Laboratory Procedures

The laboratory specimens were handled by the usual microbiologic and immunologic methods employed then in the laboratory to look for viruses, bacteria and antibodies. The attention given to timely processing and proper storage was closely managed: throat and anal specimens were placed in airtight bags and frozen within 15 minutes of collection in a portable dry-ice box. Cultures were transported, inoculated and incubated within four hours of collection, or, if this was not possible, then stored under appropriate environmental conditions. Blood specimens were also kept and stored under tight controls. Over the course of the survey differing culturing media, including tissue-culture maintenance fluid-199 and Hanks’ balanced salt solution were used depending on which worked best. HeLa cells and rhesus monkey kidney cells were used for culturing. The immunologic techniques of hemagglutination, complement fixation, and neutralization were used to identify viral agents and serological tests were made on a specific schedule.

In summary, the above clinical and laboratory procedures provided a large mass of data that enabled description of illnesses and infections according to age, sex, race distribution and their temporal interrelationships. The clinical and laboratory observations, however, yielded quite a complex mass of data. For example, the children experienced numerous irregularly grouped days of fever that made it difficult to specify time periods of illnesses. They also exhibited overlapping infections, making it difficult to separate out the date. Hence, in order to draw any epidemiological conclusions, the

illnesses and infections had to be studied independently of each other using objective criteria that during any given week were applied uniformly to all children. This required very complex methodologies, to say the least.

The Results

The intensity of the study observations and the continual flow of new children into the nursery contributed to finding a surprisingly large number of illnesses and infections. On the average each child had one new febrile illness every three weeks and a new bacterial or virus infection every 2 to 4 weeks; such prevalence, of course, was not ideal for the children, but it meant the researchers had significant data to analyze! During the course of the study, it yielded nearly 60 immunologically different viruses many of which were heretofore unrecognized or unclassified. The researchers correlated at least 10 virus serotypes associated with illness, helping to define the probable etiologic role of these various viruses. Studies on the prophylactic value of new vaccines and antibiotics were initiated.

Dr. Bell's report described the general observations on the occurrence and the temporal relationship of illnesses and infections during the first 3 years of the study from July 3, 1955 to June 28,1958. Many other reports, to be enumerated later, described more detailed observations on specific microbial agents, their role as causes of disease, the clinical nature of such diseases and the effectiveness of efforts directed toward disease control. A 6-month preliminary study (3) showed that viruses were isolated more frequently from ill nursery children than from ill older children, and the nursery children had higher attack rates from acute febrile illness than older children in the institution and higher than children of the same age group in the general population.

The high incidence of illness and infection found during the three-year study exceeded expectations to an extent that overlapping infections and illnesses complicated the analysis and presentation of results. On an average, each child was found to have a new infection with a different virus or bacterial serotype every 2 to 4 weeks, and this represented a surprisingly large number in view of the fact that many other unrecognizable infections undoubtedly occurred that were not identified because: a) adequate procedures for isolating a number of microbial agents such as group A Coxsackie viruses were never used routinely during the study, b) procedures for isolating myxoviruses were used routinely only during the latter part of the study, c) routine procedures for collecting, storing and laboratory testing of specimens on a mass scale could not be 100% efficient for every agent, and d) serological studies had not been completed, and infections which might be identified exclusively by antibody studies were not included in the report (3). During the latter part of the study when observations were most intense, some 4000 laboratory specimens per month were collected and tested for viruses and bacteria. The authors expanded their scope to include the general epidemiology of the illnesses observed, the general epidemiology of the infections and the relationship between illness and specific infection.

The authors further summarized their findings and experiences with these results that confirmed their anticipation of the high rate of illness that they expected from the study. Aside from the 107 cases of measles and 102 cases of chicken pox, departures from normal health were identified on the basis of fever >100.6F and were classified as questionable fevers (A) and definite illnesses (B or C) depending upon the height and persistence of fever and occurrence of associated clinical manifestations. The mean

weekly definite illness (BC) attack rate was 21.5 per cent. It varied considerably from week to week and varied slightly by season but was nearly uniform by age, sex and race. However, the rates for definite illnesses (B), i.e., definite fevers without associated clinical findings, increased during the early part of the study as the intensity of observations increased whereas the rates for definite illnesses (C) remained relatively constant throughout the study. The attack rates for definite illnesses, both B and C decreased with duration of residence in the nursery probably indicating increased immunity to infection, a situation that is also expected in the general population.

The high incidence of infection was illustrated by the laboratory isolation of a total of 1,718 and 2,307 child-infections with 55 virus and 57 bacterial serotypes respectively. The infection rates were similar by sex but differed by age and race for different infections. Adenovirus and enteric bacterial infection rates were notably high in the very young, probably indicating diminished immunity in the young against these agents. As noted above, each child averaged a new bacterial or virus infection every 2 to 4 weeks, and many unrecognized infections undoubtedly occurred. At least 10 new virus sero-types were discovered in these studies, and others first found elsewhere (3) were classified as a result of these studies, a major advance in virological discovery accomplished by this study.

There was a significant association between acute febrile illness and infections with adenovirus types 1, 3, and 5, myxovirus influenza A2 and parainfluenza types 1 and 3 (hemadsorption types 2 and 1, respectively; to be described in the following chapter) (3), poliovirus type 2, Coxsackie B virus type 3, Shigella sonnei phase 1, and group A beta-hemolytic streptococci types 4 and 12, and one or more of types 1, 2, 5 and 23. The

authors found these associations by using the mass of undifferentiated, definite illness rather than clinical entities, with somewhat arbitrary end-points and without using the as yet incomplete antibody studies that they thought would help eventually to determine whether the times when an agent was first isolated indicated the time of first infection. While these associations suggested an etiological relationship, Dr. Joe Bell, never one to make rash conclusions, was hesitant, at the end of 1958, to ascribe definite etiological significance to the association of illness with specific viral types until he had irrefutable proof—which he would have several years later.

From the mid-1950's till late in the 1960's the collaborating investigators wrote a series of important manuscripts. Utilizing the data that they obtained from the Junior Village studies, they delineated, defined and associated previously and newly discovered viruses with specific clinical entities (4). They described how, through this lengthy and expansive study, they had been able to isolate three new parainfluenza viruses. They were also able to characterize their biologic characteristics and to establish their association with illness (4). These isolations of new viruses and their identification with specific illnesses helped establish etiologies for large numbers of previously unidentified and undifferentiated viral respiratory infection, one of Bob Huebner's continuing primary objectives. Many new adenovirus serological types were also found during these studies, again expanding the menu of strains of this virus group that was a major cause of respiratory infections (4). Wally Rowe, who was also one of the three co-discoverers of cytomegalovirus (from adenoid tissue) (4), was able to isolate cytomegalovirus from the mouth and urine of children in the study and correlated these isolations with symptoms and clinical laboratory data (4). With the use of tissue culture methods, the laboratory for

the first time was able to isolate and describe many new serologic types of ECHO (enteric cytopathic human orphan) viruses (4), and, during the course of these studies, Leon Rosen was able to separate out similar and related types which were classified into a new group labeled REO viruses (4). Serological methods, including hemagglutination-inhibition and complement fixation were further refined to help facilitate classification and identification of some of the above agents (to be described in the next chapter) (4). Bob Chanock, who co-discovered respiratory syncytial virus (6), was able to extend his investigations of this agent utilizing the data obtained from the Junior Village studies and later in his extensive collaboration with Bob Parrott at Children's Hospital (4). (The studies on cytomegalovirus, ECHO and REO, and respiratory synctial viruses are all described in the next chapter).

In addition to overall supervision of the increased laboratory effort and recruitment of new professional personnel during the years from about 1953 to 1958, Bob Huebner oversaw the major changes of the interior structure of Building 7 on the NIH campus undertaken to accommodate the explosive number of specimens for analysis. Starting with the extensive epidemiological studies of the adenoviruses, followed by the profusion of laboratory tests associated with the Junior Village investigations, many of the animal facilities were disbanded, and their former space became occupied by thousands of tissue culture roller tubes along with their attendant apparatus. Deep freeze chests were crammed into the rest of the available space. At the same time, in addition to the space required for Wally Rowe, space had to be provided for other new, young investigators recruited by Bob Huebner to help with the ever-burgeoning workload. Bob Huebner made brilliantly intuitive decisions in recruiting the talents of Dr. Janet W.

Hartley (then a graduate student), Dr. Albert Z. Kapikian, just out of internship, Dr. Robert M. Chanock, a young but established investigator who had trained in the laboratory of Dr Albert Sabin, and Dr. Leon Rosen, who was forever grateful to Bob Huebner for providing him space (formerly occupied by a chimpanzee) when no other senior investigators in NAIAD would accept him (5). Dr. Robert Parrott spent several years from 1952 to 1954 in Building 7 until the opening of the NIH Clinical Center where he stayed until 1957 when he returned as Chairman of the Pediatrics Department at Children's Hospital (now the Children's National Medical Center of Washington, D.C.) and Head of the Research Department)

The LID-NIH continued observations sporadically at Junior Village during the 1960's, but because of sociological and political considerations, abandoned working (9) there when the institution gradually deteriorated from the above causes and from physical decay. Further studies became impossible in 1968 when the nursery group children started being transferred to foster homes following administrative action of the District of Columbia government (9). After that, the sociological and physical deterioration of the facility progressed, culminating in a series of scathing exposes by investigative reporter, Aaron Latham (7). These appeared in The Washington Post newspaper in late 1970 and early 1971. Morale was poor among the Junior Village staff members who were frustrated with working conditions and the restraints upon enforcing discipline. There was also rampant drug abuse among the older children and fear of homosexual assaults upon other children. Congressional hearings conducted by Representative Andrew Jacobs (Democrat—Indiana) recommended that Junior Village be closed down. In September 1971, the District of Columbia Council ordered the gradual closure of Junior Village. It

finally closed September 1, 1973, and the remaining children were moved to group homes (8).

In summary, the first three years of the Junior Village era provided a watershed in the clinical, etiological and epidemiological study of respiratory (virus) infection by using the model of the experience in a controlled cross-sectional population of a group of nursery children. The study indicated the massive number of recurrent respiratory illnesses in a closed childhood group caused by the closeness of physical association and the exposure to multiple different viral (and bacterial) etiologies. By extrapolation, this could also explain the frequency and ubiquity of respiratory infections in the general population where younger children experience frequent infections that diminish with age as the children develop immunity to respiratory pathogens they have encountered previously. Many new viruses were isolated during the course of the study, and the investigators elucidated many of their characteristic features. With Junior Village, Bob Huebner and Joe Bell developed an epidemiological model different from the model of their previous Coxsackie and adenovirus community studies. Once again, they demonstrated how carefully controlled studies were necessary to assign virus etiology to clinical syndromes.

Notes—The Junior Village Era

1) Note 5—Coxsackie Viruses—Herpangina.
2) Note 1—Adenoviruses.
3) Bell, J.A., Huebner, R.J., Rosen, L., Rowe, W.P., Cole, R.M., Mastrota, F.M., Floyd, T.M., Chanock, R.M. and Shvedoff, R.A. 1961. Illness and microbial experiences of nursery children at Junior Village. *American Journal of Hygiene* 74: 267-292.
4) See the chapter on The New Viruses
5) See the chapter on The Lieutenants.
6) Chanock, R.M., Roizman, B. and Myers, R. 1957. Recovery from infants with respiratory illness of a virus related to chimpanzee coryza agent (CCA). I. Isolation, properties and characterization. *American Journal of Hygiene* 66:281-290
7) Series of articles by Aaron Latham and Editors, *The Washington Post*. December 1970, January 1971, February 3,6,7,21,25, March 1, 1971.
8) Judy Luce Mann, *The Washington Post*, August 30, 1973.
9) Robert M. Chanock—Personal Communication

Chapter 8

The New Viruses

The vast number of isolations of viruses made at Junior Village during the intense epidemiological analysis of the years 1955-1958 (17) and beyond included many previously unknown and unidentified agents. These agents were not really new in terms of recent evolutionary origin; rather, they were new by virtue of their isolation and identification by the new tissue culture methods and other diagnostic techniques that had become available for their isolation and growth. The investigators not only identified a range of new viruses, they also began to construct evidence suggesting the tentative etiological associations between these viruses and various illnesses based on the mass of clinical data gathered in the initial three years of the study. While at first the exact relationship could not be conclusively determined, later studies provided the missing links. Additional Junior Village investigations, as well as other hospital-based studies, helped clarify the clinical spectrum caused by the newly described viral agents and helped establish them as the causes of specific, usually respiratory, infections primarily of children. The whole process of recognizing outbreaks, isolating viruses and connecting them to particular symptoms and illnesses was a complicated one. For example, many of the isolations actually occurred during the period of intense surveillance in Junior Village, but it took several years for the investigators to analyze the data, make the identification and then prepare and publish manuscripts on their findings.

The following describes the events involved in the identification and classification of the viruses that Huebner's research group uncovered. The history and information are

provided because they are compilations of data not readily available in a single source. The information also provides some insight into the virological methods and processes available to Huebner and other investigators in this period.

The Parainfluenza Viruses *(1)*

CA Virus

One of the most significant and interesting discoveries occurring around 1957 was the recognition that the investigators had isolated "CA Virus" from the Junior Village specimens. CA virus was a recently discovered agent; it was the second of the four *Parainfluenza* strains that were to be isolated eventually from among the Junior Village specimens. The CA virus was the first recognizable parainfluenza strain isolated from humans. During the autumn of 1955 Bob Chanock, before he came to NIH, had isolated a virus producing an unusual cytopathogenic effect, i.e. the formation of sponge-like syncytial areas, in monkey kidney tissue culture from infants with croup in Cincinnati (2). Shortly thereafter, Beale and co-workers had isolated a similar virus in HeLa cells (a human cell line in use in many laboratories) and human amnion cultures, antigenically indistinguishable, from infants with the same disease in Toledo, Ohio (6). These agents had been designated as *CA virus- croup associated virus.* The properties of the virus placed it in the myxovirus family. The virus also showed temperature dependant reversal of the hemagglutination reaction with chicken red blood cells. Although Chanock's original clinical studies (published in 1956)(2) had shown development of antibody and isolation of the virus in the sick infants, the etiological association between virus isolation and illness was thought to be only suggestive at that time. It was only later (in 1963) that

Parrott and Chanock would describe the etiologic association based on their studies at Children's Hospital (1).

The parainflenza viruses were described in the original manuscripts as *"Hemadsorption viruses 1 and 2" (1,3,4,5,7).* They were classified after discovery by the investigators (1,7) as members of the myxovirus group on the basis of the following biological, biochemical, and physical characteristics (1,7): a) hemagglutination of fowl red blood cells, b) association of the receptor-destroying enzyme with the virus, c) destruction of red cell receptors by RDE (the receptor destroying enzyme of Vibrio cholera), d) the removal of normal inhibitor from serum by RDE or periodate treatment, e) growth in the amniotic cavity of the hen's egg, f) size of 80 to 150 mμ, g) ether sensitivity and h) stability at –70C.

At the time of the original description of the hemadsorption viruses (3,4), the myxovirus group included influenza A, B, and C, mumps, Newcastle disease and fowl plague virus. The influenza viruses were the only members of the group known to cause human respiratory illness. As a result of Bob Chanock's isolation of the "CA" virus in 1955 (2), and the recovery of three additional types of hemadsorption viruses during the Junior Village studies four, new etiological agents recovered from persons with respiratory illness were classified as members of the myxovirus group (1). The investigators found that the new viruses propagated less well than influenza on primary isolation in the amniotic cavity of the hen's egg, and tissue culture appeared to be a more sensitive system for virus isolation.

Hemadsorption (Hemagglutination-Adsorption) Viruses

The new myxoviruses in the Junior Village studies were detected by a screening technique, called hemagglutination-adsorption (hemadsorption), developed by Mr. John Vogel (3,4) and Dr. Alexis Shelokov of the Laboratory of Infectious Diseases. Tissue cultures containing monkey kidney cells that had been inoculated with possible infectious agents were overlaid with a suspension of guinea pig red blood cells. The appearance of a pattern of red cell agglutination on the tissue culture cells typical of myxovirus suggested that it was that virus which was growing in the culture. This test was able to detect early infection with influenza virus or other myxoviruses with or without cytopathogenic changes in the tissue culture cells. This hemadsorption technique, used primarily as a screening procedure, helped in the isolation and serologicical differentiation of the three new hemadsorption types found during the Junior Village studies.

The Committee on Viral Nomenclature (1B), composed of prominent virologists including Bob Chanock, at the suggestion of Bob Chanock, classified the CA virus and the hemadsorption (HA) viruses as *parainfluenza virus* with the following designations: a) Sendai (Japanese strain closely related to HA-2); HA-2 Myxovirus parainfluenza 1 (parainfluenza 1); b) CA virus, Myxovirus parainfluenza 2 (parainfluenza 2); and c) HA-1: Myxovirus parainfluenza 3 (parainfluenza 3). Later, in the Junior Village studies, a fourth type isolated by Dr. Karl M. Johnson and associates (5) was designated Myxovirus parainfluenza 4 (parainfluenza 4).

More definitive information regarding the infectious role of the parainfluenza viruses (HA 1and 2) came with the occurrence of several epidemics in the winter of 1957-1958 at Junior Village when virus isolation was correlated definitely with febrile respiratory illness including croup, bronchiolitis, bronchopneumonia, rhinitis and

pharyngitis (1). The extensive cross-sectional hospital-based studies carried out by Bob Chanock and Bob Parrott, with the epidemiological oversight of Joe Bell, in the late 1950's and early 1960's helped establish the etiological association between the parainfluenza viruses and respiratory disease. In a 1962 publication (7) they stated that parainfluenza viruses were associated with a minimum of 6 to 19 per cent of respiratory tract illness in children. Parainfluenza 1,2 and 3 viruses might be found in mild rhinitis, pharyngitis and bronchitis but the more severe parainfluenza 1 and 2 infections seemed to be associated with bronchopneumonia, bronchiolitis or croup. Parainfluenza 1 and 3 infections occurred in all seasons in each year. A vast majority of adults have been infected at least once with each type. A child or an adult might be re-infected with the same agent but the presence of antibody prevented severe illness and higher levels seemed to lessen the likelihood of infection. Parainfluenza type 4 seemed to produce only mild illness (5). They theorized that an antigenically potent vaccine could prevent much serious respiratory illness in children, and, frequently administered, might even reduce the mild "colds" that result from re-infection. The production of an effective multi-valent vaccine to prevent the incidence of the majority of severe respiratory infections including the so-called common cold has still not been accomplished.

Respiratory Syncytial Virus

Another agent that played a prominent role in the research activity of the Huebner group, especially from the late 1950's through the early 1960's, was the *Respiratory Syncytial Virus.* Morris, Blount and Savage (8) first isolated this agent. They studied an outbreak of coryza (cold-like illness) in a colony of chimpanzees held under observation for 3 to 24 weeks prior to the onset of illness. They cultivated a virus which they

designated “CCA” (chimpanzee coryza agent), using Chang liver tissue culture, from one of 14 affected chimps; the remaining 13 animals developed antibody during convalescence. When susceptible chimpanzees were inoculated intra-nasally with tissue culture virus, coryza was observed after a three-day incubation period. They gained additional insight when they found that a laboratory worker who was developing coryza demonstrated a rise in antibody without isolation of virus. The investigators strongly suspected that this virus was of human origin, and that it produced infection when introduced into a susceptible population of chimpanzees by an infected human.

In 1956 and 1957 Bob Chanock and his co-workers at Johns Hopkins University, (9) made a related discovery during a study of lower respiratory illness in Baltimore infants. They recovered two agents that were biologically and antigenically indistinguishable from CCA virus. Syncytium (a sponge-like group of nucleated cells with confluent cytoplasm) formation in tissue culture was the characteristic cytopathogenic change; the virus was unrelated to other agents that produced similar changes. In view of the association with infant respiratory disease and the characteristic cytopathogenic change in tissue culture, Bob Chanock suggested changing the name of the virus from the restrictive CCA designation to *Respiratory Syncytial Virus (RSV)(9).* These early studies in Baltimore in 1956-1957 showed provisional evidence that the virus caused pneumonia in infants, but that conclusion could only be tentative because only a small number of patients had pneumonia (10).

The spadework dug on RSV by Dr. Chanock previous to the Junior Village study helped Dr. Huebner’s group. During the first three years of Junior Village (1955-1958), isolation of RSV did not occur. Bob Chanock did not arrive at NIH until July 1, 1957. In

November 1961, however, Dr. Albert Kapikian and associates (11) reported on the isolation of RSV from an outbreak of pneumonia in Junior Village that had occurred from April 24 to May 21, 1960. By this point in time, Dr. Kapikian was able to rely on the use of the Hep-2 cell tissue culture line, instead of monkey kidney (MK) cells, which increased the sensitivity of viral isolations. Studies by the LID group (13) indicated that although RSV did not hemagglutinate or grow in chick embryos, it had other biochemical and physical characteristics suggesting that it belonged to the myxovirus group. In addition, the group was able to follow up on Chanock's suspicions, and ascertained that there was a positive correlation between the presence of pneumonia and viral isolation in the outbreak.

The occurrence of the outbreak in Junior Village prompted Chanock and Parrott to look for RSV in Children's Hospital. In the late 1950's and early 1960's Bob Chanock and Bob Parrott collaborated with Joe Bell on cross-sectional hospital-based studies in which they evaluated the prevalence of RSV among patients admitted for lower respiratory tract febrile illnesses (12,13). They showed that during each year of the study RSV infections increased in the winter and were accompanied by antibody production. They were also able to delineate the clinical pattern of bronchiolitis (obstructive infection of the smaller airways) in young infants and bronchopneumonia in older infants. In addition, they observed that RSV could re-infect children even in the presence of serum antibodies (12). This was similar to the situation with parainfluenza. Their other studies in volunteers produced evidence of mild coryzal illness and re-infection even in those who possessed pre-existing antibody (14,15).

Bob Chanock and Bob Parrott continued their collaborative studies of respiratory viruses for about 20 years (16) utilizing the resources of NIH and Children's Hospital. Bob Chanock has maintained a major interest in the investigation of RSV up to the present including the investigation of vaccine development.

ECHOviruses

A continuing staple among the viruses isolated consistently in Junior Village was the *ECHOvirus* Group. In a 1961 manuscript, Dr. Joe Bell and associates (17) listed 25 serotypes of these viruses, 4 of which were newly discovered among the Junior Village patients. The "ECHO" designation is an acronym for "Enteric Cytopathic Human Orphan Virus." These agents were so-named because the original isolations by many investigators at other institutions in the early 1950's came from patients without specific clinical entities—the agents were orphans, so to speak. Many virologists called them "viruses in search of a disease." By the mid-1950s, the widespread use of tissue culture for virus isolation resulted in the recovery by other investigators of many different sero-types from a variety of summer and autumn outbreaks of illness manifesting fever, aseptic meningitis, paralysis, encephalitis, upper respiratory symptoms, diarrhea and rashes (18). Since the original isolations, etiological association was demonstrated between some of the above syndromes and specific sero-types. These viruses are now classified with the enteroviruses. They are characterized by a) cytopathogenicity for monkey and human cells in tissue culture, b) they are not neutralized by pools of the three types of poliomyelitis antiserums, c) they are not neutralized by Coxsackie antiserums against strains known to be cytopathogenic in tissue culture, d) they are resistant to the lethal effect of ether, e) they are not related to known groups of viruses

isolated from the alimentary tract, f) they do not cause disease in infant mice (except occasionally by large amounts of virus of some strains), g) they share the same small 20 to 30 mμ size of other enteroviruses, h) they are neutralized by human gamma globulin and individual human serums.

Dr. Leon Rosen, recruited by Bob in 1955, was interested primarily in studying the ECHO group of agents in the Junior Village investigations. The first sero-type that Rosen discovered and reported in his Junior Village study in 1956-1957, labeled JV-1 (later reclassified as ECHO virus type 20), occurred in association with a febrile, mild respiratory illness of short duration accompanied by frequent abnormal stools in 6 patients (19). As part of a study designed to explore the role of newly isolated viruses as causes of diseases in children, these patients were studied in detail at the Clinical Center at NIH during the period February to July 1956 along with other groups of patients in whom other agents were isolated. Examination of paired acute and convalescent sera from the infants demonstrated a rise in titer of both neutralizing and complement-fixing antibodies against the prototype JV-1 virus. Throat and anal swabs yielded virus isolations in all patients. Rosen found that virus isolation coincided with illness; that suggested but did not prove an etiological association (19).

Leon Rosen analyzed (20) the prevalence and behavior of the JV-1 virus after its introduction into the environment of Junior Village. He also completed a longitudinal study of the experience in this community with other enterviruses, including other serotypes of ECHO, poliomyelitis and Coxsackie viruses and demonstrated that the inhabitants of Junior Village had frequent and prolonged exposure to these agents (21). Yet, during the course of the studies with ECHOviruses in Junior Village there was no

evidence of aseptic meningitis or paralytic disease recorded. This was an important observation since early reports elsewhere indicated that ECHO viruses were isolated from patients with these diagnoses, an object lesson in the value of controlled epidemiological studies when attributing etiological significance to the isolation of prevalent viruses from only certain types of illnesses.

Leon Rosen also was interested in improvements in the immunologic testing for the ECHOviruses. He showed that it was possible to type ECHO viruses by the complement-fixing technique with excellent specificity thus enabling the avoidance of the more cumbersome tissue culture neutralization method (22).

REO Viruses

The *REO* viruses were another group that provided an area of exceptional interest for Leon Rosen. Dr. Albert Sabin, still associated with the University of Cincinnati in1959 (23), proposed the term REO (R-respiratory, E-enteric, O-orphan) as a group name for a number of viruses formerly designated as being identical with or related to ECHO virus type 10. These viruses were removed from the ECHO group and placed in this new classification because they share a number of important biological properties such as their size (about 60mu—larger than the enteroviruses or picornaviruses) and type of cytopathogenic effect in tissue culture that distinguish them from other ECHO viruses. They have also been found to occur naturally among various animal species.

Leon Rosen, in the course of studies on the epidemiology of reovirus infections in Junior Village (24), found that by the use of hemagglutination-inhibition (HI) techniques it was possible to segregate all available human strains (from various outside sources as well as Junior Village), as well as a number of animal strains into three distinct serologic

groups. This had not been done before. His report (24), published in 1960, described the evidence that led to the recognition of these 3 serologically distinguishable categories and indicated the group in which each of the previously described and newly isolated strains could be classified. The strains newly isolated in Junior Village were classified as group 3.

Leon Rosen and associates (25) reported on an outbreak of reovirus type 1 that occurred in January, February and March 1957 among children in Junior Village. In all, at least 43 of 73 children were infected. They found that the virus was recovered more readily from anal than from throat specimens, and that the virus could persist in the feces for a considerable period of time. All children shedding the virus developed homologous and occasionally heterologous reovirus HI antibody that persisted for at least 6 months. The virus isolation experience, but not the virus infection, was less in the children with pre-existing heterologous reovirus than it was in children with no pre-existing reovirus antibody, possibly representing re-infection with the infecting virus. Rosen noted an association between the onset of reovirus type 1 infections and the occurrence of mild febrile illness (100,6—101.5F-rectal). This finding, however, was of borderline significance as the virus isolation did not conform strictly to the Huebner postulates (see next chapter) for etiological causation. Rosen did not indicate an association between the occurrence of relatively severe febrile illnesses (rectal temperatures 101.6F or greater) and the onset of these infections. In all of these studies, with Joe Bell as co-author, only conservative evaluations and no unfounded conclusions appeared about the etiological relationship between illness and virus isolation. In a similar vein, the report of newly recognized type 3 reovirus (Abney) (26) stated that there was no definite correlation with

a specific clinical syndrome. Administration of virus to volunteers produced no illness but did produce evidence of infection manifested by virus excretion and antibody development (27).

The LID investigators thought originally that the reovirus group was non-pathogenic for animals but the viruses can be isolated in suckling mice, and there is evidence of infection in all classes of vertebrates; it is widespread in nature (28). A reovirus has been described as the cause of Colorado tick fever (29) after successful isolation from patients. Despite widespread human sero-prevalence of reovirus antibodies, otherwise, reoviruses have been described primarily in non-specific enteric and respiratory illness syndromes (24, 25, 26, 27).

Cytomegalovirus

Another pathogen of current prominence, *Cytomegalovirus*, appeared among the early isolations in Junior Village. In 1956 Wally Rowe and associates (30) recovered a cytopathogenic agent resembling “human salivary gland virus” from tissue cultures of human adenoids. This isolation was reported almost simultaneously from two other non-NIH laboratories. Smith (31) recovered the same agent from a human salivary gland that demonstrated intranuclear inclusions, and Weller (32) reported the isolation of a similar virus from the liver biopsy of an infant with the clinical diagnosis of cytomegalic inclusion disease. Wally Rowe recovered three strains of this agent that was characterized by the production of intranuclear inclusions in the tissue culture cells from spontaneously degenerating cultures of human adenoids. Occasionally early isolation of adenovirus occurred from the same tissue but in different culture tubes. One strain was studied in detail and appeared to be closely related to or identical to the viruses isolated in the other

two laboratories. Wally Rowe concluded that these agents were representatives of the so-called human salivary gland virus. He also found a high proportion of complement-fixing antibodies in human serums with an increased age incidence of seropositivity.

During the Junior Village studies (33) he detected this virus in the mouth and urine of children. Using tube cultures of trypsin-dispersed fibroblasts of human embryonic skin (obtained from the main contract supplier, Microbiological Associates, Bethesda, Maryland) to increase isolation sensitivity, he described the procedure for the detection of the human salivary gland virus in the mouth and urine. Rowe detected virus by this method in the mouths of 13 of 21 young children with serum complement-fixing antibody and in the urine of 7 of 8 virus positive children. Some children were virus positive for periods from 2 to 5 months and for as long as 15 to 24 months after antibody was known to be present. Virus could not be detected in the mouths of 26 children without complement-fixing antibody nor from 103 newborn infants or 26 adults. At that time, Rowe did not associate these viral isolations with any known or suspected clinical illnesses in the children, and long-term follow up history of the patients is not available. These findings provided laboratory confirmation of the chronicity and high prevalence of subclinical “salivary gland infection” and suggested that persistent urinary tract infection occurred with nearly equal frequency.

Inasmuch as subsequent isolations in the general population occurred in predominant association with the virus, which was established as the etiology of infant cytomegalic disease, this agent was called *cytomegalovirus*. This virus can also produce infection and illness in monkeys and other animal species. In the infant, the cytomegalovirus of man can produce a disseminated disease involving multiple organs

that may result in death. In immuno-compromised patients, as exemplified by patients with AIDS, cytomegalovirus is a dread opportunistic infection with devastating consequences.

Over forty years have elapsed since the initial and important observations of all the above newly discovered viral agents. The Junior Village experiences and the extended cross-sectional hospital-based studies that followed provided invaluable knowledge about the viruses that were isolated and their association with human infections. More detailed information about the nature of the viruses and the illnesses they cause can be found in recent texts of virology (34) and infectious diseases (35).

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Chapter 9

Volunteer and Vaccine Studies

The mass of undigested clinical and laboratory information obtained during the first few years of the Junior Village studies, prior to the later assortment of the data leading to etiological association of virus and clinical illness, led Bob Huebner to ponder on the concept of "The Virologist's Dilemma." So, what was the "Dilemma"?

In 1957, in the midst of the Junior Village activities, he wrote an essay by the same title (1) in which he attempted to address the issue. The manuscript was published as part of a viral symposium sponsored by the New York Academy of Sciences (1). In this essay Bob described some of his laboratory's recent and current experiences with the Junior Village nursery. On the basis of his previous investigations, he stated that the prevalent Coxsackie, ECHO and adenoviruses might occur in apparently healthy persons. To illustrate how he had reached that conclusion, he juxtaposed two actual scenarios. First, he described a period (September 1955) that was characterized in the nursery as an unusual "attack" of good health (meaning no fevers). During that period, the weekly routine surveys showed the association of the occurrence of an ECHO-like virus in 100% of 43 infants. This virus, or one similar to it, was isolated on at least 90 occasions. For contrast, he described the laboratory's experience during one of the more "normal" or average months, April 1956. At that time, there were 50 children in the nursery. Daily observations showed 42 bouts of fever (temperatures of 101F or higher) in 39 of the 50 children. A throat swab was taken from each child on each of four Wednesdays during April. One hundred sixty-eight of 200 throat swabs were tested satisfactorily in tube

cultures containing HeLa cells. Nineteen adenoviruses, 5 ECHO-like viruses and a single unidentified virus were recovered from the patients. In 123 tests of anal swabs, 8 specimens yielded adenoviruses, 40 showed infection with an ECHO-like virus and 1 yielded an unidentified agent. Thus, showing a necessarily close association with 42 bouts of fever, the laboratory isolated no less than 27 adenovirus agents of various types, 45 ECHO-like agents not yet classified and 2 completely unidentified agents. Bob Huebner described this as a slow month!

The dilemma, then, was how to sort out this mass of laboratory and clinical data in order to establish etiological relationships. Bob sought an answer drawing on the experience he had gained since 1949 with the investigations of Coxsackie and adenoviruses that included the study of small community outbreaks, community-wide epidemiological surveys, small cross-sectional hospital studies, and ongoing volunteer and vaccine studies. In this manuscript (1) he reviewed the postulates that Robert Koch had originally designed for bacteria. Koch, the discoverer of the tubercle and anthrax bacilli, was one of the founders, along with Pasteur, of the science of bacteriology. The postulates stated: 1) An organism isolated from a sick person or laboratory animal must be grown successfully on suitable bacteriologic media and identified, if possible. 2) The cultivated organism should cause signs of illness when injected into a suitable laboratory host. 3) The organism should be isolated from the host in pure culture and identified as the same injected organism. 4) The organism then is said to be the cause of the illness in the animal or human host.

Huebner was familiar with the work of Dr. Thomas M. Rivers (prominent virologist of the mid-20th century, Head of the Hospital of the Rockefeller Foundation,

author of a comprehensive virology text, and influential promoter of poliomyelitis vaccine development) who had revised Koch's Postulates to make them applicable to viruses (2). Building on Rivers' work, Huebner proposed a series of his own postulates, based on previous experience, for determining when a virus should be regarded as an established cause for a specific human illness. These guidelines, as described by historian Victoria Harden in her history of the impact and transformation of the original postulates, (3) were as follows: [Note: author's comments in brackets follow each enumeration.]

1) The virus must be established as a real entity that can be cultured in other laboratories. [This is one of the reasons that responsible investigators exchange newly isolated agents to see whether the organism is truly unique or whether it has been identified previously elsewhere.]
2) The virus must be shown to be of human origin and not a contaminant or virus from experimental animals. [This was a current (and future) problem when some of the laboratory rodent tissues were found to be harboring indigenous or latent viruses.]
3) The virus must be shown to produce an active infection by invoking an increase in serologically demonstrable antibodies. [This postulate is valid even in the absence of clinically demonstrable illness in some persons.]
4) The virus should be characterized early so that comparison can be made as early as possible with other agents already described or soon to be discovered. [This postulate is really a corollary of number 1.]

5) The virus should be constantly associated with a specific illness. [This is true most of the time but infections may occur with atypical symptoms or they may occur as infections without symptoms. One respiratory virus may produce symptoms caused by other respiratory agents and many viruses may cause symptoms characteristic of an individual respiratory agent. This was a major reason for Huebner's search to separate the individual agents from the undifferentiated mass of respiratory infections existing prior to his investigations into the causes of respiratory infections.]

The first five postulates relate primarily to viral attributes. Huebner's innovative contribution in the next few postulates was the provision of parameters and description of research initiatives to establish the validity of viral isolations.

6) The researchers should use double blind studies of the virus in human volunteers taking cognizance of the subjective impressions produced in both observers and subjects. [This was of utmost importance, Huebner thought, when studying poorly defined minor illnesses that were nevertheless familiar to most people.]

Then, there is Huebner's distinctive research approach and major contribution that he proposed in the following postulate.

7) He maintained that carefully conceived epidemiological studies coordinated with adequate laboratory and clinical observations were indispensable for the purpose of finally establishing the etiological role of highly prevalent viruses in human disease. He recommended the general employment of two types of studies: a) Studies of populations experiencing a disease outbreak, and b) long term

studies of communities or institutional groups. [By the time of the "Dilemma" manuscript, he had had extensive experience with epidemiological studies of this nature.]

8) [Another criterion postulated for viral identity was]: prevention [of disease] by specific vaccination; if a vaccine prepared from a suspect virus prevented a specific disease, the virus may be said to cause the disease.

9) [Finally, he postulated that sufficient]: financial support should be made available for adequately trained technical help and for epidemiological, clinical and laboratory research.

Dr. Huebner included the last postulate as a "non-scientific" principle because he felt that studies satisfying the first eight conditions could be accomplished only in those institutions and facilities where specially trained people, motivated to undertake the studies, had available adequate, dedicated, institutional, financial support.

Bob Huebner sought to achieve consistency in his criteria for establishing the newly discovered, primarily respiratory, viruses as etiological agents for specific clinical illnesses utilizing the principles stated in postulates 6 and 8. An integral part of Bob's investigative program to achieve this goal included the production of illness and/or infection in human volunteers and the demonstration of the protective effects of specific vaccines. The program extended intermittently from the time of the isolation of the adenoviruses in 1953, through the initial 3 years of the Junior Village studies and into the early 1960's. The agents, generally selected for study, usually represented newly isolated viruses thought to cause significant morbidity in infants and young children. The series of volunteer trials were performed utilizing male adults, either correctional institution

inmates (prisoners) or, especially, military recruits in whom exposure to or illness with the offending viruses produced morbidity, occasional mortality, and interference with the training process. In the early phases of the volunteer trials studying the adenoviruses, Bob had the collaboration of Dr. Thomas G. Ward who was with the Department of Microbiology, Johns Hopkins University School of Public Health. Bob also collaborated with other investigators from the United States Armed Forces and from various medical schools.

From the beginning of the individual volunteer and vaccine studies, informed consent was obtained routinely and regularly. Most of the early as well as the later programs were in federal prison facilities, and the administrative personnel and wardens were instrumental in facilitating the studies. All prisoners provided written consent. When the National Institutes of Health opened the Clinical Center, strict protocols for human experimentation were followed, and all of the volunteers signed consent forms. Inducements offered to prison inmates included relief from the prison routine and the granting of minor special privileges, including probably small stipends. Studies involving the military were mainly in Naval or Marine recruits. The Naval programs were developed in collaboration with the Bureau of Medicine and Surgery of the U.S. Navy with the active participation of naval medical officers involved in the studies. Volunteers were readily available (4), given the gentle, persuasive arguments of the Naval chief petty officers and the Marine drill sergeants.

Bob had started the initial volunteer and vaccine studies during the investigations of adenoviruses when it was recognized around 1953-1954 by Huebner, Parrott and associates that adenovirus type 3 caused pharyngoconjunctival fever. The first study

entitled “Production of Pharyngoconjunctival Fever in Human Volunteers Inoculated with APC Viruses” (adenoviruses) (5), published in 1955, involved the inmates of the Maryland State Reformatory for Males in Breathedsville. Typically, Huebner drew on his genius for collaboration and put together diverse resources to make the study viable. He obtained the assistance of Robert L. Clopper, Assistant Superintendent of the Reformatory, and the authorities of the Department of Corrections, State of Maryland. The work from Johns Hopkins, represented by Dr. Thomas G, Ward, was supported in part by a grant from the Research Grants Division, National Microbiological Institute and the Common Cold Foundation. The study showed that conjunctival and pharyngeal inoculation of tissue culture fluids containing live type 3 and type 4 (APC) adenoviruses viruses, in volunteers having little or no preexisting neutralizing antibody, produced illnesses indistinguishable from pharyngoconjunctival fever.

A companion investigation titled “Studies of Adenoidal-Pharyngeal-Conjunctival (Adenoviruses) Vaccines in Volunteers” (6), written by the same major authors in 1956 encompassed inmates of the Federal Industrial Reformatory, Chillicothe, Ohio and the Maryland State Reformatory for Males, Breathedsville, Maryland. The staffs of both institutions cooperated in this study and The Johns Hopkins University effort was supported in part by another grant from the Common Cold Foundation and by a grant from the Research Grants Division at USPHS. This study was performed with vaccine against adenovirus type 3 prepared from monkey kidney cells grown in tissue culture. The study investigated the efficacy of a vaccine against adenovirus type 3. This particular vaccine, inactivated by heat and formaldehyde to render it non-infectious, induced neutralizing antibodies against type 3 adenovirus when inoculated into young adult

volunteers. The investigators tested the vaccine's efficacy further by comparing the degree of protection offered by the vaccine among the different groups of volunteers in the study. After vaccine challenge with live type 3 APC virus swabbed on the conjunctivas (the mucous membranes lining the eyelid), volunteers with vaccine-induced antibodies experienced much greater protection against infection and illness than did vaccinated or unvaccinated persons who had not produced any detectable antibodies. This vaccine-induced protection was nearly equal to that of unvaccinated volunteers with naturally acquired antibodies. Bob and his collaborators thus demonstrated that it was possible to protect persons against adenovirus infection by immunization with a specific vaccine. The vaccine used in this study was prepared in the laboratory; vaccines used in subsequent studies were usually prepared commercially as noted below. This study set the stage for the immunization of select groups, such as military recruits, with vaccines directed against prevalent infections to which they were exposed frequently while they were in boot camp.

Drs. Bell and Huebner and the collaborating authors published the next study in "Efficacy of Trivalent Adenovirus (APC) Vaccines in Naval Recruits—Progress Report" in 1956 (7, 8). A formaldehyde inactivated adenovirus (APC) vaccine containing types 3, 4 and 7 was prepared commercially (by the pharmaceutical company, Parke, Davis) and administered intramuscularly in a single 2-ml dose to nearly 4,000 naval recruits without local discomfort, fever or general reactions. The vaccine induced a substantial neutralizing antibody response to each of the three types of virus contained in the vaccine indicating that each vaccine component produced some immunity to all three types. All evidence, deduced from examination of morbidity among recruits, visits to the base

infirmaries and incidence of respiratory infections, indicated that the vaccine, in turn reduced considerably the rate of occurrence of acute febrile respiratory illness associated with type 4 adenovirus. Given this promising outcome, the authors concluded that the adenovirus vaccines could potentially reduce the acute febrile respiratory illnesses that commonly interfered with military routine. This cooperative study was conducted by the Naval Medical Research Unit No. 4 (NAMRU-4) at the U.S. Naval Training Center (USNTC), Great Lakes, Illinois, the NIH Laboratory of Infectious Diseases, and the Department of Preventive Medicine of the University of Chicago at Chicago, Illinois (Dr. Clayton G. Loosli was a prominent investigator in infectious diseases and epidemiology. He later went to the University of Southern California. Dr. Thomas G. Ward, served as a consultant but had moved from Johns Hopkins to the Lobund Laboratories, Notre Dame University, South Bend, Indiana.)

This was probably Bob Huebner's (and Joe Bell's) initial venture with industrial pharmaceutical companies in carrying out some of his studies. All seed viruses (the small inoculums needed to prepare large amounts of virus, known as "live virus pools") used in the study were prepared at NIH. Live virus pools were prepared by Parke, Davis and Company, Detroit, Michigan, (manufacturer of the vaccine) and Microbiological Associates, Inc., Bethesda, Maryland. The laboratory at this time was absorbed completely in research on the respiratory viruses and did not have the space, personnel or equipment to make large amounts of routine reagents. It was more economical to contract out the preparation of reagents to a professional, well-equipped and competent commercial laboratory. Bob had already started his ongoing fruitful and future

relationship with Microbiological Associates in providing reagents and other resources for research.

While the study of vaccine in Naval recruits (7) was underway, Drs. Joe Bell, Bob Huebner and associates were completing a compilation of the first two adenovirus volunteer studies carried out in the prisons of Maryland and Ohio (5, 6, 9). (Warden R.P. Hagerman and Chief Medical Officers Edward Slaten, M.D. and T.P.Hackett, M.D. of the Chillicothe Reformatory and R.L. Clopper, Assistant Superintendent of the Maryland Reformatory assisted actively in the performance of the studies.)

This compilation summarized these studies and provided some additional information (9). The authors found that the intranasal instillation of adenovirus types 1,2, 3, 4, 5 and6 and the swabbing of the oropharynx with type 4 produced infection as demonstrated by a complement-fixing antibody response. However, such inoculations were followed commonly by a minor respiratory illness, chiefly manifested by an afebrile, nasopharyngeal catarrh (runny nose- nasal discharge) which could not be attributed to infection with these viruses. The reason for these findings was not explainable readily by the authors, and it was notable that the authors subsequently used the conjunctival route exclusively to produce infection with adenoviruses in volunteers.

The researchers readily produced infection and subsequent illness in susceptible volunteers by swabbing the lower palpebral (eyelid) conjunctiva with adenovirus types 1,3,4 or 5. The frequencies of infection and illness were similar when virus grown either in HeLa or monkey kidney cells was used—the cell lines used routinely in the laboratory to grow adenoviruses. The researchers demonstrated infection by recovery of the homologous (same adenovirus strain used to produce infection) virus from the eye or the

throat 5 to 9 days after inoculation and by complement-fixing antibody response. Illnesses occurred from 2 to 7 days following inoculation, generally persisted from 4 to 8 days, were occasionally febrile and often were characterized by a follicular conjunctivitis (bumpy inflammation of the lining of the eyelid). The volunteers also often exhibited pharyngitis (sore throat) with vascular injection (prominent capillaries) and lymph follicle hypertrophy (little bumps). They also complained of eye irritation and discharge, nasal discharge and obstruction, sore throat and occasionally cough and headache. Both objective signs and subjective symptoms characterized demonstrated infection, and illness occurred predominantly in volunteers without demonstrable pre-existing homologous neutralizing antibodies. Swabbing viruses onto the conjunctiva produced a higher frequency of conjunctivitis than did dropping the virus into the conjunctival fornix (hollow), possibly because greater irritation occurred with the swabbing procedure.

As in the previous studies, the laboratory prepared heat and formaldehyde-inactivated virus vaccines against type 3 adenovirus. When the researchers inoculated intramuscularly adult volunteers without detectable type 3 antibodies with these vaccines, 78% developed type 3 neutralizing antibodies, and no untoward reactions occurred. Such vaccinated persons were protected against challenge-induced illness apparently to the same extent as adult volunteers with naturally acquired antibodies.

Bob Huebner's research activities had been powerful attention getters among medical science writers and the lay press ever since his initial prominence with the rickettsialpox and Q fever investigations. When official information from NIH (Science Service, NIH press releases) about Bob's respiratory virus activities started circulating, the print media began conducting interviews with Bob, and they greeted with "irrational

exuberance" the "battle in the conquest of the common cold" (10). The problem of respiratory infections, especially the "common cold," was, and still is, one of the health obsessions of the general public because of the inconvenience, prevalence and economic consequences of frequent uncomfortable episodes. The lay press community commonly misunderstood the nature of the viruses under study. During the period when Bob and associates were performing the various volunteer studies, headlines such as the following appeared in various news publications (10): "Children's adenoids yield possible mystery virus," "APC cold vaccine tests start in Naval recruits," "Vaccine cuts up to 75% of colds in Navy recruits," "Toward the conquest of the virus," "Virus cocktail may reduce toll of colds," and "Viruses that cause one kind of cold may get family name." (8) Despite Bob Huebner's cautionary dampening of excessive optimism, he had difficulty dissuading reporters and other news writers that the conquest of the common cold was not only one more vaccine away in the future. He also had problems explaining to these eager journalists that the agents he was working on, although the illnesses produced by them superficially resembled the common cold, were not the same "virus" that caused the relatively mild symptoms of the common cold.

On May 25, 1956, principal investigators working with the adenoviruses, including Bob Huebner and Joe Bell (8), published a manuscript in *Science* in which they proposed a group name for the new respiratory tract viruses. Instead of using the hodge-podge of names such as "Adenoid Degenerative (AD)," "Adenoid-Pharyngeal-Conjunctival (APC)," "Respiratory Illness (RI)," and "Acute Respiratory Disease (ARD)," the investigators proposed for the new viral group the adoption of the term

Adenoviruses for consistency of nomenclature and to indicate the tissue from which these viruses were first isolated.

For a change of pace from the studies with the adenoviruses, Bob Huebner participated in a study published with the title "Artificially Induced Asian Influenza in Vaccinated and Unvaccinated Volunteers" (11A). In this study Bob Huebner worked with Dr. Joe Bell and his epidemiology unit in order to test the efficacy of a commercially prepared vaccine against the strain of influenza A currently prevalent in 1957. Joe Bell also had a primary interest in influenza and influenza immunization. The particular virus strain had already appeared in the Junior Village nursery. Influenza A has the tendency to undergo frequent genetic variation from year to year, and vaccines prepared from previous years' strains may not protect against a strain prevalent in the current year. For this reason, current recommendations for influenza immunization prescribe annual injections, for the most vulnerable population groups, with vaccines of the most recently isolated strains with the aim of providing the widest possible protection. In this study, the volunteers were healthy male inmates of the Patuxent Institution of the Maryland State Board of Corrections at Jessup, Maryland. Some volunteers who were administered the virus became infected but did not become ill. Others who were infected became ill with influenza ranging from the mild to the moderately severe "classic" form (chills, fever, muscle aching, sore throat and cough). Based on the evidence that this study produced, the researchers concluded that the vaccine gave moderate but incomplete protection against Asian influenza; 78% of the unvaccinated placebo group and 44% of the vaccinated group became ill with challenge-induced influenza. This investigation was antecedent to and a companion to the double-blind community study, proposed by Joe

Bell (11B), of the vaccine efficacy in a selected population in northwest Montgomery County, Maryland. Bob Huebner was not involved in this community study

Worldwide surveillance for the appearance of new strains of influenza virus occurs on a constant basis in many detection laboratories, so that the trend of more recent times has been the attempted improvement in the protective efficacy of influenza vaccines. Antiviral antibiotics administered before or early to patients during an epidemic of influenza can protect from infection or ameliorate symptoms, especially in the elderly.

In 1958 Huebner and his associates were finally able to perform some volunteer studies on one of the "new" viruses isolated several years after the Junior Village nursery surveillance had been underway. The manuscript titled "Infection of Human Volunteers with Type 2 Hemadsorption Virus" (12) described the initial volunteer investigations related to the newly discovered respiratory agents subsequently classified as myxovirus parainfluenza. Bob Chanock had isolated the prototype strain of this group in 1954 before he came to NIH, and he initially labeled the virus "CA," or croup-associated, since he isolated the virus from an infant with croup. Type 2 hemadsorption virus later became classified as parainfluenza type 2. These agents were found subsequently among the residents of Junior Village, with respiratory illness, initially in 1957, after Chanock arrived at NIH.

Further cross-sectional hospital studies (13) showed that a considerable portion of febrile respiratory illness in children during the winter of 1957-1958 was associated with the hemadsorption viruses based on the observations of Drs. Parrott (Chief now of Pediatrics and Head of the Research Department) and Chanock among patients seen in the clinics and wards of Children's Hospital of Washington, D.C. With the cooperation of

the authorities of the Maryland State Board of Corrections and Harold N. Boslow, Director of the Patuxent Institution, they were able to definitively correlate signs and symptoms of illness with virus isolation, and the development of specific antibody responses. The investigators performed this current volunteer study to determine whether type 2 hemadsorption virus could produce infection and illness in adults. The volunteers were selected from among healthy male inmates of the Patuxent Institution at Jessup, Maryland. Seven of the 32 volunteers in the study had no neutralizing antibody for type 2 virus. The remaining 25 volunteers had antibody levels of 1:4 to 1:128. A total of 25 men developed a rise in antibody level for type 2 virus after the administration of live virus. Because symptoms did not develop by the fifth day, the volunteers were released from isolation. In volunteer studies involving live viruses, the subjects remained in isolation in order to prevent the inadvertent spread of infection to other participants in the study, especially the control group; however, when symptoms of illness began to appear among the volunteers, they were returned to isolation. Their temporary release into the general prison population resulted in an unintended secondary outbreak in un-inoculated inmates with type 2 virus. The illnesses observed in volunteers and secondary cases were mild with coryza-like (mild nasal and throat irritation similar to the common cold) symptoms, and the incubation period was longer than anticipated. It was apparent then that infection and illness could occur in adult persons with pre-existent antibodies. The subsequent pattern was seen in another study, (described next), when respiratory syncytial virus was administered to adult volunteers. This pattern was different than that observed with adenovirus infection where pre-existent antibody appeared to confer immunity to infection and illness. The finding of re-infection in persons with pre-existing antibodies

to parainfluenza viruses was a unique experience for the investigators doing these initial studies, and a variety of possible explanations have been proposed to explain the phenomenon since the same observation was first made up to the present. Investigators doing the studies have stated that the presence in adults of antibodies probably prevents the serious type of respiratory infection seen in children, and when the adults become re-infected they usually develop symptoms of mild “colds”.

Around 1960-1961, a new dimension entered investigations of volunteer studies when Huebner’s group began to look at the manifestations of infection with the newly discovered respiratory syncytial virus (RSV) in adult volunteers. (The history of the virus discovery is covered in the chapter on “The New Viruses.”) The group published several manuscripts dealing with the volunteers including: A) “Correlation of Virus Shedding, Serological Response and Illness in Adult Volunteers” (14) (Respiratory Syncytial Virus) and B) “Ecology of a Newly Recognized Common Respiratory Agent, Respiratory Syncytial Virus: Combined Clinical Staff Conference at the National Institutes of Health” (15).

These studies and discussions formed part of a series dealing with a variety of issues of respiratory syncytial virus in humans. In addition, they illustrate how the researchers incorporated several new features and techniques into their investigations. The first innovation involved the use of the Clinical Laboratory of NIAID in the NIH Clinical Center to house the volunteers during the conduct of the studies. Up to this time, volunteer studies had been conducted within the penal institutions where the subjects were incarcerated. Dr. Vernon Knight, Director of the Clinical Laboratory felt that more precise, useful information could be obtained by bringing the prisoners to the Clinical

Center where the laboratory facilities were located. He also felt that the examining physicians would be able to make more detailed observations of the volunteers that would have been difficult had it been necessary for the physicians to go to the prisons. As the program progressed, it became obvious that the physical layout of the Clinical Center facilities allowed excellent precision in maintaining strict isolation and preventing crossover exchange of virus among the volunteers, the hospital personnel and other hospital patients.

The impetus for these volunteer studies came from the cross-sectional studies of respiratory infections by Chanock and Parrott (16A) at Children's Hospital of Washington, D.C. in 1958-1960 in which they had demonstrated that a high percentage of serious, febrile, respiratory infections, occurring at predictable intervals, were caused by RSV. Kapikian (16B), also, reported the first documented outbreak of RSV in Junior Village in 1961. These Huebner associates observed that natural infection in young children and infants usually resulted in severe, febrile bronchiolitis (inflammation of the small lung airways with blockage) or pneumonia (inflammation of the lung air sacs). The clinical illness produced in adults was much different. Administration by the nasal or throat route of tissue culture grown respiratory syncytial virus (RSV) resulted in infection in 33 and clinical "colds" without fever in 20 of 41 adult volunteers. The incubation period averaged 5 days, and the illness lasted an average of 5 ½ days. Production of infection and/or illness correlated positively with subsequent excretion and recovery of virus and an increase in antibody levels. Volunteers who became ill generally shed virus for a longer period and were more likely to develop a rise in antibody than infected individuals who did not become ill. RSV infection in the volunteers represented re-

infection, as all men studied had detectable RSV neutralizing antibodies prior to challenge. This finding was and has been consistent, and is reminiscent of the situation that the investigators encountered with the parainfluenza viruses; it seems to be a feature associated with both types of viruses It seemed probable to the investigators that such antibody was responsible for the mild nature of the observed illnesses in adults. Huebner speculated, on the basis of these observations (15) that a vaccine incorporating many strains of respiratory viruses to prevent severe respiratory infections would be impractical; however he thought that administering a RSV vaccine early in infancy might prevent the serious initial illness, and any subsequent illness might be a mild cold-like re-infection with RSV. This idea has, generally, not been accepted. Chanock's laboratory is still working with RSV vaccine development.

The volunteer studies of RSV at NIH were conducted in three phases. A concern emerged after the first two phases resulting in a change in the growth medium for the third phase inoculum. The third phase infectious inoculum was virus grown in tissue culture of a continuous human epithelial type (Hep-2). Prior to that the inoculum was virus grown in common rhesus monkey kidney cell culture. However, in 1960 Sweet and Hilleman (17) found that the monkey kidney cell lines contained a latent virus that when grown in "green" or grivet monkey cells produced a cytopathogenic vacuolating pattern. This was the so-called SV-40 (simian vacuolating) virus. The presence of virus in monkey kidney cell lines was of great concern to the Division of Biologics of LID because its infectious or oncogenic potential was unknown at that time, and because the current killed polio vaccine was made in monkey kidney cell cultures. It was not known at that time whether the current method of producing killed polio vaccine had eliminated

all of the SV-40 virus in the vaccine that had already been administered and what the possible consequences might be in terms of unknown infection or development of tumors. Studies of the early phase NIH volunteers showed that a small number developed low level antibodies but no evidence of illness (15). It was deemed judicious, nevertheless, by Dr. Joseph E. Smadel (15), (Chief of the Laboratory of Virology and Rickettsiology, Division of Biologic Standards, NIH), with some equivocation, to try to use SV-40 free cell lines for future volunteer studies and for use in live vaccine products such as the oral polio vaccine then under development by Dr. Albert Sabin.

In 1961 Huebner's group published a manuscript, with Dr. Al Kapikian as the lead author, titled, "Inoculation of Human Volunteers with Parainfluenza Type 3" (20). This study was part of the continuing series related to these recently isolated new respiratory disease agents. In an attempt to see whether parainfluenza type 3 would produce illness in adults, 28 adult volunteers from the Patuxent Correctional Institution at Jessup, Maryland, were inoculated with either the virus or a sterile salt solution. Twelve of the 17 volunteers inoculated with the virus and none of the 11 who received the placebo inoculum developed laboratory evidence of infection with parainfluenza 3 virus. Nine of the virus-inoculated volunteers and only one of the 11-control groups developed an acute respiratory illness. However, 3 of the 9 virus-inoculated volunteers who became ill failed to demonstrate laboratory evidence of infection with parainfluenza 3 for unexplainable reasons. The non-febrile "cold-like" illness observed in the six infected volunteers was characterized by mucoserous nasal discharge, nasal obstruction, sneezing dry cough and erythematous nasal and pharyngeal mucous membranes (red nose and

throat). The mean incubation period was 2.2 days. Most of the volunteers had pre-existing antibodies.

Although the study furnished evidence consistent with the view that parainfluenza type 3 virus could cause acute adult respiratory disease, the results were not as unequivocal as the results obtained with the previous study described above (12) using hemadsorption virus 2 (i.e., parainfluenza 2). The authors thought that the results suggested that parainfluenza 3 produced milder illness than parainfluenza 1 and 2. Serologic evidence of infection with this agent had been shown to occur in adults under natural conditions, but the authors felt that larger, more intensive controlled studies were needed to assess the clinical virulence of this virus in adults.

An extremely significant volunteer study performed under the direction of Dr. Robert Chanock appeared in 1961 with the title “Respiratory Disease in Volunteers Infected with Eaton Agent; A Preliminary Report” (18). Bob Huebner did not participate personally in this study, but many of his colleagues who had worked on related vaccine and volunteer trials did. Huebner, as Chief of LID and as a member of the National Academy of Sciences (NAS), sponsored the research and communicated the findings to the NAS. The Eaton agent had long been suspected to be the culprit that caused cold-agglutinin positive atypical pneumonia. It was thought to be a virus rather than a bacterium. In 1944, Dr. Monroe D. Eaton, at Harvard, had first isolated this filter-passing agent, in cotton rats, mice and chick embryos from patients with atypical pneumonia characterized by the presence, in many cases, of cold-agglutinins in the blood. These proteins (gamma globulins) cause the clumping of red blood cells when they are refrigerated. Atypical pneumonia refers to the febrile lung infection from which common,

easily grown bacteria cannot be isolated. During World War II these pneumonias were all thought to be of viral origin. Confusion developed when physicians observed that they could treat some, but not all, atypical pneumonias with antibiotics, initially with tetracyclines and, later, with erythromycin type antibiotics. As already noted, Huebner's group, in collaboration with the US Armed Forces, was able to designate adenoviruses type 4 and 7 as important causes of viral pneumonia. Epidemiological studies in the early 1960's provided substantial evidence that the Eaton agent was associated with human respiratory disease. The evidence was the result of serological studies conducted by Eaton and associates in patients (21), by the Huebner group in Children's Hospital childhood respiratory infections (22), and by Chanock and his colleagues in the ecology of infection and a controlled field study among Marine recruits (23). In the same Marine recruit population a double blind controlled study indicated that a tetracycline was effective therapy of pneumonia associated with the Eaton infection (24). During the course of the study Chanock was able to isolate 14 strains of the agent and grow them in monkey kidney tissue culture.

In the current study, the volunteers were Federal male prisoners but the study was performed at the NIH Clinical Center. The investigators used the Eaton organism grown in monkey kidney cell culture as the inoculum. They neutralized the small quantity of SV-40 virus contained in the inoculum by specific antiserum, and administered the tissue culture fluid to the volunteers as a coarse spray into the nose and throat. One-half of the men had undemonstrable specific antibody against the agent. In this group of 27 men, 3 developed pneumonia, 11 developed otitis media (inflammation of the middle ear), 2 developed febrile upper respiratory illness, 4 developed afebrile upper respiratory illness,

7 did not become ill, and all developed 4-fold or greater rise in specific antibody titer against the agent. Also, 12 in the group developed a rise in cold-agglutinins, a non-specific marker of infection by this particular agent. By contrast, in the group with detectable specific antibodies, one person developed otitis media, 6 developed afebrile upper respiratory illness, 18 did not become ill, none developed a rise in cold-agglutinins but 17 of the 25 showed a rise in antibody titer against the agent indicating probable re-infection. The researchers concluded that the administration of Eaton agent to volunteers with pre-existing antibodies could result in re-infection, increase in antibody level and mild illness similar to the situation when myxoviruses or respiratory syncytial virus were given to volunteers who possessed antibodies to those viruses. An unanticipated clinical finding in the volunteers given the Eaton agent was the development of a severe, hemorrhagic otitis media with bullous myringitis (blisters on the ear drum). This is not a usual finding among patients with community acquired (mycoplasma) pneumonia but may be seen as an occasional manifestation accompanying the pneumonia.

The serological method referred to previously in detecting antibodies to the Eaton agent (21-23) was the demonstration of immuno-fluorescence on the surface of the bronchial epithelium in infected chick embryos. The fluorescence appeared to be localized to tiny, round bodies that the observers speculated might be bacteria with very fastidious growth requirements. Working on this assumption, Bob Chanock, in conjunction with Leonard Hayflick and Michael L. Barile (19), succeeded in cultivating the Eaton agent on a highly refined artificial bacteriologic medium, and they classified it as a pleuro-pneumonia like organism (PPLO). It is a mycoplasma (bacterium without a cell wall), and it was named officially *M. pneumoniae.* When grown on artificial media,

the colonies are translucent and difficult to see; when viewed under the microscope the colonies look like fried eggs, sunny side up.

Among other causes of atypical pneumonias that have been recognized gradually and for which treatments are available include those caused by Q fever, the chlamydia group (pneumoniae, psittici, trachomatis) and the legionella group of bacteria with its fastidious growth requirements. It has been evident that Bob Huebner and his associates have made significant contributions in helping to unravel the many causes for the clinical syndrome of atypical pneumonia.

Bob Huebner participated in a relatively new and innovative technique to produce active immunity in military recruits described in the manuscript “Immunization with Types 4 and 7 Adenovirus by Selective Infection of the Intestinal Tract” (25). The rationale for feeding live virus to establish protection was derived from observations that adenoviruses appeared to have a greater predilection for growth in the intestinal tract than in the respiratory tract (26). The viruses used for inoculation in this study were grown in human embryo kidney tissue culture and were free of SV-40 virus (for the reasons quoted previously). In this study it was possible to by-pass the respiratory tract and to infect selectively the intestinal tract of military recruit volunteers without detectable antibodies for adenoviruses 4 and 7 by feeding live virus contained in enteric-coated capsules that dissolved in the small intestine. Except for those volunteers who apparently inadvertently self-inoculated the conjunctivae with fecal material by not paying close attention to personal hygiene, the infection remained confined to the intestinal tract and was not associated with illness. The antibody response that resulted from the selective infection was as effective as that following administration of two types of intra muscularly injected

inactivated vaccine. The authors felt that it was probable that the antibody response following intestinal infection would protect against naturally occurring adenoviral disease. When adenovirus types 4 and 7 were orally administered simultaneously, there was no evidence of virus interference, suggesting that this procedure could fulfill the need for rapid immunization against these viruses in military recruits in whom adenoviral respiratory infection caused excessive interference with training schedules. A limited study suggested that fecal excretion of adenovirus did not constitute an effective means of transmission of the agent to other adults. It was suggested that this technique of selective intestinal infection with adenoviruses should be pursued as a possible basis for immunization against naturally occurring adenoviruses. The approach of using the intestinal tract for immunization was innovative and paved the way for this mode of vaccination. It has been used since the mid 1960's for live attenuated virus vaccines, notably for poliomyelitis since about 1965, for rotavirus and typhoid fever since the 1990's.

With the conclusion of these investigations, Bob Huebner became less involved with vaccine and volunteer studies related to the respiratory viruses as he shifted his attention to tumor virology. Bob Chanock, primarily with other associates, continued to conduct additional vaccine trials. One of the most important involved the serial controlled testing of adenovirus type 4 grown in human embryonic (diploid cells derived from lung fibroblasts) tissue administered by the enteric route to large numbers of Marine recruits. The results were excellent, and the vaccines proved effective over many years in providing good protection among the vaccinated Marines against the acute febrile respiratory disease and atypical pneumonia caused by this prevalent adenovirus strain.

Shortly following the initiation of the type 4 vaccine trials, U.S. News & World Report conducted an interview with Bob Huebner and Bob Chanock (27) that appeared in May 3, 1965. The title was: A Cure For The Common Cold? The questions and the issues raised were: “Is a sure-fire “anti-cold pill” on the way? For the first time scientists have produced a capsule that wards off a virus disease that is similar to a severe cold. Tests prove it works. From this success, this question: If one cold-like ailment can be prevented what are the chances of conquering the most widespread disease of them all—the common cold”? These statements reflected the concerns and hopes of the lay public. Huebner and Chanock explained the nature of the new vaccine, the possible potential for adapting the technology to other viruses, the ongoing vaccine development program at NIH, the technological difficulty of incorporating many respiratory virus strains into a single delivery vehicle for immunization and the difference between the already discovered respiratory viruses and the virus of the common cold.

With all of the foregoing studies Bob Huebner was able to establish etiological viral causes for many human respiratory infectious illnesses by utilizing volunteer and vaccine development programs. On March 28, 1966 he received a letter from the Undersecretary of Health, Education, and Welfare (28): “Dear Dr. Huebner: It is a pleasure to inform you that Secretary (John W.) Gardner will, in recognition of your outstanding service, present you with the Distinguished Service Medal of the Public Health Service Commissioned Corps at the Department’s Fifteenth Annual Awards Ceremony. ------ The Secretary and I extend our warmest congratulations to you. Sincerely yours, Wilbur J. Cohen, Under Secretary.” On April 11, 1966 Bob Huebner received his medal with the following citation: “ For his role in the discovery of a vaccine

for the adenoviruses that has achieved an incalculable saving in human resources and economic expenditures."

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Chapter 10

Seventy New Viruses: Summation and Transition

In order to give an update on the current state of virology in 1959, Bob tabulated in the *Public Health Reports* (1) a list of 70 newly recognized viruses in man that had been recognized in the decade since 1948 when River's textbook (2) listed approximately 60 viruses that were then known to infect man. Huebner's tabulation included all the agents discovered at NIH as well as at other laboratories. The list included all the recognized groups of viruses and the various serological types encompassed within those groups. It also specified the clinical illnesses caused by the various agents. Some serological types produced more than one clinical illness, and multiple types were shown to produce the same signs and symptoms.

To recapitulate, a major problem had been the difficulty in associating the presence of virus with specific illness. During the course of the Junior Village studies, the investigators observed as many as four acute viral infections in the same child during the same week. In very young children these new viruses most often cause clinical entities that are difficult to distinguish from one another and attributing the illness to the proper agent could thus be quite difficult. The studies conducted primarily in volunteers frequently caused mild or unapparent infections that differed clinically in substantial ways from the often-severe illnesses that occurred in infants and young children. In this age group it is still difficult for even experienced pediatricians to distinguish among the illnesses caused most commonly by the Coxsackie, adenoviruses, ECHO, myxoviruses and even polioviruses.

The six-year longitudinal study of suburban communities by Bob Huebner, Joe Bell and his other associates of NIH showed that respiratory illness characterized by mild fever of more than 1 day's duration occurred approximately 5 times more often in children under 6 years of age than in persons over 17 years. The intermediate age group showed an intermediate experience. Because of the increased frequency of respiratory illness in young children, their studies of these illnesses were focused on the childhood illnesses in three different population groups: a) in the suburban community, b) in pediatric hospital wards and clinics and c) in infants and young children confined to an orphanage nursery. By the specific methodology of longitudinal and cross-sectional observations they hoped to determine more precisely the roles of viruses, and other pathogens as well, in producing acute and undifferentiated illnesses in children.

Longitudinal studies in the orphanage nursery provided Bob Huebner and collaborators with observations on thousands of respiratory and undifferentiated illnesses. Thousands of virus isolates were obtained and, for the most part, identified. At least 35 to 40 different prevalent viruses occurred regularly in this population, and substantial amounts of illnesses could be attributed to the adenoviruses, and some of the enteroviruses and myxoviruses. During nearly 3 years of observation since 1955, many of these viruses made periodic, almost predictable, reappearances at appropriate seasons. However, even under intense scrutiny, many illnesses still could not be identified as viral or bacterial infections for a variety of reasons such as poor specimen collection, loss of the agent in transit to the laboratory, loss during specimen storage, inappropriate growth medium, inability of an unknown pathogen to grow in the usual culture mediums and other unknown reasons.

As indicated previously, the hospital-based cross-sectional studies were advanced by the collaboration of Dr. Bob Parrott who had returned to Children's Hospital from NIH. With the laboratory support provided by Dr. Bob Chanock, Huebner and his colleagues were able, during the next few years, to enumerate the contributions that the new respiratory viruses made to the incidence and type of illnesses seen in local pediatric clinics and hospital wards at various times of the year. They were also able to associate clinical signs and symptoms with specific etiologies.

During this period, Bob Huebner and associates worked out some of the steps in testing various experimental vaccines. While he directed most of this work against specific viral strains, he hoped that a multivalent vaccine could be developed that would be effective against many viral organisms. He realized, however, that the logistics and the complexity of producing such a vaccine would be difficult. Moreover, any multivalent vaccine would still protect against only a fraction of the possible respiratory viruses. His associate, Bob Chanock, realized that difficulties might be encountered even with some univalent vaccines such as for respiratory syncytial virus (3). The few vaccine trials conducted were greeted in the press (4) with extreme enthusiasm as signs of progress for control of the "common cold." Bob was always careful to discount this misplaced enthusiasm by indicating that the viruses in the vaccines were agents that produced atypical respiratory symptoms, not the one usually associated with coryza (the common cold), or that these viruses only occasionally caused coryzal signs and symptoms as part of their clinical spectrum (1). In the late 1950's Andrewes and associates (5) in England, and other investigators, were able to grow *rhinovruses*, the agents that typically cause coryza as their principal physical manifestation. Bob Huebner and his associates did not

work with this group of viruses during their early intensive investigation of the other respiratory viruses. The rhinoviruses exist in more than 100 serological types. This fact helps explain the apparent lack of immunity to recurrent attacks of the “common cold.”

The years 1958-1959 were a period for Bob Huebner’s re-assessment of the direction of his future research activities. In a few brief years from 1949 Bob had succeeded in major research accomplishments with his investigations of herpangina, epidemic pleurodynia (the Coxsackie viruses), the adenoviruses, the other newly recognized viruses uncovered at Junior Village and the development of effective adenovirus vaccines. He had acquired a national and international reputation with these activities and could well afford to “rest on his laurels” for many years. Nevertheless, with his keen instinct and insight for what were becoming important new developments in virological research, and with his restless intellectual curiosity, Bob was ready to turn the unfinished work on respiratory viruses over to his associates, primarily Bob Chanock, and to embark on a different area of virus research that was to occupy him for the remainder of his scientific career.

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Chapter 11

The Lieutenants

One of the secrets to Bob Huebner's success in his research activities was his uncanny ability to select associates with intellect, investigative inquisitiveness, dedication, professional integrity and personal loyalty. Bob had the instincts of a superb talent scout; the people whom he chose to work closely with were unswerving in their devotion to him. He was also a wonderful mentor, able to nurture those qualities that enhanced their professional development and generated respect among their peers. Dr. Huebner provided ideas and encouragement for tasks that they could accomplish, and his associates in turn found stimulation and satisfaction in the successful accomplishments of specific investigative goals. According to his wife Harriet, (27) Huebner set-up goals that he felt his associates would be able to attain. He believed that nothing was more discouraging to a young investigator than to saddle him or her with a problem to which there was no easy solution or no solution at all. On the other hand, if he found someone in the laboratory who did not have the necessary feel for investigative work, he would gently but swiftly try to set that person on a different career path.

1960. Dr. Robert Huebner in the laboratory. (Office of NIH History files).

The group of individuals whose careers are summarized here is not inclusive but encompasses those who were among his earliest associates. Joe Bell, Wally Rowe, Bob Parrott, Janet Hartley, Leon Rosen, Al Kapikian and Bob Chanock all maintained

intimate and long-standing associations with Bob Huebner and benefited from his mentoring. In turn, they helped insure, with their expertise and insight, Huebner's astounding successes in the discovery and study of many new viruses in the 1950's and early 1960's.

Dr. Joe Bell

Joseph Asbury Bell (1) was born in Trinidad, Colorado March 27, 1904. He was among Huebner's oldest associates, joining Bob in 1947 when the Q fever studies were underway. He was with Bob until his compulsory retirement from the Public Health Service for physical disability on July 1, 1964. Joe Bell participated in the studies of Q fever in California, herpangina, pleurodynia, adenoviruses, the respiratory viruses of Junior Village and Children's Hospital of D.C. He had other areas of interest as well, and he received major recognition throughout his professional career for that work.

Joe Bell graduated from the Manual Training High School in Denver in 1921, attended the University of Colorado in Boulder and took a full academic course for four years, receiving his M.D. degree from the University of Colorado in Denver in 1929. During his medical courses he worked as an auto mechanic to help pay expenses and to support his new bride, Margaret Mae Mitchell. From medical school he went directly to the San Francisco Marine Hospital, serving as an intern for one year. After successfully completing the examination required by the Public Health Service, he was commissioned as an Assistant Surgeon (First Lieutenant, Army-Lieutenant, Jr. Grade, Navy) on July 1, 1930. He was then stationed for two years, from August 1, 1930 to July 18,1932, at the U.S. Quarantine Station on Angel Island in San Francisco Bay. After that he spent another year at the San Francisco Marine Hospital in internal medicine. In 1933 he

passed another examination and was commissioned Passed (Senior) Assistant Surgeon (Captain, Army). He was placed immediately in charge of the San Diego Quarantine Station and Relief Station where, in addition to his administrative duties, he was responsible for conducting the quarantine duties at the port, the medical examinations of aliens at the Mexican border and the medical and surgical care of seamen and other beneficiaries. At the end of 16 months, on November 30, 1934, he was sent for a three to four month training course at the National Institute of Health, which was then located at 25th and E Streets N.W. in Washington, D.C. Beginning in March 1935, he was assigned to the Foreign and Insular Quarantine and Immigration Division as Assistant to the Chief (Assistant Surgeon General F.A. Carmelia) under Surgeon General Hugh S. Cumming. He also served (as Dr. Cumming's deputy) as Acting Director of the Pan American Sanitary Bureau. Joe Bell was one of the group of talented physicians who sought commissions in the Public Health Service during the years of the "Great Depression" (1929 to World War II) and was moved frequently to various duty stations.

He was detailed from the NIH in September 1936 to the Johns Hopkins School of Hygiene and Public Health for post-graduate study. In June 1937 he received the degree of Master of Public Health, and for the year 1938-1939 he returned to the school as instructor in epidemiology.

From 1937 he was in the Division of Infectious Diseases, Section on Epidemiology at the National Institute of Health with Dr. James P. Leake. Bell worked with Leake on studies of pertussis (whooping cough) vaccination, and Bell succeeded Leake in 1945 as Chief, Epidemiology Section, National Microbiological Institute, National Institutes of Health.

Meanwhile he was commissioned Surgeon (Major), then Senior Surgeon (Lt. Colonel), and on account of World War II, transferred to duty with the US Army. In May 1943 he completed a 4-month course in military government and was sent to Africa. At Tizi Ouzou, near Algiers, he attended an Allied Military Government School. The same year, 1943, he was Military Director for Health for the Provinces of Enna and Caltanisetta in Central Sicily. From December 1943 to February 1944 he served as epidemiologist for the Allied Control Commission with the US Typhus Commission in Naples. He probably contracted typhus fever while engaged in this activity. Then he designed and conducted a school for training Allied Military Government officers for public health operations in Europe and served with the public health staff for military government at Supreme Allied Headquarters in London.

In 1945, he became Chief, Public Health, US Group, Central Council for Germany. In November 1945, he was officially transferred from the US Army back to duty with the US Public Health Service and was awarded the Legion of Merit by the Army.

After returning to the National Institute of Health in 1945, he continued his studies on immunization for whooping cough and diphtheria. In conjunction with this he received the degree of Doctor of Public Health from Johns Hopkins University in 1948 following acceptance of his thesis and doctoral examination. The following year he was commissioned as Medical Director (Colonel) in the USPHS and was certified as a Diplomate of the American Board of Preventive Medicine. Shortly thereafter he was appointed Head of the Epidemiology Section of the National Microbiological Institute (later NAIAD), a post that he occupied until his retirement.

From the late 1940's until the early 1960's, he collaborated with Bob Huebner on Q fever, herpangina, epidemic pleurodynia, adenoviruses, pharyngoconjunctival fever, and the Junior Village investigations and provided epidemiologic support for the respiratory virus studies of Drs. Chanock and Parrott.

It was during the latter phases of the Q fever period that I became acquainted with Joe Bell. He was a tall, impressive appearing person, always dressed immaculately in conservative business suits, wearing a gray Homburg hat perched at a jaunty angle and, looking more like a Wall Street investment banker than a field epidemiologist. He exuded confidence and gravitas. I had a closer association with him during the herpangina and pleurodynia investigations. He was always cordial but reserved. In conformity with his unwavering precision, he "chewed me out" unmercifully when, after a preliminary review of a manuscript I was writing, he called the paper unsatisfactory and unprofessional. I redrafted the manuscript several times until it met with approval as worthy of publication. He himself was a perfectionist, and he frequently delayed submitting papers until he was absolutely satisfied with the result. He waited seven years before submitting one paper on influenza vaccination. Because of this personality trait, several colleagues christened him "The Prince of Procrastinators."

Joe Bell enjoyed a reputation as one of the outstanding epidemiologists in the United States by the early 1950's. In 1953 he was called upon to head up one of the most ambitious, intensive epidemiological studies ever proposed in the United States, namely the clinical evaluation of the efficacy and safety of the Salk vaccine against poliomyelitis. On July 6,1953, (2) Dr. Harry M. Weaver, Director of Research for the National Foundation for Infantile Paralysis, wrote to Dr. Leonard A. Scheele, Surgeon General,

USPHS, and requested that Joe Bell's be appointed to assume responsibility for carrying out of a field trial of a poliomyelitis vaccine. Joe Bell had requested leave without pay from the PHS to do this project. The request was honored after a supporting statement was submitted by Dr. Victor M. Haas, Director, National Microbiological Institute (2), to Dr. James A. Shannon, Associate Director, NIH and approval given by Dr. William H. Sebrell, Director of NIH. Joe Bell proposed a slow, meticulous study with adequate blind controls. This proposal drew criticism from Mr. Basil O'Connor, President of the National Foundation, and Dr. Jonas Salk, the developer of the polio vaccine. Both men wanted a rapid study with "observed" controls (3). Joe Bell was adamant in the use of adequate controls for the poliomyelitis vaccine study, as he had done successfully in the past with his study of whooping cough and diphtheria vaccines in children. Because of the impasse with O'Connor and Salk, Joe Bell resigned in disgust. Jonas Salk turned for help to his mentor with whom he had trained, Dr. Thomas Francis, a renowned virologist and epidemiologist, of the University of Michigan School of Medicine. Dr. Francis also insisted on an adequately controlled study before launching the project under his responsibility (4). After this was agreed to, the Salk polio vaccine was finally evaluated. Joe Bell returned to the NIH to continue his collaborative studies with Bob Huebner and to expand his investigations of influenza vaccines.

Joe Bell served on many epidemiologic committees and advisory boards. In 1952, he was elected president of the American Epidemiological Society. In April 1962, he was awarded the Distinguished Service Medal of the USPHS. About 1960 he began to have a series of attacks of chest pain, some of which were shown to be myocardial infarctions. In 1962, he had an abdominal aortic aneurysm with resection of the terminal aorta and

proximal iliac arteries and replacement with a Dacron prosthesis. On July 1, 1964, he was compulsorily retired from the PHS for physical disability. During a trip around the world with Mrs. Bell, he had another severe coronary attack in October 1967 and was hospitalized in New Delhi, India for six weeks. The end came suddenly and instantaneously on October 29, 1968, as he was supervising the installation of heating and air conditioning improvements in his home. He was active mentally and physically until the end.

Several years before Dr. Bell's death, Bob Huebner, on April 4, 1961, (2) as Chief, LID, NIAID wrote to Dr. Richard C. Arnold, Assistant Surgeon General for Personnel and Training, PHS, a laudatory statement supporting Joe Bell's nomination for the PHS Commissioned Officers Meritorious Service Medal: "Dr. Bell for his contributions to preventive medicine and epidemiological research is recognized as one of the outstanding investigators in the field of infectious disease control. Through extensive field studies of epidemic and endemic diseases, and through the development and field testing of vaccines and antibiotics against common illnesses, he has contributed significantly to our understanding of the cause, mode of spread and method of preventing many bacterial, viral, and rickettsial diseases, including whooping cough, diphtheria, Q fever, influenza, poliomyelitis, herpangina, pharyngoconjunctival fever and acute febrile respiratory illnesses.

"Dr. Bell's contributions in the field of infectious diseases have been characterized by meticulous attention to detail, unusual thoroughness in the collection of facts and the logical development of conclusions and inferences." There seems no more fitting summary of Dr. Bell's lifelong achievements and contribution to science.

Dr. Wally Rowe (1926-1983)

1962. Drs. Robert J. Huebner and Wallace P. Rowe. (Courtesy of the National Library of Medicine).

Wallace Prescott Rowe (5) was born in Baltimore, Maryland on February 20, 1926. His career was one of continual accomplishments. He started his premedical college preparation during World War II, entering the College of William and Mary in Williamsburg, Virginia in June 1943. He was on an accelerated wartime academic program and finished his first year in June 1944. He spent the remainder of that summer at Johns Hopkins University prior to entering Johns Hopkins Medical School in September 1944. Briefly, he returned to William and Mary in summer 1945 to obtain more college credits, even though he had a year of medical school under his belt! He was on the accelerated wartime combined college-medical school program. He obtained his M.D. degree June 8, 1948. Over the course of the next year (July 1, 1948 to June 30, 1949) he took his internship in internal medicine at North Carolina Baptist Hospital, Winston-Salem, North Carolina, which was affiliated with the Bowman Gray School of

Medicine. He then worked as a Research Fellow at this School prior to beginning duty with the US Navy. For the few months he was there, he worked on the Aureomycin (tetracycline) therapy of mouse salmonellosis under the supervision of Dr. Manson Meads with whom he had worked on a clinical chemotherapy problem during his internship. (6)

On September 29, 1949, he reported for duty as a Lieutenant Junior Grade to the Naval Medical Research Institute, National Naval Medical Center, Bethesda, Maryland. For the next two years he worked on the pathogenesis and immunity mechanisms in lymphocytic choriomeningitis infection of the mouse (7). The first year and a half Wally worked under the supervision of Dr. Eric Traub of the Research Institute; for the remainder of the time, he worked on his research independently, assuming a supervisory role over both technical and non-technical personnel. This work resulted in a detailed research report dated May 27, 1954, which he submitted for publication to the *Journal of Immunology*. In sum, this study focused on the pathogenesis and acquired immunity in lymphocytic choriomeningitis (LCM) of adult mice with emphasis on determining the relation between virus growth and development of disease, and developing procedures for demonstrating protective antibodies in immune mice. (Lymphocytic choriomeningitis (LCM) is a virus endemic in its major host, the house mouse, with man as an accidental host. Dr. Charles Armstrong discovered and isolated this virus from the brain of a patient who had died during the 1933 outbreak of Saint Louis encephalitis. On Dr. Armstrong's role with LCM, see the chapter on "Rickettsialpox.") It demonstrated that the degree of reactivity of the host to the growth of high titer virus was an important factor in the pathogenesis of the disease and in acquired immunity, and that the reactivity was

modified by previous infection, persistence of virus in the host, x-irradiation and possibly by immune mouse serum.

Wally Rowe developed a new sensitive intra-peritoneal protection test that could detect the presence of protective antibody in the immune mice. He uncovered the interference phenomenon (presence of one strain of virus preventing infection with a second virus—an observation described by him and other investigators using different virus systems that ultimately led to the discovery of *interferon*) between LCM strains differing in intra-peritoneal pathogenicity.

As his tour of duty with the Navy came to a close, Wally finished up his work at the Naval Medical Center and applied for a commission in the USPHS (on March 31, 1952) requesting that he be assigned to infectious disease research somewhere in the continental United States. He received the commission and was released from active duty in the Navy on July 1, 1952 (5). In August 1952, Wally reported to Bob Huebner's laboratory at LID/NMI/NIH. Wally was to take over my position when I left in September to return to and finish my clinical training in internal medicine. Initial impressions are often the important ones, and my brief personal interaction with Wally was extremely favorable and pleasurable. I found him amiable and modest, and I appreciated his expressions of admiration for the work that the laboratory had just accomplished with the Coxsackie viruses. While Wally was waiting to get his tissue culture system underway so that he and Bob Huebner could begin studying respiratory viruses, he carried out a very clever experiment using a strain of group A Coxsackie virus (8). He was able to propagate viral growth and produce pathologic changes in denervated adult mouse muscle by cutting the sciatic nerve thereby providing a possible clue to the

age-dependant requirement in mice for the growth of group A Coxsackie viruses. This experimental observation to my knowledge has not been explored further.

Like this early start, the rest of Wally's research was stellar. He subsequently participated in the highly significant investigations of adenoviruses, cytomegalovirus, the Junior Village studies, respiratory viruses and polyoma virus coming out of Huebner's laboratory. On a professional level, he assumed the same sort of success, rapidly advancing through the ranks of the Public Health Service and receiving a commissioned rank. From when he entered to USPHS in 1952 through 1955 he was a Senior Assistant Surgeon (Captain). In 1956 he was promoted to Surgeon (Major); from 1957 to 1960 he held the rank of Senior Surgeon (Lt. Colonel); from 1957 to 1968 he was the Chief, Oncolytic and Oncogenic Virus Unit, Virus Section, LID-NIAID-NIH; from 1960 to retirement in 1982 he held the rank of Medical Director (Colonel); and from 1968 to retirement in 1982 he was the Chief, Laboratory of Viral Diseases, NIAID, NIH. On August 19, 1960, Bob Huebner wrote a glowing and persuasive letter to Dr. Justin M. Andrews, Director of NIAID, recommending the temporary promotion of Wally Rowe to the rank of Medical Director (2). Bob indicated that in just 8 years from 1952 to 1960 Wally had made major discoveries and received many honors because of his achievements. Wally had been offered several full professorships at prestigious universities (the University of Washington at Seattle and the University of California at San Francisco), but he refused these offers, preferring to continue his work at NIH. "He is recognized as a lucid writer, as a superior teacher and as one of the most accomplished experts in the virus field as well. Best of all his future is still largely before him" (2). It

was, indeed, and he would have more than 20 years of important contributions and insights into the nature of tumor viruses.

A partial listing of studies upon which Wally Rowe embarked alone or in collaboration with Bob Huebner, Janet Hartley and other associates include: the isolation of reoviruses in mice in nature; the oncogenic effects of adenovirus types 12 and 18; the oncogenic effects of simian virus-40 (SV-40) in hamsters; bovine papilloma; rabbit papilloma; viral tumor (T) antigens in non-infectious adenovirus produced tumors (with Bob Huebner); continuing studies of cytomegalovirus (CMV) and lymphocytic choriomeningitis (LCM) with investigation of laboratory outbreaks; the study of hybrid adenovirus-SV-40 tumors and their implications for human cancers; investigations of the so-called "helper viruses," which provided a required murine leukemia strain (this strain combined with a non-producing murine sarcoma produced infectious virus); development of a plaque assay to assess the presence of infectious murine leukemia viruses. In later years, Wally Rowe and associates demonstrated murine leukemias as chromosomal genes of the mouse. By cross breeding and back breeding with various strains of mice with and without viral leukemias, they were able to show that the mouse leukemias (retroviruses) followed the general laws and inheritance patterns of classical Mendelian genetics. By utilizing the techniques of molecular biology, they also showed that the chromosomal viral sequences could incorporate the chromosomal leukemia inducing foci in the mice (oncogenes).

For these innovative and important investigations, Wally received many honors and invitations to memberships in prestigious professional societies including the American Society for Clinical Investigation, American Association for the Advancement

of Science, and the National Academy of Sciences. As well, he received many honors including the 1960 Eli Lilly Award-Society of American Bacteriologists, the 1970 PHS Meritorious Service Medal, the 1972 Rockefeller Public Service Award, the 1974 PHS Distinguished Service Medal, the 1976 Selman A. Waxman Award in Microbiology and many others.

Bob Huebner and Wally Rowe had an extremely close personal and professional relationship extending over many years. Bob was initially a mentor and source of ideas; later, as Wally initiated his own and original research, they became close friends. When Bob moved from LID to the National Cancer Institute, the administrative officials at the National Cancer Institute (NCI) wanted Wally and Janet Hartley to move to NCI along with Bob but they preferred to remain at the new Laboratory of Viral Diseases-NIAID. However, Wally and Jan Hartley received a portion of their funding from NCI (9).

Dr. James A. Rose, (10) who was detailed in the mid 1960's from the Radiation Branch of NCI to temporary assignment in Wally's laboratory in Building 7 to study viral oncology, described the almost daily afternoon meetings between Bob Huebner and Wally. Bob's arrival was announced usually by a sound approximating the shattering of a brittle piece of clear plastic. This sound was produced when Bob reached into a box of apples kept for feeding the laboratory mice, selected an apple and took the first crunching bite. Jim Rose then described the Huebner monologue with its endless flow of new ideas, suggestions for additional research approaches and projects that Wally should do. Jim, in addition to other persons I interviewed (Drs. Robert Parrott, Robert Stevenson, James Duff, and others) for their knowledge about Bob Huebner's personality, mentioned in his first statement to me that, "Bob was a man of many ideas." Wally would listen to Bob

patiently and attentively nod his head in apparent agreement. After Bob departed, Jim Rose would ask Wally if he was going to follow-up on all the suggestions that emerged from Bob's monologue. Wally would smile benignly, and give a very non-committal reply. Wally, by then had become quite independent. Occasionally Wally's technician, Worth Capps, as a practical joke, would startle Wally into thinking that Bob Huebner had arrived by simulating the apple crunching sound. He did this by sneaking up on Wally and cutting a plastic culture tube with a heavy scissors thereby producing an almost comparable sound.

Janet Hartley (11), in comparing the achievements of Bob Huebner and Wally Rowe, described the more sophisticated intellectual level of Wally's later research attainments. She explained that Bob was a great biologist, virologist, epidemiologist, immunologist and promoter and coordinator of research activities; Wally, on the other hand understood and was able to use the techniques of modern genetic analysis as well as molecular and biochemical techniques in the investigation of tumor viruses at the cellular level. However, each man in his own way brought the full force of their personalities and skills into play in achieving success at NIH.

In the early 1980's Wally's health deteriorated (9). He developed the signs and symptoms that led to surgery for colon cancer. Despite evidence of metastatic spread, he declined the use of adjuvant chemotherapy because he did not want a treatment that might cloud his mental processes. He passed away in 1983 at age 57.

Dr. Robert Harold Parrott

Bob Parrott (12) was born in 1923 in New York City. He died December 26, 1999 of a recurrent stroke. Bob's interest in infectious diseases began in adolescence

stemming from personal medical experience. He suffered from osteomyelitis (bone infection) of the right femur (thigh bone) in the days prior to effective antibiotic treatment. After several surgeries and subsequent treatment with antibiotics the infection was arrested but left him with a shortened right leg, a limp and the need to use either a crutch or a cane in order to walk with comfort. He took his pre-medical training at Fordham University, finishing up in 1945. He graduated from Georgetown University School of Medicine in 1949, and then completed a one-year internship at the Hospital of Saint Raphael in New Haven, Connecticut. He did a two-year residency in pediatrics from 1950 to 1952 at the old facility of Children's Hospital at 13th and V Streets N.W. Washington, D.C.

It was during this period that I initially became acquainted with Bob Parrott. As a young Resident at Children's Hospital he had received the first Fellowship offered by the new Children's Hospital Research Foundation. In this capacity he served as a link between Children's Hospital and Bob Huebner's laboratory in 1950-1951 during the collaborative study on herpangina. He located most of the herpangina cases in the Out-Patient Clinic of the Hospital including the patient who harbored simultaneously the two strains of herpangina virus that was so important for the understanding of a major aspect of enterovirus epidemiology. Bob's first scientific publication (13) was the one we wrote as co-authors describing this event. I was impressed that the challenge of his physical disability did little to diminish his energy, intellectual enthusiasm or cheerful personality. After completing his residency, he joined the LID where he worked with Bob Huebner and his staff from 1952 to 1954 while waiting for the opening of the NIH Clinical Center, and the position for which he had been recruited by Huebner. He helped Bob Huebner

and Wally Rowe discover and define the early phases of the adenoviruses (14), and, as noted previously, he described the clinical entity of pharyngoconjunctival fever (15). He continued working on the Junior Village studies, and, when he left the NIH Clinical Center on July 1,1957, he continued his long term collaboration with Bob Chanock on the respiratory viruses at Children's Hospital. Shortly after becoming Physician-in-Chief at Children's Hospital, he helped orchestrate the hospital's new virus laboratory.

The old Hospital facility at 13th and V Streets N.W. was a crumbling structure, the descendant of a foundling home that had modest beginnings in the era after the Civil War. In 1977, after more than a decade of fund raising by Bob Parrott and others, and with a matching grant approved by Congress, Children's Hospital moved to an $80 million, modern, state-of-the-art facility at 111 Michigan Avenue, N.W., Washington, D.C. Located adjacent to the Washington Hospital Center, the new entity was renamed Children's National Medical Center. It now employs more than 250 full time physicians and researchers, and it continues to serve as the pediatric training facility for George Washington University School of Medicine, where Bob Parrott also served as Professor and Chairman of Child Health and Development.

Bob Parrott retired in 1985 because of physical disabilities. My contacts with him after he left NIH were infrequent but quite cordial. He was very kind towards my family and me when one of my daughters was a patient at the old Children's Hospital facility and gravely ill, with infectious complications resulting from a post-operative perforated appendix.

My first meeting with him in January 1999, when I was interviewing Bob Huebner's associates, turned into a tragic encounter because he was in the early stages of

recovery from a recent hip replacement and a stroke. He was frail, his memory for recent and remote events was dim, and he was confused. Fortunately, a mutual pediatrician friend who saw him several months later at a social gathering said that Bob Parrott's mental state had improved greatly. I saw him in late March 1999, and the improvement was remarkable. His first unsolicited comment was that Bob Huebner was "a man of many ideas." He also described how Bob Huebner encouraged him to remain in research. He recalled many incidents relating to the Junior Village era, and his period of collaboration with Bob Chanock in the long-term studies of respiratory viruses at Children's Hospital. Unfortunately, his remission was short-lived and he passed away six months later. I am grateful that I had the opportunity to meet with this remarkable man who shared in so many of Bob Huebner's accomplishments.

Other observers (12) described Bob Parrott as calm and unflappable, active on the wards of Children's Hospital, despite the pressures of fund raising and his own deep interest in research. Dr. Peter R. Holbrook (12), the Hospital's current Chief Medical Officer who had directed the Hospital's intensive care unit for 20 years, described Bob Parrott's exceptional leadership capabilities, "His gift was to hire people he thought had great promise and give them a tremendous amount of freedom to explore their fields." This attribute undoubtedly reflects Bob Parrott's exposure to the influence and philosophy of Bob Huebner.

Dr. Janet W. Hartley

Janet W. Hartley (16) was born March 25, 1928, in Washington, D.C. and received most of her pre-professional and professional training locally. She received her Bachelor of Science degree in 1949 at the University of Maryland, College Park,

Maryland, and obtained a Master of Science degree in 1951 and Doctor of Philosophy degree in 1957 at George Washington University, Washington, D.C. While working with Wally Rowe on the cytomegaloviruses (CMV), she completed her doctoral thesis: "Comparative Studies of the Human, Mouse and Guinea Pig Salivary Gland Disease Viruses (CMV)." From 1949 to 1952 Jan was a Fellow in Bacteriology and from 1951 to 1952 a Research Associate, at George Washington University. She spent the next year as an Assistant Bacteriologist at the American Type Culture Collection, which was then in Washington, D.C. In 1953 she joined Bob Huebner's laboratory in Building 7 and spent the remainder of her career up to the present time in the same location. Her career steadily advanced at NIH; starting as a Bacteriologist, (1953-1961), she became a Research Bacteriologist, (1961-1963), and then Microbiologist, (1963-1968), all at LID, NIAID, NIH; she then served as a Research Microbiologist, (1968-1983), of the Laboratory of Viral Diseases, NIAID, and was appointed Head of the Viral Oncology Section, 1983-1985, LVD, and then Head of the Viral Oncology Section, 1985-1996, of the Laboratory of Immunopathology, NIAID. In 1996, she became Scientist Emeritus in the same unit. During her career she was a member of many professional societies and was the recipient of honors and special scientific recognition. These included many awards from the Public Health Service for her long career of outstanding research accomplishments. Her research interests in virology included the biology of mouse leukemias, adenoviruses, papova viruses, corona viruses, cytomegalovirus and the virology of laboratory and feral mice.

The bulk of her career was spent working with Bob Huebner until his transfer to the National Cancer Institute in 1968 and with Wally Rowe until his death in 1983. She

also worked with Dr. Herbert C. Morse, III, when Dr. Morse became Head of the Laboratory of Immunopathology, NIAID, NIH in 1985. Bob Huebner brought her to NIH, and she expressed her thanks and gratitude to him in the following testimonial letter (17), "Dear Bob, although the idea of an NIH without you is unhappy indeed, the occasion of this letter is at least an opportunity to say a personal 'thank you'—for many things but most particularly for a day which you may not remember at all.

"It is the day of my first visit to Building 7, and my first climb to the third floor (the elevator had its off days even then) for a job interview with you. My first appointment that day had been with a very pleasant gentleman who told me—most honestly and fairly—that he was looking for a technician, pure and simple, and not for some one who was trying to stay in graduate school and who had visions of a career as a researcher. The job sounded pretty dull anyway so I was really hoping for better things from my interview with you. I was ushered into an office crowded with books, journals and papers, and you, looking rather like a large blue bear in your rumpled coveralls and with your hair every which way. And I was immediately caught up in your enthusiastic and vivid descriptions of new and beautifully logical approaches to studying the etiology of viral respiratory disease. You and Wally were just then in the middle of the excitement of working out the significance of spontaneous cytopathology in adenoid tissue cultures; I don't think even you could have predicted the extent of the body of knowledge which would emerge from the findings of that first cluster of round cells but your exceptional instinct for choosing the important thread to follow was in full operation.

"What an experience that interview was for me—the real-life exposure to the unique excitement of research, and research aimed at clear and important questions. And

you seemed delighted with the idea of having a graduate student around—'There are a hundred things you could work on.' you said, and you proceeded to outline a fair sampling of that number in rapid succession. And, to make it all immeasurably better, I got the job, and with it many years of learning from you, of stimulation by your intuition and enthusiasm, of unflagging support and encouragement, and of sharing your dedication to doing research that mattered, and in doing it with complete honesty and integrity.

"With thanks for all this and more, most sincerely, Jan"

In these few sentences, Jan Hartley captured Huebner's enlightened and liberal attitude toward women in the sciences as well as his personality, spirit, personal convictions, and the moral compass that guided Bob Huebner during his professional career.

An interview with Jan taken by Dr. Carl G. Baker elaborates further on Jan's introduction to Bob Huebner and his laboratory operation (11). It is part of a series of interviews that Dr. Baker conducted in 1995 with former investigators and administrators who were associated with activities of the National Cancer Institute. Jan was included because of her association with Bob Huebner and Wally Rowe in the work on cancer viruses. Dr. Baker asked Jan for her insights into the personalities of Bob Huebner and Wally Rowe whom he considered "interesting people."

Dr. Hartley responded: "Indeed they are. My first experience with Bob Huebner was when I came for an interview. I don't know if you know it, but in those days in Building 7, all the investigators wore blue jumpsuits. Everybody. And I met with Bob Huebner who was a big man, and his blue jumpsuit was a little too small for him, so you

had the feeling he was about to burst out of his suit and out of his office and everything else. But he was so full of enthusiasm for what they were doing with these adenovirus isolations—the use of a new tissue culture technique and finding something that had never been seen before—that you know I could think there is no place that I've been that I want to work more than this place.

"And then he took me back in the lab and introduced me to Wally Rowe, who was in a blue jumpsuit and was sitting up on a lab bench—very young, very handsome—and I met him and the two of them decided that I could come and work here. And that was one of the most exciting and happiest days of my professional life.

"But Bob Huebner was, of course, full of tremendous enthusiasm and full of great energy, and he was a motivator. He could get other people interested and enthusiastic in what he conceived as being important. And he had broad knowledge of biological matters. I mean, he had, of course, worked with rickettsias and done some fabulous epidemiology that - - Q fever and rickettsialpox and epidemic pleurodynia and just had really opened up the virus-rickettsial epidemiology field in many ways. He just went out and did things that nobody else had the nerve to do. But in the lab he was very insightful but he sometimes got a little carried away with his enthusiasm. Wally Rowe, on the other hand, was tremendously knowledgeable, a very, very brilliant mind. He understood genetics and mathematics and all aspects of medicine and virology. And he was sort of a self-taught virologist. And he was kind of an analyzer. And I was sort of a facilitator."

Dr. Baker: "Between the two of them"?

Dr. Hartley: "Well, no. I just sort of saw that things got done. You know, they were busy thinking and talking, and somebody had to get things done."

Dr. Baker: “Well, Wally Rowe was very critical in the high quality sense, and he was a very good one to have him assess something.”

Dr. Hartley: “Extremely. They made a very good pair because Bob had the unusual ideas and enthusiasm, and Wally had the ability to sort out what was doable and what should be followed up and then went ahead and did it.”

Dr. Baker: “They worked together on some things, but they also had other work that was separate from each other.”

Dr. Hartley: “Yes. They worked fairly closely together for the first number of years I was here. They interacted all the time. Wally always had a little project of his own, but generally they were looking at slightly different aspects of similar questions”.

In this interview Janet Hartley expanded further on some of Bob Huebner’s personality and attributes and the working relationship between him and Wally Rowe. She minimized her own role in their joint collaborations. In addition to the intellectual input, she physically did most of the actual “bench” work of their investigations. She is still very active and goes into the laboratory almost every day.

Dr. Leon Rosen

Leon Rosen (18) was one of Bob Huebner’s peripatetic associates who, during his professional career, amassed an awe-inspiring curriculum vitae and bibliography. His interests in microbiology were many and varied, and, according to Bob Chanock (19), he “never knew Leon to be wrong scientifically.” Through the good offices of Bob Chanock, I had an opportunity to meet and to interview Leon Rosen one Friday morning in July 1999 during one of Leon’s infrequent visits to Bethesda from his home in Paris, France. The physical crowding in Building 7 limited space for a quiet interview, but we were

fortunate to be able to use the office of Dr. Al Kapikian who happened to be on vacation at the time.

Leon Rosen was born October 4, 1926, in Los Angeles, California. He graduated with a Bachelor of Arts degree in Medical Sciences from the University of California in Berkeley in 1945. He received his MD degree from the University of California, San Francisco in 1948. While in medical school he rotated through the emergency room of the San Francisco County Hospital and became good friends with Dr. Alex Shelokov (my fellow intern in Boston who followed me to NIH in 1950) who was going to medical school at Stanford University. Leon and Alex's careers overlapped at the Laboratory of Infectious Diseases in Building 7. Leon received the degree of Master of Public Health in Epidemiology in 1950 from Berkeley and the degree of Doctor of Public Health in Epidemiology from Johns Hopkins University in 1953. From 1946 to 1947, he was a research assistant at the G.W. Hooper Foundation, University of California, Bakersfield, California. After medical school he spent a year as a rotating intern at the Gorgas Hospital, Panama Canal Zone. After this stint he went to Berkeley for his degree of Master of Public Health. In 1950 he joined the US Public Health Service and from 1950 to 1955 he was a Staff Member of the Laboratory of Tropical Diseases, NIH. He was stationed in Papeete, Tahiti, where he worked on filariasis and other tropical diseases. He also married his wife there. She was of French and Native Tahitian heritage. The Pacific area drew him back again in later years. From Tahiti he went to Johns Hopkins University for his Doctorate in Public Health. Following this he was sent to Panama for almost 2 years to work on dengue fever. He had to leave Panama because of financial reasons. In 1954 he sought a position at the Laboratory of Tropical Disease Unit in

Bethesda, Maryland. Dr. G. Robert Coatney, Head of the Laboratory, discouraged Leon because Leon was still working with dengue fever; this did not fit the Laboratory's current program that was then focused primarily on the investigation of malaria. Also, there was no space available to accommodate him.

In 1955, Leon came to Bethesda where he sought a position with other investigators at the Laboratory of Infectious Diseases. He was rebuffed repeatedly. No one had room for him because "space was tight." After all these rejections, Leon said that he was eternally grateful to Bob Huebner for finally offering him a position in the Viral Unit. For work space Bob assigned him a "small" animal room that was used formerly to house a chimpanzee for which the unit had no further use. Leon was able to finish up his work with dengue fever in about six months and then devoted his energies to the work of the unit. Technical help was also at a premium; he was finally assigned a depressed, broken-down laboratory technician who suffered from anxiety and frequent memory lapses. Later, when Leon was eventually fully integrated into the Junior Village studies, he was given adequate working space and technical assistance.

When the Junior Village studies began, Leon's primary area of responsibility was study of the fecal specimens from which he literally "mined gold." He isolated, characterized and made original observations on many new viruses, including previously un-described serological types of echoviruses, the discovery that higher serological types of adenoviruses caused hemagglutination of red blood cells, immunologic studies of ECHO, Coxsackie, and poliomyelitis viruses, longitudinal studies of enteroviral infections in children, discovery of new types of reoviruses and their serologic grouping

by hemagglutination-inhibition, and reoviruses in cattle. He also wrote many review manuscripts.

In 1962 he moved from Bethesda to Hawaii where he became Head of the Pacific Research Section, NIAID in Honolulu (this was funded by NIH through Bob Huebner). He remained there until 1978 and retired from the Public Health Service when the Section was about to be disbanded. He became associated with the University of Hawaii as Director of the Pacific Research Unit, Research Corporation of the University of Hawaii from 1978 to 1980. The NIH also funded this unit. From 1980 to 1994 he was the Director, Arbovirus Program, Pacific Biomedical Research Center, University of Hawaii, Honolulu, Hawaii. After his move to Hawaii he completed unfinished Junior Village manuscripts and returned to the investigation of parasites as well as other viral diseases. He described the entity eosinophilic meningitis caused by the parasite *Angiostrongylus cantonensis* in a series of manuscripts. Since 1983 he has been associated with the Pasteur Institute in Paris, France and is currently on its Advisory Committee.

Leon Rosen had tremendous admiration for Bob Huebner who provided the "jump start" for his career, and he was grateful to Bob for offering him a position in the laboratory when no one else would have him. He had great respect for his intellect (18). "Bob was always moving ahead and had a great feel for what was important in research. When one project was finished, Bob would move on to the next project. One day an associate came into Bob's office to discuss a new laboratory observation and Bob remarked, 'but that's last year's virus.'" Leon was impressed also with the abundance of Bob's ideas; one of their laboratory colleagues put it this way (18), "Ideas pop out of Bob Huebner's head faster than fleas jumping off a dog." A number of investigators spent

years chasing Bob's ideas. Leon's relationship with Bob was characteristically cordial. Any disagreements were dealt with amicably and usually in a good –humored fashion. Leon, also, has continued to feel warmly toward his former colleagues at LID and returns as time permits to visit with those who still remain in Building 7.

With his habitations on either side of the Atlantic Ocean, Leon remains actively engaged and continues an extremely fruitful and respected career.

Dr. Albert Z. Kapikian

Dr. Al Kapikian (20) arrived at NIH July 1, 1957. He earned his Bachelor of Science degree *cum laude* in 1952 from New York's Queens College. While there he was also a standout baseball pitcher setting a record of eleven consecutive victories. He went to Cornell Medical School graduating in 1956. In medical school several of his professors, Drs. Edwin Kilbourne and Walsh McDermott, both of whom were involved with infectious diseases and clinical practice, influenced his career interests. At their suggestion he selected for his senior year term paper the newly described adenoviruses. They advised him to write to Bob Huebner for additional references about adenoviruses and for up-to-date information. After Bob received Al's note, he invited Al to come to NIH to observe tissue culture techniques and to learn more about adenoviruses and virus cultivation. Much to Al's amazement, the famous Dr. Robert J. Huebner personally conducted an unvetted senior medical student through the laboratory, showed him tissue culture tubes with cytopathic changes in the infected tissue, and explained viral techniques in detail with visual demonstrations.

Al wrote a term paper that received an excellent grade. He sent a complimentary copy to Bob, whom he had acknowledged and thanked in the paper for his help. Bob was

impressed by Al's obvious intellect, industry and enthusiasm and offered Al a position at LID-NIH after his internship. Al, at that time, was actually more interested in internal medicine and cardiology. From 1956 to 1957 Al had a rotating internship at Meadowbrook Hospital, Hempstead, Long Island, New York. During the internship year Bob called Al repeatedly with the offer of an appointment at NIH to the extent that his fellow interns would tell Al, "That Dr. Huebner called again" on the occasions when Al could not take the call. Al was almost ready to accept an assistant residency in medicine when he received another persuasive call from Bob. Al finally decided to try the opportunity to work for a short period at NIH.

Al arrived at the NIH July 1,1957, the same day that Bob Chanock also arrived, and the day that Bob Parrott left the NIH-Clinical Center to take up the reins at Children's Hospital. Bob Huebner assigned Al to the Epidemiology Unit under Joe Bell, and he worked in the Clinical Center on the General Population Group Studies with Dr. Thomas Reichelderfer (who later went to the District of Columbia General Hospital to become Chief of Pediatrics). Al was thrust immediately into many epidemiological studies under the supervision of Joe Bell including the ongoing studies at Junior Village in Washington, D.C. Al was amazed that the first manuscript that bore his name as a co-author appeared in November 1957 (21) just a few months after his arrival at NIH in July 1957.

Despite his work in the Epidemiology Unit, Al took advantage of his presence in the Clinical Center to go on teaching rounds with some of the outstanding cardiologists present at that time at the Clinical Center; Al was the only person who came into Building 7 with a stethoscope in his laboratory jacket pocket. After having been at NIH

for 4 to 5 years, Al was in the Building 7 elevator one day with Bob Huebner. Bob, in his gentle way, but with his unmistakable ability to make a point said to him, " Al, what do you think you will learn from the culture tubes with that stethoscope"? Al recognized the message, realized then that he had to make a definitive career choice, put the stethoscope away in the bottom drawer of his desk and never took it out again. As Al said to me at a later date (20), "Bob Huebner persuaded me to relinquish the stethoscope."

Al continued with research activities related to Junior Village through the 1960's. In 1967, he became Head of the Epidemiology Section, Laboratory of Infectious Diseases, several years after Joe Bell's retirement in 1964. Some of his major accomplishments were still in the future. In the early 1970's he became one of the pioneers in the development of immune electron microscopy. This is a technique of visualizing fastidious organisms whose growth could not be recognized by otherwise conventional methods. It consists of combining fluids containing viral organisms with the appropriate specific antibodies and observing under the electron microscope aggregates or single particles of virus surrounded by antibody. In this way Al was able to demonstrate fastidious respiratory disease agents and, for the first time, to visualize the agent of Norwalk disease (non-bacterial gastroenteritis) and the virus of Hepatitis A (in association with S.M. Feinstone and Robert H. Purcell). By immune electron microscopy he was able to distinguish, by immunologic means, Hepatitis A from Hepatitis B (22). In 1974 he detected human rotavirus in the stools of infants and young children with diarrhea, the first visualization of the virus that had been discovered in Australia in 1973. Since then he has worked on rotavirus, the most important cause of severe diarrhea in

infants and young children, responsible for almost one million deaths annually in developing countries.

Using a “Jennerian” (23) approach (e.g. cowpox vaccination against human smallpox disease), Al worked with his Laboratory of Infectious Diseases (LID) team for many years to develop a live attenuated quadrivalent vaccine that included one rhesus monkey strain of rotavirus. After a series of carefully controlled field trials, he developed a successful vaccine. In 1998 this became the first rotavirus vaccine to be licensed in the United States, and a year later it was recommended for use in routine immunization of young infants. However, shortly after its introduction for general use, it was withdrawn by the Centers for Disease Control and the FDA because of reports of intestinal obstruction caused by intussusceptions [Footnote—The slipping of one part of an intestine into another part just below it, noted chiefly in children-usually in the ileo-caecal region.]. The statistics for this observation and the withdrawal of the vaccine have been challenged (19,20)), and the situation is still awaiting resolution as of this writing.

Al Kapikian has had a distinguished career, and he has received many professional and academic honors. When he first came to NIH he joined the commissioned corps of the USPHS. He is currently in the Senior Biomedical Research Service. Despite having been at the Laboratory of Infectious Diseases since 1957, he still demonstrates undiminished enthusiasm, and he is in his laboratory every day.

Al had a close personal relationship with and great regard for Bob Huebner both before and after Bob’s move to the National Cancer Institute in 1968. Some of Al’s sentiments were best expressed in a personal note sent October 18, 1982 on the occasion of Bob’s retirement (24): “I have been privileged to know five ‘standouts’ whom I would

categorize as ‘great people’ in the full sense of the meaning ‘great’ according to my own private definition. One of these five is my first laboratory chief, Bob Huebner, who recruited me for LID in 1957 near the end of my internship at Meadowbrook Hospital.

“Bob, your talents as a scientist are well known to all as your contributions to infectious diseases are legendary. We who had the opportunity to work in your laboratory were indeed fortunate, for your influence shall always remain with us. However, with all your achievements and all your brilliant scientific observations, there are two qualities which I shall always cherish, and these are your humility and humaneness.” Al goes on in appreciation of Bob’s breadth of non-scientific intellectual interests and his apparent athletic skills. Bob demonstrated this skill at one of the many farm picnics where the grace and competence of Bob’s playing at the baseball shortstop position impressed Al who was, in his youth, a star baseball athlete in college.

Al Kapikian feels that he is a continuation of a tradition that was fostered by Drs. Charles Armstrong and Bob Huebner. He firmly believes that in the Laboratory of Infectious Diseases “Tradition fosters excellence.” This is exemplified by the numerous accomplishments of the talented people recruited by Drs. Armstrong and Huebner.

Dr. Robert M. Chanock

1965. Drs. Robert J. Huebner and Robert M. Chanock in the laboratory. There are tissue culture tubes in the foreground. (Office of NIH History files, contributed by R.M. Chanock).

Bob Chanock (25) has had a remarkable and productive career in scientific research and administration punctuated by repeated original and important observations and discoveries, successful leadership of the Laboratory of Infectious Diseases since 1968 and crowned by numerous awards and recognitions. Many of these achievements have been alluded to previously.

Bob was born in Chicago, Illinois July 8,1924, went to high school there, received his degree of Bachelor of Science in Physiology from the University of Chicago in 1945, and stayed there at the medical school from which he received his MD degree in 1947. After a year of internship at Highland-Alameda Hospital in Oakland, California, he returned to the University of Chicago School of Medicine for a 2-year residency in pediatrics. At the time, Dr. Howell Wright, who earlier had been associated with Dr.

Albert Sabin when they were simultaneously at the Rockefeller Institute, suggested to Bob that he should consider a fellowship with Dr. Sabin who was then at the University of Cincinnati. Bob began his research career as a Fellow of the National Research Council and National Foundation for Infantile Paralysis at Children's Hospital Research Foundation in Cincinnati under Dr. Sabin. Initially, Bob published several important papers (25) with Dr. Sabin on the hemagglutinin of St. Louis encephalitis virus. Unfortunately, Bob was redrafted into the US Army in 1952 to complete the fulfillment of his military obligation. Sabin arranged for him to be assigned to the Virology Section of the 406 Medical General Hospital in Tokyo, Japan where the setting was one of "close and continuous collegial interchange that catalyzed his scientific growth and independence. A most wonderful experience that forged close and long lasting friendships."

When Bob returned from the Army in 1954, he rejoined Sabin's laboratory as an Assistant Professor of Research Pediatrics. Despite the laboratory's need for rapid expansion in looking for promising candidates for attenuated live poliovirus vaccine strains, Sabin suggested that Bob dissociate himself from this effort and strike out on his own to pursue investigations in the area of respiratory disease viruses. Despite misgivings in an already overcrowded research field, Bob met with initial success when he isolated a new agent from an outbreak of life-threatening croup in children in Cincinnati. This was the originally named "CA virus", later called "a hemadsorption virus" and finally classified as human type 2 parainfluenza virus. This discovery started Bob on his research path in the field of respiratory viruses.

Against the advice of Dr. Sabin that he should remain in Cincinnati, Bob accepted a position in 1956 as an Assistant Professor of Epidemiology at the Johns Hopkins University School of Hygiene and Public Health. During his year in Baltimore he made another breakthrough discovery in isolating from infants with pneumonia and bronchiolitis, respiratory syncytial virus, a prominent cause of infant mortality and morbidity. Despite this success, Bob described the time as the unhappiest and the lowest period in his life. He could not tolerate the chairman of his department, and he had little opportunity for interaction with his peers.

Several years earlier Sabin had intervened in Chanock's behalf when Bob was in the Army training for his assignment to the Far East. Sabin had arranged for a meeting between Bob Huebner and Chanock at a medical symposium. The meeting was brief and perfunctory because Bob Huebner was in a hurry to meet a group of Angus cattle breeders to negotiate the sale of one of his bulls. Four years later in 1956, when Chanock was at Johns Hopkins, Bob Huebner approached him at a conference in New York City and, without prior warning, invited Chanock to join him at LID. Almost as a reflex, and without a moment's hesitation, Chanock accepted the invitation in a most positive manner.

The accomplishments of Bob Chanock after joining LID have been more fully outlined in the previous chapters; they included the development of the live enteric-coated adenovirus vaccines for military recruits (still in current use), the discovery of three additional serologic types of parainfluenza virus, the growth of mycoplasma pneumoniae on solid bacteriologic media and the collaborative studies of respiratory diseases at Children's Hospital of Washington, D.C. with Bob Parrott and Joe Bell.

Some of the above work occurred during the early years with Huebner, from 1957 to 1959. In 1959, Bob Chanock became Head of the Respiratory Virus Section of LID when Bob Huebner began to devote the bulk of his time investigating oncogenic viruses in collaboration with Wally Rowe and Janet Hartley. Chanock became Chief of LID, and he continues in that position up to the present time, when Bob Huebner left to join the National Cancer Institute in 1968. As Chief of LID, Chanock was charged with the responsibility of maintaining the long term goals of the Laboratory which included, in his own words (26): "resisting the siren call to shift to a reductionist approach when confronted with the dazzling and unending progression of new opportunities for biological insights made possible by technological advances." The shift to the more sophisticated techniques of genetic engineering and molecular biology occurred later in the various investigations of LID when these techniques were needed as tools for further study and understanding of the continuing viral projects, and when the techniques became available for use by personnel with special training.

During Bob Chanock's tenure as Chief, the major investigations included the search for an effective respiratory syncytial virus vaccine, the pioneering work of Al Kapikian on the immune electron microscopy visualization of non-bacterial virus gastroenteritis agents, the basic studies of Al Kapikian and Robert Purcell on hepatitis, initial investigations of simian immunodeficiency virus and the development of the rotavirus vaccine. To all of these efforts Bob Chanock contributed encouragement and intellectual input.

Bob Chanock was one of four former colleagues who arranged the retirement celebration for Bob Huebner on October 18, 1982. He contributed the following

statement (24): “Dear Bob, Dr. Armstrong was the far sighted laboratory chief who brought you to LID. You admired him enormously and passed that respect on to Wally (Rowe), Jan (Hartley), Leon (Rosen), Al (Kapikian), myself and others. Early on Leon dubbed you ‘the boss,’ an accurate and amusing title, which on first consideration seemed inappropriate. Doubts of its appropriateness vanished as we used the title in its most affectionate guise.

“The five of us profited immeasurably from your insight, your vast knowledge of virology and especially your faith in us which you expressed with generous enthusiasm for our special interests. Despite occasional setbacks, you met the challenges of the laboratory with ‘true grit.’ People rarely turned over in LID when you were the chief. With a rangy grace you listened carefully to each member of the laboratory staff, and these individuals remained deeply loyal to you. You realized far better than most of your contemporaries that scientific teams would replace solitary giants in the future of science. This trend did not bother your ego. Your gift of sharing the haunting doubts and thrilling possibilities of our work was more infectious than the viruses we studied. Your own enormous spirit found the planning of massive studies easy. Projects with a twist or dimension unlikely to occur with other scientists came tumbling out of your head—a comprehensive longitudinal study of childhood viral infections [Junior Village-EAB], large collaborative efforts involving workers throughout the USA [Virus Cancer Program –EAB], trailers for the effective isolation of hazardous viruses [Virus Cancer Program, NIH Poolesville, Maryland facility—EAB], etc. Perhaps the average citizen should regret that you chose science. I am certain that you could have developed unimagined solutions to the problems of ailing industrial giants such as Chrysler Corporation [rescued by Lee

Iacocca in the 1980's—EAB]. My mind boggles as I imagine you in David Stockman's position [Director of the Office of Management and Budget, Reagan administration—EAB]. The jobless rate of 10.1% would never have occurred.

"All of us at LID owe you an unpayable debt. Your moral and ethical compass has always had an unfailing true orientation. It has served as a beacon to your many professional associates and collaborators, who like you, have never used privileged information to gain undeserved advantage in priority for scientific discovery. This is a wonderful gift to have given those of us fortunate enough to have worked with you. You probably never thought about the unerring high moral and ethical standard that you set, since it is so basic to your character.

"You showed us that it was possible to lead by example, rather than by fiat. You proved that one could lead by generating enthusiasm and raising your co-workers to a high level of excitation. Finally, you demonstrated that one could lead without compromising the self-esteem or dignity of your associates.

"My personal debt to you is inestimable. You rescued me from the Johns Hopkins School of Hygiene at the lowest point in my professional career, a time when self-doubts and anxiety reached their highest levels. I can only say thank you, thank you.

"With the deepest affection and admiration"

These vignettes summarize the careers and the relationships to Bob Huebner of his associates at the Laboratory of Infectious Diseases. It is interesting to note the commonality of the themes expressed by these talented scientists about the character of Bob Huebner's personality, and the impact that it had upon them A few other persons

who interacted with Bob were less generous in their appreciation of the talent, accomplishment and personal attributes that were expressed so eloquently by his early associates. Those detractors, however, are few and far between.

1) Material for the biographical sketch of Dr. Joseph H. Bell was derived primarily from: A) His personnel service record in the U.S. Public Health Service Commissioned Officers Corps. B) Joseph Asbury Bell, 1904-1968. A Biographical Appreciation, 1969. *American Journal of Epidemiology*, Joseph A. Bell Memorial Issue 90: 463-470. C) Personal recollections.
2) Letter in Dr. Bell's personnel file.
3) Smith, J.S. 1990. *Patenting the Sun. Polio and the Salk Vaccine*. First edition. William Morrow and Co., Inc. New York pp. 198-205.
4) Ibid. Pp. 225-229.
5) Material for the biographical sketch of Dr. Wallace P. Rowe was derived primarily from: A) His personnel service record in the U.S. Public Health Service Commissioned Officers Corps. B) Curriculum vitae. C) Bibliography. D) Personal recollections.
6) Meads, M., Rowe, W.P., and Haslam, N.M. 1951 Alterations in the bacterial flora of the mouth during therapy with Aureomycin. *AMA Archives of Internal Medicine* 87: 533-540.
7) Rowe, W.P. 1954 Studies on pathogenesis and immunity in lymphocytic choriomeningitis infection of the mouse. *Naval Medical Research Institute Report* NM 005 048.14.01.

8) Rowe, W.P. 1953. Propagation of group A Coxsackie viruses in denervated mouse muscle. *Science* 117: 710.

9) Dr. Janet W. Hartley—Personal communication.

10) Dr. James A. Rose—Personal communication.

11) Interview with Dr. Carl G. Baker, former Director of the National Cancer Institute, June 20, 1995, as part of a series of interviews on the administrative aspects of the (Special) Virus Cancer Program.

12) Material for the biographical sketch of Dr. Robert H. Parrott was derived from: A) Interview March 24, 1999. B) Obituary, Washington Post, December 27, 1999. C) Bibliography. D) Personal recollections.

13) See the chapter on - Coxsackie Viruses—Herpangina.

14) See the chapter on—Adenoviruses.

15) See the chapter on—Pharyngoconjunctival Fever.

16) Material for the biographical sketch of Dr. Janet W. Hartley was derived from: A) Curriculum vitae. B) Bibliography. C) Several interviews and conversations 1998-2000.

17) Letter from Janet W. Hartley to Robert J. Huebner October 18, 1982 on the occasion of his retirement from NIH; among his personal papers.

18) Material for the biographical sketch of Dr. Leon Rosen was derived from: A) Curriculum vitae. B) Bibliography. C) Interview July 10, 1999.

19) Robert M. Chanock—personal communication.

20) Material for the biographical sketch of Dr. Albert Z. Kapikian was derived from: A) Bibliography. B) Many interviews and conversations.

21) Bell, J.A., Ward, T.G., Kapikian, A.Z., et al. 1957.Artificially induced Asian influenza in vaccinated and unvaccinated volunteers. *JAMA* 165: 1366-1373.

22) Kapikian, A.Z. et al 1975. Detection and identification by immune electron microscopy of fastidious agents associated with respiratory illness, acute non-bacterial gastroenteritis, and hepatitis A. *Perspectives in Virology* 9: 9-45, discussion, pp. 45-47. Academic Press, Inc. New York, San Francisco, London.

23) A) Kapikian, A.Z., Chanock, R.M. 1996. In Fields, B.N., Knipe, D.M., Howley, P.M., Eds. Fields *Virology*, Third edition, Philadelphia, Lippincott-Raven pp. 1657-1708. B) March 19, 1999. Rotavirus vaccine for the prevention of rotavirus gastroenteritis among children. Recommendations of the Advisory Committee on Immunization Practices (ACIP). *Morbidity and Mortality Weekly Reports* (MMWR). Vol. 48. No. RR-2 (Recommendations and Reports).

24) Among the personal papers of Robert J. Huebner.

25) The material for the biographical sketch of Dr. Robert M. Chanock was derived from: A) Ligon, B.L. 1998. Robert M. Chanock, MD: A living legend in the war against viruses. (Biography). *Seminars in Pediatric Infectious Diseases*. Upper and Lower Respiratory Tract Infections. Part 3. Feigin, R.D., editor; Wald, E.R. and Dashefsky, B., guest editors. 9: 258-269 W. B. Saunders, Company. B) Bibliography. C) Many interviews and conversations.

26) December 1996 Board of Scientific Counselors' Review of LID/NIAID/NIH.

27) Harriet Huebner—Personal communication

Chapter 12

Polyoma Virus - Explorations in Oncology

The results obtained with the Laboratory of Infectious Diseases virus studies during the 1950's produced a profound change in Bob Huebner's philosophical orientation about the role of viruses in the causation of human illness. He began to speculate (3) that frequent exposure to viruses during youth might lead to disposition toward chronic and degenerative diseases later in life and published these ideas for the first time in 1959. The viruses might very well generate gradual changes that would remain hidden for years until they emerged in the form of disease. Huebner's theory was merely conjectural at that point, as no evidence yet existed for such a hypothesis in 1959. About the same time Bob was also becoming aware of the increasing interest and research in animal tumor viruses occurring at NIH and at other laboratories. New information about the properties of these viruses stimulated Bob's imagination as a virologist and epidemiologist and impelled him to investigate whether there might be a relationship between these agents and the development of human cancer. Various studies in the 1950's (3) showed that the methods for working with tumor viruses appeared to be only slightly more difficult than those for some of the ordinary non-tumor viruses, and they did not seem to present severe technical problems for laboratory manipulations. These studies indicated that animal tumors grew to high titer, and that they produced antibodies that could be measured by conventional immunological techniques. Some of them grew in tissue culture, and some of them grew in suckling rodents. Their manifestations were generally hyperplastic (excessive proliferation of cells) rather than

cytolytic (causing destruction of cells), and the viruses frequently remained latent for several months; but these were not unusual properties. Only their ability to produce tumors set them apart from other viruses. From his own experience, he was aware of the frequency of human viral pathogens occurring in various animal species and vice versa. Based on the information gleaned from those studies and his own knowledge, Bob deduced that the next obvious question to ask was whether some viruses could cause human cancer. Up to that time, Bob felt, this question had not been answered satisfactorily. Subsequently, for the remainder of his career, Bob focused on the study of animal oncogenic viruses in an attempt to answer the question of whether such studies might provide clues to a possible viral etiology for human cancer.

In July 1, 1957, major personnel changes occurred in the Laboratory of Infectious Diseases that allowed Bob to change the direction of his research activities. Wally Rowe, Janet Hartley and Leon Rosen had already been integrated into the studies coordinated by Bob Huebner on adenoviruses and at Junior Village. In July, Drs. Robert M. Chanock and Albert Z. Kapikian arrived at LID, and Dr. Robert H. Parrott left LID (1). On the day that Dr. Chanock arrived at NIH, Bob Huebner introduced Chanock to Bob Parrott and suggested that the two should get to know each other better despite that Parrott would no longer be with the Lab. He was leaving NIH to become Chairman of the Department of Pediatrics at Children's Hospital in Washington, D.C., and Director of Virus Research in the newly established research unit of the hospital. (The two men apparently took Huebner's advice; they collaborated successfully together for almost 20 years (2), making major advances in knowledge about the nature, epidemiological behavior and clinical attributes of the newly discovered respiratory viruses prevalent in infants and young

children.) Bob Chanock immediately became an integral part of the laboratory, and rapidly became indispensable both to its research and administration, so much so, that in 1958, Bob Huebner, by now Chief of LID, made Bob Chanock Head of the Respiratory Unit. Chanock would devote most of his future efforts to the investigation of these respiratory viruses—gradually taking over the direction of this research at the Laboratory, as Bob Huebner turned his attention to an exciting and daring new subject: oncogenic viruses and their potential to cause cancer in humans.

Viral oncology had a very rocky start. In 1908, Ellerman and Bang (25) described the first true "cell-free" transmission of cancer—the transfer of avian erythromyeloblastosis. In 1910-1911, Rous (5) transmitted avian sarcoma to chickens. The scientific community at that time, probably because of its innate dogmatic conservatism, refused to accept the concept that a cancer could be transmitted by a virus. (In 1966 Rous would win the Nobel Prize for his fundamental discovery). While Shope described rabbit fibroma in 1932 (22) and rabbit papilloma in 1933 (23), and Bittner in 1936 produced the first report of a mouse cancer virus—the mammary tumor agent (24), the scientific community in general remained skeptical about tumor virology. During the intervening years up into early 1960's, a dedicated group of tumor virus investigators, including Rous, Shope, Beard, Kidd, Bittner, Andervont, Bryan, Duran-Reynals, Oberlin, and Syverton kept the flame alive. They continued their characterization of tumor viruses and developed methods for quantifying them and studying their behavior both in the laboratory and in nature (25). The discovery of additional animal cancer viruses including leukemia viruses in mice and the versatile mouse polyoma virus provided a major impetus to the sudden widespread interest in viral oncogenesis. A major milestone in the

history of viral oncology occurred when Gross (6) discovered and was able to pass mouse leukemia virus consistently to other mice in 1951 with cell-free filtrates. Stewart (7) confirmed Gross' discovery in 1953. Also discovered during this period were the reproducible virus leukemias which were shown to be transmissible to adult mice by Friend in 1957 (26), Moloney in 1960 (27), and, later, by Rauscher (28). The latter two discoveries originated from the National Cancer Institute. By the late1950's extensive studies were occurring with the animal cancer viruses, most prominently with the avian viruses. To a certain extent, the notion of cancer causing viruses had assumed more credibility.

Huebner, working with Wally Rowe and Janet Hartley, decided to begin their excursion into viral oncology by studying the occurrence of *Polyoma Virus* in nature. Dr. Ludwik Gross (4) had initially isolated this virus in 1953 from cell-free leukemic filtrates obtained from infected AK mice. Gross was an expatriate Pole who had served in the United States Army during World War II. After failing to get a position at the National Cancer Institute he joined the Veterans Administration Hospital in the Bronx, New York City with the understanding that he could use an unoccupied room in the basement and devote one afternoon a week to his research. The scientific community greeted Dr. Gross' work on mouse leukemia initially with extreme skepticism in much the same fashion scientists had reacted to Peyton Rous' discovery of the virus that caused avian sarcoma in 1910 (5). Dr. Gross was also handicapped by lack of "scientific credentials." His work was not accepted generally until he was able to produce leukemia regularly by passage through very young (day-old) suckling mice (6). His success was due to the use of these very young mice rather than to the genetic breed. This was a major breakthrough in virus

cancer research. During the course of his studies, when he injected leukemic cell-free filtrates into susceptible 1 day-old suckling C3H inbred mice, he noted that some mice developed leukemia whereas others developed hitherto unrecognized parotid gland tumors. (The study of animal cancer viruses has been influenced to a great degree by the host genetic factors in susceptibility to various viral agents). Dr. Gross suspected that two agents were present in the leukemic filtrates. By means of differential ultra-filtration, ultra-sedimentation and heat experiments, he was able to separate out a smaller, heat resistant agent from the larger, heat sensitive leukemia agent. The former was the polyoma virus, so-named (by Stewart and Eddy), because when firmly established in a suitable tissue culture medium it grew in high titer and produced multiple tumors of various histological types in suckling mice and hamsters.

Dr. Sarah Stewart of the USPHS in 1953 discovered independently the polyoma virus described initially by Dr. Gross (7). She found the virus during attempts to transmit AKR mouse leukemia to newborn mice by inoculation of cell free filtrates of leukemic tissue. Dr. Stewart, along with her friend and collaborator Dr. Bernice Eddy, expanded on Gross' initial work, and made many important new observations, devising methods for growing the virus and for identifying it by immunologic means. Their scientific and administrative supervisors and other investigators in the labs where they worked treated both women shabbily. Their work was denigrated for not being "scientifically controlled." Dr. Eddy was a "whistle blower" par excellence. She discovered that the Salk polio vaccine submitted to NIH for safety control contained live virus but her information was squelched, thus allowing the outbreak with the Cutter Laboratory polio vaccine. Later she found that the polio vaccine grown in monkey kidney cell culture

contained the SV-40 virus, which produced cancer in suckling hamsters. She was severely reprimanded and prevented from publishing the information until another laboratory reported the same finding. Drs. Sarah Stewart and Bernice Eddy (USPHS Laboratory of Biologic Standards) (8) made the major discoveries that the polyoma virus could be propagated in tissue culture with production of cytopathic effects in mouse embryo cultures, and that infected tissue culture fluid injected into new born mice induced multiple tumors, both multi-centric in origin and of multiple histological types (9). The most frequent tumors produced were mixed tumors of the salivary glands and respiratory tract mucous glands, subcutaneous sarcomas, mammary tumors, osteogenic sarcomas and thymic epitheliomas, but many other cell types were also involved. In addition, the virus was capable of inducing tumors in other species, producing hemangio-epitheliomas and sarcomas in the hamster, sarcomas in the rat, and fibromas in rabbits as shown by Eddy, Stewart and co-workers (10).

Perhaps the most important technical breakthrough in the study of tumor viruses was the establishment of avian sarcoma virus and polyoma virus in tissue culture systems (29) and the rapid exploitation of these discoveries by other investigators as well as the intramural investigators at NIH. [Footnote—The success for establishing tissue culture systems for mouse tumors depended on the cultivation of mouse embryo lines and chick embryo lines for avian tumors. Cell lines of chicken fibroblasts (any cell from which connective tissue may be developed) were also used for avian tumor viruses.] The major observation that tissue culture-grown polyoma virus had a ready ability to cross species lines and to produce tumors in mice as well as hamsters, rabbits and guinea pigs marked a conceptual breakthrough (10A, B, C). These findings demolished the prior notion of the

absolute species specificities of tumor viruses (29B, 30, 31). As an epidemiologist, Bob Huebner was aware of the inter-species sharing of many of the non-tumor viruses between animals and humans. Now, the possibility that tumor viruses could spread between animals and humans became an intriguing area of speculation that directed many of Bob Huebner's future activities.

Bob Huebner was attracted to polyoma virus as a subject: he felt a variety of important questions remained to be answered and that its natural history needed to be elucidated. Some of these questions included: 1) was this exclusively a mouse virus or were other species naturally infected? 2) What was the distribution of infection among mice, how was it transferred and maintained, and was it an infection of a particular genetic strain of mice, and 3) what was the relation of polyoma virus to spontaneous neoplasms in the mouse and other species, including man? (11).

Fortunately, it was discovered that traditional virology techniques including tissue culture, use of suckling, weanling and adult mice, and adaptation of standard immunologic methods could be employed to elucidate the epidemiology and ecology of polyoma in its natural setting in various mouse populations. Bob and his collaborators therefore undertook the development of sensitive and reliable techniques, especially the immunologic methods, which would be most sensitive in carrying out the study of polyoma infection among populations of mice in various settings.

Bob Huebner, Janet Hartley and Wally Rowe discovered that polyoma virus hemagglutinates red blood cells of many species, and that the hemagglutinating activity was associated intimately with the tumorigenic property (12). In separate experiments, Drs. Eddy and Stewart made the same discovery. The development of the

hemagglutination procedures made possible the development of a hemagglutination-inhibition test for detecting antibody responses. In addition, it was possible to develop complement fixation (13), tissue culture neutralization and tumor neutralization tests that were of particular value for establishing the degree of specificity, sensitivity and reliability of the hemagglutination-inhibition test (14). With the use of these antibody tests, they established that the virus was antigenic, that it produced antibodies. Both newborn and adult mice responded regularly to inoculation of small doses of live virus by developing antibodies. The production of antibody in weanling mice following intra-peritoneal injection of virus-containing material was a highly sensitive method of detecting polyoma infectivity, more sensitive, it was found than prolonged observation of mouse embryo tissue cultures, the standard method then employed. Huebner and company used this indirect immunization test, or "mouse antibody protection test," as their standard method of detecting and titrating polyoma infectivity (14). One 50% infectious dose for weanling mice or tissue culture corresponded to 300 physical particles in most preparations (15), a rather small dose of virus.

The Huebner group also studied some of the virus' physical and chemical characteristics. Based on these characteristics, polyoma has been incorporated into the *Papova* group of viruses that also include *pa*pilloma and *va*cuollating viruses (18). The virus was found to be spherical, about 44 mμ in diameter in dried preparations, and in size and appearance very similar to the Shope papilloma virus (16). It was also found to be a DNA virus (its viral genome was composed of the nucleic acid DNA). Like the Shope virus, it was highly resistant to environmental influences including heat, ultra-violet light and many disinfectants (17) and resistant to ether, like other members of the

papova group. It was found to be similar also to the simian vacuolating virus—SV-40 (18). Huebner's group found that antibody response was highly specific; no cross serological reactions were observed in tests against a wide variety of mouse viruses, human viruses and tumor viruses of various species (12). Antibody in mice was highly durable, apparently persisting for life in the vast majority of infected animals thereby providing a useful marker for animals studied during initial and subsequent population surveys.

This information was the result of very careful, well designed and controlled experiments that provided the tools for the study of the mouse polyoma problem (11). With the development of well-evaluated, sensitive and specific procedures for detecting virus and antibody, it became possible to determine the extent of spontaneous infection in various populations of laboratory and wild mice and the implication for human exposure and infection.

The initial studies were performed utilizing mice from different laboratories (including Jackson Memorial Laboratory, Bar Harbor, Maine) including those within and from outside NIH. The studies also included various strains of inbred mice. The percentage of positive serological tests varied considerably within the mouse colonies; they found a correlation between positive serological tests and the extent of infection within a colony. Infection spread within areas where experimental work was performed with the virus, causing extensive environmental contamination. Suckling mice were most susceptible to experimental infection, and in turn readily spread infection to nursing mothers. Infected animals also shed virus in large quantities through their saliva and urine for many months, and this accounted for the high degree of environmental contamination.

The researchers found that endemic infection with polyoma virus did not occur in animal colonies that were kept in isolation and not in proximity to experimental studies; for example, in the breeding colony of NIH, endemic infection with polyoma virus did not occur (19). The researchers also observed that spontaneous tumors among mice in laboratory colonies and among mice in the wild were uncommon, probably because the mice in these settings did not live long enough to reach a ripe old age when tumors might be expected to occur (19).

Following the initial survey of laboratory stocks, Bob Huebner decided to get additional information about polyoma prevalence from "The Horse's Mouth" (25A), and he quoted a favorite aphorism, "There is no higher order of information than that provided by nature herself." He and his group undertook studies to determine the prevalence of polyoma among wild mice. They trapped mice extensively in the tenements on several streets in the Harlem section of New York City. The incursion of Bob Huebner and his team into Harlem was duly noted in the publication *"Medical News"* on May 11, 1960; the text was accompanied by: pictures showing field epidemiology in action and crowds of interested spectators. While Bob Huebner and the survey crews uncovered polyoma infection, the distribution was not uniform. It was highly localized. Certain streets contained buildings that had extensive evidence of infection, but often only specific floors were involved and only certain groups of mouse colonies contained infected mice (19).

At the beginning of the Harlem study (19A), Huebner's group had some question whether or not the common house mouse was indeed the natural host for this agent, since their earlier sporadic tests of wild house mice were negative. They found that most of the

smaller colonies of mice were free of polyoma and remained free over a period of more than a year's surveillance. However, they discovered that polyoma-infected mice were found in many of the large mouse colonies infesting Harlem for at least a year. Continuous studies over the course of a year showed that virus infection persisted, with an almost 25% rate of infection among the mice in the many distinct colonies studied.

Huebner stated that three epidemiological factors seemed most important in the persistence of polyoma in the Harlem mouse populations: (1) the large population of mice in Harlem tenements obviously furnished adequate supplies of young susceptible mice thereby guaranteeing continuous opportunities for new infections; (2) extensive contamination by the urine of infected mice of the tenement environment (virus was demonstrated in sweepings from closets, kitchen cabinets and other areas showing evidence of mouse activities), and the hardiness of the virus provided numerous durable environmental sources of infection; and (3) the communal nest, a social nicety preferred by mice and put to considerable use in the dense mouse population of Harlem, provided both concentrated focal areas of infection and intense exposure of many mice. In some of the positive Harlem apartments, it was possible to trap as many as 20-60 mice during a 24-hour period, and it was possible to demonstrate polyoma virus directly in mouse nests and in nesting materials found in the tenement closets and kitchen cabinets.

Although Bob Huebner's group felt that these factors explained the prolonged persistence and widespread distribution of polyoma virus in urban households and commercial mouse breeding establishments, they still had no satisfactory explanation for the presence of polyoma in so many different ecologies. For this reason, early in 1960 Bob and associates (20) initiated surveys for polyoma infection in mice on farms and in

grain and feed mills located in small towns in Montgomery and Frederick Counties, Maryland, a grain and livestock-producing area north of Washington, D.C. that also supplied a large part of the milk for that city. They felt on the basis of their initial preliminary data that they might have found the basic natural cycle of mouse polyoma. Five of 16 farms and each of three grain and feed mills in the same general area disclosed mice positive for hemagglutination-inhibition antibodies to polyoma, indicating the presence of mice positive for and excreting the virus. In August 1960, they initiated continuing surveys for polyoma infections in mouse populations on one of the 5 positive farms and in 3 feed mills located in a small town in the same general area.

The one farm where serial surveys were performed was a dairy farm. Mice were trapped in several different areas of the farm at various seasons. Some mice were tested repeatedly to look for antibodies. Mice positive for polyoma were found in areas that were protected from the cold and from predators. These areas were in the haylofts and cereal bins that provided safe havens for breeding and nesting. The hay, grains and other nesting materials were also positive for polyoma virus. Some mice found negative on earlier surveys were later recaptured and found to exhibit evidence of infection, undoubtedly acquired from the environment (20).

Mouse activity in the grain and feed mills was largely confined also to the protected areas where nesting and breeding occurred. These areas had the most infected mice. These areas were usually in the grain storage bins where evidence of environmental contamination was also present. Interestingly, some of these mills provided feed for laboratory animals; this could account for infection of some of the mouse colonies studied for polyoma antibody prevalence by the Huebner group.

These investigations of Maryland rural ecologies thus showed that polyoma-infected mice could be found in abundance in grain-storage areas on farms and in feed mills. Virus was also demonstrated on cereal grains and in mouse nests in those areas where serologically positive mice were taken alive in traps.

Bob Huebner and his colleagues reasoned that unless these findings in Maryland ecologies were unique—and there was no reason to believe that they were—it would appear that grain agricultural ecologies contained the basic natural cycles of polyoma virus infection, and that this extensive natural source might be responsible for the widespread infections found in laboratory, production and urban colonies of the common mouse, *mus musculus.*

Drawing on these findings, Bob Huebner, (19,20), mused that the age-old association of the mouse, both wild and commensal, with grain storage areas might provide explanations for certain well known zoonoses, and that new investigations could possibly yield evidence of unsuspected new ones. Furthermore, Huebner concluded that polyoma could produce a chronic, latent infection with only rare production of tumor under laboratory or non-laboratory conditions (in the wild). In this respect it could behave like other latent non-tumorigenic mouse viruses that produce chronic infection such as lymphocytic choriomeningitis (11), mouse salivary gland virus (11), Theiler's viruses (11), the L. Kilham virus (11), the mouse adenovirus (11), the mouse thymic agent (11), ectromelia (11), mouse hepatitis (11), and the pneumonia virus of mice (11). Indeed, Wally Rowe and Janet Hartley had encountered several of the above listed latent non-tumorigenic viruses in the laboratory during their polyoma studies.

As a result of all the above studies Bob Huebner and associates summarized the epidemiological patterns of mouse polyoma as follows: mice that became infected as newborns or young sucklings constituted the major source of spread through a colony (through urine and saliva). They readily infected their mothers and cage mates, and the cage mates, in turn, could serve to infect other mice. However, the mice that became infected as adults much less frequently served as sources of infection. The introduction of experimental procedures in which virus-containing materials were inoculated into young mice constituted a very efficient amplification procedure to maintain and disseminate the virus throughout the colony because of the large quantity of virus excreted by suckling mice that resulted in environmental contamination.

To explain the continuance of infection in colonies such as the commercial mouse colonies and the wild mouse populations in which there was no exposure to experimental procedures, it appeared probable that several mechanisms were active. In the commercial colonies large numbers of mice were housed in such close contact that even the small amount of excretion by the weanling mice could be sufficient to maintain the infection. In the smaller laboratory colonies, smaller numbers of mice could excrete enough virus to maintain the infection and contaminate the environment with the virus. Newborn mice would not be infected often because the statistical chance of acquiring infection during the first few days of life was quite small, and in a colony with a high level of infection many newborns would be protected for a period of time by maternally transmitted antibodies (19).

Huebner and associates articulated another tempting hypothesis to explain environmental contamination. In the wild mouse populations the nesting areas became

contaminated with urine of infected babies, and, because of its high stability, the virus could remain in the nests and infect repeatedly subsequent litters, which in turn would reseed the nests with infected urine. By contrast, in the laboratory and commercial colonies in which the bedding and containers would be changed frequently and sterilized, virus infection could not be so easily induced.

An interesting anecdote relating to the Harlem investigation involved Dr. Isadore Brodsky who worked temporarily under the auspices of the USPHS and was assigned to Bob Huebner and Wally Rowe during the polyoma period. In personal correspondence written for the Bob Huebner's retirement *Festschrift*, Dr. Brodsky wrote as follows on October 26, 1982 (21): "Bob Huebner called me into his office and informed me that mice had been trapped in apartment buildings in Harlem on West 132 Street and West 102 Street. The mice were found to be positive for polyoma antibodies. Bob turned to me and said, 'Iz, What is the next step?' I quickly replied, 'Go to New York and bleed (that is, obtain a sample of venous blood to test for antibodies) all the people in those apartment buildings to see if they have antibodies against the polyoma virus'. Dr. Huebner said, 'You're right on target'. I then asked Dr. Huebner if he would clear it with Dr.Greenberg, who was then Commissioner of Health for New York City. He told me not to worry, and that he would take care of it. I made one cowardly move in that I took a visiting nurse with me from NIH as well as an official U.S. Public Health Service truck. I went to West Harlem as well as to East Harlem and systematically bled all of the people that I could find in those apartment buildings. Of course, both the nurse and I always proceeded to obtain informed consent even in those days. Before I bled a tenant of any of those buildings, I would always flash my I.D. card from the USPHS. Incidentally, I found

out subsequently that most of the volunteers felt that I was doing a venereal disease survey. At the end of the day I had collected 60 samples. From the City Health Department labs, I called Dr. Huebner to ask if everything had been cleared with Dr. Greenberg. 'No, I haven't done it,' he said, 'but don't worry, you are not a good epidemiologist until you have been thrown in jail at least one time.'" This anecdote illustrates some of the amusing and humorous aspect of field epidemiology as well as Bob Huebner's apparently relaxed attitudes toward bureaucratic and regulatory red tape.

As a final comment on the result of this unique type of investigation (19,20), Bob Huebner felt that studies of the natural occurrence of viruses furnish information of the very highest order, coming, as it were, from the "horse's mouth." He planned to extend his group's studies of possible polyoma infection to surveys of various animal species, particularly those having repeated exposure to known infected environments. He also planned to extend natural history studies, as new laboratory tools were developed, to other mouse viruses, particularly the leukemia viruses, and subsequently to similar viruses of chickens.

He emphasized that his studies were of natural polyoma infection and never once mentioned any tumors in mice. The fact was that no polyoma tumors were observed, and none were expected. The polyoma strains from laboratory colonies, urban tenements and rural ecologies could not be distinguished from each other, and the New York City Harlem strains produced the same kind of cancers in laboratory mice as the original prototype strains of polyoma isolated from laboratory mice by Stewart and Eddy (7,8,9). Huebner and his associates did not find any immunologic evidence that mouse polyoma caused human disease.

In the early 1970's several viruses (32) were isolated from humans and classified as papova viruses of the polyoma type. These human viruses did not react immunologically with mouse polyoma. The first human virus was the so-called JC virus, isolated initially, from the brain of a patient with progressive multi-focal leucoencephalopathy. This disease is a diffuse, ultimately fatal infection, relentlessly destroying the white matter of the brain. It occurs in immuno-compromised hosts and is seen most frequently now in patients with AIDS. The second agent, BK virus, was cultivated initially from the urine of a renal transplant patient. The primary infections with JC and BK viruses occur in childhood as determined by antibody studies. Re-activation of BK virus has been associated with hemorrhagic bladder infections, stenosis of the ureter and some urinary tract illnesses. Both viruses persist in kidney epithelium and lymphocytes, and may become re-activated when patients become immuno-compromised. So far, these viruses have not been associated with malignancies.

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Chapter 13

Domestic Life, Life on the Farm (1)

For the first several years after arriving at NIH Bob moved his family to Maryland from Virginia in order to be closer to work. From 1944 to 1947 they lived at 3121 McComas Avenue, in Kensington, Maryland. When I came to NIH in August 1948 a secretary in the NIH housing office described Kensington as a "little community out in the country" but it has since then acquired an in-town cachet. In the meantime, Robert James (Jim) arrived August 7, 1945 and daughter Virginia Rose (Ginny) joined the family on June 30, 1947. Later the Huebners were able to move into one of the duplex Georgian-style houses on the NIH campus that serve as living quarters for some regular USPHS commissioned officers. The Huebners lived there until early 1951 when they moved out to their newly purchased farm of 165 acres in Ijamsville, Maryland. While the family was still "in quarters" at NIH, Roberta Sue (Susie) put in her appearance as a family member on July 10, 1949. She was the sixth child.

Summer 1950. Picnic on the south meadow of the Huebner farm. In the middle is Berdi Huebner surrounded by Huebner children. On the right is Aunt Catherine Huebner. (Office of NIH History files, contributed by E.A. Beeman).

Several reasons prompted the move to the farm. Although the quarters at NIH were comfortable, they were not sufficient for a large and growing family. Berdi, who had a farm upbringing and background, felt that the children would benefit from growing up in a rural environment, and she provided the primary influence for the move to the farm. Bob agreed with the above reasons and also envisioned definite economic advantages to living on the farm. The family was still not complete. Edward Nelson (Ed) arrived September 30, 1951, Mary Louise (Lou) on September 1, 1954, and Richard Daniel (Danny) prematurely on August 5, 1960.

The farm was located in the rolling, hilly piedmont area of Frederick, County, Maryland. A meandering stream (Bush Creek) runs through the lower south meadow. The farm was quite run down at purchase time. The farmhouse was over 50 years old and very dilapidated. The indoor plumbing was broken. The barns and outbuildings were in poor repair. They named their new acquisition “Hidden Hills Farm.” The first order of business was to construct an outhouse and to repair the barn. Later they repaired and installed new indoor plumbing. Over the years they improved the kitchen, patched up the roof and painted the trim. As Bob rationalized at an early date with Berdi, “Remember, You’re gilding a skunk cabbage not a lily” (2). After the indoor plumbing was installed they still retained the outhouse for many years because, with 11 to 12 family members and many guests, someone always needed to use the sanitary facilities.

After moving to the farm in 1951 the family planted a kitchen garden. This has been an annual practice up to the present time. Bob also hired a contract farmer to plant field corn and hay for the small herd of Holstein cattle that were to be raised and sold later for beef. However, the family suffered an early financial crisis when the barn, which

had just been stocked with the first year's recently harvested cattle feed, burnt to the ground. The very likely cause was spontaneous combustion because of the new mown hay that had been packed tightly in the barn up to the rafters. This event occurred in the fall of 1951 while Bob was in Texas investigating the outbreak of epidemic pleurodynia. He returned home on an emergency basis. He sold the small herd because he could not afford purchasing feed for the animals over the winter until they could be brought to auction. He started raising animals again the next year. In lieu of a barn that was not built until several years later, he stored the animal feed under large plastic sheeting weighted down with used automobile tires.

Another crisis, but this one of a medical nature, also occurred shortly after the move to the farm. Bob, who was still an astute clinician, observed that some of the older children appeared pale. Medical study showed that the children had been born with hereditary spherocytosis inherited from Berdi as an autosomal dominant trait. [Footnote—Hereditary spherocytosis is an inherited trait transmitted predominantly to about 75% of the offspring of an afflicted parent. There is a defect in the bi-concave structure in the normal red blood cell resulting in a rounding and weakness of the cell. The abnormal cells are sequestered and destroyed prematurely in the spleen releasing hemoglobin that is converted to bilirubin (bile) in the liver. This may be deposited in large amounts as bilirubin gallstones in the gall bladder. Events, such as infections, may lead to what are known as "hemolytic crises" that result in massive acute destruction of red blood cells and acute anemia with greatly increased release of hemoglobin from the destroyed cells. Splenectomy (surgical removal of the spleen) usually results in symptomatic cure of the anemia](6). During the years 1952 through 1955 Berdi and the

affected children underwent splenectomies. Mary Louise (Lou) was the only child not involved with the disease. Danny, who was born prematurely, had his splenectomy at a later date. The surgeries were uneventful, but Gerry became very ill prior to her splenectomy. She developed a severe hemolytic crisis associated with acute cholecystitis and sepsis secondary to the deposition of stones in her gallbladder. She recovered after emergency cholecystectomy and splenectomy.

I visited the farm several times in the first years after purchase. My earliest recollection of the children in the farm setting was of the oldest girls, Betty and Kay, in oversized, muddy boots doing their chores around the farm. From the very beginning the older children had their assigned tasks for household and animal duties. In the early years and thereafter they tended the kitchen garden to supply the family table. Initially the Huebners had a few Holstein cattle, and after several years they began to raise Angus beef cattle as a breeding herd. From the beginning of the breeding activities the older children and the next siblings in line were required to raise and to care for "prize" animals that were to be presented at county, regional and state fairs. They had to feed the animals, water them twice daily and keep them groomed. They had to keep track of their weights. They were responsible for keeping the fences in repair and keeping the gates closed. A stray bull coming from another farm could wreak havoc with a year's worth of breeding. During the course of the twenty years in the cattle breeding activity Bob and the children improved the characteristics of the breed with resultant improvement in its stamina and size of the herd.

The Huebner cattle, primarily the bulls, consistently won prizes at the various fairs where they were exhibited. The farmhouse was festooned liberally with the many

blue ribbons won by the prize animals. About one-third of the prize money was given to the children for their own separate bank accounts for accumulation to help pay later for college tuitions. Toward the end of the breeding era the Huebner herd had a national reputation. Many sales were made to agricultural and farm schools throughout the country. The entire herd was sold in 1973 when Bob decided to discontinue the breeding program. During the period of breeding activity he became extremely well known, respected and popular in the industry. For several years he was head of the breeders' association. As in his other activities he applied energy and initiative in learning about the cattle industry, and he became very proficient in conducting this enterprise.

Bob's scientific and animal husbandry accomplishments attracted several articles in the lay and farm press (2.3). Bob described that what started as a "hobby to amuse the children" had turned into a profitable cattle business. Bob philosophized that a non-farm background was advantageous for a neophyte because he might not come to agriculture with pre-conceived notions. Bob credited his success in cattle breeding to holding down costs, scientific record keeping and genetic breeding. He quipped, "Actually, most people think we're a little late to talk about 'planned parenthood' after nine kids" (2). After the barn burning in 1951 the Huebners went into Angus cattle with the resolve to breed the most productive cattle in the state of Maryland. "This is the only hope for a small operator," Bob said, "to offer a unique and highly valuable item. We had tried feeding for slaughter and found it was fun but there was no profit in it. You can't make a go of a small farm producing run-of-the-mill stuff you have to sell by the pound or the bushel. We had to have something to offer so exceptional that people would pay us three or four times the going price. So we decided to come up with a very select seed stock operation.

"We went the Angus route", he explained, "because we knew there were a number of small purebred Angus herds in Maryland that were actually making money. And we knew we could get good cows without paying a fortune for them. There were fads in the Angus cow families, and if you were willing to buy individuals from the less popular families you could get them in the mid 1950's for $150 to $250 per head" (2).

The Huebners introduced 4 purebred heifers and a bull to their new barn. The cows, though purebred and registered, were from a successful commercial herd operated by Elmer Hodges, a pioneer Maryland breeder. Under the experienced guidance of the breeder, Mr. George Porteus, a highly respected veteran of the business who helped make Angus history on the breeding farms and at the stock shows in the early 1900's, the Huebners selected the best bulls they could afford. With judicious purchases, culling of non-productive cows and bulls and replacements from their own breeding they were able to build their herd to around 40 head of prime cattle. Their most fortunate purchase was bull "492-17" which sired many of their champion animals and was largely responsible for the reputation of the herd. His offspring were sold to many of the agricultural schools for their breeding programs (3).

Bob introduced a scientifically developed feeding program that would produce predictable and consistent weight gain at various time periods after birth. One of the great assets of the Huebner cattle was their reliability and predictability so that the purchasers were getting a guaranteed quality product. He sold individual animals to famous personages such as President Dwight D. Eisenhower (for his Gettysburg farm) and Admiral Lewis E. Strauss, former Head of the Atomic Energy Commission. The reporter for the *Business Week* article (2) reported they had purchased bulls whereas they had

actually purchased heifers. Bob had an interesting correspondence with the editors of this magazine gently chiding them for their reporter's not knowing the difference between bulls and heifers (4,5).

Summer 1950. Bob Huebner clearing brush on the south meadow of the farm. (Office of NIH History files, contributed by E.A. Beeman).

Bob's reputation as a cattle breeder acquired international overtones, and it spread to foreign scientists who visited the NIH. The Soviets always wanted to see the Huebners' "collective farm." Bob was always very hospitable to these visiting scientists, and they were frequent guests at Hidden Hills Farm. In the mid 1960's when Soviet scientists were on temporary service at NIH, plans for the United States' participation in a 1967 international virology conference in the Soviet Union were sketched out on the banks of Bush Creek in the south meadow. This may have been the conference when Dr. Alexis Shelokov (7) saw Bob carrying a bag filled with bull semen in Moscow for distribution or sale to some of the Soviet cattle breeding farms. (Dr. Shelokov, born of

White Russian parentage in Harbin, Manchuria, usually accompanied the NIH delegations to the Soviet Union as an interpreter).

Bob found the transition from scientist to cattleman intriguing and important. "The worst thing you could do to be a successful farmer is to be born and brought up on a farm (despite Berdi Huebner's background). Ours is a fresh, objective approach. And it is surprising how much is carried over between the farm and the laboratory." The similarity between Bob's approach to cattle breeding and the laboratory is a remarkable reflection of the approach to his research efforts as seen in the analytical thought processes that he brought to both.

The Huebner (1) children offered their own perspectives on "Life with Father" on the farm. They described Bob's relationship toward his children as strict and authoritarian. He demanded adherence to the discipline of farm life and the family enterprise in which they were all engaged. Only several children did not conform to this standard. Berdi supported him in this approach. Bob, however, was kind and non-punitive. He did not show much outward affection, but he had a loving relationship with his children. He was instrumental in developing their appreciation of the arts, good literature and educational pursuits. When he was home he instructed them actively in many areas related to non-farm learning experiences. He taught the girls ballroom dancing and the waltz.

He was very supportive of his children. Danny, the youngest Huebner, recalls that when he was about 7 or 8 years old one of the cows in the meadow thought that Danny was coming too close to her calf, and she tried to protect the calf by attacking Danny. When Bob saw what was happening he interposed himself between the cow and Danny,

thus allowing Danny to escape back to the house. Bob sustained several severe collisions with the cow until he could extricate himself from her anger and her horns.

Early 1960s. Dr. Robert Huebner and one of his prize bulls. (Office of NIH History files, contributed by R.M. Chanock).

Betty, the oldest Huebner child, recalls another interesting aspect of Bob's interaction with the children. One winter while Betty was readying a heifer for presentation at the local fair, Bob returned from Bethesda to the farm on a weekend. He

said she was grooming and trimming the animal incorrectly. Betty made minimal changes. When Bob returned to the farm the following weekend he was apparently pleased with the result; however, he wanted 5 additional heifers trimmed the same way—but in one day after he came back from some errands. Betty started working on the animals in the barn. It was an exceptionally cold winter's day. She completed grooming four animals but her fingers became so cold, numb and immobile that she could no longer manipulate the trimming shears. She appealed to Berdi who said that she had to finish the last animal as she had been instructed. At this point some modifications from the usual farm practices were adopted, and it was agreed that the animal could be brought into the warm house so that the grooming task could be completed. The heifer, though, had grown quite large and could not be brought through the kitchen door. However, by applying greased cookie tins to the animal's flanks and with the help of her siblings Betty was able to maneuver the heifer into the kitchen where the grooming job could be completed. The family was apprehensive about Bob's early return and his reaction to the solution of the problem. They expected an eruption of volcanic anger. When, indeed, he did return earlier than expected, he laughed uproariously evidently appreciative of the humor of the situation and pleased with his daughter's initiative and ingenuity. He rushed to get his camera so that he could immortalize the image on photographic film. As to be expected, the animals were prizewinners.

On February 10 and 11, 2001, I had an opportunity to spend a weekend interviewing Jim Huebner when he visited me at my vacation dwelling at Longboat Key, Florida, from his home in Sanford, Florida (outside of Orlando). I was interested in his role as the oldest Huebner son. He was the fourth of the nine siblings and was preceded

by Betty, Kay and Gerry. His sisters teased him and subjected him to physical and psychological abuse that he survived. He described himself as a mischievous youngster and a very active toddler. At age 2 or 3 years, despite the benign environment of living "in quarters" on the NIH campus, he did manage to fall through a second floor bedroom window but, fortunately, landed safely on the bushes below. A few months later he also managed to be left behind at a gas station when a distracted Bob was driving the family out to California during the Q fever studies. After about one-half hour later, Bob became aware that the sound level in the station wagon was somewhat lower than he remembered, and, after counting heads, he realized that Jim was missing. The family turned around, retrieved Jim from the gas station, and the journey continued "uneventfully" thereafter.

Jim said that he had a happy childhood. He had companionship from many siblings and playmates, fun with farm activities, playing and swimming in the creek and exploring the woods. At around 8 years he and the other siblings had to assume their share of chores around the farm, chief among which was tending and nurturing the family enterprise of the Angus breeding herd.

As Jim grew older Bob also enlisted him in some of the ecological studies and the mouse trapping around the farm. At age 14 years Bob took him on the field trip to Harlem, New York City, to help trap mice during the polyoma virus studies. Jim said that on this trip Bob was "prudent" with the government's money because he deposited Jim and a companion at the Harlem YMCA for $2.00 a night. Bob sought more comfortable quarters elsewhere.

Jim indicated that Bob was very kind and caring but authoritative. One thing that Jim missed in his father was the opportunity to have more prolonged individual conversations with Bob. With the limited time that Bob spent at the farm during the weekends, it was difficult for him to divide the time among nine children.

Jim remembered the large number of foreign, and especially Soviet, visitors to the farm. Bob was very cordial to them, and he used the farm extensively for hosting them. In his later teens, Jim had the major responsibility for managing the annual "bull roasts." For this event he dug the pit, set the fire, butchered the animal, roasted the meat and helped serve sometimes up to 300 people.

Jim also described some of Bob's athletic abilities. Bob became extremely skillful at pitching horseshoes. He was also an excellent tennis player, in fact, beating Danny's high school tennis coach. Bob also played some golf but with more enthusiasm than skill. Later, when he was developing Alzheimer's disease, he still had a good golf swing and directed the ball skillfully even though he was having difficulty occasionally finding the golf ball.

Jim also observed that Bob abandoned his religious faith gradually. Jim remembered that the family also began losing the habit of going to church regularly and that the family members were not allied with any particular religious denomination.

Jim left the farm around the time of the Vietnam War. Bob sold the Angus herd in 1973 primarily because there were not enough siblings at home to do the chores that were necessary to maintain the herd.

According to Susie (1), the early years of life on the farm were not idyllic and were not without their difficult aspects. Money was always an issue: the Huebners tried

to juggle the multiple problems of raising nine children, starting a new home on an old farm that had few modern amenities and was in need of extensive renovations, starting a cattle breeding enterprise with no prior experience—and all with the limited salary of a USPHS medical officer!

Life on the farm did offer, however, some economy in major living expenses. They were almost self sufficient in food. The children did not require much clothing since they wore parochial school uniforms, and garments were handed down from one child to the next. Toys were in rare supply. The children did not require them or other diversions such as television since they were busy with their homework or their farm and household chores. There was no sense of deprivation, however, because there was constant and abundant activity to keep them occupied. As farm children they had their large animals and also the smaller creatures prevalent around farms. They learned and became proficient in the operation of all the farm machinery. They developed an appreciation for the environment and for nature conservancy. They have all developed into well-educated adults successful in their respective homes, business and professional lives except for Ed who was killed tragically on the farm in a hunting accident at age 39.

Despite his dominance in family life Bob was not present physically on the farm during most of the week. When he was not on the farm he spent the rest of the week in Bethesda either staying in the laboratory or in the apartment of his laboratory technician, John D. Estes. He usually returned to the farm on weekends. At these times the family experienced his presence keenly. He was able to exercise control, and he usually outlined plans for Berdi and the children for the coming week during these weekends in the country. His energy was restless and boundless; the children did not find time hanging

heavily on their hands when he was home. Occasionally during the winter when he was completely snowbound he became fidgety, and he could not find enough to keep himself busy. Ordinarily getting from the main Ijamsville road into the farm was difficult enough. The road leading to the farm was ½ mile long, the width of one automobile, lined with gravel, dusty in warm, dry weather, muddy in wet weather, hilly, twisting and with some deep dips. Bob was usually able to get to Bethesda during the winter. Occasionally he would have to travel on horseback accompanied by several of the children in order to get to the main road where he either had a ride or where he had left his Volkswagen Beetle. He went through the fields avoiding the farm road where the snow frequently accumulated to the depth of 10 to 12 feet in the dips that were not passable. He disliked these trips to his automobile because he was not fond of horses. On these trips he presented an unusual equestrian image. He would have two pillowcases hanging over either side of the saddle pommel. In one pillowcase he would have his papers and books, and in the other, he would have the chains for his Volkswagen. The farm road required constant repair and upkeep. On one occasion, shortly after the memorial service for Bob, when I went to the farm to interview several of the children, Ginny was operating the road grader and Susie was moving aside debris from the road while she told me about many of aspects of farm life noted above.

Although Bob was able to make the farm and breeding activities successful, he was described as being personally clumsy and somewhat inept in the actual physical tasks around the farm. His talent was to mobilize, instruct and motivate the family members to accomplish their common goals. This was really, again, a reflection of the way he

operated in the laboratory where he was able to motivate others to cooperate in well planned and imaginative research efforts.

1960. Drs. Albert Sabin and Robert Huebner relaxing on the porch of the Huebner farm house. (Office of NIH History Files, contributed by R.M. Chanock).

Susie Huebner Creamer concluded that, despite living on the farm, she and her other siblings experienced a very atypical farm life. This was attributed to Bob's professional prominence and his frequent hosting at the farm overnight famous visiting scientific peers from the United States and abroad. The children's exposure to a wide range of educational and cultural ideas was also very unusual for a typical farm environment. Bob was extremely hospitable. There was a steady stream of members of the laboratory and visiting dignitaries to picnics, barbeques, and bull roasts at the farm.

Even at the present time the farm is the site of family gatherings at holidays and other significant occasions. The farm was originally 165 acres but the family partnership sold 5 acres to Mary Louise (Lou) who built a house there and stables her horses on the farm. Ginny lives at the farm full time. Susie and Danny live nearby in Frederick, Maryland.

1) Much of the information in this chapter was provided by interviews and conversations at various times with the Huebner siblings, primarily Susie ("The Family Historian"), Betty. Danny, Jim and Ginny.
2) A) Research, February 24, 1968. A man in search of cancer's cause. *Business Week*. P. 90. Among the personal papers of Robert J. Huebner. B) March 1968. Farmer-scientist breeds a theory. *Medical World News* p. 70. Among the personal papers of Robert J. Huebner. (Based on information in 2A)
3) Koch, C.R. Spring 1968. Seed stock is our business. Quality cattle spell success for a small breeder. *The Farm Quarterly*, 22 E. 12th Street, Cincinnati, Ohio 45210. (Reprinted for Hidden Hills Farm). Among the personal papers of Robert J. Huebner.
4) Letter from Robert J. Huebner to John L. Cobbs, Editor, Business Week. March 6, 1968. Among the personal papers of Robert J. Huebner.
5) Letter to Robert J. Huebner from Howard L. Lewis, Assistant Research Editor, Business Week. March 12, 1968. Among the personal papers of Robert J. Huebner.
6) Golan, D.E. 2000. Hereditary spherocytosis in *Cecil's Textbook of Medicine*, 21st Edition, Goldman, L. and Bennett, J.C., Editors, W.B. Saunders Company, Philadelphia, etc. Pp. 868-870.
7) Alexis I. Shelokov—Personal communication

Chapter 14

The Tumor (T) Antigens

Bob Huebner's investigation of polyoma virus was the beginning of a two-decade unique approach to the study of tumor viruses. Until 1961, his research activities had proceeded in almost linear progression with the help of talented associates. Over the next decade and beyond, his endeavors branched out and encompassed a large, dispersed network of collaborators, both local and national. During the 1960's he also achieved international prominence as the result of his previous discoveries in non-tumor viruses succeeded by his innovative approach to working with tumor viruses. He became a frequent traveler overseas participating in conferences and giving lectures. His first major overseas excursion occurred in May 1961 when he went to the Soviet Union as Chairman of the first American Delegation on Virus Diseases accompanied by a group of prominent American virologists, including Bob Chanock with Alex Shelokov as interpreter (1A, B).

May 6, 1961. Group picture of six American virologists together with their Soviet counterparts in Moscow. Abraham Ribicoff, Secretary of Health, Education, and Welfare in the John F. Kennedy administration, sent the group, led by Robert J. Huebner, on the first exchange visit with the Soviet virologists in 1961. Seated left to right: Bela Kaplan, interpreter; R.J. Huebner, LID/NIAID/NIH; M.P. Chumakov, Director of the Soviet Poliomyelitis Institute; E.H. Lennette, Director of the California State Health Laboratory; W.McD. Hammon, Dean of the University of Pittsburgh School of Hygiene and Public Health. Standing left to right: R.M. Chanock, LID/NIAID/NIH; F. Davenport, University of Michigan; S.G. Drozgov; unidentified scientist; V.I. Agol; unidentified scientist; U. Chumakova; Ms. Lechinskaja; A.I. Shelokov, LID/NIAID/NIH; A Avakian. (Office of NIH History Files—contributed by R.M. Chanock).

May 1961. Dr. Robert Huebner talking with Dr. Victor Zadanov, Soviet Deputy Minister of Health. (Office of NIH History files).

The Delegates' mission was to examine research facilities and discuss matters of mutual virological interest with their Soviet counterparts. This mission was the forerunner of additional meetings between groups of American and Soviet virologists. In addition to Bob Huebner, the other members of the Delegation were Drs. Edwin H. Lennette (Chief of the Viral and Rickettsial Laboratory, California State Department of Health), William McD. Hammon (Professor, University of Pittsburgh School of Medicine), Fred M. Davenport (Professor, University of Michigan School of Medicine), Robert M. Chanock and Alexis I. Shelokov both of the Laboratory of Infectious Diseases, NIAID, NIH. Shelokov, the translator (1A,B), described their activity as "making rounds on the original vodka-caviar circuit. Our host at the (Virological) Institute was the celebrated Professor E.N. Pavlovskyi, founder of landscape epidemiology, impressive in

his gold-braided General's uniform of the Soviet Army Medical Corps. After a while we heard him telling you (Bob Huebner) in Russian something about *Mus musculus*, and you telling him in English about *Mus musculus* carrying the SV-40 (or perhaps polyoma) from the Ukraine to New York. The Soviet scientists and the American delegates exchanged winks and smiles realizing that, in your mutual admiration and excitement, the two of you managed to have effectively communicated even though you were speaking in different tongues. What we did not know until later was that Professor Pavlovskyi's hearing aid was not working that day."

Other episodes made the trip memorable for the members of the American Delegation. Despite the hospitality of their Soviet hosts, elements of the Soviet security apparatus turned out to be less hospitable as indicated by their suspicion of Alex Shelokov's White Russian background. Alex's parents fled Russia in 1917, found refuge and settled in Harbin, Manchuria. He was detained upon arrival in the Soviet Union for interrogation. Bob Huebner and Ed Lennette were furious at this breach of civility, and they remained outside the group's hotel refusing to register inside until Alex was returned safely to the rest of the Delegation. According to Dr. Hammon (1C), while the members were staying in the hotel, they were reminded to be careful of their conversations after being warned that their rooms might be "bugged." Apparently minor changes in travel plans precipitated major problems for Soviet bureaucracy and service personnel. One member confused the kitchen staff at the hotel by ordering "one fried egg for breakfast" instead of the two hard-boiled eggs that the kitchen served ordinarily. Unfortunately, all members of the Delegation had received no specific guidelines about rendering gratuities to the waiters, with resulting disappointment and frustration among the servers. The

Delegates also had problems on departure when there was a mix-up on overweight luggage, and they had no more Soviet currency to pay for this unforeseen annoyance. The Delegation, however, was afforded splendid opportunities to attend horse races (no betting allowed), to view the May Day parade in Red Square, and to hear and see the Bolshoi Opera and Ballet. Over the years, Bob Huebner maintained ties and contacts with Soviet scientists who exchanged periodic visits with him.

During the transition period between the polyoma studies and the exciting discovery and recognition of the "Tumor Antigens" in the early 1960's, Bob Huebner carried out his previously stated intention of studying tumor and non-tumor viruses in their natural setting. In collaboration with Janet Hartley and Wally Rowe, Bob recovered reoviruses from wild and laboratory mice in 1961 (2). Reoviruses, as noted previously, were isolated from some children in the Junior Village studies (3) and had been described initially by Albert Sabin (3). Using the standard virus isolation and serologic survey procedures developed for these agents, they recovered four isolates of reovirus type 2 from urban and rural wild mice. These mice were some of the same animals trapped in Harlem, New York City, and in the dairy farms and grain mills of Frederick County, Maryland, during the polyoma surveys. They found seven strains of reovirus type 3 from normal laboratory mice and from mice with transplant induced mouse leukemias. They found that antibody to type 2 was focally distributed in wild mice but was not encountered in laboratory mice. A low titer serum inhibitor of type 3 hemagglutination and infectivity, possibly representing specific antibody, was prevalent in laboratory mice.

This work expanded knowledge of the variety of viral prevalence in different mouse population groups and this indicated the necessity for caution in attributing

significance to sporadic isolations of mouse viruses in experimental investigations. This study probably influenced Bob in his future studies to use mice that were free of viruses not under investigation and to house the animals in individual isolation trailers to prevent accidental introduction of extraneous mouse viruses.

To extend the study of other viral agents occurring in nature as well as those that produced tumors in their natural hosts, Bob Huebner and associates collaborated on some studies of rabbit papilloma (warts) virus (first described by Shope in 1933) along with Dr. David White, a United States National Academy of Sciences Fellow associated with the School of Bacteriology, University of Melbourne, Australia (4). Rabbit papillomatosis had been a problem in parts of Australia. The collaborators described methods for the accurate assay of rabbit papilloma virus and its antibody. They examined some of the relationships between tumors, infectious virus, antigen and antibody in naturally and experimentally infected rabbits. The results they achieved consisted primarily of improvements in the manipulation of the virus without implication for control of the virus.

In additional studies of the papilloma group (5) the LID team, along with Bob Huebner, described the tumor formation in bovine tissue culture caused by bovine papilloma virus. This virus was of personal interest to Bob because some of his prize Angus breeding cattle were afflicted with bovine warts. The changes in the tissue culture cells occurred first and were most easily recognized in a semi-continuous diploid (two copies of each gene) cell line of fetal bovine conjunctiva (the membrane lining the exterior of the eye) (strain DBC). As with previous long-standing resources arrangements, Microbiological Associates, Inc. of Bethesda, Maryland, provided all the

tissue culture reagents including the cell lines. The earliest changes noted in the cultures were the appearance of short, thin spindle-shaped cells in contrast to the normal epithelial-like appearance of the non-inoculated tissue culture cells. The immunologic data, the chloroform resistance of the agent, the filterability and sedimentability of the agent and the occurrence of positive results with wart (papilloma) extracts from cattle in three geographical areas all indicated that the agent responsible for transformation in the tissue culture was actually the bovine papilloma virus.

Previously, studies of the wart viruses had been hampered by lack of convenient assays. None had been propagated in tissue culture. Since transformation had been induced in these experiments by virus dilutions as high as 1:1000, the authors postulated that the system they described could provide a relatively sensitive assay for the viruses. In their studies, the authors found that infectivity was not associated with the transformed cells but rather with the associated fluid medium. The DBC cells did not appear to provide a method for propagating the virus, and, in this respect, the cell virus relationships resembled some of the transformation systems described for other papova viruses

The authors suggested that the high percentage of DBC cells transformed by the bovine wart viruses might be a particularly useful model system for the study of cellular transformation by viruses. This observation plus other similar examples of tumor viruses "lost" in transformed tissue may have sparked Bob Huebner's interest in deciphering the fate of the missing viruses. This interest was to be rewarded soon in the upcoming unfolding experience with the "tumor antigens."

Around 1962, Bob Huebner began recruiting additional personnel to expand his laboratory's capabilities for collaborating on work on tumor viruses occurring in commercial scientific laboratories outside of NIH. Also in 1962, he entered into collaboration with Dr. John L. Sever in an effort to refine serological techniques for many non-tumor viruses (6). Bob Huebner intended to use these improved techniques in mass human epidemiological surveys as a way to determine prevalence of anti-viral antibodies in patients with and without cancer. Dr. Huebner had first met Dr. Sever, who had received his M.D. and Ph.D. degrees from Northwestern University in Chicago, when Drs. John Utz and Dorland Davis had introduced them in 1960. Dr. Sever was on a two-year sabbatical leave from Northwestern when Bob invited him to join LID; he offered him a position, as an observer until working space should open up in the laboratory. Sever "followed Wally Rowe around for six months" until a small area was found for him in an animal room with a chimp named "Elvis" who, he always claimed, hated him (8). Despite the hostility from that quarter he was able to start work on refining of serological techniques. In 1963 he published a paper with Bob Huebner and others entitled "Serological Diagnosis 'En Masse' with Multiple Antigens" (6) in which he described the preparation and use of 97 hemagglutinating and complement-fixing antigens for epidemiolologic investigations of the role of viruses in diseases of man and animals. After finishing up the serological work, the Huebner-Sever collaboration continued with studies involving rubella (German measles)(7A-D). Rubella, generally a relatively benign disease of children, can have a devastating effect on the fetus during pregnancy. Intrauterine infection can result in the so-called "congenital rubella syndrome" leading to early termination of pregnancy, or in surviving infants, eye

cataracts, deafness, heart malformations or central nervous system damage. Later when the NIH became interested in the health of pregnant women and babies, Dr. Sever transferred to the Section on Virology, Perinatal Research Branch, National Institute of Neurological Diseases and Blindness. Despite Huebner's primary involvement with tumor viruses, he continued collaborating with Sever through the late 1960's on a variety of rubella serology, clinical, volunteer and vaccine development studies. Sever retired from NIH to become Head of Infectious Diseases at Children's Hospital (Children's National Medical Center), Washington, D.C.; he simultaneously held a professorship of Pediatrics and Obstetrics-Gynecology at George Washington University. The Sever-Huebner collaboration was productive and helped advance knowledge about rubella infection (7A-D).

As Bob moved further into the tumor virus studies, space in the laboratory was at a premium, as exemplified by Dr. Sever's early experience; Bob found he no longer had the room to carry out all the projected experimental work. The laboratory still had the increased numbers of staff, and residual storage problem that had come with the expansions made during the respiratory virus studies at Children's Hospital and Junior Village and which were still continuing. Bob also wanted to expand into other tumor virus projects. One solution to the space problem was to work more closely with commercial laboratories. The main impetus for entering into a contractual relationship with a commercial laboratory was that it could produce more rapidly and in greater quantity the reagents—tissue culture cells, serum antibodies, etc.—used in viral studies. But it had something else to offer—work space for Huebner's new associates. He worked out arrangements only with those commercial laboratories that had space, qualified post-

graduate research personnel and adequate technical facilities; these arrangements were at first an expeditious attempt to solve particularly pressing space and personnel problems but in a few years he adopted the arrangements as a unique Huebner practice. This practice of farming out associates—off the NIH campus to private facilities—would come under severe criticism later when anonymous investigators reviewed the Virus Cancer Program in the mid 1970's. For the time being, however, it served Huebner's needs when public funding and institutions could not (9).

As part of Bob Huebner's efforts to develop a comprehensive laboratory approach to the study of oncogenic viruses, he looked for opportunities to recruit personnel who had experience in working with the avian (chicken, birds, turkeys etc.) viruses—the agents that had been under continuous investigation for many years. Many of the early investigators (mentioned previously), who had kept the flame alive for the study of tumor viruses, had focused mainly on the avian strains and had made considerable progress in determining the biological behavior of these agents. A major breakthrough occurred with the ability to cultivate the avian viruses in tissue culture, and knowledge about the avian viruses began to accumulate rapidly. Comparable experience with the rodent viruses tended to lag a little further behind. With this mandate in mind, Dr. Huebner recruited Dr. Padman Sarma, one of the most promising "avian experts" who just happened to be a veterinarian with a doctorate in philosophy. Huebner had met Dr. Sarma in 1962 at a panel on animal viruses offered at a veterinary meeting (10). Dr. Sarma (who previously had gone by the name: P. Subramanyan) had presented a paper on avian leucosis that impressed Bob. Dr. Sarma came to work at NIH in 1962 in the capacity of a visiting scientist as he had neither a permanent United States visa (he was a recent immigrant

from India) nor a green card for permanent employment. This was only a minor inconvenience that did not prevent Sarma from becoming a valuable addition to Bob's team in Bethesda. Trained in classical virology, he worked initially on avian leucosis, and then after gaining satisfactory work status, he went to work on related contract projects at Microbiological Associates, one of the Laboratory of Infectious Diseases' major contract facilities. Dr. Sarma continued his career at NIH for about 30 years studying a variety of aspects related to retroviruses. During his tenure there, he made many significant contributions to scientific knowledge about the avian oncogenic viruses.

In the early 1960's, Bob Huebner's activities, with Wally Rowe and Jan Hartley in cancer viruses, attracted the attention of National Cancer Institute officers who had started planning an expanded program of research on the possible relation of animal cancer viruses to the development of human cancers (see next chapter). In the midst of Bob Huebner's early collaboration with NCI, a new discovery occurred in 1962 that startled investigators studying cancer viruses and prompted a new urgent direction in Bob Huebner's research efforts. Dr. J.J. Trentin and associates (11) at Baylor University in Houston, Texas reported that adenovirus type 12 produced malignant tumors when injected into newborn hamsters. This was a very disturbing observation since none of the adenovirus strains had been associated previously with malignancy or pathogenicity in studies with other laboratory hosts. It had broad implications for the widespread prevalence of adenovirus infection in the human population. Huebner and his associates had already demonstrated the frequency and ubiquity of adenovirus respiratory infections in many population groups; a natural fear following Trentin's observation was whether the widely disseminated adenovirus infections might give rise to cancer in humans. In

addition, it was now thought that military recruits who were immunized with an effective live adenovirus oral vaccine could be at increased risk (12). Trentin reported the induction of cancer in NIH Syrian hamsters (Mesocricetus auratus [Golden]) by the prototype strain of adenovirus type 12 supplied by Bob Huebner's laboratory, where it had first been isolated. The Huebner laboratory had sent a portion of the prototype adenovirus type 12 to the Viral and Rickettsial Registry of the American Type Culture Collection (ATCC) as a reference standard, and the ATCC supplied some of this reference material to Dr. Trentin.

After learning of Trentin's observations, Bob Huebner, along with Wally Rowe and William T. Lane (13) immediately set out to do their own studies. They confirmed the observations of Trentin's group; in addition, they found that adenovirus type 12 strains (other than the prototype), and the prototype strain of adenovirus 18, also produced cancers in hamsters. The other 26 serotypes available in Bob Huebner's laboratory did not produce cancers in hamsters. Bob indicated that of all the human adenoviruses, types 12 and 18 were unique in that they were the only ones that did not produce hemagglutinins for human red blood cells (hemagglutination phenomenon). Trentin, as well as Bob Huebner, failed to demonstrate infectious (or "free") virus or replication of adenovirus 12 in the tumors of the hamsters developing cancers. Using various sensitive human cell cultures in attempts at isolating adenoviruses, Huebner similarly failed to recover adenovirus 12 although he experimented with a variety of methods such as taking tissue samples at various time intervals from various sites of inoculation in the hamsters. Nevertheless, his efforts were not totally fruitless. At this point he demonstrated his unique approach and intuitive grasp of investigative problems.

He was able to demonstrate complement-fixing (CF) antibody responses in the serums of a majority of hamsters developing cancer following injection with the type 12 prototype, thus indicating infection. The experiments showed that antibody responses occurred more frequently and at higher titers in cancerous hamsters than in cancer-free hamsters injected with the same virus. He concluded, therefore, that cancer was associated with infection from adenovirus type 12, and that the infectious process was responsible for the development of antibodies rather than the antigenic stimulus from the small infectious inoculum.

Commenting conservatively on the significance of the above work, Bob Huebner stated that the results were quite preliminary in nature, but that they carried important implications for investigators concerned with the development and the field-testing of adenovirus vaccines for human use, for those interested in mechanisms of viral oncogenesis, as well as for those concerned with the etiology of cancer. The close relationship of adenovirus 12 complement-fixing antibodies with tumor development in hamsters suggested that serological surveys of cancer patients might provide valuable information concerning the possible role of adenoviruses in human cancer. Thus, not only relative prevalence of antibodies but also relative titers might prove useful. He also concluded that the oncogenic effects of early passage type 12 isolates and of the type 18 prototype in hamsters suggested that this property was present in naturally occurring strains of adenoviruses 12 and 18 and was not an artifact produced by laboratory manipulation (13). For the next several years Bob Huebner designed studies and enlisted associates in various research facilities to help answer the questions raised by the above new information. These investigations continued until the results of molecular tests for

adenovirus tumor antigen in human cancers came out uniformly negative (in the mid-1960's, see subsequent chapters), and the serological tests of the blood of cancer patients for adenovirus antibodies showed no difference in incidence from the blood of non-cancer controls (in the mid-1960's, see subsequent chapters). Adenoviruses proved not to be a cause for human cancers.

On the basis of these first initial observations on the lack of replication of free virus in adenovirus induced tumors in newborn hamsters, Bob Huebner, in another major investigation, was able to demonstrate the presence of complement-fixing antigen in tumor tissue, the so-called "tumor (T) antigens," in these animals and in other species with other tumor viruses. He reported these findings in a paper entitled "Specific Adenovirus Complement-Fixing Antigens in Virus-Free Hamster and Rat Tumors" written in collaboration with Wally Rowe, H.C. (Chick) Turner and W.T. Lane (14). Chick Turner was the supervisor of the LID Serology Unit and had been collaborating actively with Bob Huebner since the period of the rickettsial research in the 1940's. He continued to work closely with Bob in many subsequent studies that required accurate and sophisticated serological testing.

In this initial study with Rowe, Turner and Lane, Huebner demonstrated type-specific complement-fixing antigens in cancers induced in hamsters by adenoviruses 12 and 18 and in newborn rats by adenovirus type 12. The study also showed that primary and transplanted tumors were devoid of infectious virus in over 50 separate tests. The specific complement-fixing tumor antigens occurred with remarkable regularity in transplanted types 12 and 18 tumors and in serial tissue cultures of type 12 hamster tumor cells. The tumor antigens were not sedimentable (in the high-speed ultra-centrifuge) and

were found to have other properties similar to type-specific or “C-soluble” antigens [Footnote- Pereira (17) had separated the adenovirus antigens by paper chromatographic analysis into A) group-reactive (in the complement-fixation test); B) cell-separating; and C) type-specific antigen fractions (17)] produced in tissue cultures during replication of adenoviruses 12 and 18. Antibodies induced in hamsters and rats carrying primary tumors and serial transplants of tumor cells reacted with type-specific antigens produced in KB and HEK (human embryo kidney) tissue cultures by replicating adenovirus types 12 and 18. During this same study Huebner quoted unpublished data from his laboratory indicating that the group also found complement-fixing antigens in hamster tumors produced by the SV-40 virus and the Schmitt-Ruppin variant of avian Rous virus without evidence of demonstrable infectious virus in the tumors. Huebner also referred to past and present work performed by other laboratories (14) reporting the presence of antigen-containing virus-free cancers produced by other tumor viruses.

In his discussion of the group’s tumor antigen study, Bob Huebner quoted from the concluding address Renato Dulbecco (14) (a Nobel Laureate) gave at the 1962 Cold Spring Harbor Symposium on Basic Mechanisms in Animal Virus Biology. Dulbecco had commented on evidence concerning “new” antigens in cells transformed by polyoma virus as follows: “At this moment it is impossible to decide whether this new antigen is determined by a gene of the virus persisting in the transformed cells, or by a gene of the cell activated by the infection”. Huebner decided that, in the case of adenovirus tumor systems in hamsters and rats, it was not only possible, but necessary to decide in favor of Dulbecco’s first hypothesis, namely, that the complement-fixing antigens were formed in response to a viral genome continuously present in the tumor cells. Huebner stated that,

"The continuous presence of type-specific viral-like antigens in the absence of the virus itself provided high-order evidence in favor of the conclusion that they were coded for by viral information incorporated into the genetic apparatus of the cancer cells." Still, he did not totally exclude an alternative hypothesis postulating an inheritable "new" cell antigen (16), brought about by virus action on cellular genetic material. The development of such an antigen, however, could not explain all the phenomena observed, particularly the virus-neutralizing antibody produced in hamsters carrying large adenovirus induced tumors for long periods. The presence of specific virus-related antigens in hamster and other animal tumors induced by three different groups of viruses (adenovirus, papovavirus and avian leucosis) suggested that similar antigens might be demonstrable in some naturally occurring "virus-free" tumors of animals and man. [Note- The Huebner group study (14) was presented during the 1963 Symposium on Virus-Cancer Relationships, Wendall M. Stanley, Chairman, under the title "Evidence for the Intrusion of Adenovirus 12 Genetic Information in the Genome of Induced Tumor Cells," National Academy of Sciences.]

In a paper written with Wally Rowe and Leonard D. Berman in 1963 entitled "Observations on a Specific Adenovirus 12 Antigen in Virus-Free Adenovirus Transplants" (15), Bob Huebner speculated that although they had not demonstrated that the production of the specific adenovirus complement-fixing antigen was directly linked with the cancerous behavior of the cells, "the regular association of this antigen with transplanted, actively growing tumor cells (in the absence of complete virus) implied in these cells the replication of genetic information derived initially from adenovirus 12. It was stated further that this might prove to be the first specific evidence of the intrusion of

a specific viral DNA into the genome of transplantable tumor cells. In those animals in which tumor did not grow, no antibody was produced." The accumulation of additional experimental data thus seemed to reinforce Bob's concept of the replication and transmission of tumor viruses through the genetic mechanism of the host.

For the next several years Bob Huebner continued to study the phenomenon of the tumor antigens and to refine his concepts of the implications of tumor antigens in relation to cancer genetics. As a result of the continuing investigations (18), Bob Huebner's ideas about the genetic transmission of tumor viruses through the genome of the host were beginning to crystallize which ultimately would lead to his formulation of the oncogene theory. His new investigations (18) in tumor viruses, new human cancer epidemiological surveys, and increasing cancer-related activities lead to his eventual permanent association with the National Cancer Institute.

1) A) Dr. Alexis I. Shelokov—Personal communication, August 23, 2001. B) Letter from Dr. Alexis I. Shelokov, October 18, 1982 among the personal papers of Dr. Robert J. Huebner. C) Letter from Dr. William McD. Hammon, August 8, 1982 among the personal papers of Dr. Robert J. Huebner
2) Hartley, J.W., Rowe, W.P. and Huebner, R.J.1961. Recovery of reoviruses from wild and laboratory mice. *Proceedings of the Society for Experimental Biology and Medicine* 108: 390-395.
3) See the Chapter on—The New Viruses and The Junior Village Era.
4) White, D.O., Huebner, R.J., Rowe, W.P. and Traub, R. 1963. Studies on the virus of rabbit papilloma. I. Methods of assay. *Australian Journal of Experimental Biology* 41: 41-50.
5) Black, P.H., Hartley, J.W., Rowe, W.P. and Huebner, R.J. 1963. Transformation of bovine tissue culture cells by bovine papilloma virus. *Nature* 199: 1016-1018.
6) Sever, J.L., Huebner, R.J., et al 1963. Serological diagnosis “en masse” with multiple antigens. *American Review of Respiratory Diseases* 88: 342-359.
7) A) Schiff, G. M., Sever, J.L. and Huebner, R.J. 1963. Rubella virus: Neutralizing antibody in commercial gamma globulin. *Science* 142: 58-60. B) Sever, J.L., Schiff, G.M. and Huebner, R.J. 1964 Frequency of rubella antibody among pregnant women and other human and animal populations. *Obstetrics and*

Gynecology 23: 153-159. C) Sever, J.L., Monif, G.R.G., et al 1966. Clinical and sub-clinical rubella following intra-dermal inoculation of rubella virus into human volunteers. *American Journal of Epidemiology* 84: 163-166. D) A series of additional rubella manuscripts with Robert J. Huebner as a co-author.

8) Interview with Dr. John L. Sever, December 21, 1999.

9) See the chapter on—Critics Anonymous.

10) Interview with Dr. Padman S. Sarma, May 3, 1999.

11) Trentin, J.J., Yabe, Y. and Taylor, G. 1962. The quest for human cancer viruses. *Science* 137: 835.

12) Couch, R.B., Chanock, R.M., Cate, T.R., Land, D.J., Knight, V. and Huebner, R.J.1963. Immunization with types 4 and 7 adenovirus by selective infection of the intestinal tract. *American Review of Respiratory Diseases* 88: 394-403.

13) Huebner, R.J., Rowe, W.P. and Lane, W.T. 1962. Oncogenic effects in hamsters of human adenovirus types 12 and 18. *Proceedings of the National Academy of Sciences* 48: 2051-2058.

14) Huebner, R.J., Rowe, W.P., Turner, H.C. and Lane, W.T. 1963. Specific adenovirus complement-fixing antigens in virus-free hamster and rat tumors. *Proceedings of the National Academy of Sciences* 50: 379-389.

15) Huebner, R.J., Rowe, W.P. and Berman, L.D. 1963. Observations on specific adenovirus 12 antigen in virus-free adenovirus tumor transplants. Viruses, Nucleic Acids and Cancer. *Proceedings of the 17th Annual Symposium on Fundamental Cancer Research* Pp. 564-570.

16) Habel, K. 1961. Resistance of polyoma immune animals to transplanted polyoma tumors. *Proceedings of the Society for Experimental Biology and Medicine* 106: 722-725.

17) Pereira, H.C. 1960. Antigenic structure of non-infective adenovirus materials. *Nature* 186: 571.

18) See succeeding chapters.

Chapter 15

Collaboration with the National Cancer Institute

Collaboration

As Bob and his associates steadily concentrated their activities on tumor virus studies, they began to attract the attention of some of the administrative personnel of the National Cancer Institute. Encouragement in these endeavors came from high ranking administrative officers of the National Cancer Institute (NCI) who had begun to realize that the burgeoning interest in animal tumor viruses might have important bearing on the etiology of human cancer. The NCI Officers most interested in Bob's cancer research were Dr. Carl G. Baker, as Director of Etiology, NCI, and Dr. Kenneth M. Endicott, Director of NCI until 1969. In the early 1960's, Bob increasingly relied on the National Cancer Institute to financially support his expanding activities in the cancer virus field as his funding from NIAID began to dry up. Increasingly conservative and subject to fiscal restraints, NIAID limited the resources it devoted to cancer-related research. Fortunately, Bob could turn to the NCI for additional support when the NCI initiated a viral oncology program of its own which culminated in the establishment of the Special Virus Leukemia Program in 1964. From the beginning, Huebner was involved in developing the program, planning its organizational structure, coordinating the research efforts, and determining its goals. Despite that his official home was still the NIAID, Huebner was a vital force that got the cancer program up and running.

Dr. Carl G. Baker (Director of the National Cancer Institute from 1969 to 1972), in his unpublished manuscript "Administrative History of the National Cancer Institute

Viruses and Cancer Programs, 1958-1972," (1) provides a good description of the development of the NCI's viral cancer activities and the role that Bob Huebner played in them. He outlines their early development and the resultant expansion that climaxed with the formation of the Special Virus Leukemia Program, later called the (Special) Virus Cancer Program. He describes in exquisite detail the scientific accomplishments of the program, which employed animal tumor viruses as investigative tools for carcinogenesis. Baker shows how these special programs through the generous infusion of funds for research spurred the development of molecular biology and biotechnology. He documents some of the administrative aspects of the program with which Bob Huebner was associated as well as Bob's scientific accomplishments in the program. Some of his organizational successes included the development and standardization of the required quality resources: tissue culture cell lines; virus preparations; antibodies; special animals and animal model systems; hazard containing facilities; banks of human tissues and sera and other resources; low temperature storing equipment; and special instrumentation for use by all the participants in the program network. Later in the program most of these resources were available commercially, but in the early years they were not available in sufficient quantities and many of the materials were of insufficient quality (1).

By the late 1950's, even before Bob Huebner arrived on the scene, the National Cancer Institute had become interested in oncogenic viruses. Encouraged by the research community's rapidly increasing interest in oncogenic viruses and the discovery of new animal viral tumor agents, the National Cancer Institute promoted an expanded program to study animal tumor viruses and their possible relation to human cancer. To establish the program, administrators had to first come up with additional funds, as the regular

annual budget could not support the new program. In 1958, the NCI sought an appropriation from the United States Congress targeted specifically for investigation into viral cancer research. The Congress eked out $1 million dollars (1C) additional to the regular annual budget. Once they had the financial means, NCI administrators turned to developing the structural supports necessary to oversee a new research program. NCI made good uses of the resources available to it from both within and outside of NIH. NCI enlisted expert outside advisors, as well as expert NCI staff, who jointly analyzed the main problems to solve, determined strategies and goals, identified the resources required to do the research and outlined the administrative structures needed to implement a viral oncology program. In February 1959, the NACC (National Advisory Cancer Council) of the National Cancer Institute newly established the Virus and Cancer Panel and charged it with the responsibility for facilitating research in this important area. The Council looked to in-house advisory panels, already in place, to help provide guidance. Members from the Virology and Rickettsial Study Section of NIH consulted with the Panel on the best ways to develop the new program. As soon as the planning bodies were set up, the administrators immediately began to encourage applications for investigative research. The new Virus and Cancer Panel encouraged prominent virologists in the United States, as well as from certain foreign laboratories, to submit applications for research funding (1C). The Panel members especially encouraged investigators with poliomyelitis research backgrounds to apply for funding; most of them had extensive investigative experience that drew on viral research methodology. Moreover, the tempo of poliomyelitis research was slowing now that effective vaccines had been developed successfully and were in production.

By 1960, the outlines of a viral oncology program were beginning to settle into place. Bob Huebner's increasing interest in animal cancer viruses coincided with that of the National Cancer Institute, and his early efforts in that field came to the attention of its key administrators. Bob Huebner became a regular attendee at the meetings of the Virus and Cancer Panel, beginning with the fourth meeting of the group on January 27, 1960; thereafter, he increasingly took part in the continuing organizational and coordinating efforts, eventually becoming an integral part of the planning force. Dr. Robert E. Stevenson, who was then Chief, Cell Culture and Tissue Materials Program and Executive Secretary of the Cell Culture Collection Committee of the Panel, was greatly impressed with Huebner's intellectual and scientific accomplishments. Following the sixth meeting in February 1961, Stevenson proposed a resolution that the NCI provide funds for expanding facilities, personnel and financial support in support of Huebner's cancer activities within the LID. The Virus and Cancer Panel submitted this proposal to the NACC, which was approved shortly thereafter.

Around 1962, Bob Huebner became more actively engaged in organizing, planning, and coordinating the NCI's efforts to assess the possible role of animal tumor viruses in the etiology of human cancer. Bob's reputation as an outstanding, productive investigator was very well known. Not as well known, however, to the NCI administrators was his ability to construct excellent, incisive memoranda on laboratory and organizational operations. Many of Bob Huebner's reports and memoranda show the precise attention to detail and process that made him such a capable administrator (1). He had the right touch for using administrative channels such as memorandum writing to mobilize real results, and he had a talent for conveying the full force of his scientific

accomplishment to his superiors who were not involved on a day-to-day level with the laboratory. Particularly illustrative of this is his August 22, 1962 memorandum entitled "The Oncogenic Virus Program in the Laboratory of Infectious Diseases (in NIAID)" (1) sent to the Directors of NIAID (Dr. Justin M. Andrews) and NCI (Dr. Kenneth M. Endicott). The memo showcased his organizational and scientific talents. Huebner wrote the directors that he and his colleagues, Wally Rowe and Janet Hartley, had decided to devote their efforts to viruses and their relationship to cancer. He summarized his earlier work in the LID, pointing out that the LID had made significant contributions to the field of viral oncology. In the laboratory they had focused their activities on characterizing the biological properties of tumor viruses and adapting conventional viral techniques for use in defining their natural behavior. In the field, they had innovatively attempted to get at the oncogenicity of viruses by using an epidemiological approach similar to that used in rickettsial diseases, Coxsackie virus diseases and the respiratory diseases. In addition to direct searches for tumor viruses, Bob Huebner and co-workers (2), with the aid of Microbiological Associates' contracts, had been able to standardize serological procedures for 120 human and animal viruses including the well known oncogenic viruses. The latter included adenoviruses types 12 and 18, bovine, rabbit and canine papillomas, polyoma and SV-40 viruses (the adenovirus and papova virus groups). The LID had also demonstrated the value of sero-epidemiological surveys of cancer patients. Through the surveys, the LID had also identified group and specific antigens for all known myxoviruses (influenza, para-influenza, mumps, Newcastle disease), poxviruses, adenoviruses, reoviruses, cytomegalovirus, enteroviruses and others. In addition to outlining these achievements, Huebner's memo clearly, yet diplomatically, described

how obstacles were now hindering the research despite the successes. One of the things that Huebner dexterously communicated in the memorandum was the unfortunate deterioration in working conditions that had accompanied the otherwise propitious growth in research at LID over the past 10 years. Huebner mentioned that the NCI had helped alleviate some of the problems, in particular the chronic shortage of space and staff at LID that was a primary obstacle to completing research tasks. Through the efforts of Dr. Stevenson, the NCI had also furnished some contract funding as well as 10 staff positions. (1c)). However, Huebner pointed out, LID still had to resort to contracting with commercial organizations, and, even then, Huebner and his group's minimum spacing requirements were barely met. As a solution, Bob Huebner astutely proposed a new building (Building 37) as a way of handling the space problem. Ultimately, this memorandum, written even before Huebner had transferred to NCI, seems to have set in place a chain of events that eventually resolved many of the problems that had plagued him for so many years. Nearly 6 years would pass, however, before it was possible to transfer Bob and his key laboratory personnel physically from LID to a new building in NCI (1B, 3).

Following Huebner's August 22,1962, memorandum, a planning meeting was held on September 5, 1962, which consisted primarily of NCI senior staff (Kenneth Endicott, Carl Baker, Michael Shimkin, W. Ray Bryan, Paul Kotin and Robert Stevenson) as well as Bob Huebner (1A). The group agreed that in order to carry out the expanded virus and cancer efforts projected for the future, NCI would have to significantly expand resources, services and training; specifically, the program would need to be able to facilitate and accommodate growth in: 1) animal production, including

large animals; 2) long term animal holding; 3) virus identification services; 4) histo-pathology services (central services and special technologies); 5) electron microscopy; and 6) training. The group envisioned an ambitious program requiring extensive funding and the participation of many investigators and many facilities to provide the necessary resources. In anticipation of the funding problem, Bob Huebner expressed the opinion that the grants or intramural (in-house at NIH) programs could not fulfill the above needs. The group also determined that it would be important to fund various aspects of the programs by the contract mechanism as well as the research grants mechanism in order to maintain co-ordination among the various groups that would be participating in the programs. Many investigators, both within and outside of NIH, were disturbed by this concept of directed research, but the authors of these programs maintained that they would not tell the individual investigators how to conduct the research in their own laboratories (1).

During this period, the National Institutes of Health had at least two methods of providing funds for extra-mural (outside of NIH) research. In the first method, the grants or "investigator initiated" method, applicants submitted a written application to the Division of Research Grants. The application was reviewed by a study section of prominent scientific peers to judge the merits of the projected research. When the funds were granted, they were subject to continual review and site visits by a member of the appropriate study section. Contract funding was the other mechanism NIH used to support research. An institute established a contract for specific research or services needed for a given program. Investigators were encouraged to apply for or were asked to accept funds for a project. There was also a rigid review process in place for contracts.

The academic community has traditionally favored the grant mechanism of funding because it allowed for independence of scientific inquiry whereas the contract approach has been thought characteristically to be more appropriate for developmental or "targeted" research programs (see the chapters on "Politics and Cancer" and "Critics Anonymous"). Despite the difference of opinions, NIH maintained that the use of contracts offered a solution to the problem of scarce resources.

The planning group discussed possible contractors who might be able to help including: Microbiological Associates, Bethesda, Maryland; Pfizer Pharmaceuticals; Bionetics Laboratories, Rockville, Maryland; Hazleton Laboratories, Walkersville, Maryland; Flow Laboratories, Rockville, Maryland; Melpar Laboratories; AEC (Atomic Energy Commission)-Union Carbide; Fort Detrick, Frederick, Maryland; Pennsylvania State University, State College, Pennsylvania; University of Pennsylvania, Philadelphia, Pennsylvania; University of Tennessee-Oak Ridge Laboratories (for ultra-centrifugation studies). In view of the contract mechanism that had been employed in NCI's chemotherapy program, the group thought that some of the Cancer Chemotherapy National Service Center (CCNSC) contracts could be modified to help meet the needs of the Virology Research Resources Branch (VRRB) and Huebner's work. The planning group decided that a new animal building would not be necessary, as a single building would potentially invite cross cotamination with the many infectious agents under study. They decided, mainly on Bob's recommendation, that 10 to 15 trailers, each about 100 square feet in area, separated from each other, would allow Bob Huebner to begin expansion of high priority work. The trailers would be set up at the Poolesville, Maryland rural facility of NIH and would function, essentially, as viral containment units. The

purpose of this arrangement was not only containment of infectious agents within the units, but also prevention of infectious agents entering the units and introducing contamination among the test animals. In addition, Bob allowed only one trusted technician, William T. Lane, to care for these study animals as a further precaution in preventing contamination (1A).

In early October 1962, the NCI organized The Human Cancer Virus Task Force to succeed the Virus and Cancer Panel and held its first meeting on the 25th.. W. Ray Bryan, Associate Director for Program, NCI, was appointed Chairman; the other members included James Grace; two virologists, Frank Horsfall, from Rockefeller Institute in New York and Joseph Melnick, from Baylor University; NCI administrators Paul Kotin and Robert Miller; and Robert Stevenson from the NCI as Executive Secretary. Huebner—still at NIAID—was also appointed a member. This new Task Force was established in order to provide a more comprehensive effort to address the cancer problem and assess progress, make recommendations for continued investigative directions and to determine the ongoing needs of the programs. (1A) It also assumed the responsibility for disseminating information about the new program. Over the next few years, the Task Force held periodic meetings and seminars so that participating investigators in animal tumor viruses could share research results and exchange information. Bob participated prominently in these meetings, which were held initially at Airlie House, a convention center in Warrenton, Virginia, and, later at Hershey, Pennsylvania (1C). The (Special) Virus (Leukemia) Cancer Program took over the functions of the Task Force in 1964. The first meeting of the Task Force was October 25, 1962, and the group met at periodic intervals. Throughout the NCI's Virus and Cancer Programs, Dr. W. Ray Bryan was also

responsible for developing data on the projected needs of Bob Huebner and his co-workers.

As part of his continuing involvement with the NCI's programs, Bob Huebner was asked to submit to the NCI Director a memorandum setting forth his projected viruses and cancer program at LID and resources needs for a five-year period including coordination with planned projects of the Human Cancer Virus Task Force (1A). Huebner's memorandum, submitted November 6, 1962, was another model document, that clearly articulated the scientific basis for the program and the managerial requirements for key programs. Bob, at this time, was still doing his own laboratory studies, administering LID as its Chief, planning a network of collaborating investigators for NCI's virus cancer studies, and trying to coordinate the activities of the small NCI cancer unit at Fort Detrick, Maryland, with the newly envisioned program. The full funding package for the Frederick facility (including contracts for its management, for developmental work, and for the cost of resources such as tissue cultures, experimental animals and other serological materials) came in at total $791,500 for fiscal year 1963. [Footnote-The "Frederick facility" located in Frederick, Maryland was a portion of Fort Detrick sections collaborating with the NCI and employing NCI personnel. Fort Detrick functioned as a biological weapons development center for the United States Armed Forces. On October 17, 1971, President Nixon would convert the former biological warfare facilities to house research activities on the causes, treatment and prevention of cancer, companion legislation to the National Cancer Act also signed in 1971.] (4). He was also beginning various activities to get the program moving. Bob's memo stated that a contract had been signed with the private company Microbiologic Associates for

undertaking virus characterization, identification (or typing) and viral diagnostic services using complement-fixation, cross neutralization and hemagglutination-inhibition tests on various groups of viral diagnostic reagents. About 50 per cent of the effort would be spent developing and applying virus typing techniques and 50 per cent on production of satisfactory viral diagnostic reagents. At this time co-carcinogenesis studies with several cancer viruses were included in the plans, but they would never be pursued; Dr. Paul Kotin (of NCI), who would have been in charge of such studies, moved in 1965 to head the Environmental Health Programs in North Carolina. Bob Huebner's long-term vision projected a tripling of personnel (50 professional and 150 technical workers) and increased space—25,000 square feet for tissue culture work, 5000 square feet for pathology studies plus outside space for animal quarters. Huebner pointed out the likely need for elaborate and expensive facilities to insure the safety and protection for those working with and exposed to oncogenic viruses. His anticipation was prescient given what was known at that time. Only later on would it be understood that there was minimal or no danger of acquiring infection from oncogenic viruses undergoing experimental study. He attached to the memorandum a list of animal tumor viruses and indicated natural and other hosts; nucleic acid types (DNA or RNA); cellular location of viral inclusions; and other features. He grouped the viruses by seven families: 1) the papova group (papilloma, polyoma, SV-40), 2) adenoviruses (types 12 and 18), 3) the pox virus group (herpes-2 and later Epstein-Barr virus [the cause of Burkitt's lymphoma, nasopharyngeal carcinoma and infectious mononucleosis], 4) the mouse leukemia group, 5) the avian leucosis group (including Rous sarcoma), 6) mouse mammary carcinoma (the Bittner agent), and 7) frog kidney carcinoma (discovered by Balduin Lucke′). The

administrators of the National Cancer Institute were deeply impressed with this demonstration of Bob' organizational insights, his ability and initiative in acting on the plans he had conceived and his obvious mastery of virus-related knowledge.

The Human Cancer Virus Task Force continued to meet periodically with timely input and suggestions from Bob Huebner. As might be expected, much of the discussions concerned procedures and administrative details. By the end of 1962, the NCI viruses and cancer activities, especially those of the Virology Research Resources Branch (VRRB), were well defined in terms of philosophy, main objectives and organizational patterns. Moreover, the various resources needed to move ahead with the research—and which needed strict standards of quality control—were beginning to be produced in sizable quantities. Private commercial contractors produced the resources and then made them available to the investigators. Once the basic plans and procedures were in place, the Task Force also began to formulate ideas about the additional steps that would need to be taken in the coming years.

On January 8, 1963, Bob Huebner wrote yet another thought provoking—and action goading!—Memorandum to the Human Cancer Virus Task Force members and NCI staff. He provided suggestions on what the administrators and researchers should concentrate on in the future to ensure the viability of the program and how the multiple researchers funded by the program should engage with one another (1A). He pointedly commented that instead of talking about work, the Task Force should actually get to work. He criticized the Task Force for spending too much time discussing details about equipment and personnel instead of new ideas and approaches to research problems. His final paragraph offered a succinct and sharp-sighted analysis of the situation: "After all

the only justification of this Task Force will be what it accomplishes in the end, and, to this end, it must spend most of its time and effort on building programs and on achieving program objectives; this, strangely enough, means working. The chief function of staff is to facilitate this end by assuming as much responsibility as possible for administrative details leaving the investigators to get on with their work. This is also the best way to serve the best interests of the NCI staff, since it will insure maximum opportunities for achieving something worthwhile. In a five year race against time, the bearings must be greased, not filled with repetitive consideration of gritty details concerning specific justification." In order to get this done, he suggested that the Task Force staff make specific priority selections within the broad scope of possible investigative activities. Bob felt that the participating laboratories should each pursue different directions of their research while collaborating on approaches requiring joint efforts. He thus outlined and defined the pattern that the future collaborative efforts among the researchers would indeed follow. The other members of the Task force, in general, agreed with Huebner's approach. Yet, despite his perceptive insight into how the researchers funded by the program might best collaborate to achieve the maximum results, he was overly optimistic in predicting that five years would be enough time to establish a definitive link between viruses and human cancer and finish the race.

On January 24, 1963, Bob sent another memorandum to the Task Force entitled "Sero-epidemiological Surveys of Human Cancers for Anti-viral Antibodies" (1A), this time focusing on the prevalence of antibodies against tumor viruses in various population groups. He proposed establishing, in three phases, a "Cancer Serum Center" for surveying serums taken from patients with representative diverse types of cancer for

antibody reactions to various viruses. Such a center should prove useful to the Task Force for serological confirmation and epidemiological testing of specific hypotheses deriving out of current efforts to identify human cancer viruses. An overall center could not be established at that time because of inadequacy of facilities, reagents, personnel or informational storage. However, a beginning could be made with a pilot program in the Laboratory of Infectious Diseases, NIAID, coupled with a supply of specimens from the Sloan-Kettering Institute (New York City) added to the leukemia specimens already being received from the Leukemia Task Force activities of Dr. Gordon Zubrod of the Laboratory of Clinical Investigation of NCI. This would constitute Phase 1. Phase 2 would involve double-blind controlled studies, and Phase 3 would encompass broader investigations in populations with greater varieties of characteristics. Phase 1 would require only advisory and moral support from the Task Force and a modest amount of funds for Sloan-Kettering Institute. Phases 2 and 3 would require additional resources.

Bob Huebner not only wrote memorandums to the Task Force, suggesting what course it should take, but he also contributed to the various reports coming out of the Task Force. On February 5,1963, a status report on the activities of the Human Cancer Virus Task Force was presented to the NCI Scientific Directorate (1A). The statement summarized concisely up to 1963 the status of the viruses-cancer research effort and the progress made by the Virology Research Resources Branch (VRRB) and the Human Cancer Virus Task Force. Projected program efforts were also identified. The report noted that the high degree of collaboration between facilities and investigators, who shared materials and information, had previously only been common in wartime.

The Task Force's activities initiated a growth in biomedical resources that later led to a vast array of commercially available materials, indeed to a whole new industry. In 1963, the quantity and variety of resources funded with contract funds were determined by defined requirements of planned research projects for the program and not funded simply at the behest of an individual investigator. Take, for example, the case of reagents. Most virology investigators did not think that large amounts of reagents could be made of sufficient quality by commercial organizations under contract. The NCI assured them that the reagents could be tested by the same methods they themselves used, and, if they did not meet the investigators' requirements, the reagents would not be used. On numerous occasions during the interviews he conducted in the mid-1990's with current and former NCI personnel (5), Dr. Baker (former NCI Director, 1969-1972) pointed out that prior to the institution of the program in the 1960's, individuals' laboratories could barely produce enough reagent to test for quality control and still have amounts left over for the research. Dr. Baker used the example of John Moloney to illustrate the success of this commercial approach: much to his astonishment, Moloney had found in the course of research that Pfizer Laboratories could make large quantities of the mouse virus in greater amounts and with equal purity than he—the discoverer of the mouse virus—could in his own laboratory!

Despite the benefits perceived by Bob Huebner and the Task Force for such shared access to resources, most or many academic investigators objected to Government research money going for this collaborative research instead of for support of individuals' project research. Since "targeted research" (or "problem-solving research") aimed at attacking the cause of cancer involved large-scale multi-discipline efforts in addition to

the projects of individual investigators, the Task Force felt that the Federal Government laws regarding contracts needed modification if it was to carry out its mandate.

Armed with data about what would be necessary to achieve the aims of the Task Force, the Director of NCI (Dr. Kenneth M. Endicott) sent a memorandum (1A) (drafted by Dr. Carl Baker and Zelda Schiffman) to the Director of NIH (Dr. James A. Shannon) on February 28,1963, entitled "Applied Developmental Research and Research Services—Need for Legislation." This memorandum presented a brief summary of NCI's position, the background, the need and the changes in contract law that NCI would like to see enacted. Contract authority more nearly like that of other Government agencies, such as NASA (National Air and Space Administration), the Department of Energy and the Department of Defense, could allow NCI to meet its needs for additional space, positions and added managerial capability. Dr. Baker would later lament, after no action was taken on this memorandum (1A) that, despite clear justification of the need for legislative changes, this memo disappeared into a bureaucratic morass. It was not until 1964 that the NCI was finally able to obtain appropriations from Congress to begin fully funding the Special Virus Leukemia Program (later named the Special Virus Cancer Program, and then the Virus Cancer Program (VCP)).

Ultimately, NCI offered the right sort of administrative and investigative milieu, and Bob Huebner collaborated with NCI with exceptional enthusiasm. He was as eager to join the NCI as the NCI Directorate was eager to have him (and also Wally Rowe and Janet Hartley). Bob realized that the NCI would have the funding that would enable him to establish the ambitious program that he envisioned for the future in collaboration with the Human Cancer Virus Task Force. He provided thoughtful and imaginative leadership

in establishing an extensive network of highly qualified investigators whom he could fund through the contract mechanism. In this endeavor he was supported fully by the senior personnel of NCI, especially by Drs. Carl Baker and Kenneth Endicott. Because of the extent and resourceful establishment of his well-coordinated but scattered collaborative investigators, Dr. Baker labeled Bob “his General George Patton” (6) as a tribute to Bob’s organizational skills. Huebner was an efficient and capable administrator well valued by NCI officials, and his research efforts resulted in significant advances in cancer research that contributed to the success of the program. With his collaboration with the NCI, Bob Huebner was entering one of his most active and productive professional periods. It also proved to be the most controversial.

The Special Virus Leukemia Program

Bob Huebner maintained a busy schedule, presiding over a very active animal cancer virus program within LID and with collaborating contract laboratories; he also began the recruitment of a nationwide network of virologists and other professionals interested in cancer virus research. Many investigators and institutions, some with only loose or tenuous associations with NCI programs or funding, were also actively engaged in cancer virus activity. By July 1964, Dr Kenneth M. Endicott, Director of the National Cancer Institute, reached the conclusion that the results of research on acute leukemia, both in animals and in humans, had shown enough promise that additional funds, separate from the authorized annually appropriated NCI funds, should be sought for this area of research, especially for virus research. Dr. Endicott, on July 16, 1964, sent to Dr. James Shannon, Director of NIH, a memorandum, “ Needs for Funds in Acute Leukemia,” which was based on a detailed rationale provided by NCI administrators Drs. Ray Bryan,

Frank Rauscher, and Carl Baker; Dr. Gordon Zubrod reviewed it before it was sent. The memorandum, lengthy, full of detailed facts and scientific observations, was designed to provide justification for the request for additional funds. Following NIH protocol, Dr. Endicott asked Dr. Shannon for permission to seek additional funds from the United States Congress in the amount of $10,000,000 and furnished him with specific details about how the funds were to be used. Dr. Shannon granted permission, and Dr. Endicott presented the request to the Congressional Appropriations Committee asserting that there existed sufficient knowledge and technical capability to plan and implement an intensified and coordinated program. Congress subsequently approved funding of the program (1A) (2C). The program plan was approved by the NCI Scientific Directorate on October 6, 1964 (1), and at a meeting on October 14, 1964, the National Advisory Cancer Council (NACC), recommended unanimously "that the NACC and NCI Board of Scientific Counselors go on record as enthusiastically endorsing the scientific plan of attack and the scientific management program as outlined to us and, therefore, the making of speedy progress in this problem of the causation of acute leukemia and the means of eradicating acute leukemia."(7)

During the early pre-appropriation discussion phases of the Task Force, The NCI administrators, especially Dr. Carl Baker and Bob Huebner, realized that the extensive expansion and the massive infusion of funds would require a systems management approach to integrate and coordinate the activities of all the participants in the program. They envisioned as models the Army's World War II Manhattan Project (development of the atom bomb) and NASA's (National Air and Space Administration) space exploration program. After the Appropriations Bill passed, Dr. Endicott told Dr. Baker and Mr. Louis

M. Carrese (Deputy Associate Director of Program, NCI), "OK, you guys have been talking about the need for program planning, plan me a $10 million program on viruses and cancer-leukemia." Dr. Endicott appointed Dr. Baker, Mr. Carrese, and Dr. Frank Rauscher to form a science-management team operating from his office to plan, develop and manage the program. During September 1964, the three of them engaged in intensive planning activities (1).

The Special Virus Leukemia Program (that later became the Special Virus Cancer Program) thus began in 1964. The $10 million in additional funds received from Congress were designed to support special efforts, such as epidemiological studies in cancer, and to compliment the extensive research through the medium of grants, but especially contracts, in support of work by other, primarily extra-mural, investigators. Utilizing the planning approach that they called "The Convergence Technique" (8), Carrese and Baker initiated the program, with Bob Huebner assuming a major role in its getting off the ground. (Carrese and Baker published an article on this technique in the April 1967 issue of *Management Science*.) The philosophy of the approach was to build a network of investigators who each focused on their own specific projects (funded through the contract mechanism) related to the virus leukemia problem; their activities would be integrated in order to avoid duplication of the same project goals and laboratory procedures. The main objectives of the program were to: (1) determine whether viruses comparable to those then known to induce cancers in laboratory and domestic animals might also be etiological agents of human cancer, and (2) to develop effective vaccines or other means for the prevention and/or control of human cancers when such etiological agents might be found. The main assumption or working hypothesis on which the overall

program was based was that at least one virus is an indispensable element for the induction (directly or indirectly) of at least one kind of human cancer and that the virus or virus genome persists in the diseased individual (1). One of their most important goals was to produce standardized resources and reagents that could be used with confidence by all the investigators involved in the program, both intra-mural at NIH and extra-mural outside of NIH. The program's name was changed later to the Special Virus Cancer Program because it also came to include the study of solid tumors as well as leukemias.

The Special Virus Leukemia Program was renamed in 1964 the Virus Cancer Program (VCP) with Dr. Frank Rauscher appointed as its Scientific Director and Dr. Carl Baker as Director of Etiology. Bob Huebner, though still officially with the Laboratory of Infectious Diseases-NIAID, was encouraged to enlist other investigators to participate in the program. In the late 1950s, well before the initiation of this program, the NCI had started recruiting investigators of poliomyelitis such as Albert Sabin and Joseph Melnick to undertake studies in animal cancer viruses since research in poliomyelitis was receiving decreased financial support and interest. Bob Huebner proceeded with his usual energy and enthusiasm to recruit an extensive network of investigators in all regions of the United States. A large group was concentrated on the West Coast, a smaller group on the East Coast. As noted previously, Dr. Carl Baker dubbed Bob his "General Patton" because of Bob's ability to coordinate the projects and activities of these scattered new associates. Initially the investigators near the East Coast met periodically in meetings held at the Airlie House, a conference center in Warrenton, Virginia. Popular annual meetings of the entire group at Hershey, Pennsylvania, where papers and results of ongoing investigations were presented and discussed, succeeded these gatherings. When

the West Coast group became established, Bob would travel to the area at regular intervals to meet with the various people for consultation. This was usually in the format of an informal meeting for the exchange of ideas and information. This group was designated as the Pacific Coast Virus Group (PACTVIGR) (9).

A partial list of investigators and participating institutions is as follows: (8, 10)

- University of Washington- Dr. Helstroms
- California State Department of Health- Dr. Edwin Lennette, Project Director, assisted by Dr. Paul Arnstein, assigned by the US Public Health Service through Bob Huebner.
- University of California at San Francisco- Dr. J. Michael Bishop and later Dr. Harold Varmus, (supported by contracts and grants)
- Stanford University- Dr. Henry Kaplan (radiation induced cancer in mice)
- Salk Institute, La Jolla, California- Dr. Renato Delbecco
- Scripps Institute, La Jolla, California- Drs. Frank Dixon and Richard Lerner
- University of Southern California, Los Angeles, California- Drs. Murray Gardner, Robert McAllister, Peter Vogt, Saraia Rasheed and associates.
- Oakland Naval Biological Laboratory, Oakland, California- Dr. Walter Nelson-Rees
- Peiralta Laboratory, Oakland, California- Dr. Adelaide Hackett

Facilities in other parts of the United States included:

- Jackson Memorial Laboratories, Bar Harbor, Maine- Drs. Hans Meier and David D. Myers, inbred mouse colonies
- St. Louis University School of Medicine- Dr. Maurice Green
- Sloan Kettering Institute, New York City, New York

- Florida Life Sciences Laboratory- Dr. Jack Frankel
- Los Alamos Laboratory, Alamagordo. New Mexico- Dr. Sy Kalter, primate facility
- Baylor University, Houston, Texas- Dr. Joseph Melnick
- Wistar Institute, Philadelphia, Pennsylvania- Dr. Hilary Koprowski
- George Washington University, Washington, D.C.-Dr. Ariel Hollinshead
- Microbiological Associates, Bethesda, Maryland- Dr. Aaron E. Freeman, research on contracts and preparation of reagents.
- Flow Laboratories, Rockville, Maryland- Dr. Ray Gilden, research on contracts and preparation of reagents.
- Several pharmaceutical manufacturers.

From 1964 to 1968 Bob Huebner coordinated the research activities of most of the above investigators and contributed the benefit of his abundant ideas to their work, notably with Dr. Maurice Green at Saint Louis University and Dr. Murray Gardner and associates at the University of Southern California. His involvement took the form of active collaboration as well as acting as the project officer for many of the contracts in the program (10). He continued this activity during this interim period while he was still at LID-NIAID, and he extended it after he moved over to the NCI in 1968.

Huebner- Transfer to the National Cancer Institute

The decade of the 1960's was a period of intense investigative collaboration between Bob Huebner (and his associates) and the NCI. Many important observations were made about the oncogenic viruses the value of which was recognized both nationally and internationally. Dr. Kenneth M. Endicott, Director of the National Cancer Institute, formally invited Bob to transfer from NIAID to NCI. There were political and

administrative overtones to this invitation. Bob had been receiving large amounts of funding from NCI for his own investigations and for those of Wally Rowe and Janet Hartley. Wally and Jan had been invited also to transfer to the NCI, but they preferred to remain in their own facilities in the Laboratory of Infectious Diseases-NIAID (1). The NCI was also funding through the contract mechanism the extensive network of investigators recruited by Bob for the Virus Cancer Program. Bob was also the project officer for many of these investigators. The enormity of Bob's activities and his growing professional prominence was causing unease in the Office of the Director of NIAID. For several years prior to the transfer, Bob's relationship with the Director, Dr. Dorland J. Davis, had cooled because of philosophical differences about the direction of Bob's research activities and his unorthodox methods for the funding of those activities. Despite Dorland Davis' lavish praise to the press of Bob's work (12), it was, therefore, with some pleasure and relief that the Office of the Director welcomed Bob's departure from NIAID.

Bob Huebner was supposed to have transferred in 1967, but at that time there were no physical facilities within NCI to accommodate him and the personnel that he was proposing to bring with him. Dr. Endicott asked Dr. Carl G. Baker (then the Director for Etiology for NCI's Viral Cancer Program), to try to obtain space and facilities within NCI for Bob's activities. Dr. James Duff of NCI was assigned as a liaison person to assist Bob's transition from NIAID to NCI. When the facilities were in place, Dr. Endicott (13) (14) announced on October 24, 1968, that Bob had been appointed Chief of NCI's Viral Carcinogenesis Branch, and that he would also serve as Chairman of the Solid Tumor Virus Program Segment of the Institutes' Special Virus Cancer Program. Dr. James Duff

was to be Vice-Chairman of the Segment and to assist with the administrative and scientific aspects of the Segment's activities. Chick Turner and John Estes, Bob's trusted and capable laboratory associates moved over to the NCI with him. Administrative and organizational changes also occurred at the Laboratory of Infectious Diseases (LID-NIAID). In 1967, during Bob's transition period, a new unit had been created within LID called the Laboratory of Viral Diseases (14) that Bob headed until he moved to NCI. When Bob moved, Wally Rowe became the Chief of the Laboratory of Viral Diseases and remained in that position until his death in 1983. Bob Chanock was appointed Chief of the Laboratory of Infectious Diseases when Bob moved to NCI, and he has remained as Chief up to the present. The Solid Tumor Virus Program was a new integrated coordinated research assault upon the viral aspects of cancers of special interest such as bone, muscle, and kidney and bladder (12) (13). It paralleled to some extent the Institute's older effort to determine the role viruses might play in causing human leukemia and lymphoma. Solid tumor virus research in NCI laboratories in Bethesda was being funded in 1968 at $1,000,000 and contracts with outside investigators were being supported in the amount of $5,500,000. The work was also being conducted in collaboration with a large number of research grantees.

In a news release (13) issued by the Public Health Services, Dr. Endicott recapitulated and summarized the important contributions made by Bob Huebner that warranted his top-level appointment to the NCI at that time. Much of the current search for viruses causing human solid tumors or leukemia was based on discoveries previously reported by Bob and his co-workers. Dr. Endicott explained that viruses known to cause leukemia in animals could be recovered from the tissues they infected but virus causing

solid tumors replicated little or not at all in the animal systems studied to date (1968). Instead, as Bob showed in 1963, new chemical substances known as virus-specific cellular ("T") antigens arose in the malignant cells and served as telltale "fingerprints" of the causative agent. The new cellular components, coded for by viral genes, could be detected by appropriate serological techniques. These were then being applied to studies of human tumor tissue in an effort to determine if any viruses known to cause animal tumors, such as the adenoviruses, were implicated in the development of human diseases (see the next chapter).

Another of Bob's findings, Dr. Endicott thought, might eventually have application to the human problem. It had been known for some time that a relationship existed between viruses causing leukemia and those causing solid tumors in chickens. In 1966 Bob found that a similar situation existed for leukemia and solid tumor viruses of rodents, in that a leukemia virus could act as a "helper" to a solid tumor virus providing essential components of a protein outer coat or envelope and thus completing the infectious virus particle (see the next chapter).

Dr. Endicott concluded by listing some of Bob's many awards and honors. Over the course of his years in research, he had received recognition on many occasions for his accomplishments. On April 11, 1966, Bob was one of the recipients of the Distinguished Service Medal of the Public Health Service Commissioned Corps "in recognition of his distinguished accomplishments in the field of virology with particular emphasis for his role in the discovery of the adenoviruses and the development of adenovirus vaccines that had achieved an incalculable saving in human resources and economic expenditures." (A full listing of awards, honors and lectures is in the appendix.) He had been cited by

numerous scientific organizations for his work on rickettsial diseases (rickettsialpox and Q fever) and for studies with Coxsackie viruses (herpangina and epidemic pleurodynia) and parainfluenza viruses in childhood respiratory disease. His awards included the Pasteur Medal and the Ricketts Medal. Bob was a member of the National Academy of Sciences, the American Epidemiological Society, the American Association for Cancer Research, and many other honorary scientific societies. He was a member of the Scientific Advisory Board of the Jane Coffin Childs Memorial Fund for Medical Research (Princeton University) and served as a consultant to the World Health Organization Expert Committee on Virus Research. He also served on other national and international advisory groups. He delivered many honorary lectures including the Eli Lilly Lecture in 1957, the Harvey, R.E. Dyer and Carl Puckett Lectures in 1960, and the James D. Bruce Lecture (and Award) of the American College of Physicians in 1964.

With this glowing summary of Bob Huebner's career to date, Bob was welcomed officially into the National Cancer Institute in 1968.

1) A) Baker, C.G. 2005. *Administrative History of the National Cancer Institute Viruses and Cancer Program, 1958-1972*. Unpublished manuscript in the files of the NIH Historical Office. B) Several interviews with Dr. Carl G. Baker, 1999-2001. Former Director of the National Cancer Institute, 1969-1972. C) Interview April 27, 2001 with Dr. Robert E. Stevenson, formerly with the National Cancer Institute and associated with the Resource Procurement for the Virus Cancer Program. The early history of the Virus Cancer Program is contained in the personal files of Dr. Stevenson donated to and now located in the archives of the University of Maryland Baltimore Campus' Albin O. Kuhn Library as part of the American Type Tissue Collection Papers. Dr. Stevenson is also one of the persons assisting Dr. Baker in interviews for the National Cancer Institute's Oral History of the Virus Cancer Program.
2) See note 6 in the chapter on—The Tumor Antigens.
3) Interview by Dr. Carl G. Baker February 5, 1995 of Dr. Norman Anderson who worked on centrifuge development at Oak Ridge National Laboratory.
4) National Cancer Act of 1971. Public Law 92-218, 92nd Congress. S.1828, December 23, 1971. Reprinted from the *Journal of the National Cancer Institute* 48: 577-584.
5) Interview September 6, 1995 of Dr. Carl G. Baker by Dr. Robert Stevenson.

6) Dr. Carl G. Baker—Personal communication.

7) Rettig, R.A. 1977. *Cancer Crusade, The Story of the National Cancer act of 1971*. Princeton University Press, Princeton, New Jersey. P.71.

8) Carrese, L. and Baker, C.G. April 1967. The Convergence Technique: A Method for the Planning and Programming of Research Efforts. *Management Science* 13: B420-B438.

9) Communications, undated, from Harriet Huebner in her capacity as Robert J. Huebner's administrative assistant from about 1962 till his retirement in 1982.

10) Review of Annual Reports of the National Cancer Institute 1968—1972. Reports of the Chairman (RJH), Viral Carcinogenesis Branch and Solid Tumor Segment.

11) Personal communication—Dr. Janet W. Hartley (undated).

12) April 1, 1968. A yes or no on virus and human cancer. Scientific Research: *Washington Science News* p. 30.

13) A) U.S. Department of Health, Education and Welfare (HEW), Public Health Service, National Institutes of Health, National Cancer Institute. News release for October 24, 1968. B) *The NIH Record* October 29, 1968.

14) National Institute of Allergy and Infectious Diseases (NIAID). *Intramural Contributions*, 1887 –1987. Edited by Greenwald, H.R. and Harden, V.A. U.S. Department of Health and Human Services (HHS), National Institutes of Health, National Institute of Allergy and Infectious Diseases. October 1987.

Chapter 16

Hybrids, Helper Viruses, Adenovirus Testing, Field Studies

From 1964, when the Special Virus Leukemia Program was established, until 1968, when he finally transferred to the NCI, Bob Huebner was on a merry-go-round with so many multiple responsibilities to juggle. He continued to actively administer the Laboratory of Infectious Diseases as its Chief and remained actively engaged in his own laboratory investigations and field studies. By this time period, his research was related exclusively to cancer research.

One of the areas in which he focused his research in this period was the laboratory phenomenon of the possible hybridization (see glossary) and tumor growth potentiation of several cancer inducing viruses. In 1964, Huebner wrote a paper with Bob Chanock and others (1) describing the induction by adenovirus type 7 of tumors in hamsters having the antigenic characteristics of SV-40 virus. This was a disturbing observation at that time for several reasons. First, SV-40 virus contaminated many monkey kidney cell culture lines including those used for the preparation of killed and live poliomyelitis vaccines, killed vaccines used to immunize military recruits against adenoviruses, and killed vaccines against various viruses and live viruses used for volunteer studies. The second major concern was the report from another laboratory that some adenovirus type 7 strains, a major cause of acute respiratory disease (ARD) and atypical pneumonia, could cause tumors in hamsters. The military also administered adenovirus type 7 by the oral route as a component of a living vaccine to protect recruits, and in light of this new

information, it was possible that the vaccine might actually have a deleterious affect on recruits.

Huebner described the study as follows (1): “Tumors having the virus-specific antigenic characteristics of those produced by SV-40 virus developed in 27 of 36 hamsters injected as newborns with adenovirus type 7, strain L.L., a strain isolated and grown continuously in the laboratory in monkey kidney tissue cultures. The antigenic character of these tumors was particularly interesting because the 28th passage inoculum, which produced them, contained no detectable SV-40 virus, the latter having been eliminated from the L. L. strain at 23rd passage with the use of hyperimmune SV-40 serum.

“The SV-40 tumor antigens were demonstrated in the complement fixation (CF) test with the use of serums from tumorous hamsters which contained virus-specific antibodies to SV-40 tumor and to similar cell-associated antigens found in cells infected with SV-40 virus; conversely, CF antibodies to SV-40 tumor and cell associated antigens were demonstrated in the serums of hamsters carrying the L. L.-induced tumors.” Tumor antigens of adenovirus type 7 were also present in the same passage tissues as the SV-40 tissue antigens. Huebner attributed these findings to the probable hybridization between the genomes of SV-40 and adenovirus type 7.

Bob Huebner and associates, in another study, described the potentiation of adenovirus type 12 grown in African green monkey kidney cell cultures pre-infected with SV-40 virus. They summarized the study as follows (4): “The growth of adenovirus type 12 in African green monkey kidney was significantly enhanced by SV-40 pre-infection as indicated by the development of increased virus infectivity and CF antigen. After six

tissue culture sub-passages, the oncogenicity of the resulting virus in newborn hamsters was also remarkably potentiated and accelerated.

"The potentiation of oncogenicity was not due to a mere mixing of SV-40 virus and adenovirus particles but developed only after additional growth of the two viruses together for several subcultures. Tumor antigens characteristic of both viruses were demonstrated in all primary tumors induced by the postulated hybrid virus and remained present in tumors carried through five transplant passages. The oncogenic and T antigen determinants were eliminated by adenovirus type 12 antiserum but not by antiserum to SV-40 virus, thus suggesting that SV-40 genetic information was contained in some of the adenovirus capsids." They postulated that the mechanism for these results were similar to those of the prior study involving adenovirus type 7 and SV-40 virus. (1).

These two studies and the prior concerns about SV-40 virus (see the chapter on Vaccine and Volunteer Studies) raised the frightening possibility that SV-40 could enhance the oncogenic potential for people exposed to a live, attenuated poliomyelitis vaccine made in SV-40 infected monkey kidney cell culture. Later controlled epidemiological studies of this issue fortunately indicated that the vaccines did not embody enhanced oncogenic potential after all. The studies (2) showed no increased evidence of neoplasms in the recipients of these vaccines. An even later controlled study of serum antibodies (3) showed no increase in exposure to adenoviruses among cancer patients

Given that experimental studies had uncovered the cancer-producing properties of adenoviruses, Huebner was persuaded that further investigations along these lines would be the most fruitful avenue of research. Huebner continued to focus attention on these

agents as potential causes of human cancer. One of the first investigators that Bob recruited for the Special Leukemia Cancer Program was Dr. Maurice Green of the St. Louis University School of Medicine, Bob's alma mater. Dr. Green was the Director of the School's Molecular Virology Institute. Bob had helped Maurice Green establish the Institute in 1964 with NIH funds. Bob, who was the NCI's project officer for Dr. Green, backed the Molecular Virology Institute's effort to the point where NIH grants and contracts provided $900,000 of the Institute's million dollar annual budget during the later 1960's (5). The relationship was very cordial with each man finding a compatible intellectual companion. This was Bob's first encounter with an investigator who was able to blend molecular virology with chemistry and immunology. He and Maurice Green worked closely together for many years on DNA oncogenic viruses, primarily the adenoviruses, and the development of tests to determine whether the T antigens of adenovirus infection could be detected in human cancer tissues. Bob's previous efforts had been directed toward the immunologic methods employing primarily the complement-fixation test. This method was technologically not difficult and, if it had been applicable to the study of T antigens in patients' tissues, would have made the study of these cancer viruses as simple as the study of influenza or poliomyelitis viruses. Instead, Dr. Maurice Green and his associates, working under several NCI contracts, developed a DNA-mRNA (messenger RNA) hybridization test for detecting the viral "finger prints" (T antigens) in virus free tumor cells. This was a method of detecting mRNA coded by the DNA of the tumor in the absence of the free infectious component of the latter. This test when applied directly to human tumors had the same specificity as the complement-fixation test for T antigens and was thought to be perhaps even more

sensitive. As Dr. Green explained his test for the lay press (6), he started with a sample of animal oncogenic adenovirus DNA and reacted it with human cancer RNA. This, in turn, was reacted with radioactive adenovirus mRNA. If the cancer RNA contained mRNA, it would react with DNA in the first step and inhibit the subsequent reaction of the radioactive RNA to the virus DNA. If the second reaction was inhibited, then that meant that the human cancer RNA contained adenovirus mRNA. Dr. Green felt that with this test he and his associates could run studies on 1,000 cancers a year.

With the availability of these tests to determine the presence of T antigens in human cancers of the 13 human adenoviruses oncogenic in animal hosts, Bob Huebner, in 1967, launched a $3.5 million intensive cooperative effort on the part of NIAID and NCI to determine whether or not adenoviruses were also a primary cause of cancer in humans (6A). The study included 10 other organizations and involved thousands of test specimens from cancer patients across the United States. The 10 organizations participating in the effort included: Merck and Co., St. Louis University, Flow Laboratories, the Salk Institute, the Wistar Institute, the Jackson Laboratories, George Washington University, California State Department of Health, University of Southern California and Microbiological Associates. The results of the study were basically negative. On the basis of this phase of the study, the conclusion was that the adenoviruses did not play a role in the causation of human cancer.

The second phase of the study was reported later and was based on collaboration undertaken after Bob Huebner moved to NCI in 1968. The resulting publication was entitled " Serological Surveys of Human Cancer Patients for Antibody to Adenovirus T Antigens" (3) The collaborative testing program was carried out under the Solid Tumor

Virus Segment of the Special Virus Cancer Program of the National Cancer Institute (NCI). The research program was designed in accordance with a plan outlined by a small working group established by the Viral Carcinogenesis Branch of the National Cancer Institute (the organizational framework for Bob's enterprises within NCI) and included Bob (who served as chairman), Albert Sabin, Edwin Lennette and Joseph Melnick. The Solid Tumor Virus Segment, of which Bob was also the Chairman, included Albert Sabin, Joseph Melnick, Maurice Green, Edwin Lennette, Renato Dulbecco, Herbert Rapp, Wallace Rowe, Charles Boone and James Duff as Vice-Chairman. The results included both complement-fixation (CF) and immunofluorescent assays (FA). The complement-fixation tests were carried out in the laboratories of Drs. Bob Huebner, Raymond V. Gilden, Herbert Rapp, and Edwin Lennette, while the FA tests were carried out in the laboratories of Dr. Fred Rapp, Joseph L. Melnick and John Riggs.

Three hundred and eighty-nine serums from advanced solid tumor cases and matched controls were tested for CF and FA antibodies against the various T antigen fractions (A, B, C, and later D). The results showed that the absent or minimal prevalence of antibodies against the tumor antigens in the serums of the cancer patients did not differ from the prevalence of antibodies in the control group.

The researchers concluded from these studies that the adenoviruses, producers of widespread respiratory illnesses in infants, children and young military recruits, were unlikely candidates as viral causes of human cancer. Bob Huebner, much to the consternation of many collaborating colleagues, made the decision to abandon continuing intensive investigation of the adenoviruses as likely etiologies for human malignancies. Instead, Bob decided to concentrate his efforts on the investigation of the RNA oncogenic

viruses that had widespread representation in the animal kingdom, both in laboratory hosts and in nature. He did continue, however, to support Maurice Green's basic molecular studies of the adenoviruses (7).

The amount of money expended on the negative adenovirus studies became a subject of critical review later (see the chapter on "Critics Anonymous"). However, Bob Huebner felt that the high prevalence of adenovirus infections in the population, coupled with the demonstrated oncogenic effect in animals plus the enhanced oncogenic effects of adenovirus-SV-40 hybrids, demanded a thorough, well controlled epidemiological and laboratory-based study, utilizing whatever resources were needed, to determine whether the adenoviruses were agents responsible for human cancers. He was following the principles that he had enunciated earlier (see the chapter on "The Virologist's Dilemma"). His wife Harriet, in our conversations, said that once Bob Huebner had a definitely negative result on one research approach he did not hesitate to abandon further studies and move on to new approaches. The negative results of the adenovirus cancer investigations in humans caused him to concentrate his subsequent efforts on the RNA mouse and avian viruses as research models for determining potential candidate etiologies for human cancer.

In the late 1960's, the cancer investigators, including those at the NIH, became aware of a herpes-like (DNA) virus associated with a specific form of lymphoma and naso-pharyngeal carcinoma described originally by Dr. Dennis Burkitt in certain African populations (8). In England Dr. Epstein and his laboratory assistant Miss Barr visualized and later isolated the virus that now bears their names (9). Later, the Henles in Philadelphia showed that this virus was also responsible for the illness infectious

mononucleosis (10). Subsequent studies have shown that some DNA viruses, few in number, could be implicated as causes of human cancer. These include hepatitis B that may result in hepato-cellular carcinoma, human herpes virus type 2 and various serological types of human papilloma virus that cause cancer of the female genital tract. Recently (11), the newly discovered human herpes virus type 8 has been shown to be associated with and to be the probable cause of Kaposi's sarcoma, a malignancy that is one of the identifying criteria for the diagnosis of AIDS. Many frustrating years were to pass until, finally, Dr. Robert Gallo, inspired by Bob Huebner's ideas and enthusiasm about RNA viruses, the development of new molecular techniques, and the ability to grow the susceptible cell lines, was able to isolate the (RNA) retroviruses HTLV-1 and 2 (human T-cell lymphotropic virus), the etiological agents of T-cell lymphoma and hairy cell leukemia respectively.

In his vigorous pursuit of other biologic and cancer causing effects of the RNA viruses, Bob Huebner and his associates became aware of the so-called "helper viruses" and thought that they might possibly eventually have application to the human problem. It had been known for some time that a relationship existed between viruses causing avian leucosis and those causing solid tumors in chickens, as illustrated by transforming, non-infective forms of Rous sarcoma. In 1966 Bob and associates found that a similar situation existed for leukemia and solid tumor viruses (sarcomas) of rodents, in that a leukemia virus could act as a "helper" to a solid tumor virus, providing essential genetic components of a protein outer coat and thus "completing" the free infectious virus particle (12).

Working forward from this background knowledge about the avian leucosis-Rous sarcoma system, Janet Hartley and Wally Rowe (13) found that the in vitro focus-forming effects of Moloney sarcoma viruses (MSV) depended on the presence in the same cells of two virus particles, a defective MSV particle and a fully infectious Moloney leukemia particle. These studies suggested that murine leukemia viruses served as “helpers” for a defective MSV particle in much the same way that avian leucosis viruses helped to complete defective Rous sarcoma virus (RSV) infectious particles but with the difference, that in the MSV system in mouse cells, helper virus might be required for cellular alteration (transformation) as well.

Sarma, Vass and Bob Huebner (14) described a virus-free sarcoma induced in hamsters by the defective Bryan strain of RSV (Rous sarcoma virus), the cells from which when propagated in mixed tissue cultures with chicken embryo fibroblasts, transferred the non-infectious RSV genome to the latter. When the mixed cultures were super-infected with avian leucosis viruses, fully infectious RSV was released. On the other hand, when uninfected mixed cultures were implanted in the wing web of leucosis free chicks, virus-free sarcomas having the avian karyotype were produced. When cells from these sarcomas were grown in tissue cultures, they behaved as typical non-producer sarcoma cells. The addition of avian leucosis viruses to these avian cells yielded infectious RSV. In this system, the “non-producer” hamster and chick cells contained large amounts of complement-fixing (CF) and immunofluorescent-stainable (FA) antigens believed to represent the internal protein moiety (genome) of the virus but the genetic information for the outer envelope had to be supplied by the leucosis “helper” virus.

In another article, (12) Bob Huebner, Janet Hartley and Wally Rowe and their associates W.T. Lane and W.I. Capps described fibrosarcomas induced in hamsters by the Moloney sarcoma virus that carried the defective MSV genome but not infectious MSV, murine leukemia virus, or mouse leukemia group-reactive CF antigen. By adding standard murine leukemia virus such as Rauscher (15), Friend (16), Moloney (17), or Gross (18) strains to mixed cultures of MSV-induced hamster tumor cells and normal mouse fibroblasts, they obtained fully infectious pseudo-types of MSV having the immunologic characteristics of the helper leukemia viruses. Sarcomas containing the infectious pseudotype viruses were also readily produced when newborn Swiss mice were injected with MSV hamster tumor cells mixed with various murine leukemia viruses.

They concluded that the hamster MSV-induced rhabdomyosarcoma carried in vivo and in vitro was free of infectious virus and CF (group) antigen. When hamster tumor cells were grown in contact with mouse cells either as mixed tissue cultures or by inoculation into newborn mice, in the presence of various murine virus strains, focus forming and sarcomagenic viruses were readily recovered. Preliminary studies indicated that these focus forming viruses had the envelope antigens of the helper virus and thus represented newly created pseudotypes of MSV.

They suggested that the term MSV be retained as the designation of the original sarcoma strains (Moloney's and Harvey's), and that the pseudotypes produced with the Moloney, Rauscher, Friend or Gross Passage A leukemia viruses be designated MSV(MLV), MSV(RLV), MSV(FLV) and MSV(GLV) respectively. These designations were adopted and used in subsequent publications. They also concluded that it would be

important to determine whether or not the avian and murine models would provide useful patterns for studies of the etiologies of sarcomas and leukemias of other species including those that might be observed in man.

As a follow-up on these observations, Bob Huebner reviewed the murine leukemia-sarcoma complex and compared its biological behavior to the avian leucosis-sarcoma complex (19). The avian and murine complexes both showed focus formation in tissue culture, defective sarcoma genomes, rescue with leukemia virus in vitro and in vivo; they demonstrated type-specific envelope antigens and group-specific internal antigens; they demonstrated replication by budding at plasma membranes (by electron microscopy); their host ranges were determined by the helper virus envelopes; their pathogenesis was determined by the sarcoma genome and not by the helper virus; and both complexes were susceptible to interference by leukemia viruses with sarcoma virus replication. The only major difference was that in the avian complex it was possible to transfer the genome from non-producer sarcoma cells without helper virus.

Bob Huebner concluded in the article that the newer in vitro techniques for detecting and assaying the leukemia and sarcoma viruses of chicken and mice had produced radical changes in previous concepts concerning the natural behavior of these viruses. Like many other viruses, leukemia virus infection appeared to be widespread, yet in most natural circumstances it rarely resulted in clinical disease during the normal lifetime of the infected animal. Indeed, Bob continued, one might conclude from the studies of the prevalence of avian leucosis viruses and mouse leukemia viruses that they represented the most common infections of the mouse and chicken. If this were so, as he had pointed out in the past for other pathogenic viruses, ecological studies of a limited or

uncontrolled nature could be expected to uncover such infections as frequently in association with good as with ill health, despite the fact that under certain natural conditions they did cause rarely clinically observable disease.

Huebner also observed in the article that with the tools at hand, meaningful field studies could now be done to determine more specifically the roles of the avian and murine leukemia-sarcoma viruses in producing leukemias and other neoplastic diseases in their natural hosts. The many similarities exhibited by these natural models in two different classes of animals suggested that they might represent expressions of a general biological pattern likely to be expressed also in man and his domestic animals. Bob Huebner intuited that the murine model might mimic more closely the model for the possible human acquisition of leukemia, and he concentrated his efforts and those of his associates for the next ensuing years on studies of the murine leukemia-sarcoma viruses. In this vein he enlisted the help of Dr. Murray B. Gardner and associates in the extensive field studies in California.

As part of Bob Huebner's efforts in establishing a network of investigators on the West Coast of the United States in the late 1960's, one of his most fortuitous recruitments was that of Dr. Murray Gardner. Dr. Gardner was a young pathologist on the faculty of the University of Southern California School of Medicine in Los Angeles recruited in 1968 to head up an interdisciplinary research team to augment the Virus Cancer Program. He had a rather ordinary and lackluster career until his ambition became energized by his encounter with the enthusiasm and charisma of Bob Huebner (20). The charge to Dr. Gardner's group was to determine whether RNA tumor viruses in the form of infectious agents or inherited viral genes, or both, were involved in the cause and pathogenesis of

naturally occurring cancer in out-bred animals such as wild mice, domestic cats, dogs and humans. Once the natural history of such agents was understood, preventive measures such as vaccination might be possible. The initial encounters and interaction with Bob Huebner had a profound and permanent effect on Murray Gardner who became an unabashed fan of Bob's (20). It became an exciting opportunity. It also opened a new fascinating world of comparative biology that had been missing during Dr. Gardner's prior training. As a result of his enthusiastic participation in this program, Gardner became one of the outstanding and prominent investigators of RNA viruses in their natural setting.

Gardner's research group at the University of Southern California (1968-1980) sought to determine the biologic significance of type C viruses (e.g., mouse leukemia, sarcoma, and avian leucosis, sarcoma) and type B (e.g., mouse mammary tumor) in humans and their commensal house pets (such as parakeets, cats, dogs, wild mice and wild rats). The group's mandate was to determine whether such viruses 1) existed in humans and contributed to cancer, either as infectious virions or as latent inherited virogenes that were potentially activated by aging, by DNA tumor viruses or by chemical or physical carcinogens; and 2) spread between animals and humans. Foremost among the possible chemical carcinogens under scrutiny was the ambient air (i.e., smog) in urban Los Angeles County. Five years of prior research by Dr. Gardner had not disclosed any detectable cancer causing effect on inbred mice of life-long exposure to Los Angeles smog, but RNA tumor virus activity was not measured in that study. Now, with the possibility that infectious type C or B virus might be found in humans, the Los Angeles area with its diverse population groups and environmental pollution seemed the ideal

place to study the viral natural history and to test RNA viral vaccines for protection against the associated cancers. Gardner used wild mice in the study. Wild mice (Mus musculus), the progenitors of laboratory mice used for cancer research, were considered a particularly relevant animal model because, like humans, they were out-bred, not exposed to laboratory pathogens, and were subject to similar environmental exposures (21).

The multi-disciplinary team that carried out the above studies included: Earle Officer, Suraiya Rasheed, Vaslov Klement, Martin Bryant, Howard Charman, Bob Rongey, Pradip Roy Burman, B.J. Pal, Brian Henderson, John Cassagrande, Ron Ross and Malcolm Pike at the USC School of Medicine. Dr. Robert McAllister at Children's Hospital in Los Angeles gave Murray Gardner immeasurable help and added credibility. In addition, Dr. Hugh Edmondson, Chair of the Department of Pathology at USC at that time, and his wife Dorothy, who purchased a building to house the team, gave financial support and immeasurable encouragement. Bob Huebner also arranged for financial help through the contract mechanism, provided his own ideas and input and sent his own technician, John D. Estes, to help with some of the studies.

Gardner's group collected human tumors, other tissues and serums from about 16 hospitals in Los Angeles County and supplied these materials to Virus Cancer Program investigators throughout the United States. They sent weekly shipments of human tumors to Adelaide Hackett and Walter Nelson-Rees at the Oakland Naval Biologic Laboratory for tissue culture and for chromosome analysis. In addition, Los Angeles veterinarians provided access to many pet animal tumors. Gardner's group started a human tumor registry that eventually included all hospitals in Los Angeles County and put pins on the Los Angeles County map to look for cancer clusters. They collected smog particulates,

tested their mutagenic properties using the Ames test and for their transforming activity in rodent cells, and compared smog levels with the geographic distribution of cancer in humans, pet animals and wild mice. They looked in households and neighborhoods for clusters of cancers in humans and animals in an effort to discover horizontal spread of RNA viruses across species (such as FeLV=feline leukemia virus) or activation of endogenous viruses in different species by common environmental carcinogens.

The NCI supported the USC group from 1968 to 1980. Murray Gardner documented the work of the group in several comprehensive reviews (21). He summarized the accomplishments of the group as follows: 1) They discovered and characterized infectious type C and type B RNA viruses (later to be named retroviruses) in wild mice, including natural history and pathogenesis of associated lymphoma, breast cancer and neurological disease. They demonstrated the feasibility of control measures. 2) They discovered and characterized infectious retroviruses in wild rats and domestic cats and evaluated the natural history, including horizontal transmission of feline leukemia virus via saliva. 3) They discovered noninfectious endogenous retroviruses in cats, mice and rats. 4) They discovered *fes* and *fgr* oncogenes in feline sarcoma viruses and the Rasheed rat sarcoma virus. 5) They demonstrated the absence of infectious retroviruses in humans, helping to balance many false reports at that time. 6) They found no evidence of cross-species infection of human with feline leukemia virus, amphotropic (replication in the cells of one or more than one species in addition to its natural host) mouse leukemia virus or primate retroviruses. 7) They found no evidence of infectious retroviruses in dogs, parakeets, squab, New World rodents or apes. 8) They established a county-wide Human Cancer Registry in Los Angeles for ongoing epidemiological

studies. 9) They showed that the potential carcinogenicity of Los Angeles smog could not be correlated with the incidence, prevalence or geographic distribution of naturally occurring human or animal cancer. 10) They discovered and characterized the prototype Epstein-Barr virus-related herpes virus (H. pongo) of the orangutan. 11) They discovered and investigated an outbreak of chicken sarcoma coincident with the implementation of the Marek (a herpes virus causing paralysis and lymphoid tumors in fowls) disease vaccine. 12) They provided useful resources (e.g. animal viruses, cell lines, tissues, and smog particulates) to Virus Cancer Program scientists.

These fruitful activities, extending through the life of the Virus Cancer Program, provided additional insight into the role and prevalence of the animal cancer viruses in their natural setting and their lack of relationship to the causation of human cancer. They also helped establish Murray Gardner as an outstanding investigator in the biology of animal cancer viruses who had helped uncover their epidemiological behavior and their molecular virology characteristics. With his tremendous admiration for Bob Huebner and his valuable contributions to the VCP, they provided mutual pillars of support for each other.

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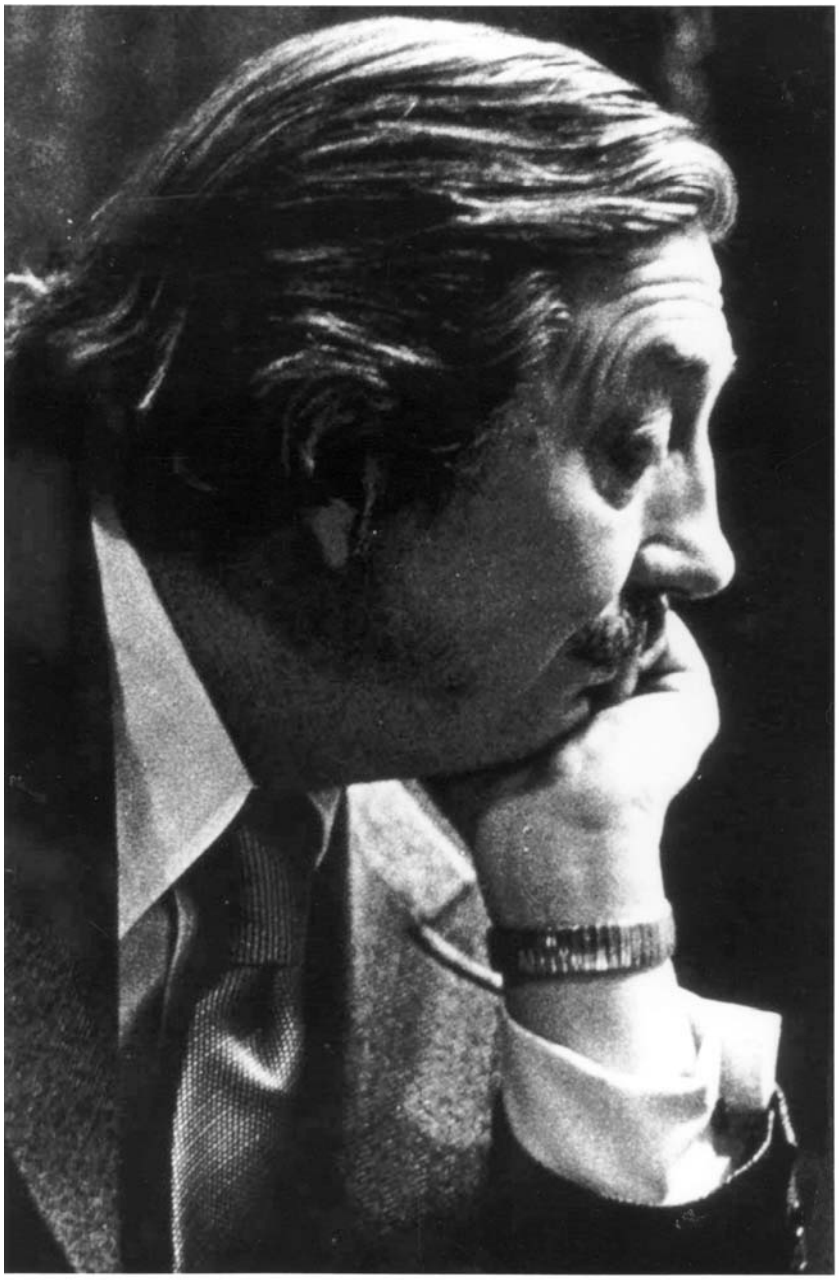
Chapter17

The Oncogene Theory of Huebner and Todaro; Reverse Transcriptase



1970s. Formal Portrait of Dr. Robert J. Huebner. (Office of NIH History files).

Beeman, *Robert J. Huebner, M.D.: A Virologist's Odyssey*, 2005.

In 1969, Bob Huebner postulated a theory of pathogenesis to account for the development of cancer among diverse species of animals. Huebner formulated this theory based on the observations that he had made over the course of his studies of polyoma virus and his subsequent investigations of the avian and murine RNA viruses, as well as on the work emanating from many other laboratories. With this theory, which became attractive to many, Huebner revolutionized prevailing notions about carcinogenesis. He also introduced the concept of the "oncogene"; this term, created by Huebner, has become standard in the vocabulary of molecular and cancer biology. Huebner defined the oncogene as the genetic material in the virus that was responsible for the transforming or cancer-causing property of the virus. He also associated the oncogene with the rest of the viral genetic material (virogene) that was responsible for viral replication and other metabolic functions. According to Huebner, all this genetic material—containing both the oncogene and virogene—was transmitted from generation to generation but resulted in cancer only occasionally in the host. Huebner initially presented his pathogenesis theory in a manuscript written in collaboration with many associates (1) entitled "'Switched Off' Vertically Transmitted C-Type RNA Tumor Viruses as Determinants of Spontaneous and Induced Cancer: A New Hypothesis of Viral Carcinogenesis," which he delivered at a private international symposium of the National Center of Scientific Research in Royaumont, France June 3—5,1969. The concepts presented there generated intense excitement among the symposium attendees.

At the Royaumont Symposium, Bob Huebner described the intellectual and experimental path that he had traveled to reach his new theory. He explained that not only did he base his ideas on the cumulative research findings and observations, but also on

the general observations of naturally occurring events in the animal kingdom that he had made over the years. Coupling the concrete experience of his work in the laboratory with his philosophical musings on the role that cancer might play in nature, Huebner had drawn inspiration for his pathogenesis theory. Explaining how he had reached his conclusions, Huebner told his audience that "the natural prevalence of cancer in virtually all animals from frogs to man seemed to suggest that cancer was a natural biologic event; occurring primarily after the reproductive period, it was quite compatible with the survival of the species and might even serve this end by providing an obsolescence device helping to ensure termination of individual life, a needful eventuality for survival of species. Thus any environmental factors, including viruses, deserving serious consideration as 'causes,' were required to operate within the realities imposed by the indeterminate yet stochastic [Huebner used this term repeatedly, which means "random"] 'built in' factors that conspired to determine the spontaneous occurrence of cancer."

In order to help his audience comprehend his theory, Huebner first detailed the history of virus cancer research during the course of the previous decade. Outlining the relationship between viruses and cancer, Huebner described how up until the middle to late 1960's, the only human cancers that scientists thought might possibly be linked with a virus were the entities of Burkitt's lymphoma and nasopharyngeal carcinoma. These entities were later attributed tenuously to the herpes-like DNA, newly isolated Epstein-Barr virus. Epstein-Barr was later identified by Werner and Gertrude Henle as the etiological agent of infectious mononucleosis (4). (Subsequently, researchers linked other DNA viruses definitely to hepatocellular carcinoma, female genital tract cancers, and Kaposi's sarcoma (5).) In the animal kingdom, despite extensive sero-epidemiological

and experimental tumor induction studies in the early 1960's of the "oncogenic" DNA viruses including polyoma (1,6), SV40 (1,6) and adenoviruses of many species (1,6), none had been established as significant causes of spontaneous cancers in their natural hosts. In fact, experimental and sero-epidemiological studies indicated that, except for polyoma, these viruses were unable to induce cancers when injected into the newborns of their natural hosts (1). (The possible involvement of SV40 virus, or close relatives, such as polyoma and papilloma, in human cancer is still being debated.) Even the data on polyoma viruses—potentially the most oncogenic virus—did not seem to indicate that polyoma was responsible for the high prevalence of cancer. The injection of polyoma virus into newborn mice had been shown to induce cancer, but other studies of the natural infections of mice with polyoma had shown these mice to be singularly free of oncogenic consequences (1,6); this was consistent with Huebner's observation that polyoma occurred randomly in nature. Indeed, recent studies of spontaneous cancers in most specific pathogen-free laboratory mice had excluded polyoma viruses as possible causes. Herpes-type viruses, since they had not been found in laboratory strains of mice, were generally excluded as possible etiological agents of lymphoma and other cancers in these animals.

The findings of these various research efforts convinced Bob Huebner that DNA viruses and other horizontally spread viruses (viruses such as the adenoviruses spread through respiratory, fecal-oral, saliva, or urinary routes) were probably not significant causes of natural cancer. This conclusion led Huebner and his associates to ask a very pertinent question: Did any of the other known oncogenic viruses have properties and/or behavioral patterns consistent with the well known random occurrences of the

"generalities" of cancers? Based on their own and others' experiences they hypothesized that the only candidates for the causation of cancer were the RNA tumor viruses of the C and B types (based on their distinctive appearances under the electron microscope). Respectively, the prototypes of these agents were the sarcoma and leukemia viruses of the chicken and mouse described by Vilhelm Ellerman and Oluf Bang (6), Peyton Rous (6), and Ludwik Gross (6) and the mouse mammary tumor virus originally described by John J. Bittner (6). The appearance under the electron microscope set the Bittner agent apart as a B-type RNA virus in contrast to the other agents that were C-type RNA viruses. The B-type particle occurs both within and outside of the host cells, is spherical, 100-105 nm (nanometers) in diameter, and contains an eccentrically located dense region surrounded by a pale zone with a well-defined boundary (membrane) (20). The C-type particle occurs as a semi-circular object seen budding from the cell membrane of the host's cells. Huebner has described it as resembling a Chinese fortune cookie.

In the mid-1960's, Huebner told the Royaumont audience, new serological (1) and in-vitro (1) cell culture techniques became available to researchers. Huebner and others who were investigating the viral/cancer link drew on these techniques in their studies of the prevalence, modes of transmission and oncogenic expressions of the C-type RNA tumor viruses. These studies yielded new findings that supported Huebner's new belief that C-type RNA viruses were the causes of many, if not most, spontaneous and laboratory induced cancers. Indeed, much about this idea had already proved to be demonstrable in the laboratory. Studies by many groups, including Huebner's own, had now established the C-type viruses as significant natural causes of cancers in mice (1), chickens (1), cats (1), and probably also in hamsters. The unique C-type virus particles

had also been observed by electron microscopy in tumors of rats (1), swine (1), snakes (1), guinea pigs (1), monkeys (1) and, possibly humans (1). Thus, 3 classes of vertebrates were known at the time of his presentation to have at least some natural experience with this virus type. Except for the B-type mammary tumor virus of the mouse and perhaps various papilloma viruses, both only having very limited oncogenic potential, they believed that the C-type virus represented the only well-established oncogenic group of viruses that could be considered seriously in the etiology of the "generality" (i.e., prevalence) of naturally occurring cancer.

At the Royaumont symposium, Bob listed the then known (June 1969) C-type viruses (including non-isolated agents seen typically only under the electron microscope) and described some of their biologic and immunologic properties. The list included the established avian and mouse C-type RNA sarcoma viruses; the C-type RNA leukemia viruses; the murine (mouse) sarcoma pseudo-type viruses produced by rescue procedure (the "helper viruses"); and the overt and covert phenotypic expression of C-type RNA tumor viruses); demonstrable virus genome expressions in mice of various high incidence and low incidence genetic strains; response to various types of physical and non-carcinogenic chemical manipulation; and many other experimental studies. He also listed methods of virus detection: tissue culture, serology, pathology, tests for virion [virus particle] antigens, non-virion antigens, anti-virion antibody, activation of phenotypic and oncogenic expression by radiation, carcinogens, and aging. Huebner told the audience that they were proposing a new theory based on the accumulated information and experimental data that postulated a determining causative role for the C-type RNA viruses in the generation of spontaneous and induced cancers.

The theory, he said, had as its central hypothesis the postulate that the cells of many and probably *all* vertebrates carried vertically transmitted (i.e., inherited) RNA tumor virus genomes genetically through the germ plasm (egg and/or sperm). These virus genomes served as indigenous sources of oncogenic information in the oncogene—that portion of the viral genome capable of transforming normal cells to become cancerous. They envisioned the virogenic (phenotypic) and oncogenic (tumor) expressions of the indigenous genome as most commonly "switched off" (i.e., cancer not expressed) or repressed during the early and mid-life stages, a situation less commonly maintained late in life when cancer is characteristically most prevalent. The exception was in certain highly inbred strains of animals where the C-type viral genome was spontaneously "switched on" (i.e., cancer expressed). They postulated further that the oncogenes and the virogenes (the gene sequences that can code for virus production) visualized as various independent operons (a cluster of genes transcribed together to give a single molecule of mRNA) of the total virus genome, must have separate independent repressors, the latter coded for by "regulator" genes of the host cells. The concept of cell regulation in cancer by specific repressors was suggested originally by Andre′ Lwoff (7) based on the observations that Francois Jacob and Jacques Monod made on the bacterial cell (8). The early work in molecular biology was performed using simple one-cell organisms such as bacteria. Lwoff, Jacob and Monod were contemporary colleagues at the Pasteur Institute in Paris; they shared jointly the Nobel Prize in Biology or Medicine in 1965 "for their discoveries concerning the genetic controls of enzymes and virus synthesis."

At Royaumont, Huebner described his group's extensive studies of expression of the C-type RNA tumor viruses, chiefly in the chicken and the mouse. He and his

colleagues had repeatedly found that the absence of detectable *infectious* C-type virus in tumors originally caused by the oncogenes of a C-type RNA virus certainly did not mean that the genome of the C-type virus was no longer present. On the contrary, when tested properly with sensitive serological and cell culture techniques or by genome de-repression and rescue techniques, the hidden genome generally could be demonstrated directly by the detection of viral antigen (T antigen) subunits and/or it could be unmasked. It was also quite clear, Huebner maintained, that except for certain situations when the host gene was controlled and certain inbred mice were found to have vertical transmission of infectious virus, most naturally occurring C-type virus genomes were transmitted in a switched off or partially switched off covert fashion. Thus, 99% to 100% of all natural expressions of the C-type RNA viruses were found to be absent in non-inbred feral mice, as well as in many inbred resistant mice. This phenomenon was not only true of feral mice but also of hamsters, rats, guinea pigs, swine, cattle, monkeys and humans. Yet, all had been observed by various investigators to have occasional demonstrable expression of the C-type RNA virus. Electron microscopy occasionally demonstrated electron microscopic evidence suggestive of C-type particles. This latter observation was suggestive, but not conclusive, evidence of the presence of C-type viruses.

Huebner and company took the theory further. They further argued that expressions of the RNA cancer virus genes (operons), like those of other cell genes, were controlled by a system of repressors coded for by the regulator genes of the cell. According to their hypothesis, mutant genes, genetic defects and exogenous inducing agents such as radiation, chemical carcinogens and mutagens and the aging process itself

all tended to reduce the power of the oncogene repressors. This de-repression lead to partial or complete expression of a universally inherited RNA tumor genome, the most significant consequence of which would be the occurrence of cancer. The chief determinant of the histological type of cancer that ensued would then depend on the cytological type of the differentiated cell in which the de-repressor activity occurred or was induced and, of course, on the strength of repressors involved in those cells.

Bob and his associates believed that the value of their hypothesis lay not only in possibilities for testing its validity in a growing number of animal test systems, but also in the fact that it could well stimulate a concerted search for repressor substances specific for both oncogene and virogene. The success of such a search might then lead to a totally new approach to the prevention and/or control of cancer—the ultimate objective. The process of these new viral techniques, Huebner told his audience, provided for “movable” oncogenes (between species and individuals) that could now be identified and assayed in human as well as in animal cells grown in-vitro. These techniques made it possible to embark on such studies as expeditiously as possible. Hence, a host of further studies at the National Institutes of Health should be started to explore the nature of the C-type oncogenes, their genetic and molecular structure, their biochemical functioning and the way in which they interact with ordinary cell genes (1,3). He also felt that the nature of the C-type viruses themselves and their reaction with cellular DNA should be investigated (1,3).

Huebner later on recalled that during the preparations for the Royaumont symposium, he briefly told Dr. Andre′ Lwoff, who organized the meeting, what he was going to say. Dr. Lwoff, the Pasteur Institute's Nobel Laureate, found the concepts so

compelling that he put Bob first on the program. Lwoff's reading was prescient: the presentation generated a great deal of excitement and interest among the symposium attendees. From then on, according to other investigators who attended the meeting, the other paper discussions were merely "footnotes" (commentaries) to Bob's presentation (9).

Dr. Robert J. Huebner. (Office of NIH History files).

Huebner anticipated, indeed almost predicted the direction of new important findings about the nature of oncogenes and the RNA tumor viruses. Yet, despite the excitement with which Huebner's presentation was greeted by the symposium attendees, the concepts failed to receive a broader reception—at least initially—because they received insufficient publicity. The media and other members of the scientific community did not immediately pick up initially on the subject. Nevertheless, Huebner continued to discuss this theory at other scientific meetings in the intervening months, including the fall meeting of the National Cancer Institute in Cherry Hill, New Jersey (9). The first full articulation of his concept to reach a wider audience was published in late 1969 in the *Proceedings of the National Academy of Sciences*, with the title "Oncogenes as Determinants of Cancer" (2). This manuscript discussed much of the key data originally presented at the Royaumont symposium. The abstract succinctly summarized the article: "Evidence from sero-epidemiological studies and from cell culture studies supports the hypothesis that the cells of many and perhaps all vertebrates contain information for producing C-type RNA viruses. It is postulated that the viral information (the virogene), including the portion responsible for transforming a normal cell into a tumor cell (the oncogene), is most commonly transmitted from animal to progeny animal and from cell to cell in a covert form. Carcinogens, irradiation and the normal aging process all favor the partial or complete activation of these genes. An understanding of how normal cells and normal animals prevent expression of endogenous viral information would appear to offer one of the best hopes for control of naturally occurring cancer." The publication attracted widespread attention, stimulating a great deal of excitement and discussion.

Over the next several years, Bob and his associates accumulated additional observations and presented further findings to promote the concepts of the theory. They published another study in the *Proceedings of the National Academy of Sciences* in 1970, which expanded on the earlier concepts (21), entitled "Group Specific Antigen Expression During Embryogenesis of the Genome of the C-type Tumor Virus: Implications for Ontogenesis and Oncogenesis." Bob collaborated with Drs. Gary J. Kelloff and Padman Sarma; W.T. Lane and H.C. Turner of the National Cancer Institute; Drs. Raymond V. Gilden and Stephan Oroszlan of Flow Laboratories, Rockville, Maryland; Drs. Hans Meier and David D. Myers of the Jackson Laboratory, Bar Harbor, Maine; and Robert L. Peters of Microbiological Associates, Bethesda, Maryland. The study documented the presence of viral genomic material during early embryonic life and provided additional evidence for vertical genetic transmission.

More specifically, the group theorized in the article that an inherited cancer gene could be present in an embryo before birth and that it could be a growth factor in the development of the embryo. The investigations on which the article was based showed that the inherited genetic material common to the oncogenic C-type RNA viruses could be detected as a group specific (gs) antigen in healthy embryos of both laboratory bred and wild mice. Most antigens, either naturally occurring or introduced by investigators into an animal's body, stimulate the production of antibodies against themselves. However, the gs antigen demonstrated by Bob and his colleagues in all strains of mice did not. This lack of antibody production was a result of the animal's tolerance for and acceptance of the antigen as self.

As an animal does not normally produce antibodies against parts of its own body, the finding of viral genetic information (gs antigen) in one or more tissues in most of the mouse embryos studied was another indication, that the RNA tumor virus might be present at the earliest stage of development. This logically led to the conclusion that this virus material might be part of the animal's genetic inheritance.

Furthermore, the group speculated, the detection of such a "footprint" of the virus early in the embryonic life of an animal (which might develop cancer only later in life) suggested that the C-type RNA virus gene might be a necessary factor in normal growth. It might, for example, provide the basic message to cells to divide and replicate.

The highly sensitive laboratory tests painstakingly developed previously by Bob and his colleagues, including complement fixation, fluorescent antibody, and gel-diffusion techniques were indispensable for the studies reported in this manuscript. With them, Huebner was able to show detectable amounts of gs antigen in mouse embryos of the BALB/C, NIH Swiss and other laboratory strains as well as several wild mouse populations bred in captivity. Evidence of the viral genetic material seemed to be present in mouse tissues that continued to grow after birth: ovaries, testes, thymus, liver and parts of the intestinal wall. This gave further support to the idea that the virus gene had a role as a growth factor.

In general, younger rather than older embryos more readily demonstrated the group specific antigen, particularly in mouse strains that showed little evidence of RNA tumor virus after birth. Gs antigens were also found in the embryos of mouse strains that had low leukemia rates and were free of infectious virus. In older wild mice, the presence of gs antigen frequently correlated with the incidence of a wide variety of spontaneous

cancers. This probably reflects the occasional prolonged survival of mice in the wild and the increased potential for cancer with advancing age. Bob's Huebner's group along with scientists in other laboratories demonstrated gs antigen activity in chicken embryos and found immunologic tolerance to similar antigens in hamsters and cats. Traces of type-C virus in snakes, rats, monkeys and in human cells preserved for laboratory testing had also been seen with the electron microscope.

Bob postulated that the phenomenon found in mice might apply also to man. His prior research with other classes of lower animals led him to extravagantly extrapolate the theory to include "all or most vertebrate cells." He based this hypothesis on the widespread prevalence of the C-type RNA viruses in nature and the lack of a precise time frame for their appearance in the animal kingdom. He speculated that viruses were of ancient origin and were transmitted vertically in their animal hosts for countless generations. Later on, the elucidation of the nature of the oncogene would help, but not entirely resolve the issue of whether or not the oncogene was of recent or ancient origin.

The goal of Bob and his group, as a next step, was to hopefully find the repressor substance (s) that through most of all life kept cancer activity "switched off." Huebner believed it was logical to search for such "repressors" (a term introduced by the French Nobel Laureates Jacob and Monod, in reference to control mechanisms in molecular biology) and to find ways to stimulate or synthesize them when they began to fail. In April 1972, Dr. George Todaro and Huebner (3) published a manuscript entitled "The Viral Oncogene Hypothesis: New Evidence," that had originally been presented at a National Academy of Sciences Symposium on "New Evidence as the Basis for Increased Efforts in Cancer Research." Todaro was a newly arrived research associate, with an

established investigative background, who shared much of the manuscript preparation. In the essay, they summarized many of the recent studies including their own studies investigating the group-specific antigen during embryogenesis; the production of virus from single cell clones of mouse tissue cultures; and the demonstration of virus from mouse embryo studies (10). They went on to propose a model for the control of oncogenes and virogenes by regulatory repressor genes. They also discussed the inductions of tumor activation by the chemical carcinogens bromodeoxyuridine (BrdU) and iododeoxyuridine (IdU) in clonal cell lines, thereby demonstrating the effect of mutagens in activating the oncogene. They compared their oncogene theory with the "pro-virus" or "proto-virus" theory of Howard Temin (3), a cancer investigator at the University of Wisconsin; Temin's theory on the link between virus and cancer was stirring some controversy. Huebner, as described previously favored an ancient origin for the "inherited" oncogene; Temin, on the other hand postulated an entity, controversial in the scientific community, the "pro-virus," a DNA intermediate, intracellular, viral precursor stage of more recent origin. In addition, the manuscript discussed a major development that had occurred in 1969-1970: the discovery of the enzyme "reverse transcriptase" by Temin and David Baltimore. The Temin and Baltimore discovery had disclosed how a C-type RNA virus genome could be incorporated into the DNA genome of the host, and the discovery gave additional support to the concept of the genetic inheritance of RNA tumor viruses. The discovery of reverse transcriptase would have a profound effect on cancer virus research and molecular genetics.

In the 1972 article, Todaro and Huebner asserted that the oncogene hypothesis was an almost inescapable conclusion given the data available from animal, cell culture

and molecular biology studies (rapid strides had occurred in molecular biology among tumor virologists). To update their concept of the oncogene, they wrote: “A special aspect of the oncogene theory is that it provides a unifying concept to explain a diversity of phenomena. Rather than the effect of agents such as radiation, chemical carcinogens, and the normal aging process being exclusively random and unpredictable, the hypothesis suggested that these agents exert their oncogenic action directly on the oncogenic information (the oncogene) present in all cells and that cancer results from the destruction of the normal repressor systems that keep both the oncogenic and virogenic information in check in the normal cell. Since type-C viruses carry oncogenic information as part of the virus genetic information, *the most reasonable assumption was that the oncogene is a portion of the virogene (the endogenous genetic information for making a type-C virus) or alternatively, that the virogene is capable of picking up the oncogenic information with high frequency.”* (emphasis added). This last speculation would be of extreme importance to the eventual elucidation of the actual nature of the oncogene. Huebner did not yet precisely know, however, the nature, the origin and the oncogene’s role in the production of cancerous transformation. The need to acquire this additional knowledge by other methods of investigation led Bob Huebner to recruit into the Virus Cancer Program Dr. J. Michael Bishop’s group and to enlist their support for studies of the nature of the oncogene. Dr. Bishop was a molecular virologist in the biochemistry department of the University of California San Francisco Medical School. Bob Huebner had made his acquaintance during the many meetings of the Pacific Coast Virus Interest Group (PACTVIGR—see previous chapters) and had become very impressed with Bishop’s professional expertise.

Dr. Robert J. Huebner. (Office of NIH History files).

The "Viral Oncogene Hypothesis" as proposed by Huebner and Todaro, based on many field and laboratory observations, was a reasonable explanation to account for the occurrence of cancers in animal caused by the vertical transmission of genetic viral information. Wally Rowe, Janet Hartley and their associates, as well as other investigators provided parallel corroboration for the genetic basis of viral cancer

causation in animals. In the 1970's and early 1980's, Wally Rowe and his team (11) described the genetic factors at play in the natural history of mouse leukemia virus infection. Using molecular techniques, they demonstrated mouse leukemia virus as part of the chromosomal genes of the mouse. The concept that RNA viruses could be transmitted genetically into the DNA chromosomal tissues of mice and other animals contradicted accepted scientific theory. Since 1953, when James Watson and Francis Crick described the structure and mechanisms of DNA functions, a central tenet of molecular biology had been that DNA transmits genetic information through RNA and not vice versa. However, the accumulating observations of Bob Huebner and others working with the RNA tumor viruses indicated that these agents were incorporated into the host's chromosomal DNA. This led to the prediction that a mechanism had to be present to allow genetic transfer from RNA to DNA to occur. In 1970 researchers proved that speculation right, and they learned how RNA viral genome was incorporated into host chromosomal DNA through the process of reverse transcriptase; this discovery was a revolutionary advance in molecular virology.

In 1970, two separate manuscripts describing the isolation of viral RNA-dependant DNA-polymerase from RNA viruses appeared in the British journal *Nature*. One was authored by Howard M. Temin and Satoshi Mizutani (12) and the other by David Baltimore (13). In 1975 Temin and Baltimore, along with Renato Dulbecco, would receive the Nobel Prize for Physiology or Medicine for their "discovery concerning the interaction between tumor viruses and the genetic material of the cell." Dulbecco, with whom both men trained at one time, would also be honored for his work on the relationship of the molecular basis of DNA viruses to cancer (14). The scientific

community accepted the discovery of viral RNA-dependant DNA-polymerase with unrestrained enthusiasm.

For many years, Temin had advanced the hypothesis that in order for RNA viruses to transform cells or to replicate, they required a DNA intermediary that he designated as a "pro-virus." Some of his experiments showed that the chemical, actinomycin D, an inhibitor of DNA, would prevent the replication of RNA viruses. His theory was not accepted readily, due to a perceived lack of experimental data, until the discovery of the enzyme. The virus that he worked with was the Rous sarcoma virus. Using the techniques of enzyme chemistry and implementing extensive controls (12), he was able to show that the genetic material of Rous sarcoma virus contained a polymerase (an enzyme catalyzing the production of many copies or polymerization) that used viral RNA as a template to produce corresponding (copy) DNA that could be incorporated into the host genome. This enzyme was at first designated "RNA-dependant DNA-polymerase." Later, the term "reverse transcriptase" was coined by Tooze, a biochemist, textbook author and editorial writer for the journal *Nature* (15). The RNA viruses that used this mechanism of replication were designated subsequently as "retroviruses." [Footnote-Temin's work was supported by a US Public Health Service research grant from the National Cancer Institute (not Viral Cancer Program funds). He held a Research Career Development Award from the National Cancer Institute. He had a faculty appointment as Professor in the McArdle Laboratory for Cancer Research at the University of Wisconsin, Madison, Wisconsin.]

David Baltimore described his theory in the same issue. Interested in testing Temin's theories, he had worked simultaneously along the same lines and used identical

laboratory techniques. He isolated reverse transcriptase initially from Rauscher mouse leukemia virus and then from Rous sarcoma virus (13). [note-Baltimore's work was supported by grants from the US Public Health Service and the American Cancer Society and was carried out during the tenure of an American Cancer Society Faculty Research Award. At the time he performed the research he was Associate Professor, Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts. In the manuscript, Baltimore acknowledged Drs. George Todaro, Frank Rauscher, and Richard Holdenreid for their assistance in providing the mouse leukemia virus through the Viral Resources Program of the Virus Cancer Program. Dr. Todaro was on temporary assignment at the time at the Meloy Laboratory in Northern Virginia, later Rockville, Maryland.]

As a conclusion to his manuscript, Temin described the import of his findings, "These results demonstrate that there is a new polymerase inside the virions (virus particles) of RNA tumor viruses. It is not present in supernatants of normal cells but is present in virions of avian sarcoma and leukemia RNA tumor viruses. The polymerase seems to catalyze the incorporation of desoxyribonucleotide triphosphates into DNA from an RNA template. Work is being performed to characterize further the reaction and the product. If the present results and Baltimore's results with the Rauscher leukemia virus are upheld, they will constitute strong evidence that the DNA pro-virus theory is correct and that RNA tumor viruses have a DNA genome when they are in cells and an RNA genome when they are in virions. This result would have strong implications for theories of viral carcinogenesis and, possibly for theories of information transfer in other biological systems" (12).

Within a short period, many investigators isolated and categorized reverse transcriptase from most of the known retroviruses. In 1974 (16), a symposium on nomenclature proposed the name “Retroviridae” (retroviruses) for a family of reverse transcriptase viruses and classified the various members according to genus and sub-genus. The discovery of reverse transcriptase helped to firmly establish the validity of Bob Huebner’s concept of the vertical transfer of RNA viral genes. Huebner, his associates and other cancer virus researchers now had a new laboratory tool to employ in their further investigations of tumor viruses. The Virus Cancer Program at NCI continued to support research programs in viral oncology that included reverse transcriptase applications as well as the resultant progress in the methods and techniques of virus-related biochemistry and molecular biology (18,19)

In the early 1970’s, the Virus Cancer Program continued to sponsor research looking for a viral cause of human leukemia and other cancers. Bob Huebner coordinated this intense flurry of activity. Toward the end of the late 1970’s, however, it became obvious that the retroviruses were not going to be likely candidates for the causation of human cancers or leukemias. With the demise of the Virus Cancer Program in the late 1970’s, many investigators abandoned the study of retroviruses with the notable exception of one very active laboratory group at the National Cancer Institute (17) led by Dr. Robert Gallo. Gallo, although he did not work directly with Bob Huebner, admired and was influenced by Bob’s vast experience and many ideas about retroviruses and their possible relationship to human cancers. Gallo pursued his quest for candidate human retroviruses by trying to establish self-perpetuating lines of cells from leukemia patients and searching for reverse transcriptase as a marker for retroviruses. He had a temporary

setback when a candidate virus proved to be a laboratory contaminant with a non-human primate strain. While the classification of human lymphocytes was still in its infancy, Gallo discovered that one type, the T-4 lymphocyte (a type of immune system cell responsible for cell-mediated immunity that makes T-cell receptors instead of antibodies), could be propagated continuously by using as a growth factor the cytokine (T-cell tropic factor), Il-2 (interleukin-2). In 1980, Gallo and his group isolated the first human retrovirus labeled "HTLV-1" (human T-cell leukemia virus) from the T-cells of a patient with a malignancy called "T-cell leukemia," or lymphoma. Later, he isolated a second virus from a patient with the "Sezary syndrome" or "hairy cell leukemia"; this virus, labeled HTLV-2, was similar to but immunologically distinct from HTLV-1. These retroviruses are now implicated in human cancer. It has also been found that they are transmitted horizontally, rather than vertically. In addition to making these findings, Gallo co-discovered the retrovirus HIV (human immunodeficiency virus), the cause of AIDS (acquired immunodeficiency syndrome). The initial pharmacological treatment for this infection was a class of medications called "anti-retrovirals" to describe their function as inhibitors of the HIV reverse transcriptase.

The oncogene theory proposed by Huebner represented the insights accumulated from many years of extensive, well-controlled observations and experiments performed by himself and his associates. The theory proposed major evidence for the genetic pathogenesis of cancer—evidence that is of current relevance for diagnosis and potential treatment. The data accumulated during the studies of the cancer retroviruses provided a major impetus to the discovery of the mechanism of genetic retroviral transmission. The discovery of reverse transcriptase provided a new potent tool for molecular biology

research and an application for the study of retroviral infections. The concept of the oncogene was perhaps the most outstanding intellectual achievement accomplished by Bob Huebner.

1960s. Bob Huebner making a point. (Office of NIH History).

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Chapter 18

Politics and Cancer

In 1969, a year following the election of a new Federal administration that was largely unsympathetic to government sponsored medical scientific research and that was under the continuing financial pressure of the Vietnam Conflict, the National Cancer Institute became subject to the partial loss of funds budgeted for continuing research. In response to this situation, Bob Huebner wrote the so-called “Moon Shot” letter on December 9, 1969, to the new Secretary of Health, Education and Welfare (HEW), Robert Finch. In this letter Huebner presented the reasons that the National Cancer Institute should continue to receive federal appropriations. Huebner discussed the military approach to solving problems, which, coupled with huge sums of government money, and resources, had resulted in technological breakthroughs. To make his case that large sums and resources should be similarly dedicated to making a breakthrough in cancer, Huebner pointed to the results achieved by big government funding of science with the NASA space program and the Army’s development of the atom bomb. Authoring the letter as the Chief of the Viral Carcinogenesis Branch, National Cancer Institute, Huebner described the detrimental impact the “budget cuts” (1) would have on cancer research, pointing out how current research was already being compromised and how opportunities to make further advances would be eliminated. Huebner then threw the political punch and called for an effort that was at least the equal of the attempt to send a rocket to the moon (hence, “the moon shot letter”). Huebner’s entreaty was compelling, a

well-reasoned argument designed to capture the attention of the highest government officials:

"The gradual erosion of funds ear marked for viral-cancer research which had been included in the 1971 DHEW budget is a tragic denouement to what promised to be a brilliant new opportunity to make a significant breakthrough in cancer etiology and possibly control. I had hoped for about $7 million additional for the Viral Carcinogenesis Branch program alone; extending our observations in mice, chickens, hamsters and cats to monkeys and man will be very expensive. Millions of dollars were and are still required to provide newly developed reagents and test systems for detecting the switched off C-type virus genome in the four species listed above. [note: To help officials understand what he meant, he also enclosed with the letter several copies of an explanation of the new "Oncogene Theory."]

"The attack we envision on basic etiology and prevention of cancer cannot be maintained unless we can think and operate along 'Big' science NASA-like lines. Many of those (including myself) who have been immersed in studies of this problem for many years believe that, like the moon landings, control of cancer can be achieved. It seems equally clear to us that if this is to be accomplished, it can only be done with an effort comparable but not equal to moon shot proportions. We think the effort, viewed in any context, should be worth several hundred million dollars. The talent needed in this effort is available and eager; all that is lacking is the will and support of the Administration.

"But the current situation is much more than being unable to mount such an effort. The existing new program [Virus Cancer Program] has been cut, and the projected

increase authorized is being whittled away—presumably by persons who have little knowledge of what such cuts are affecting.

"I would be delighted to have an opportunity to discuss these matters and to elaborate further on even more recent data that indicate the time is ripe for a concerted attack on the virological, molecular, genetic and immunologic factors in cancer. The new theory we have proposed is subject to test. I believe that in five years its application to the human problem could be determined, but this is not likely to happen if the pace of our research and that of others is to be reduced."

With his assessment of the situation, Bob uncannily anticipated the political events associated with the National Cancer Act of 1971 that were to unfold. The letter passed through the appropriate channels and eventually made its way to Secretary Finch, who apparently gave the letter his personal attention. Bob did have an opportunity for further contact with the Secretary for additional discussion. According to Dr. Murray Gardner, Bob's associate at the University of Southern California in Los Angeles (2), Bob met with the Secretary on several occasions and was able to persuade Mr. Finch to stop smoking—a significant step for cancer control. The story may be apocryphal, but Harriet Huebner confirmed that more than one meeting occurred, saying that the business of the later meetings was usually conducted on a social basis over cocktails.

The above letter indicates an evolution of Bob Huebner's thinking about the need and magnitude of governmental funding for research funding involving projects of indeterminate or unknown solution. Many years previously, early in his research career, Bob expressed the view to me that he was dubious whether solution to a problem such as cancer control would yield to the type of effort that had been employed to develop the

atom bomb. Yet, that viewpoint is understandable, considering that Huebner's research focus on non-cancer virology was less expansive in those years and that basic knowledge of cancer biology was limited. The change in his thinking probably came about as the result of his perception that the nature of the cancer process was coming into clearer focus due to accumulating experimental data compiled by himself and many other investigators.

On August 4, 1970, Bob Huebner circulated among his associates a "Statement of Objectives" (3) in which he outlined the current status of cancer research and direction for future efforts. He wrote the following: "Given support in a national program against cancer: By 1976 we should have established the basic causes and contributing factors in the generality of cancers in man, and, as a consequence, will have in hand or available an armamentarium of both preventative and therapeutic measures.

"At the molecular level we should have well defined and isolated the DNA and RNA sequences (cancer inducing operons) that code for specific cancers.

"At the cellular level we should have full knowledge of the host cell-gene regulation systems that control repression and expression of the tumor-inducing genes that are inherited by the cells.

"At the level of the individual person, the normal immunologic policemen that maintain order in the body will be helped by and conditioned to either prevent or to eliminate cancer cells.

"At the human community or ecological level, the exogenous physical and chemical factors, which precipitate cancers, will be well identified and hopefully on the way to being eliminated from the environment.

"Why are we confident that these four objectives can be achieved?

"New discoveries at each of these four levels relating to cancer in a number of animal species, most notably mice hamsters, cats, and chickens, have opened doors to entirely new concepts and approaches that promise to find the basic answers to the generality of cancer and, for the first time, permits a unitary theory of cancer causation."

Huebner then described the discoveries and concepts that arose from recent breakthroughs in the studies of the RNA tumor virus genomes. These included the studies giving rise to the new oncogene theory, the revolution in the current concepts of the RNA viral genome as a result of the recent discovery of reverse transcriptase, the description by two groups of French investigators of repressors and inhibitors of tumor viruses at the tumor cell level, the new and relatively rapid detection and assay methods for cancer-inducing environmental factors, and the establishment of a comprehensive virological, epidemiological and ecological research program in Los Angeles County centered at the University of Southern California. The enumeration of the preceding studies summarized the salient status of virus cancer research up to that time.

Huebner's timing in releasing this "Statement of Objectives" in August 1970 is speculative. It is unclear whether the timing was spontaneous on his part or whether it was in response to the April 27, 1970, US Senate Resolution 376 that authorized a "Panel of Consultants [non-governmental] on the Conquest of Cancer" as a prelude to the legislative process that resulted in the passage of the National Cancer Act of 1971.

There is an old adage that states, "There are two things that a person should not watch being made—the first is the manufacture of sausage, and the second is the making of legislation." A prime illustration of the wisdom of this adage is the Byzantine process

that accompanied the passage of this legislation. Richard A. Rettig (4) portrayed this tortuous process, describing in detail the origins, provisions and five-year follow-up of this law in his book "*Cancer Crusade. The Story of the National Cancer Act of 1971*." In the preface he states, "In political terms, the Act is of interest because it indicates how a small but powerful elite composed of private citizens mobilized sufficient political resources to secure passage of legislation opposed by the National Institutes of Health and by most of the biomedical scientific community. In policy terms, the Act captures much of the current conflict between the public and its elected representatives eager to see life-saving and life-prolonging results flow from biomedical research and, on the other hand, a scientific community acutely conscious of the long time and great uncertainty characteristic of the process by which medical research is translated into clinically useful results. These reasons justify our attention to this statute."(5) These conflicts are still relevant today when special interest groups try to influence legislation and the medical establishment in order to divert limited resources for favored illnesses.

In late 1970 a relatively obscure group called the National Panel of Consultants on the Conquest of Cancer presented a report entitled *National Program for the Conquest of Cancer* to a hearing of the Committee on Labor and Public Welfare of the United States Senate. The hearing had been hastily called; the committee chairman (Dr. Ralph D. Yarborough—Democrat, Texas) was concluding his Senate career because of an earlier defeat at the polls (he lost the Democratic primary to Lloyd Bentsen) and the event received little press coverage (6).

"Yet one year later, on December 23,1971 President Richard M. Nixon signed into law the National Cancer Act of 1971, the legislative result of the Panel's report.

Between the hastily called Senate hearing in December 1970 and a well attended signing ceremony in December 1971, cancer research legislation had occupied a prominent place in the priorities of the President, the Senate, the House of Representatives, the medical-scientific community and the interested public. The outcome of that year of legislative struggle and controversy was a much expanded national cancer program, a program that held out the promise of major progress in the war against cancer and the concurrent possibility of failure to deliver on that promise" (6).

The creation of the Senate Panel of Consultants on the Conquest of Cancer and its report resulted primarily from the efforts of Mary Lasker, medical philanthropist, and her group of "Benevolent Plotters" (8). Rettig (9) has provided a concise summary of Mary Lasker's career and the influence she wielded on health and medical legislation by virtue of her political affiliations, great wealth and the prestige of the Albert and Mary Lasker Foundation of which she was the president. She had powerful allies among prominent physicians, medical scientists and national politician legislators. In the 1950's and 1960's, the politics of biomedical research were the politics of the appropriation process (10). Mary Lasker successfully exerted pressure on the process through the influence of Congressional leaders such as Representative Thomas Fogarty (Democrat—Rhode Island) and Senator Lister Hill (Democrat—Alabama). Both politicians were key to the politics of health legislation: Fogarty was a member of the House Appropriations Committee and Chair of the Subcommittee that reviewed budgets of the Department of Labor and the Department of Health; Hill was the Chair of the comparable Senate subcommittee at the same time. Lobbying pressure by Lasker and legislative moves by Fogarty and Hill occasionally resulted in budgetary chaos and the disruption of

appropriation requests from other government agencies. Despite Mrs. Lasker's obvious great charm, talent, energy, many interests and strong convictions, she was a controversial figure in many quarters (11). Dr. James Shannon, Director of NIH, provided aid and encouragement to Lasker's lobbying efforts, but she had difficulty with other NIH administrators. According to Rettig, "She had never seen eye to eye with the leadership of the National Institutes of Health, some of who may have viewed her initiatives with strong misgivings. Her emphasis on categorical [author's footnote: i.e., applied as opposed to basic] research perhaps never found widespread acceptance among many academic medical scientists. The National Cancer Act of 1971 came to be viewed by some as one more controversy generated by Mary Lasker." (11)

In 1968-1969, developments shaped a new political context that greatly impacted the course that cancer research funding would take over the next decade. First, a leadership vacuum occurred in Congress among those members active in health legislation. Representative Tom Fogarty died, and Senator Lister Hill retired in 1968. The decline in a strong advocate group located within the halls of congress jeopardized the high levels of government appropriations that NIH had been receiving as a whole. In 1969, the new Republican administration under Nixon reduced the appropriation for NIH cancer and medical research. This action had mobilized Bob Huebner to send his "moon shot" letter to HEW Secretary Robert Finch. Mary Lasker had become concerned about the loss of leadership in congressional health legislation. In addition, her own perception of the failure of President Lyndon B. Johnson's Commission on Heart Disease, Cancer and Stroke (12,13) (of which she was an appointee) to reverse a decline in funds for cancer research (primarily in chemotherapy trials) led her to search for a new cancer

initiative (12). Dr. Solomon Garb, M.D., a physician in Chicago, published in 1968 a book (14), *Cure for Cancer, a National Goal*, which helped crystallize her thinking. Among some of the members of the medical establishment and among the lay public, a concept became prevalent that if the government could split the atom and send men to the moon, then similar types of effort and financial expenditure could find a cure and control cancer. After discussing this idea with her close associates and the lobbyists in her employ, notably Colonel Luke Quinn, Lasker and company formed the Citizens' Committee for the Conquest of Cancer in 1969. They began to beat the public drum in hopes of influencing the new administration and sponsored a full-page advertisement in the New York Times, on December 9, 1969 (15). It was a highly effective ad, designed to chide the President into action with a reminder about America's ability to accomplish any goal—however seemingly fantastic—and promise of a cure if only the administration would focus on the long term. Bold print declared in the title, "Mr. Nixon: You Can Cure Cancer," and finer text underneath recalled the moon landings and suggested that with will, comprehensive planning and money the country could conquer cancer by America's 200th birthday in 1976.

Lasker's efforts had an impact. During 1969, she was able to persuade Senator Yarborough, who succeeded Senator Hill as Chairman of the Labor and Public Health Committee, to propose a resolution to establish a Panel of Consultants that would report proposals for new cancer legislation. Since Senator Yarborough had decided that health was an area that needed emphasis, he also took on the chairmanship of the Health Subcommittee. Lasker had at first lobbied for Senator Edward Kennedy (who would inherit the position later). Yarborough, as senior Democrat on the Committee, felt

obligated to take the assignment. After some discussion and smoothing of ruffled feathers, Senator Yarborough (16) settled down to business, and, on March 25, 1970, introduced Senate Resolution 376 to establish a Panel of Consultants on the Conquest of Cancer. The Resolution was adopted unanimously by voice vote April 27, 1970 (17).

On the House side, Representative John J. Rooney, at the request of Col. Luke Quinn, Mary Lasker's congressional lobbyist, introduced House Concurrent Resolution 526 (17) on March 4, 1970. The resolution declared that: "it is the sense of the Congress that the conquest of cancer is a national crusade to be accomplished by 1976 as an appropriate commemoration of the two hundredth anniversary of the independence of our country." Adding only a minor amendment, the Congress adopted unanimously House Concurrent Resolution 675; the House passed it on July 15 and the Senate on August 28, 1970. In this fashion the Congress committed itself, with encouragement from Mary Lasker and her colleagues, to a dubious goal—however noteworthy - with an impossible time constraint.

The Panel of Consultants was chosen with great care to accommodate the broad spectrum of liberal and conservative political philosophies regarding health legislation held by members of Congress. The Panel was composed equally of prominent medical-scientific leaders and of laypersons who were leaders in their respective professions and businesses. In its makeup, the panel, with its coalition of varied interests was reminiscent of the way that most Lasker political campaigns were organized (18).

Mrs. Lasker turned to Laurence Rockefeller (19), Chairman of the Board of Rockefeller Brothers, Inc., because of his wealth, prestige and prominence in the Republican Party, for assistance in selecting Republicans for the Panel. Rockefeller was

instrumental in choosing Benno C. Schmidt for the Panel. Schmidt, a Republican lawyer and highly successful businessman, had been a partner of J.H. (Jock) Whitney and Co., a New York investment firm, since 1946 and managing partner since 1959. Schmidt was from Texas, was active in state politics and had ties to the conservative Democratic wing led by John Connelly, the former Governor of Texas. Surprisingly, Senator Yarborough knew Schmidt well from their previous association in the Texas Law School where both men had taught. Yarborough had admiration for Schmidt's talents, and he asked Schmidt to serve as the Chairman of the Panel. After initial reluctance, Schmidt agreed to become Chairman at the urging of Mary Lasker and Rockefeller. Thereafter he applied his considerable skills in helping to shepherd the Panel's recommendations through the legislative phase, and, later, as Chairman of the President's Panel, he helped provide administrative guidance for the provisions of the National Cancer Act. According to Rettig, "The appointment of Schmidt as Chairman of the Panel of Consultants on the Conquest of Cancer, therefore, brought to the Cancer Crusade a man with extensive experience in both private and civic activities. Here was an individual of recognized leadership, capable of dealing with large somewhat unwieldy committees of prominent accomplished citizens" (20).

The members set about selecting staff for the Panel. On June 1, 1970 Robert F. Sweek was appointed director of the special staff of the Senate Committee on Labor and Public Welfare (21) that had been set up to work with the Panel of Consultants. It was agreed that the staff should be housed at the National Cancer Institute. Sweek had sought the appointment actively. He had been program manager for the Atomic Energy Commission (AEC) liquid-metal fast-breeder reactor program. Sweek had heard Dr.

Garb, the author of the book who brought cancer to the public's attention, on a radio broadcast in December 1969 comparing the need for a national cancer program to the space program. Sweek had just come from a graduate seminar at American University that featured a discussion of the transfer of modern management techniques from large-scale technological programs to domestic programs. Inspired by this idea, he wrote Garb suggesting that such skills might be applied to cancer control. After corresponding and meeting with Garb, then Luke Quinn and Mary Lasker, Sweek indicated that he would be interested in becoming staff director of the Panel. Having made a favorable impression, Mary Lasker passed Sweek's name on to Yarborough, and Sweek was appointed to the position. Carl Fixman was chosen as Sweek's deputy.

Following the organization of the Panel and the special staff, the first formal meeting of the Panel was held June 29, 1970. Senator Yarborough opened the meeting by reiterating that the conquest of cancer by 1976 would be a fitting tribute for the country's bicentennial. He asked for a completed report by the end of October (22). Benno Schmidt then assigned sub-panels to begin working on the various aspects of the report. He also began a series of meetings held in September and October with congressional leaders of both parties to begin acquainting them with the work of the Panel and to provide preliminary information about the major proposals of the report.

The Panel held that cancer was the number one health concern of the American people and, that current level of funding for cancer research was inadequate for finding a cure, especially as recent scientific advances had opened promising new areas for fruitful investigations. The panel made three far-reaching recommendations, each to correct what it viewed as an existing defect in the current situation. The first recommendation (and

perhaps the most controversial) was the establishment of a new government agency—the National Cancer Authority—that would absorb all the functions of the National Cancer Institute (NCI) and would be completely independent of the National Institutes of Health (NIH). The Panel maintained that this move would permit greater freedom of action and remedy the state of ineffective administration, alleged to exist in the relationship between NIH and NCI, by establishing "clearly defined authority and responsibility" (7). Secondly, the Panel recommended the development of a "comprehensive plan for the conquest of cancer" that would continue and expand current research, increase manpower and expand facilities, strengthen existing cancer centers and create new ones, and fulfill an unmet need for "a coherent and systematic attack on the vastly complex problems of cancer"(7). The third recommendation was sure to please researchers: the Panel recommended a greatly expanded budget for cancer research from the $230 million appropriated for 1971 to $800 million to 1 billion by 1976 in graduated increments (7).

The first recommendation for an independent agency was highly controversial (23). Mary Lasker had proposed this concept most forcefully because of her perception that scientifically and administratively the National Cancer Institute's efforts to "conquer cancer" had been ineffectual. There was initial lack of unanimity among the Panel about this proposal but the Panel finally endorsed this position (24).

It is uncertain to what degree Mary Lasker and the Panel were acquainted with the expanded efforts and the extent and accomplishments of the Virus Cancer Program as well as the other initiatives in chemotherapy that the National Cancer Institute had undertaken in the 1960's. There was certainly no basis for the harsh criticisms about NCI's ineffectuality in the field of cancer research, especially given the lack of basic

information about the nature of cancer biology at the time. This contrasted sharply to the known fundamental knowledge about nuclear physics that enabled the production of the atom bomb and the rocket science that enabled sending men to the moon.

A second sticking point among the Panel members was the philosophy of "targeted" (applied) versus creative (basic) research, with its emphasis on the use of the contract versus the grant to finance the "crash program" to find cancer's cure. The academic physicians in the Panel favored the "basic" research approach reflecting the preference of the medical-scientific community, but they were overwhelmed by the "systems-management" approach of Sweek and Fixman. Dr. Carl Baker became Acting Director of the National Cancer Institute on November 10, 1969, after Dr. Kenneth Endicott left to become Director of NIH Bureau of Health Professions, Education and Manpower Training; Dr. Baker became Director of NCI on July 13, 1970, just as the Panel was getting underway. He had extensive contact with Sweek and Fixman since their offices were adjacent to his at NCI. Baker and Sweek exchanged respective planning documents from the NCI and the AEC. Baker, along with Louis Carrese, (see earlier chapter) set up a systems-management approach, the so-called "Convergence Technique" to help administer the direction of the Virus Cancer Program. Baker studied the AEC plans carefully, and he and his staff "had ample opportunity to explore with Sweek and Fixman the full range of questions about research and management" (25).

"Sweek and Fixman brought with them an engineers' preference for a centralized, project-management approach to the cancer program. They saw themselves as experts at the large scale technological programs, including programs having a high degree of scientific uncertainty, and they found The National Cancer Institute's research-planning

and management strategy undeveloped at best" (25). In other words, Sweek and Fixman were of the opinion was that their systems-management was superior to Baker and Carrese's unsophisticated systems-management.

The Panel soon faced some unfavorable publicity. Apparently the preference of some of the Panel's staff for targeted, directed research versus basic research was discussed with a reporter covering the Panel's activities for the Blue Sheet (a pharmaceutical trade journal) in the summer of 1970 with overtones critical of Sweek (26). Before the Panel's final report came out, *Science Magazine* picked up the story. In its October 16, 1970, issue, it reported that the Panel was likely to recommend that "planning and management techniques" developed by the AEC and NASA be applied to cancer. The article focused on Sweek as, "an aggressive systems-management expert" who, it alleged, would have a major influence on the Panel's recommendation (27). Benno Schmidt became concerned that Sweek was acquiring a public profile that would alarm the scientific community. Schmidt tried to reassure the physician-scientists on the Panel, and he rewrote Sweek's portion of the Panel report using more conciliatory language and recommended a more balanced approach to research administration (28).

The Panel was undivided in its agreement on the third recommendation of the report that there should be a rapid increase in appropriated funds for the new cancer initiative. The Panel's report was finished by October 30, 1970. The final business for Benno Schmidt was mollifying Mrs. Lasker. She apparently did not approve some of the provisions in the draft of the final report and had prepared alternative language. Schmidt was able to reach amicable compromises with her regarding the language of the report.

All there was left to do was to send the Panel's report to Congress. On Friday morning, December 4, 1970, Senator Ralph W. Yarborough, now a lame duck senator, performed one of his last acts on behalf of cancer legislation (29). He called a meeting in the hearing room of the New Senate Office Building for the Committee on Labor and Public Welfare to hear the report of the Panel of Consultants on the Conquest of Cancer. Benno Schmidt presented the overall report and recommendations of the Panel. Dr. Mathilde Krim, a basic scientist research associate whose husband, Arthur Krim, had formerly been treasurer of the Democratic National Committee, presented the scientific report of the Panel. The hearing attracted little new publicity since it appeared to be a "non-event" in the 91st Congress's rush to complete its agenda prior to the January 1971 opening of the 92nd Congress. Yet, one year later on December 23, 1971, President Nixon signed the National Cancer Act of 1971, the legislative result of the Panel's report, following a period of intense activity politically advantageous to him.

December 2, 1970. Eliot Richardson, Secretary of Health, Education, and Welfare in the Richard M. Nixon administration, presenting the Rockefeller Public Service Award to Dr. Robert J. Huebner (Office of NIH History files, from the private papers of Dr. R.J. Huebner).

The legislative cauldron began to boil, stirred by the paddle of presidential politics, in early 1971. Dr. Robert Marston, Director of NIH, was opposed vehemently to the removal of the National Cancer Institute from NIH and the formation of an autonomous new agency. His objections, reflecting those of the majority of scientific and educational bodies in medicine-science, were that such a move would disrupt the cooperation with other specialties and the cross-fertilization of cancer research from other scientific disciplines. He also felt that the approach to cancer research should be a balanced one with grant-funded or non-directed research as a major component. He had concerns about the administrative problems at NIH involved with the sudden influx of large amounts of appropriated funds for cancer research (30).

A new political equation entered the picture with the succession of Senator Edward M. (Ted) Kennedy, (Democrat-Massachusetts) as the Chairman of the Senate Subcommittee on Health following Yarborough's departure. Ted Kennedy was the youngest remaining son of the Kennedy political family. President Nixon viewed Kennedy as a potential (although unlikely as a result of recent scandal) rival in the 1972 presidential election. To gain political advantage, Nixon maneuvered to pre-empt any potential benefit that Kennedy might accrue for 1972 from health legislation leadership. Realizing that Kennedy would probably introduce health legislation in 1971 and despite the Administration's previous budget austerity for NIH and medical research, on January 22, 1971, Nixon asked for funds to solve the problem of cancer in his State of the Union Address. In addition to other goals for the "New American Revolution" (the Republican political agenda), Nixon made the following statement: "I will also ask for an additional $100 million to launch an intensive campaign to find a cure for cancer, and I will ask

later for whatever additional funds can be effectively used. The time has come in America when the same kind of concentrated effort that split the atom and took man to the moon should be turned toward conquering this dread disease. Let us make a total national commitment to achieve this goal" (31). Thus, President Nixon leapt to the front of the parade for the conquest of cancer by encouraging the passage of legislation, out of, in part, personal political motives.

During the succeeding months the Senate and the House of Representative held hearings on the Panel report. Numerous witnesses with medical-scientific backgrounds representing various educational institutions expressed opposing viewpoints. Proponents of the Report of the Panel of Consultants recommendations also presented their arguments. Senator Kennedy chaired hearings for two days in March 1971 when his Senate subcommittee heard persuasive arguments for and against the Report by the interested parties. Harry M. Rose, M.D., uttered probably the most astute, although brief, opinion about cancer legislation that was given over the course of the hearings. During the March 1971 Senate hearings, Rose, representing the American Association of Immunologists to the FASEB (Federation of American Societies for Experimental Biology) public affairs committee stated; "Cancer will not be abolished by legislative fiat, nor by administrative control, nor by the mere expenditure of money" (32). Subsequent events over the following years bore out the validity of Dr. Rose's opinion. After the hearings Senator Kennedy prepared a bill for action by the Senate, S. 34, which contained basically the recommendations of the Panel. In mid-April 1971, the prominent syndicated newspaper columnist, Ann Landers, wrote a powerful column urging readers to deluge their senators with letters to vote for this bill. The senators' offices were

inundated in the resultant avalanche of mail (33). Some members of the cancer research community later felt that this ultimately helped push legislation through Congress. At the 58th Annual Meeting of the American Cancer Society Ann Landers received a special citation for "extra-ordinary service" to cancer through her column (34).

Trying to get S. 34 passed was not an easy fight. Despite the external pressure, the Senate, in debating the merits of the bill, had misgivings about some of the provisions, especially the idea of an autonomous new cancer agency. After due deliberation, the Senate adopted a compromise bill, S.1828 (35). Political maneuvering continued at the White House with the President stating that he wanted to take "personal charge" of the program. The Administration's positions and policies kept shifting and fluctuating with the new HEW Secretary, Elliott Richardson, playing "Artful Dodger" for the Administration by failing to articulate clearly what the Administration's policy would be.

The momentum for the legislation then swung to the House of Representatives. The Chairman of the Health Sub-committee was Paul G. Rogers (Democrat-Ninth District of Florida). The Administration and the Panel underestimated Mr. Rogers' impact on the ultimate legislation. He was dubious about the Senate version, and he sought assistance from the Association of American Medical Colleges in drafting an improved version of the Senate bill. In September 1971 he also held four weeks of hearings on the bill to try to evaluate critically the arguments for and against the proposed legislation. In October 1971 the House Sub-committee on Public Health and Environment reported their version, HR 11302, to the Committee on Interstate and Foreign Commerce by unanimous vote. The Commerce Committee considered HR 11302 and reported the

bill to the House by vote of 26 to 2. On November 15 the House of Representatives adopted HR 11302 350 to 5. On December 1 and 7 the Joint Senate-House Conference Committee met and reported compromise legislation to both houses of Congress. On December 9 the House, then, on December 10, the Senate adopted the compromise legislation. On December 23, 1971, in an elaborate crowded White House ceremony, President Nixon signed the National Cancer Act of 1971 as a "Christmas gift to the American people" (36). The politicians and the non-medical attendees were happy. Among the bio-scientists and cancer researchers the mood was one of uncertainty and apprehension.

The legislation (37) allowed the National Cancer Institute to remain within the National Institutes of Health. The new Act authorized the President to appoint the Director of the National Cancer Institute, and also the Director of the National Institutes of Health, and provided that the NCI budget be submitted directly to him. It also provided for a new three-member President's Cancer Panel and a new expanded National Cancer Advisory Board (NCAB) to replace the old National Advisory Cancer Commission (NACC). The NCAB was made up of presidential appointees who reported directly to the president in order to assess the performance of NCI under the provisions of the Act. In this way President Nixon placed "the full weight of the Presidency behind the National Cancer Program" and took "personal command of the Federal effort to conquer cancer" (38). The Director of NCI had full authority and responsibility for the conduct of research and other activities of NCI under the Act. Provisions were made for the establishment of new cancer centers. One of these was at Fort Detrick, Maryland; on October 18, 1971, President Nixon had announced that facilities there would be converted from biological

warfare to cancer research purposes. The Act also provided for a major infusion of appropriated funds for cancer. From fiscal year 1971 the appropriation of $233 million increased by annual increments to $815 million for fiscal year 1977.

Throughout 1971, in anticipation of the enactment of the pending legislation, Dr. Carl Baker (39,40) had mobilized an extensive group of scientists; he outlined major objectives and approaches for various goals in cancer management and assigned these goals to groups of investigators according to their interests and qualifications. In this way a massive program of cancer related activity was to be made ready for the enactment of the National Cancer Law. Rettig said it best when he wrote, “In anticipation of this legislative outcome, Carl Baker initiated an NCI-directed effort that must rank as the largest, most extensive planning effort ever undertaken within bio-medical research” (39).

The political effect of the new law had a major impact on the NCI and, later, on the research activities of Bob Huebner, as will be shown in the next few chapters. The President’s Cancer Panel was to consist of one non-science person and two scientists. The President appointed Benno Schmidt as the Chairman of the Panel. The President’s Cancer Panel and the National Cancer Advisory Board came to exercise major oversight functions over the NCI, and Schmidt, by virtue of his managerial abilities, became the dominant figure for both bodies. The Panel acted as an “executive committee” and the NCAB as a “board of directors” for NCI. In previous years Carl Baker had angered Mary Lasker when she was on the NACC and later, the NCAB by opposing her wishes to have these bodies exercise control over individual contract applications. He infuriated her further with his opposition to having NCI removed from NIH. Schmidt chose to be more

accommodating to Lasker to the extent that he helped remove Baker from NCI. Benno Schmidt, who was instrumental in selecting members of the NCAB (one of whom was Mary Lasker), acted as an agent of the Board and announced to Carl Baker in early May 1972 that Dr. Frank J. Rauscher, then Director of the Virus Cancer Program was replacing him as Director of NCI. Dr. Baker then left NIH. Despite his efforts to work with the new cancer imperatives and to ensure that they come to fruition by developing a systems-management model, Baker was evicted from the National Cancer Institute where he had provided many years of productive and faithful service. In this case, the political power of outsiders overrode the expertise of Dr. Baker, who with his background in science as well as administration would surely have continued to provide a valuable contribution to the new cancer program and serve as a much-needed moderating voice of reason. Dr. John B. Moloney then replaced Dr. Rauscher as the Director of the VCP. The NCI and its programs apparently functioned smoothly through the cooperate efforts of Benno Schmidt as "Chairman of the Board" and Dr. Rauscher as "Chief Executive Officer." Later, as part of its oversight functions, the NCAB revisited the management concerns of the contract-supported directed-research programs, especially of the Virus Cancer Program, following external criticisms from the scientific community. This led to the establishment of an ad-hoc committee in March 1973; a report issued by the committee later on would have a major effect on Bob Huebner's activities (41).

In looking back on these events, two historical developments seem to stand out. The first has to do with changes in the structure of power that influenced scientific research. In the period described in this chapter, an "outsider" element began to have a huge impact not only on funding, but also on the purpose and direction that cancer

research would take. Elite members of the public such as Mary Lasker—who had no scientific training—as well as individuals from the medical community like Garb, who did not have a strong affiliation with NIH, gained a voice of authority that shaped the world of cancer research and funding. In addition, a new cohort of political advocates stepped into the leadership gap left by Congressmen Hill and Fogarty. It also seems that "the public" began to wield more influence over NIH through the use of advocacy groups—which in turn impacted on NCI. Ultimately, it may be that these events—along with others described by Rettig—signaled a shift in the balance of power as NIH became more politicized: NCI, Huebner, and the viral oncology programs were affected by this both directly and indirectly.

The second historical development has to do with a shift in intellectual approaches to research. As the events described above illustrate, the notion of goal-oriented research gained credence in the period under discussion. To a certain extent, this notion held that medico-scientific research could just as easily be a technical applied field as could engineering or physics. It was deemed appropriate that members of a scientific field gear research towards achieving specific goals. In this case, cancer was identified as the definitive problem to be solved and the goal was to obtain a cure. If enough resources were thrown at the problem, a solution—a cure for cancer—could be "built" just like a rocket. The growing credibility of this notion, in many but not all quarters, had all sorts of implications for how cancer research got constructed and who and what got funded. This idea impacted on the research community in part as a result of the growing influence of non-researchers. It also resulted from the rise of systems analysis as Dr. Baker demonstrates in his work on the administrative history of NCI's viral oncology programs.

The broad cancer program, however, did not have a clearly defined technological product—such as a hydrogen bomb or a rocket—as its end goal, but something much more diffuse than that. Many exciting and provoking questions remain about these developments, such as the applicability of the model used by military programs to the medical/scientific field. It may be that in medico-science research it just was not that easy, at the time period under discussion, to implement an applied research model. Finding a cure for cancer was a tall order.

Benno Schmidt made this last point in different words. To evaluate the impact of the National Cancer Act's first five years, in August 1977, Benno Schmidt (42) wrote an article in the *Journal of the National Cancer Institute* entitled, "Five Years into the National Cancer Program: Retrospective Perspectives—The National Cancer Act of 1971." In this article Schmidt reviewed some of the progress, significant advances, and internal and external criticism of the program. Cancer had still not been conquered, he admitted, but all were making a valiant effort: "The scientific and medical community and all of us connected with the program must continue to explain at every opportunity to the American people and to the Congress that the cancer program is a vast undertaking that will require long-term support and great patience. We are still far away from being able to put either a date or a price tag on the ultimate conquest of cancer. We are making progress in our understanding of the disease, and there is no question that the benefits of our research are increasingly available to the American people in the form of better treatment as time goes by. But it is a long road that will require patience and constancy on the part of the Congress, the Administration and the public. In fact, at this stage of our

progress, it is true in a very real sense that ‘ . . . the goal is the course we travel together, and the end is only the beginning.’”

1) U.S. Government Memorandum, Robert J. Huebner, Chief, Viral Carcinogenesis Branch, National Cancer Institute, National Institutes of Health to The Secretary of Health, Education and Welfare (Mr. Robert Finch), f on the Subject of Budget Cuts. December 9, 1969. Copies to: Dr. Roger Egeberg (Assistant Secretary of Health); Dr. Jesse Steinfeld (USPHS Surgeon General); Dr. Robert Marston (Director of NIH); Dr. Robert Berliner (Deputy Director for Science, NIH); Dr. Carl G. Baker (Director of NCI); Dr. Frank J. Rauscher (Director of the Virus Cancer Program, NCI); Dr Kenneth Endicott (Former Director of the NCI). A copy is also in the personal files of Dr. Carl G. Baker under the heading "The Moon Shot Letter."
2) Personal communication—Dr. Murray B. Gardner.
3) Memorandum received from the personal files of Dr. Raymond V. Gilden.
4) Rettig, R.A. 1977. *Cancer Crusade. The Story of the National Cancer Act of 1971*. Princeton University Press, Princeton, New Jersey.
5) Ibid. p. XIII.
6) Ibid. p. 2.
7) Ibid. p. 3.
8) Ibid. p. 20.
9) Ibid. Pp.. 19-24.
10) Ibid. p. 25.

11) Ibid. p. 24.

12) Ibid. p. 40.

13) Ibid. p. 78.

14) Garb, S. 1968. *Cure for Cancer. A National Goal.* Springer Publishing Company, New York.

15) Rettig, ibid. p. 79.

16) Ibid. p. 80.

17) Ibid. p. 82.

18) Ibid. Pp. 83-86.

19) Ibid. p. 85.

20) Ibid. p. 89.

21) Ibid. p. 89.

22) Ibid. p. 91.

23) Ibid. p. 93.

24) Ibid. p. 94.

25) Ibid. p. 98; the subject is also described in Baker, C.G. unpublished version, 2005. *An Administrative History of the National Cancer Institute Virus and Cancer Program, 1958-1972*. Pp. 418-420.

26) Ibid. p. 99.

27) Ibid. p. 100.

28) Ibid. p. 101.

29) Ibid. p. 102.

30) Ibid. Pp. 115-116.

31) Ibid. Pp. 125-126.

32) Ibid. p. 172.

33) Ibid. Pp. 175-176.

34) Ibid. p. 267.

35) Ibid. Pp. 194-198.

36) Ibid. Pp. 277-280.

37) The National Cancer Act of 1971. Public Law 92-218. 92nd Congress, S. 1828. December 23. 1971. Reprinted from the *Journal of the National Cancer Institute* 48: 577-584.

38) Rettig, ibid. p. 279.

39) Ibid. p. 299.

40) Baker, ibid. Dr. Baker described in extensive detail the mobilization of the scientists, the scope of the planning and the approaches to the program.

41) Rettig, ibid. p. 300. See also the chapter on—Critics Anonymous.

42) Schmidt, B.C. 1977. Five years into the National Cancer Program: Retrospective perspectives—The National Cancer Act of 1971. *Journal of the National Cancer Institute* 59: 687-692.

Chapter 19

Critics Anonymous, Contracts Versus Grants, The Zinder Report

As long as the Virus Cancer Program existed, critics (primarily the academic-bioscience community) expressed major disagreements with the methods of the Program's funding. The critics favored the grant-funded mechanism for research, which they asserted was characterized by careful peer review, intellectual freedom, and the potential for superior scientific accomplishments. The critics were disdainful of contract funding because of their perception that it provoked results-oriented objectives at the expense of creativity, inferior intellectual quality of the research, and a lack of meaningful peer review. In reality, the Program funded a significant, but variable percentage, of research projects through the grant mechanism.

Tension existed among the academic and biomedical scientific community, the National Advisory Cancer Council (NACC) and the administration of the National Cancer Institute for many years from the mid-1950's about the proper use of contracts versus grants for the funding of research. The conflict became more intense during the initiation and implementation of the NCI Cancer Chemotherapy Program in the late 1950s and early 1960s when the program began to be funded primarily by contracts. The NCI Directorate, mainly Dr. Kenneth Endicott, felt that the Cancer Chemotherapy Program was a form of results-oriented, management-directed effort for which the contract funding approach was most appropriate. In 1962, President John F. Kennedy issued a directive to all federal agencies on conflict-of-interest of outside advisers. Prior to 1962, the Cancer Chemotherapy Program had numerous committees and

subcommittees reviewing the merits of individual contracts. Endicott seized on President Kennedy's directive and interpreted the existing contract-review procedures as being inconsistent with the directive. Endicott eliminated the many existing review committees and assigned their function to a group of senior NCI scientists. Thus, in conformity with the government rules for awarding contracts, non-governmental advisors or consultants became excluded largely from participation in deciding the allocation of contract funds (1). In 1965, NACC members Mary Lasker and Dr. Sidney Farber, reflecting the recent evaluation of the Wooldridge Committee Report (2) (a committee appointed to evaluate the NIH), questioned whether Americans were getting their money's worth from NIH expenditures for medical research; they were extremely critical of the scientific merits of the Cancer Chemotherapy Program and its funding mechanism, and they were perturbed about the loss of control of the NACC in the funding process. They urged strongly that the NACC should review individual contracts. At a meeting of the NACC on August 13 and 14, 1965, Dr. Endicott and other NCI staff tried to mollify Mrs. Lasker and Dr. Farber by explaining the current practices for contract funding, but apparently they were not satisfied (3).

Mrs. Lasker appealed to Senator Lister Hill (Democrat, Alabama, Chairman of the Senate Appropriations Subcommittee on Labor and Health, Education and Welfare) and persuaded him to add specific language to an appropriations bill that would mandate contract review for the NACC. Faced with this restrictive challenge, Endicott and NIH Director Shannon went to Representative Thomas Fogarty (Democrat, Rhode Island, Chairman of the same Subcommittee in the House.) Fogarty said that he would need a letter from Secretary of Health, Education and Welfare John Gardner before he could

intervene (3). Shannon went to Gardner (3), and on October 20, Secretary Gardner (4A,B) wrote to Mr. Fogarty saying that contract review by NACC introduced a fundamental change in policy that he would be reluctant to see introduced. He said that he would institute a study to examine the question and would report back to Mr. Fogarty, but that for now, he recommended no immediate implementation of the language of the appropriations bill. The restrictive language was removed from the Senate bill by the joint House-Senate conference committee, pending submission to the Congress of the Secretary's report not later than the end of February 1966.

Secretary Gardner appointed a committee (4), chaired by Dr. Jack P. Ruina that produced the Department of Health, Education and Welfare "Report of the Secretary's Advisory Committee on the Management of the National Institutes of Health Research Contracts and Grants, Washington, D.C., March 1966" (otherwise known as "The Ruina Report") (5). The Ruina Report defended the rationale for using contracts for funding directed, results-oriented research projects in contrast to grants for the investigator-initiated, peer-reviewed research projects. Dr. Ruina, as the President of the Institute for Defense Analysis, was very familiar with both the grants as well as the contracts type of support for research and development as both were used in the Department of Defense. Although Ruina's experience was in a different institutional context and a different field of science, he brought a valuable perspective on research funding that translated to the NIH setting. The Ruina Committee report released in March 1966 included the following conclusions and recommendations: advisory councils were not required by law to approve individual contracts and should not be required to do so; the grant mechanism was inappropriate for directed research or development programs, and the contract should

be used for such programs; programs for directed research—including objectives, justification, expected funding levels, management plans, and types of contractors—should be submitted to the appropriate advisory council for review and approval prior to initiation, termination, or substantial change in scale or direction of effort; once initiated, execution of such a program should be the full responsibility of the project manager. The report recommended that the NIH should take significant steps to make career opportunities and status for program managers more attractive [author's footnote: The NIH was having problems at that time recruiting enough capable professional personnel at attractive salary levels.]. The report also recommended that 'a strong management' structure for directed research should be established independent of the intramural or extramural research efforts, a structure already put in place by Dr. Endicott at NCI (4).

Dr. Endicott presented the results of the Ruina report to the NACC in the spring of 1966 and effectively thwarted Mary Lasker and Dr. Sidney Farber in their attempt to mandate that the NACC should review every Cancer Chemotherapy contract for approval. Dr. Endicott's philosophy using contract funding of directed research for categorical purposes received strong support from the recommendations of the Ruina Report. This support encouraged Endicott to continue extending the use of the contract mechanism to develop two other directed-research programs, one in chemical carcinogenesis initiated in 1962 and, especially, the new program in virology begun in 1964 in which Bob Huebner, encouraged by Dr. Carl Baker's systems management approach, played such a prominent role.

Perhaps to alleviate some of the unease in the Public Health Service and in the NIH about the burgeoning directed-research programs in the National Cancer Institute,

Bob Huebner, then still Chief of the Laboratory of Infectious Diseases, NIAID, sent a memorandum to Dr. William H. Stewart, Surgeon General, USPHS on January 24, 1967 (5). The purpose of the memorandum was to explain the rationale for the research approach to a prevalent disease category of major public health importance. Bob Huebner was, as indicated already, an active participant in the Special Virus Cancer Program at that time. In the following excerpt from the memorandum, Huebner described the complementary roles of basic and applied research: “The unprecedented support to basic research by the NIH in recent years depended more than anything else on the missions described and/or implied in the programs of our categorical institutes. Mission-oriented research does not compete with or threaten basic research, as articles in recent medical and scientific journals suggest. They are mutually dependent, one for justification, and the other for sustenance. Whatever is really excellent in scientific achievement is almost always both basic and mission-oriented.” Huebner also concluded that the non-scientific community might have difficulty distinguishing between the two. “One important point regarding funds for science brought out by Weinberg (6) is that one must distinguish not only basic but mission-oriented science from political and social action programs in which scientific discoveries are merely applied; all too often our political leaders fail to make this distinction.”

Huebner then presented his reasons for the mission-oriented approach that was used predominantly in the Special Virus Cancer Program. “In order to really solve many of the complex human disease problems, we have no choice but to adapt to the ‘Big Science’ approach.”

"If the NIH is to achieve its ambitious goals, its director and the rest of its leadership must periodically redefine and fully accept its mission. Well-planned national research programs cannot be mounted and carried out within the framework of academic type structures. What are needed are structures tailored to serve well-planned scientific missions. The recent reorganization of the National Cancer Institute represents a major step in this direction."

During the period of 1969 to 1971, Bob coordinated with his usual energy, efficiency and enthusiasm the efforts of his scattered colleagues in the Special Virus Cancer Program (SVCP), most of them working under contract. This period was also a time of fiscal, physical and personnel constraints at NCI for which Bob was energetically trying to find expeditious solutions. A number of talented young physicians interested in cancer research preferred coming to NIH to fulfill their military obligations in the Public Health Service rather than in Vietnam. At this time laboratory and office space was in short supply within NCI. Bob Huebner handled this problem by assigning these young men to contract facilities such as Microbiological Associates, Flow Laboratories, Meloy Laboratories, and others, to work on ongoing projects until they could be accommodated within the new intramural NCI quarters (Building 37) on the NIH campus.

In NCI's annual fiscal report on work accomplished in 1971, Dr. Carl Baker (7) described in detail the multiple extramural SVCP programs scattered throughout the country to illustrate Bob's leadership qualities in formulating, organizing and managing large multi-disciplinary programs targeted to solving disease problems. In addition, Bob was also supervising his own laboratory and coordinating an intramural program at NCI. Baker felt that "an effort of this scope and size carried out in an integrated fashion was

perhaps unique in biomedical research" (7). In addition, Dr. Baker mentioned the many recent awards bestowed on Bob for scientific accomplishments, pointing out how Huebner had been able to produce quality, innovative research even with his many administrative responsibilities.

Despite the awards and recognition bestowed on Bob for his scientific accomplishments, his administrative activities engendered envy, resentment and criticism within the academic-bioscientific community, not withstanding the official justifications (the Ruina Report) for the research funding approach to the problems of cancer control. These criticisms began surfacing in the late 1960s and over the next several years became more prominent. For many investigators, directed-research or program-management was anathema. Skepticism about Huebner's theories of viral oncogenesis exacerbated criticism of the directed-research program. Academics feared loss of research independence, the absence of stringent peer review, and poor intellectual quality of the research. The community was more comfortable with the grant method of funding; proponents argued that this method was superior because of its investigator-driven research idea origins, the extensive peer review of research quality by presumably unbiased study sections, and non-interference in the conduct of research in the laboratory. These arguments are meritorious; however, the proponents seemingly did not share the sense of urgency felt by Bob Huebner and the NCI Directorate (Drs. Endicott and Baker) to use a directed-research approach in order to accelerate the investigation and elucidation of the role that viruses might play in the causation of cancer. Others also expressed a sentiment of exclusion, a feeling of not having access to the largess of funding available to the recipients of research contracts. Many felt that the NCI's method

of awarding contracts to researchers to examine specific problems was part of an exclusive "old boys' network" and that the contracts were given primarily to favorites. Later, in the early 1970s, the criticism became more mean-spirited when critics implied that the contractors were investigators of second-rate scientific talent. The criticisms, limited at first to a few cancer investigators, eventually found a wider audience as editorials in some of the scientific literature, primarily the journal *Science.*

An editorial written by Robert Bazell (Intern on the News and Comment staff of *Science;* he is currently Network Health Reporter for NBC Television) (8) in October 16, 1970, though mild in tone, was critical of the pending cancer legislation (ultimately passed in 1971). Bazell, reflecting the sentiments of the bio-scientific community, was also critical of Bob Huebner's new oncogene theory, the large amount of money under Bob's control for directed-research, and the rationale for the Special Virus Cancer Program. His concluding remarks were, "Many cancer researchers believe that the basic knowledge is still lacking and that failure in a massive short-term effort will lead to difficulties in obtaining funds in the future [EAB—this would not happen]. Also, a project with a moon-shot type of approach tends to be a search for a 'magic bullet' which, many argue, cannot exist for cancer and will tend to keep the public from taking simple demonstrably useful steps such as dieting or stopping smoking"---"At the present time it appears unlikely that there will be a cure for cancer by 1976 even though Congressman Rooney's resolution [calling for a cure for cancer] passed the House unanimously."

The editorial comments made by Nicholas Wade (staff member of the News and Comments section; later he became a medical science writer for the New York Times)

published (9) in *Science* December 24, 1971, (the day after the signing of the National Cancer Act of 1971) attracted a wide readership. The article, entitled "Special Virus Cancer Program: Travails of a Biological Moon-shot," was a generally negative critique of the SVCP reflecting the sentiments of the scientific community. He began the article by attempting to evaluate the progress made by the SVCP since its inception in 1964. He drew the conclusion that the program had not made much progress in its objective of determining the link between viruses and human cancer. Moreover, Wade portrayed one part of the scientific community as having grave reservations about the competency of the researchers. Wade quoted one anonymous critic as holding that although the public might have a perception of progress against cancer, "the SVCP is held in rather lower esteem among the scientific community, particularly by the best qualified to assess the program's contribution." Others, Wade claimed, found that "The SVCP has been extremely ineffective and maybe was having even a negative effect." Wade quoted another scientist as saying that he had heard "nothing but complaints about the SVCP. Its main trouble is that it does not have enough of an intellectual base; it has Huebner's enormous energies, one very good person—George Todaro—but most of the contractors are pretty mediocre." He quoted scientists who said that many eminent virologists in recent years had had contracts with the Program but that the work in many contracts was still substandard. The scientists interviewed for this article all refused to have their names published, citing the risk of being denied funds "because NCI has a history of vindictiveness." Vindictiveness was certainly not part of Huebner's personality.

Wade, unfortunately, includes in his article quotes from anonymous individuals who seem to have been excluded from participation in the program and who could not

conceal their bitterness. One “eminent” virologist on the West Coast complained, “The SVCP is a masquerade; they make continuous proclamations of progress to justify the vast amounts of money being spent. But the nature of the program is that it excludes people who are highly critical. It has created a kind of stampede in which everyone rushes lemming-like in the same direction and critical discussion, points of obvious contradiction are ignored.”

Wade’s article then described the origin of the SVCP and the reorganization of viral oncology in the NCI. He suggested that the way viral oncology was reorganized and administered led to overlapping and duplicating activities among the various divisions. The NCI Scientific Director for Viral Oncology was Dr. John B. Moloney and there were three Branch Chiefs, 1) Dr Robert Manaker for Viral Biology, 2) Bob Huebner for Viral Carcinogenesis and 3) Dr. George Todaro for Viral Leukemia and Lymphoma. Wade suggested that the three branch chiefs conducted some in-house research that was distinct in theory but not in practice from the extra-mural research supported by the SVCP. That is, he concluded that the research done on the NIH campus was a mere reiteration of the research being done by those institutions funded by contracts (or vice versa). Wade also suggested that a fair fraction of SVCP funds were used to support industrial facilities that served simply as extensions of the branch chiefs’ in-house research facilities. One focus of criticism from the academic community was the unusual power wielded by the three branch chiefs’ control over massive amounts of funds. “Probably few individuals in the history of biological research have had such unfettered control over so much money.” (9)

Wade then made several critical comments about potential conflicts of interest that might arise in the way that the segments were administered. Wade first described the

SVCP organization: it was divided into eight segments, each of which was supposed to have a specific research objective. Bob Huebner was Chairman of the Solid Tumor Segment. Each segment was presided over by a chairman and an advisory working panel that reviewed contracts. “Project managers” did the actual hands-on work for the contract. “Project officers” supervised contracts directly. Wade criticized segment chairmen who sometime acted as his own project officer, particularly for contracts that were extensions of his in-house research.

However, the strongest objections of the academic community, as described by Wade, were to the contract mechanism of supporting research and to what was perceived as the program’s insulation from outside advice. For the most part, this condemnation was leveled by those anonymous souls who were excluded from the program. Although all contracts awarded by the SVCP were reviewed by the segment working groups on which outside scientists were represented, these committees, the same critics said, merely functioned as rubber stamps for decisions already made by SVCP administrators who had too much power.

Despite the SVCP’s use of some grants for research projects, Wade claimed that the NIH mistakenly relied almost exclusively on the practice of supporting grants for research while the SVCP relied primarily on contracts for research that should be more properly supported by grants. He also quoted the same sources as saying that the major advances in cancer virology came not from the SVCP and that any significant advances could have been done by grants at one-sixth to one-tenth the cost.

Wade also described what he called “overlapping” programs administered by the three branch chiefs. Wade cited two problems with this overlapping. First, he critiqued

these programs as almost identical; he quoted one source as saying, “There is hardly any real difference in subject matter between the three chiefs—The real difference is one of style.” The source then tossed a bone to Huebner: “Huebner feels that scientific input should come from in-house, meaning largely from him, and he is exceptionally good at suggesting ideas for people to do and at seeing that his contractors communicate with each other. So, his segment is really well controlled.” This statement was a generally positive assessment of Bob’s activities in the midst of an otherwise critical editorial. Wade felt that except for the exclusion of outside review, Bob Huebner and George Todaro ran good programs because they were still active in the laboratory. Another problem was the awkwardness with which some SVCP administrative personnel handled premature claims by some contractors of human cancer viruses. These claims were disproved later by NCI after vigorous scrutiny.

Wade cited a second problem with the segments: a serious lack of management control as evidenced in the segment working panels that were supposed to review all SVCP contracts. Rauscher and Moloney, Director and Associate- Director of SVCP, respectively, pointed to the existence of these panels as evidence of outside review. While most of the panels indeed drew half of their members from the NCI staff and half from the outside, in practice, according to Wade, it was almost impossible for the outside scientists to vote down a contract of which they disapproved. Wade believed that the practice of having contractors as panel members was another awkward feature that militated against their acting as sources of independent advice. The membership of Bob Huebner’s working segment panel as approved up the line administratively by Drs. Moloney, Rauscher and Baker (NCI Director) consisted almost exclusively of scientists

who had sizable contracts in Bob Huebner's segment. Wade held that the panel's one "independent voice" was Dr. Wally Rowe.

Wade's article described a dichotomy in which there was a rivalry in approach and philosophy in recruiting scientists for the program. In the years prior to the Wade article, the administrative triumvirate of Manaker-Moloney-Rauscher recruited scientists of renown to manage projects of high scientific standards. The Huebner-Todaro effort seemed to have more direction and drive based on the research interests of both men. The contracts under Bob Huebner's direction were thought, as viewed by others, to be simply extensions of his in-house laboratories. The unusual arrangement of Bob Huebner's acting as both an administrator and as an active scientist was thought to be a mixed blessing. On the credit side was that he was well regarded in both roles. One critic said, "The real trouble with the program is that it has only one Huebner, not five or six. Huebner's contracts have been more successful because he is a good manager and because he has very good intuition, which is important in science." The scientists depicted in Wade's article directed additional criticism against Bob. These included the perception that Huebner awarded, or denied, research support, depending on his personal interests and the idea that he was motivated by his eagerness to be involved in "getting the best part of every (research) pie." They also remarked on the "publicity tinged" style of his operation and of his practice of signing research publications written by contractors in far-distant laboratories. Yet, these criticisms were off the mark. Huebner's contractors tended to add his name as a courtesy. Moreover, he often contributed significant ideas, of which he had many, according to Drs. Murray Gardner and Raymond Gilden. Huebner would not allow his name on a manuscript to which he had not made a contribution. [For

example, he refused to have his name put on my research publication—see the chapter on Herpangina.] Huebner was not averse to a little publicity; however, it was the Public Affairs offices of NIH and NCI that initiated reports to the press media about the innovative nature of Huebner's efforts in "Conquest of Cancer"—a subject which was and still is a matter of undiminished interest to the lay public. According to Wade, the general style of Bob's operation engendered certain sourness toward the SVCP in academic circles. Much of the hostility caused by Bob's domination tended to fall unfairly on the heads of other administrators. One scientist connected with the program commented, "The real culprit for all this is (Dr. Carl) Baker."

Wade concluded his article with an enumeration of some of the successes of the program. Among the successes he included two lines of research that the Huebner-Todaro part of the program backed heavily, in the previous 1&1/2 years, on the reverse transcriptase enzymes possessed by RNA viruses and the group-specific antigens of C-type (gs) viruses of which more than 100 had been discovered. He also listed the resources program that provided viral reagents to investigators, the research support for the Henle's on EB virus and infectious mononucleosis, and the elimination of human adenoviruses as etiological agents in the causation of cancer. However, reflecting the views of the bio-science community, Wade suggested that the SVCP should allow more outside advice, provide more grant funding, start a sensible training program for young researchers and switch more resources to basic cell biology. In view of the new National Cancer Law of 1971 he felt that the above choices were politically foreclosed and that people should be prepared for a long wait for a solution to cancer control.

Wade's article generated anger and outrage among the participants in the Program at whom the criticisms were directed. One of the people who Wade interviewed for the above article was Dr. John Moloney (10), the NCI Scientific Director for Viral Oncology. At the time of the interview, Dr. Moloney had thought that Wade was a "pleasant young man" and gave him information freely about the SVCP. When the article appeared, Dr. Moloney said he was angry and felt betrayed because he believed that Wade presented a biased and unbalanced account of what the SVCP was trying to do, a feeling shared by many other participants in the Program.

Another editorial (11) appeared in the April 1973 issue of *Science and Government Report*, a month after the establishment of the so-called "Zinder Committee." This ad hoc committee had been appointed to conduct a review of the SVCP of NCI. The article was in the Government Report section and described the NIH reaction to the ongoing controversy about contract management. The report indicated that contract procedures were being reviewed closely in the wake of mounting criticism over alleged laxity and favoritism in the burgeoning contract programs. [Author's footnote: These criticisms applied to research on Heart Disease as well as Cancer.] The criticisms were coming from a variety of sources including the academic world, the press and Ralph Nader's Health Research Group. Top officials of the NIH attributed most of the complaints to ignorance about how the contract programs operated rather to any actual abuses. The critics were not impressed with this explanation, and, in addition, reiterated the Wade's charges contract work was often "second class research"; contracts gave "poor value for the dollar" (some cynics said the only difference between a grant and a contract was that a contract costs more); contracts were used for aggrandizement of

program officials' scientific stature, particularly when NIH officials were allowed to author scientific papers related to projects on which they awarded contracts; and contract proposals were reviewed at NIH by groups that lacked the perspective needed to pass sound scientific judgments on the merits of the proposal.

As a member of the Advisory Committee to the Director of NIH, Dr. Marian Koshland, Professor of Bacteriology and Immunology at the University of California at Berkeley, presented similar views at the Committee's February 23, 1973, meeting. Her comments were subsequently published in a report on the meeting. Dr. Koshland characterized Bob Huebner as an example of a particularly powerful individual who controlled all aspects of the contracts under his jurisdiction to the detriment of the science. These criticisms apparently stung Bob very deeply at this particular time. In a copy of this article among Bob's personal papers there is a message to administrative assistant Harriet (prior to their marriage), handwritten in the margins, asking her to have some of his prominent contractors respond to the article. He suggested a list of contractors: "Dixon (Frank Dixon, Scripps Institute), Kaplan (Henry Kaplan, Stanford University), Green (Maurice Green, St. Louis University), Hayflick (Leonard Hayflick, Stanford University), Gardner (Murray Gardner, University of Southern California), Bishop (J. Michael Bishop, University of California, San Francisco—later a Nobel Laureate)." These individuals were hardly "second rate." It is unclear whether Huebner's office actually contacted these persons or whether they responded to the Government (Koshland) Report.

The Koshland article asserted that the NIH (as well as the National Heart and Lung Institute), spurred by the criticisms mentioned, was recommending some changes

and a re-examination of the contract mechanism of funding. It also noted that there was not apt to be any significant change in the contract mechanism of funding at the National Cancer Institute, the target of most of the criticism from biomedical researchers. The article continued, stating that the National Cancer Act gave NCI considerable autonomy, and that top NIH officials were not certain that they had the authority to tell the cancer program how to handle its contracting procedures. For another thing, the cancer program's national board (NCAB- National Cancer Advisory Board which replaced the NACC after 1971) had already heard a presentation about cancer contracting procedures, and, according to Dr. Robert W. Berliner, NIH Deputy Director for Science, the NCAB "concluded that everything is under control." As part of the program planning formulated by Dr. Carl Baker (12) for the National Cancer Act of 1971, the contract-supported directed-research programs of NCI became a concern of administrative management. Administrators focused on the Virus Cancer Program (VCP- Formerly SVCP), the strongest contract program. The persistent external criticism from the scientific community and internal NCI discussions prompted the NCAB under the chairmanship of Mr. Benno Schmidt to address the problem. On March 5, 1973, Dr. Frank Rauscher, now the Director of NCI, appointed at the request of the NCAB an ad hoc committee to conduct a review of the Special Virus Cancer Program of the National Cancer Institute and deliver a report of its findings and recommendations for the future (13,14). The committee was to review the scientific and management aspects of the virology program. This committee was entitled the "Zinder Committee" after its Chairman, Dr. Norton Zinder of Rockefeller University. The Committee consisted, in the majority, of men who were not working primarily in cancer virus research. The other members of the

committee were: Drs. James Darnell, Vittorio Defendi, Robert Good, Keith Porter, James Price, Wallace P. Rowe, Aaron Shatkin, Chandler Stetson, Richard Tjalma (later resigned) and Maurice Guss (Executive Secretary, NCI). The activities and report of the Committee are described in the body of the report itself (14), in Rettig's book (13) and in two editorials in the journal *Science* by Barbara Culliton (15). The Committee presented a preliminary report to the NCAB in December 1973; it received mixed reviews, reflecting the diverse membership of the Board. A final unchanged critical report was submitted to the NCAB at its meeting in March 1974.

The Committee addressed the various criticisms and recommended remedial solutions. Members accepted the scientific rationale of research on viral causes of human cancer, but did not favor the objective of developing antiviral vaccines. The Committee noted that the ignorance of the basic disease mechanism of cancer was so great that the analogy to infectious diseases was deemed inappropriate. In addition to reviewing the scientific merits of the SVCP program, the Committee considered the question of whether the contract mechanism was the best way to support viral oncology research.

The Committee was opposed vehemently and almost unanimously. The committee agreed with the critics that the contract proposal review process was dominated by VCP officials (or segment chairmen), and that there was a possible conflict of interest in including potential and actual contractors in the review of proposals, and that the program lacked scientific vigor and was inaccessible to the larger virology community. All of these factors, members found, impacted negatively on the research. The contract-research program, moreover, represented an extension of the intra-mural research work of some VCP scientists leading to duplicative efforts and access to excess

funding. “We are quite sure,” the Committee wrote (16), “that much more would have been accomplished if equal support had been provided on a competitive basis to many more laboratories with greater capability and experience in particular areas.” (16)

The Zinder Committee recommended “opening up” (16) the VCP program specifically through the establishment of an NCAB oversight committee, a reconstitution of the segment advisory working panels that reviewed contracts, and the elimination of contract work that was clearly an extension of VCP scientists’ intra-mural research. The NCAB did establish a subcommittee, chaired by Dr. Harold Amos of Harvard Medical School, to monitor the program’s response to the Zinder Committee recommendations. The VCP, on its part, established an advisory committee of non-program scientists to provide advice on broad directions, resource allocation, promising lines of scientific inquiry, and application of research findings. The contract review process was also modified to increase the rigor of review of individual contract proposals. The Amos subcommittee in its report to the NCAB in June 1975 indicated its general approval of the program changes (16).

As a result of the VCP review the NCI moved to tighten the review of all contract research programs and moved to implement more grant-funded research. Dr. Rauscher, in a 1974 article in *Science* (17), pointed out that the ratio of research grants and contracts had shifted from 50-50 to 55-45 in the three-year period from 1972 through 1974. He also emphasized a desire (of the NCI and VCP) to be responsive to the “concerns and advice from the scientific communities of the country.”

The response of the persons criticized in the Report and in the antecedent editorial comments was one of anger and resentment to what they perceived as misconceptions

about themselves and the VCP. Once the final Report was presented, Bob Huebner apparently accepted the Report with equanimity. The Report did not interrupt the pace of his or his associates' activities (18). Dr. John Moloney, Director of the VCP, began to implement some of the recommendations and administrative changes outlined in the Report (19,15B). In 1975, all the contracts in the Virus Cancer Program were moved into the Collaborative Research Branch headed by Dr. Robert Manaker (20). The contracts in Bob Huebner's office were moved into this Branch along with Dr. Jim Duff, who had been associated with Bob since 1968 as Vice-Chairman of the Viral Carcinogenesis Branch and Solid Tumor Segment. Gradually, funding shifted from contracts to grants over the next few years accompanied by a decreased flow of funds to the VCP. In December 1978, Dr. Arthur C. Upton, who replaced Dr. Rauscher as Director of the National Cancer Institute, relieved Dr. Moloney as Director of the Viral Cancer Program. The Program slowly came to a halt and was finally terminated when the new NCI Director, Dr. Vincent T. DeVita, drastically slashed the funds and reorganized the intramural laboratories (21). Many of the NCI investigators, determining that retroviruses were not going to become major causes of human cancer, also left the Program in the early 1980's (22). Others concentrated their efforts in exploring the molecular biology approaches to cancer etiology by methods developed and funded during the years of the Virus Cancer Program.

1) Rettig, R.A. 1977. *Cancer Crusade. The Story of the National Cancer Act of 1971*. Princeton University Press, Princeton, New Jersey. Pp. 64-73.
2) A) Baker, C. G., *An Administrative History, etc.* see previous chapters. p. 253. B) Rettig, R.A. Ibid Pp. 66-67. Both references cite portions of the Woolridge Committee Report. Biomedical Science and its Administration: *A Study of the National Institutes of Health*, The White House, Washington, D.C., February 1965. Rettig Pp 37, 39, 40.
3) Rettig, R.A. Ibid p. 67.
4) A) Rettig Ibid p. 68. B) Baker Ibid Pp. 252-255.
5) Baker ibid Pp. 278-280.
6) Weinberg 1965. Basic research and national goals. *Proceedings of the National Academy of Sciences* March 1965.
7) Baker Ibid Pp. 464-472.
8) Bazell, R.J. 1970 (October 16). News and Comments. Cancer Research: Senate consultants likely to push for planned assault. *Science* 170: 304-305.
9) Wade, N. 1971 (December 24). News and Comments. Special Virus Cancer Program: Travails of a biological moon-shot. *Science* 174: 1306-1311.
10) Personal communication—Dr. John B. Moloney.
11) Government Report April 1973. *Science and Government Report* 13: 28-29. Kalorama Station. Box 21123, Section E, Washington, D.C.

12) Baker Ibid Pp. 478-500+.

13) Rettig Ibid Pp. 300-301.

14) Report of the Ad Hoc Review Committee of the Virus Cancer Program ("Zinder Report") submitted in draft to the National Cancer Advisory Board (NCAB), November 26-28, 1973. Final report submitted to the NCAB, March 18-20, 1974. The Report was kindly obtained from the files of Dr. John B. Moloney.

15) A) Culliton, B.J. 1973. Cancer select committee calls virus program a closed shop. *Science* 182: 1110-1112. B) Ibid 1974. Virus Cancer Program. Review panel stands by its criticism. *Science* 184: 143-145.

16) Rettig Ibid p. 301.

17) Rauscher, F.J., Jr. 1974. Budget and the National Cancer Program. *Science* 184: 873-876.

18) Personal communication- Harriet Huebner.

19) Personal communication—Dr. John B. Moloney.

20) Personal communication Dr. James Duff

21) National Cancer Institute Oral History Interview with Dr. Vincent T. DeVita, Jr., M.D. June 5, 1997, Pp. 31-37. Interviewer was Ms. Gretchen A. case, History Associates Inc. 5 Choke Cherry Road. Suite 280. Rockville, Maryland 20850-4004.

22) Personal communication - Dr. Gary Kelloff

Chapter 20

Personnel Relationships, Additional Activities of the Viral Carcinogenesis Branch and the Virus Cancer Program

From the earliest days of his scientific investigations, Bob Huebner surrounded himself with compatible associates to help with his extensive undertakings. He was selective in his choice of associates. He would assess their capabilities before allowing them to work independently without supervision. As indicated previously, if he found that a young investigator did not possess the talent to work in the laboratory, he would gently ease him out of the laboratory into other activities or suggest that he should pursue a different career. In the case of extra-mural investigators with established reputations and accomplishments this vetting process was unnecessary, and Bob, after hearing them speak at meetings or after studying their publications, recruited them for what they could contribute to the overall SVCP. According to Dr. John B. Moloney (1), Bob had a "fantastic way with young people." He inspired and motivated them, and he was responsible for contributing to the training of many young investigators who later went on to attain high academic rank and/or scientific prominence. He nurtured their potential abilities.

During the transitional period in the 1960's and prior to his transfer from the Laboratory of Infectious Diseases to the National Cancer Institute, Bob Huebner brought Dr. Padman Sarma into his orbit. First, he outsourced him to one of the commercial laboratories, and then brought him into the intra-mural program when space became available in the new NCI facility, Building 37. Among Dr. Sarma's significant early

accomplishments was the discovery of the group-specific (gs) antigen induced by the unapparent growth of various avian leucosis viruses. Dr. Sarma also developed a method of measuring the antigen by means of a complement-fixation test given the acronym COFAL (complement-fixation of avian leucosis) (2). In additions, he adapted certain strains of the avian leucosis viruses to grow in tissue culture (3). The COFAL test was effective in demonstrating Rous-associated virus in the stocks of the Bryan strain Rous sarcoma virus, and it was useful for the detection and assay of naturally occurring leucosis virus in viremic sera, chicken embryos, and tissues and secretions of chickens with avian lymphomatosis (4). Preliminary data indicated that the COFAL test might be useful for the direct demonstration of viral antigens in tissues of naturally infected chicken embryos and chickens (4). The data also indicated that the test could also potentially be more useful than the test then in use that measured the resistance-inducing factor (RIF) of Rubin (5), which demonstrated pre-infecting avian leucosis in tissues preventing the establishment of infection with Rous sarcoma virus. Dr. Sarma, in his early association with Bob Huebner, provided needed support in the study of avian tumor viruses. Later, Dr. Sarma also performed studies with the mouse leukemia viruses and the feline (cat) leukemia viruses. He also participated in studies of the vertical transmission of tumor viruses that helped lend some of the experimental support for Bob Huebner's concept of oncogenes (6).

Dr. Sarma retired from NIH several years ago after a successful research career. At the time of Bob Huebner's retirement in 1982, Dr. Sarma wrote to Bob describing how important their long partnership had been to him both personally and professionally (7): "Dear Bob, As I write this letter to you to wish you happiness in your retirement, my

mind goes back to 20 years to reminisce over all my years of pleasant and productive association with you and all my personal growth during these years.

"You were a father figure to me. You demanded the same high goals and high performance that my father set for me, and you made me achieve them. I am glad that you initially gave me the opportunity to join you, and I am even more grateful for the opportunity to be part of your team. This meant a lot to me, and I tried to do my best to deserve this honor.

"It was sadness that I felt when you had to leave your office as my chief in 1977, and now I will see even less of you. My only consolation is that you will be happy in your retirement and will keep in touch with yours truly. Your loyal and devoted admirer and friend."

Bob Huebner continued his ongoing association with Dr. Maurice Green at St. Louis University (8). Although Bob's field studies had eliminated the adenoviruses as causes of human cancer, he still continued to support Dr. Green's study of the molecular biology of the adenoviruses through the Virus Cancer Program. Bob still maintained a close and ongoing relationship with Dr Murray Gardner and his group at the University of Southern California (8).

From about 1967 onward many young physicians became attracted to the National Institutes of Health and the Public Health Service as an alternative to serving in the other military services during the Vietnam conflict. Even prior to that Bob Huebner was actively recruiting promising young investigators. In early 1965 Bob gave a commitment for a 2-year post-doctoral appointment (to start in 1968) to Dr. Gary Kelloff (9) who was then a medical student at the University of Colorado. Kelloff had just

completed a very successful sophomore research project that had resulted in a publication on avian tumor viruses. Such success as a researcher so early in his medical career had impressed Huebner. When first contemplating doing a research project, Kelloff had approached Dr. Donald King, Chairman of the Pathology Department. The department had strong research capabilities with many competent members including tumor virologists. Dr. King put Kelloff in touch with one of the tumor virologists, Dr. Peter Vogt, who was already well known in the field of avian tumor virology. Remembering later how well the project had gone, Kelloff made it all seem easy: "I talked to Peter Vogt. He had a project all figured out for a 2-year eager medical student, and he was so well organized—he then showed me what to do—that in a few months under his guidance I had a published article in *Virology* (10) on the avian tumor viruses looking at what was then called the group-specific antigen."

Huebner offered the post-doctoral appointment on the basis of this publication. This was a very unusual offer to someone so early in his medical career. Kelloff asked Bob to postpone his appointment for another year so that he could complete his clinical training in order to fulfill the requirements for the medical (internal medicine) boards. Bob, however, was impatient, and he said, "We've got a lot of work to do here. The trouble with you MD's, and I'm one, is you get a stethoscope around your neck and then we cant get you away. You'd better get down here. We've got work to do" (9A).

Gary Kelloff reported to NIH on July 3, 1968, assuming that he was going to have a weekend to unpack. Instead, Bob Huebner told him to come out to the "ranch" (farm) because, "We've got a lot of work to do, and I want to talk to you." Gary drove out to the farm on July 4 and Bob subjected him to a continuous 7-hour monologue on what work

had to be done in the Virus Cancer Program. Bob gave him all the background relaying how the focus of activity was shifting from the investigation of adenoviruses as causes of human cancers to the RNA retroviruses that were widespread in many animal classes of mammals and vertebrates. He talked about how the various new mouse leukemia viruses had been discovered recently; how Wally Rowe and Janet Hartley had described the development of the new focus (plaque) assay for quantifying the RNA viruses (11); and about how the new "helper" viruses that he, Rowe, and Hartley had uncovered were so important because they (8) enabled the liberation of infectious sarcoma virus from infected but non-producing free virus tissues. Bob also explained that these new techniques helped facilitate work with the retroviruses, especially in completing neutralization tests and in the preparation of immune reagents.

Kelloff accomplished a great deal during his 3-year post-doctoral appointment. In his first years at the laboratory, Kelloff worked with Bob and other members of the Viral Carcinogenesis Branch (12) on a study of the group-specific antigen expression during embryogenesis of the genome of the C-type tumor virus. Following this, he participated in the study of the immunologic tolerance of mice to RNA tumor genome expression (12). These studies provided some of the laboratory support for the formulation of Bob Huebner's oncogene theory. Kelloff's primary focus though was on isolating possible endogenous RNA virus from hamsters. He successfully isolated hamster-specific C-type viruses, which he described in a 1970 publication (9A).

In the early 1970's, after his post-doctoral appointment, Kelloff worked closely with Ray Gilden and Stephan Orozlan at the Flow Laboratories preparing specific anti-viral antibodies in order to aid in the identification of newly isolated or unknown viruses.

This research was part of the Resources Segment of the SVCP. He worked directly with Bob Huebner in the intramural program for much of the 1970's focusing on studies of retroviral immunology. Huebner and others actually developed retroviral vaccines that worked in mice (9B, 31). These vaccines could immunize mice and not only prevent any developing infection from an exogenous virus challenge, but also prevent or delay the mice from exhibiting spontaneous cancer from endogenous virus. Kelloff continued to participate in the studies on viral immunology through most of the 1970's. Ironically, this aspect was the only one of the scientific approaches in the SVCP with which the Zinder Committee (13) found fault; the committee concluded that these studies had no relevance to the problem of human cancer therapy, despite the very promising findings with mice. In the 1980s, when the retroviral studies turned from immunology and viral phenomenology to molecular biology, Gary Kelloff devoted much of his time to the production of monoclonal antibodies to the (onco) proteins coded for by the newly discovered oncogenes. He also studied their immunobiologic effect. After the demise of the Virus Cancer Program he later gradually drifted into the field of chemoprevention and evaluated the chemical inhibition of gene function, an activity that has kept him occupied into the new millennium.

Some of the other physicians who were recruited into the SVCP around 1967 included George J. Todaro, Stuart A. Aaronson, Edward M. Scolnick, and Jay A. Levy. Bob Huebner's foremost goal during this period was, of course, the search for a cancer virus in humans. That search had been stimulated by the increasing observation of retrovirus infection in various rodent species. To this end he was also interested in the development of methods to promote virus isolation and to further define the cultural,

physical, chemical and molecular characteristics of these agents. It was, therefore, advantageous to develop and characterize permanent cell lines that might be used to study virus replication (12). Earlier at New York University Professor Howard Green and his graduate student George J. Todaro had developed contact-inhibited cell lines from out-bred Swiss mice. [Footnote:—Normal cultured animal cells usually grow as a thin monolayer on the surface of a culture dish; they grow in an orderly manner, do not crawl on top of each other or pile up into heaps. Once the available surface is covered and they are touching neighboring cells on all sides, they stop dividing, a phenomenon known as contact inhibition. Cancer cells by contrast continue to divide and pile up into heaps. From Clark and Russell's *Molecular Biology*.] Between 1960 and 1970 Todaro and his colleagues, by then at NCI, (14) developed two such lines from inbred Balb/C and NIH Swiss mice. These new cell lines were also suited for studies on replication-defective retroviruses. The infectious complex of an oncogenic RNA retrovirus, as exemplified by a sarcoma virus, and its virus helper the leukemia component, was difficult to study, and a systematic method for separating the sarcoma virus from its helper was not available. By using the new cell lines, Stuart Aaronson and Wally Rowe succeeded in isolating helper-free Moloney and Kirsten sarcoma virus (15).

Todaro and Aaronson both arrived at NIH almost simultaneously in 1967. Dr. Robert Stevenson (16), who was then head of the Resources program for the SVCP, received a call from Professor Harold Green now at the Massachusetts Institute of Technology. Stevenson later recounted the how the conversation went: "Dr. Green said, 'I have this very good graduate student—he has no interest in going to Vietnam nor to the Indian Service [Medical care was provided by the Public Health Service]. Would you hire

him'?—I said 'What's his name'?—He said, 'George Todaro' and he sent George down---- I got George down to Bethesda and talked to him, and I just asked him straight out, I said, ' George, why do you think you'd be of more use in this program than you would be out in the Indian Service and so forth'?—And he says, 'I know how to do research, and I know what I want to do.' And he was no clinician. So, we hired George Todaro" (16).

Initially Todaro and Aaronson were ensconced at the Meloy Laboratory in Northern Virginia. While there, Todaro participated in the provision of the Rauscher leukemia virus to Dr. David Baltimore for the research that led to Baltimore's discovery of reverse transcriptase. For a while Aaronson and Todaro spent time in Wally Rowe's laboratory. Based on the research they did there they published a study in 1969 with Janet Hartley (17) on the "spontaneous" release of mouse leukemia virus from mouse embryo cells after long term *in vitro* cultivation. This study provided additional evidence for the genetic transmission and latency of retrovirus infection in mice.

Bob Huebner was impressed with Todaro's intellect. Other staff members of the National Cancer Institute (including Waldmann and Baker) were likewise impressed with his research and intellectual talents (18). Bob used these skills in the formulation and the writing of the "oncogene theory," a concept with which Todaro was in strong agreement (19). Todaro did the bulk of the writing of their later joint statement of the theory (20). Later in the 1970's, Todaro controlled a large portion of the appropriated funds in the VCP when he became Chief of the Viral Leukemia and Lymphoma Branch. He continued his investigative studies in-house after Building 37 was built. He stayed with the Program until its demise due to diminution of funding and administrative re-organization in the

early 1980's. He left NIH and became associated with the Department of Pathology at the University of Washington in Seattle.

Stuart Aaronson also had a distinguished career at the National Cancer Institute with many significant published contributions. When Bob Huebner retired in 1962, Aaronson succeeded him as Chief of the Laboratory of Cellular and Molecular Biology, NCI. He left NIH in 1994 to become associated with the David H. Ruttenberg Cancer Center at the Mount Sinai Medical Center in New York City.

Edward M. Scolnick is an outstanding scientist of international reputation who became one of Bob Huebner's circle of associates (21). Early on in his career, Scolnick preferred a research berth at NIH in place of medical duty as a draftee during the Vietnam War (21). He had heard about NIH, was interested in a research career and, on the recommendation of various medical faculty professors, applied for a position in the Heart Institute in the laboratory of Marshall W. Nirenberg (a Nobel Laureate who received the prize for his elucidation of the genetic code). During his interview he had a stimulating conversation with Nirenberg about genetics, a subject in which Scolnick had always been interested. Scolnick was accepted but when he reported for duty to the laboratory, he found that Nirenberg's interest had shifted to the neurobiology of a certain type of worm. Nirenberg offered Scolnick the opportunity of working directly with him on neurobiology or working with C. Thomas Caskey, who headed up a small group in the laboratory that was still working on the genetic code. Scolnick ended up doing research on the genetic code for two years (21).

While working on the genetic code, Scolnick and the group made some very important and novel biochemical discoveries in protein chemistry related to their project.

In helping to make these discoveries, Scolnick had undergone an epiphany and became convinced that he wanted to pursue research as a life-long career. In as much as the research in the genetic code was concluding, he felt that he wanted to try another area in research, preferably related to genetics. Scientists who had been involved in bacterial genetic research were talking about working in animal virology instead of the bacterial virology that had predominated. Scolnick heard about a course at Cold Spring Harbor on animal virology, and, with Marshall Nirenberg's approval, the Laboratory arranged for his attendance.

Scolnick learned a great deal at Cold Spring Harbor. He came back to the Heart Institute very excited after his exposure to the new methods in animal virology. He was offered a position to stay in that laboratory, but Marshall Nirenberg was dedicated to his neurobiology worm work and did not have the resources to set someone up to do animal virology. Scolnick looked about for other opportunities, learned what was happening in the National Cancer Institute in tumor virology and heard about the collaboration of Bob Huebner and George Todaro in tumor virology.

Scolnick had an interview with Todaro at the Meloy Laboratory in Springfield (Northern), Virginia, the contract laboratory for the VCP and came away from the meeting impressed with the facilities, the projects and the staff. He chose to give up his permanent position at NIH for a staff associate's job in the National Cancer Institute, located at the Springfield laboratory, with the promise that the job could be turned later into a permanent position. The chance to work at NCI seemed worth the commute made every day from suburban Maryland to Northern Virginia in a small, used automobile that broke down frequently on the infamous Washington beltway.

Scolnick shared an office with Stuart Aaronson and Wade Parks, another NCI associate, at the laboratory. Initially, Scolnick was interested in how RNA viruses replicated. A fortuitous event occurred shortly after Scolnick's arrival at the Springfield laboratory that helped answer his question about RNA virus replication. One day Todaro came into the office announcing that David Baltimore had called asking for a large supply of purified Rauscher mouse leukemia virus because he and Temin had just discovered an enzyme in Rous sarcoma virus. Baltimore thought the enzyme might be the secret to the puzzle about how RNA viruses replicated - reverse transcriptase. Scolnick thought that while this discovery explained how the viruses replicated, it still did not explicate how the viruses transform cells and cause cancer. David Baltimore visited the laboratory shortly after that, discussed his findings and other topics in oncology and virology. The researchers in the laboratory tried to take advantage of the Baltimore-Temin discovery of reverse transcriptase and use it as a means to find a human RNA virus. Scolnick spent a year in pursuit of this search and also learned how to exploit reverse transcriptase as a laboratory tool in studying RNA viruses.

By the end of the year, he had concluded that there was no quick way to find RNA viruses using the reverse transcriptase method, so he decided to return to his original interest in understanding the genetic structure of one of the RNA viruses. He chose the Kirsten sarcoma virus. The Kirsten leukemia virus discovered some years previously by Werner Kirsten (22) who was based at the University of Chicago. These agents had different biologic properties than the previously discovered murine leukemia and sarcoma retroviruses, and Scolnick tried to figure out what the genetic differences

were by using the crude molecular hybridization methods that were available before DNA cloning was developed.

Toward the end of Scolnick's second year in the Meloy Laboratory, Wally Rowe, Janet Hartley and their associates visited the laboratory to get more of the cell lines developed by the laboratory (contact-inhibited cell lines). Rowe and Hartley had discovered that they could induce latent occult RNA tumor viruses from the cells by using the potent carcinogens iodo- and bromodeoxyuridine (IODR, BUDR) (23). Still hoping to find a human leukemia virus, Todaro and Aaronson teamed with Rowe and Hartley to follow up on that observation and worked together on a project in Building 7 at NIH (21).

Ed Scolnick did not want to follow that course of research and attempted to have his own laboratory set up somewhere else in NCI. Bob Huebner intervened at this point much to the gratitude of Scolnick. Wade Parks (another NCI associate working at the Springfield laboratory who later became Professor of Pediatrics and Microbiology at the University of Miami, Miami, Florida) and Scolnick decided to move together from Virginia. Bob Huebner worked out an arrangement with the Meloy Laboratory in Rockville, Maryland. The laboratory owned another building, an old barn, which had housed cows and sheep. Bob arranged for contract money. The barn was completely renovated and equipped with all the necessary laboratory apparatus including incubators, tissue culture hoods, and the necessary hardware and glassware including what was needed to do biochemistry. Scolnick and Parks moved their current projects from Virginia to Maryland and started a laboratory from scratch as apart of the Meloy

operation. They worked together there and at the NIH campus for the next few years until Parks moved from the area.

Scolnick continued his studies on the genetic composition of the Kirsten sarcoma virus and then looked at the Harvey sarcoma virus and finally the Friend leukemia virus. He moved into Building 37 of the NCI and subsequently became Chief of the Laboratory of Tumor Virus Genetics, NCI. He worked primarily in the intra-mural program of the NCI Virus Cancer Program. In the late 1970's after the discovery of the *src* proto-oncogene by Bishop and Varmus (see below) at the University of California, San Francisco, Scolnick discovered the *ras*-oncogene in the Kirsten and Harvey sarcomas and the *ras*-protein that was encoded by the oncogene. In the early 1980's Scolnick collaborated with and provided generous support to a group of investigators at the Massachusetts Institute of Technology headed by Robert Weinberg (24). This group found that the *ras*-oncogenes from the mouse sarcomas were identical to the *ras*-sarcoma isolated from a human bladder carcinoma. This was the first demonstration of a mutated human proto-oncogene in a human cancer. This observation lead to further the understanding of cancer development in humans (24).

All this happened around the time that Ed Scolnick accepted a job offer from the Merck Company. He left NCI (as Chief of the Laboratory of Tumor Virus Genetics) to become Senior Vice-President, Research at Merck, Sharp, and Dohme Research Laboratories in West Point, Pennsylvania in 1982. For a while he and some of the people he brought with him continued to work on the *ras* system. He has been interested recently in chemical changes associated with Alzheimer's disease. In 2003, Scolnick is still

President of Merck Research Laboratories and Executive Vice-President for Science and Technology at Merck and Company in West Point, Pennsylvania.

Dr. Jay A. Levy (25) was among the group of young physicians who arrived at NIH in 1967. He was a staff associate of Bob Huebner for three years. Dr. Levy graduated from Columbia University College of Physicians and Surgeons in 1965. He came to NIH after finishing an internship and residency in medicine at the University of Pennsylvania. He first encountered Bob Huebner when he went to hear Bob speak about "Footprints of Tumor Viruses" while he was still in medical school. He was impressed when Bob answered his questions in broad detail. Dr. Levy was favorably impressed when he met Bob again at the time of his visit to NIH in 1965 during his senior medical school year. At that time Dr. Levy decided that he wanted to work with Bob, and he was "really happy to work" in his laboratory. Bob apparently became very fond of Jay Levy. Dr. Levy had the usual introduction to the Huebner farm and to individual members of the Angus cattle-breeding herd. He and Bob played competitive tennis matches with great enthusiasm. Dr. Levy participated in a number of research studies with Bob Huebner and other of Bob's associates during his tour at NIH. Dr. Levy then joined the faculty of the University of California San Francisco School of Medicine. Bob Huebner maintained contact with him through the periodic meetings of PACTVIGR (Pacific Coast Virus Interest Group). When interest in the retrovirus tumor viruses waned, Dr. Levy took up the study of HIV/AIDS and became one of the authorities in this field. He currently holds the rank of Professor in the Department of Medicine and the Cancer Research Institute, UCSF. He is the author of the text "HIV and the Pathogenesis of AIDS," Second Edition, American Society for Microbiology, Washington, D.C., 2000.

Dr. Levy was one of the organizers for Bob Huebner's retirement celebration. In his letter to Bob at this time Jay Levy described some of the above events, and he said, "It is a great pleasure to share in this day honoring you, one of the world's greatest scientists. I have always considered my meeting you as both a tremendous stroke of good fortune and an inevitable plan of destiny. By my path meeting yours, I was able to benefit from the tremendous insight, scientific knowledge and creative research that have highlighted your career and punctuated your brilliant achievements. I feel deeply privileged to have been associated with you and very proud to be considered one of your students.

"Thank you, Bob Huebner, for encouraging my curiosity, enthusiasm and joy of scientific thought. I have sought to follow in your footsteps and be a tribute to you. You have made broad strokes in many areas of science; I had the privilege of being with you during your tumor virus days. In this field, you have paved the direction of the world toward recognizing the importance of virogenes and oncogenes. Your creative thinking, innovative scientific approaches, and ability to organize a research program helped establish the international efforts now dedicated to retroviruses.

"You are a legend in your own time and will be long remembered for your contributions to our knowledge in many scientific fields including infectious disease, immunology, cell biology, virology and pathology. To me you are remembered as a creative scientist, a respected teacher and a sincere friend. With warmest regards, Jay." Several other investigators collaborated with Bob Huebner over the years from the 1960's to the late 1970's. Among them were Raymond V. Gilden and Johng S. Rhim. Ray Gilden (26) was a Stanford University trained immunologist who was brought from the Wistar Laboratory in Philadelphia to the contract Flow Laboratories in Rockville to

provide the needed expertise in the preparation of immunologic reagents and to supervise some of the immunologic studies at the laboratory. Ray Gilden collaborated with Bob Huebner on many projects related to retroviruses until 1981, a year before Bob retired. Ray worked at Flow Laboratories from 1965 to 1975 and then moved to the NCI facilities at Fort Detrick, Frederick, Maryland. Bob and Ray remained very close professionally and personally during the years of their association.

Johng Rhim (27) graduated from Seoul (Korea) National University Medical School in 1957. He interned at the University Hospital for one year. He undertook further post-graduate medical training in the United States, including research experience at Children's Hospital in Cincinnati, Baylor College of Medicine, Houston, Pittsburgh Graduate School of Public Health and Louisiana State University, New Orleans up through 1964. Between 1964 and 1966, he was a visiting scientist in the National Institute of Allergic and Infectious Diseases (NIAID). Bob Huebner recognized Rhim's talent and potential. When it became clear that Rhim could stay no longer at NIH because of immigration visa problems, Bob Huebner was able to get a work permit that allowed Rhim to obtain a position doing research at Microbiological Associates in Bethesda. He served there as a Project Director in cancer research from 1966 to 1978. After naturalization as a United States citizen, he moved over to NIH where he held the position of Senior Investigator in the NCI from 1978 to 1998. Johng Rhim collaborated with Bob on many projects (27). He was a prolific writer and authored numerous publications dealing with various aspects of retroviruses. Rhim was especially grateful to Bob for rescuing him from professional occupational limbo. Rhim treasured the hundreds of memoranda he received from Bob over the years. In his testimonial letter (27) on the

occasion of Bob's retirement, Johng Rhim wrote, "Dear Bob, I have known you for sixteen years—actually, two-thirds of my life in the United States. These have indeed been unforgettable, rich and productive years for me. Like the last leaf upon the tree, I am among the last people to be associated with you and your work--- though I have learned many things from you, perseverance may be the lesson you taught me best!"

Many other budding investigators became associated with Bob as his reputation expanded. They are acknowledged in the extensive bibliography to which Bob contributed either as the senior author or as a co-author (28).

Following the publication of the oncogene manuscripts (12) and the discovery of reverse transcriptase by Temin and Baltimore (12), Bob recognized the importance of the molecular approach to the study of retroviruses and their relationship to oncogenesis. Bob had also felt that there should be no conflict in the "basic" as opposed to the "directed" approach in the Virus Cancer Program (29). With the view toward providing the "basic" approach, he began to recruit or invite investigators who had molecular biology expertise into the Program. He also encouraged this approach in some of the intra-mural scientists at NCI. Among the outside investigators who became involved with the Program were Renato Dulbecco of the Salk Institute in LaJolla, California, Sol Spiegelman of Columbia University, New York City, and J. Michael Bishop and Harold Varmus of the University of California, San Francisco.

From the mid- to the late-1970's, in addition to the active supervision of his intra-mural investigations and his functioning as the project officer for his remaining extra-mural contracts, Bob was involved deeply in immune protection studies in animals (31). In recognition of this work, starting in September 19, 1974, Bob began a 3-year VIP

(Variable Incentive Pay-special salary program for senior USPHS Commissioned Officers) agreement. In a VIP re-certification of the original agreement in September 1976 he received the following evaluation: “Dr. Huebner has been and is currently engaged in studies on prevention and modification of naturally and chemically induced cancer by passive antibody and viral/viral protein vaccines. His success in the murine system is being extended to primates and, hopefully soon to humans (EAB-speculation). His work is of the highest priority, and his performance (is) consistently superior. He is a physician/scientist of extraordinary originality and productivity” (30). The re-certification was signed by Drs. Louis R. Sibal for John B, Moloney, Chief of the Virus Cancer Program, Frank R. Rauscher, Director of NCI, and Philip S. Chen for the Director of NIH. This evaluation was in some disagreement with the assessment by the Zinder Committee of the value of the immunology studies in progress at NCI (30).

Bob’s career, the laboratory and his professional relationships took on an international flavor in the 1960s. As his reputation became widely known, his laboratory was able to attract many young investigators, first from Western Europe and Israel, and then from India and Southeast Asia. He maintained a lively correspondence with many overseas investigators and collaborated freely with virologists behind the Iron Curtain during the period of the Cold War with the Soviet Union. He had contact with Dr. Jan Svoboda in Prague, and he traveled extensively overseas including several trips to the Soviet Union. He hosted many Soviet scientific counterparts both at NIH and at the farm that the Soviets enjoyed visiting, calling it “Huebner’s Collective Farm.”

In 1977 Bob’s professional status changed. He had been at NIH since 1944 and with the Public Health Service before that in World War II service. He was close to

retirement age, and questions were being raised about the subtle intellectual changes noted in him as the result of his advancing Alzheimer's disease. These changes were noticeable, but were deemed by NIH and NCI administrators insufficient to prevent his continuing appointment as the chief of his laboratory. While Huebner continued his duties as chief, he did retire from the Commissioned Corps of the Public Health Service and received an honorable discharge. In light of his distinguished past service, he received a Civil Service appointment so that he could continue working at NIH. In as much as the nature of retroviral research was changing, the Laboratory of RNA Tumor Viruses was renamed the Laboratory of Cellular and Molecular Biology, and Bob continued as its Chief, with the title of "Expert," until his retirement in 1982.

Notes—Personnel Relationships, Additional Activities of the Viral Carcinogenesis Branch and the Virus Cancer Program

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3) Personal communication—Dr. Padman S. Sarma.
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7) Letter from Dr. Padman S. Sarma, September 29, 1982, among the personal papers of Dr. Robert J. Huebner.
8) See the chapter on—Hybrids, Helper Viruses, Adenovirus Testing, Field Studies.
9) A) Interview of Dr. Gary Kelloff on April 19, 1995, by Dr. Carl G. Baker as part of a series of interviews of persons associated with the Virus Cancer Program of the National Cancer Institute and/or with Dr. Robert J. Huebner. On file in the National Institutes of Health Historical Office. B) Several personal interviews with Dr. Kelloff by the author.
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12) See the chapter on The Oncogene Theory of Huebner and Todaro; Reverse Transcriptase.
13) See the chapter on Critics Anonymous; Contracts versus Grants.
14) Scolnick, E.M. 1984. Virogenes to Oncogenes, in Stetten, D., Jr., *NIH. An Account of Research in its Laboratories and Clinics*, Academic Press, Inc., p. 371.
15) Aaronson, S.A. and Rowe, W.P. 1970.Non-producer clones of murine sarcoma virus transformed BALB/3T3 cells. *Virology* 42: 9-19.
16) Interview of Harriet Huebner and Dr. James Duff conducted by Dr. Robert Stevenson, formerly of the National Cancer Institute, on July 18,1995 as part of a series of interviews related to the Virus Cancer Program of NCI. On file in the NIH Historical Office, Bethesda, Maryland.

17) See note 11 in the chapter on The Oncogene Theory of Huebner and Todaro.

18) Personal communication—Drs. Thomas Waldman and Carl G. Baker.

19) Personal communication—Harriet Huebner.

20) See note 3 in the chapter on—The Oncogene Theory of Huebner and Todaro.

21) The information on Dr. Edward M. Scolnick was abstracted from the interview conducted June 24, 1998, by Ms. Gretchen A. Case of History Associates, Inc. as part of the National Cancer Institute Oral History Project. On file in the NIH Historical Office, Bethesda, Maryland.

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25) Dr. Jay A. Levy, information from NIH records and NCI yearbooks. Also from a letter by Dr. Levy September 22, 1982 among the personal papers of Dr. Robert J. Huebner.

26) Interview with Dr. Raymond V. Gilden April 19, 1999 and several telephone conversations.

27) Interview with Dr. Johng S. Rhim May 2, 1999. Letter from Dr. Rhim, October 10, 1982 among the personal papers of Dr. Robert J. Huebner.

28) See the bibliography of Dr. Robert J. Huebner.

29) See note 5 in the chapter Critics Anonymous; Contracts versus Grants.

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Chapter 21

The Oncogene Unveiled

In the Summary Report of the Viral Carcinogenesis Branch in the1972 Annual Activities of the National Cancer Institute (1), Bob Huebner wrote, “The first order of business is to identify the nature and the origins of the cancer-inducing oncogenes in cells.—It is a matter of record that the VCB-STS (Viral Carcinogenesis Branch-Solid Tumor Segment) programs have assumed leadership in developing the technological tools needed for identification and quantification and assays of the natural and induced expressions of the genes of the RNA tumor viruses in normal and neoplastic cells.” This statement reflected Bob Huebner’s recognition that the time had come to examine the oncogenes minutely, beyond biological observations, at the molecular level in order to determine their nature and origins. He had been recruiting additional investigators with expertise in molecular virology into the Virus Cancer Program with the view of using highly refined technological methods to accomplish these ends.

Many members of the cancer virology community had adopted the oncogene theory as a working hypothesis. There was general agreement that the retroviruses (RNA tumor viruses) were transmitted primarily in vertical fashion by inheritance. There were questions, however, about whether transformation had occurred in some ancient time or was of more recent occurrence. In the manuscript (2) outlining the oncogene theory that they published in the *Proceedings of the National Academy of Sciences*, Todaro and Huebner emphasized the concept of the virus-derived pre-historic linear transmission of the oncogene as part of the retroviral genome. They contrasted this concept with Temin’s

view that the “provirus” (or “proto-virus”) component of the retrovirus was of relatively recent origin (2). Bob Huebner was somewhat extravagant in his idea that all vertebrates, including man, probably had retroviral genes as part of their genetic inheritance. However, in his presentations (2, 3), he questioned when and how the retroviruses (the virogenes) acquired the transforming factor (the oncogene) that resulted in the production of cancer. This void in knowledge prompted Huebner to continue studies to find answers to these questions.

Around 1971, in order to answer some of these questions, Bob Huebner expanded the roster of scientists with complementary expertise to help with the SVCP activities. Many of these investigators were located on the West Coast and were interested primarily in the avian sarcoma retroviruses. The most prominent among them were Dr. J. Michael Bishop and, later, his associate Dr. Harold E. Varmus. Dr. Bishop’s name appeared for the first time as a participant in the VCP in the Summary Report of the NCI in 1972.

Dr. Bishop is well known for his work on retroviruses and his molecular biological approach. He came from a non-medical and non-scientific background. His father was a minister. After majoring in history at Gettysburg College in Pennsylvania, he decided that he wanted to do something “altruistic” (4) so he went to Harvard Medical School where he developed his interest in research, especially molecular biology. He spent his fourth medical school year in virology research. He graduated medical school in 1962 and spent two years as an intern and assistant resident at the Massachusetts General Hospital. In 1964 he was appointed as a research associate at NIH where he pursued his interest in virology and viral oncology. He remained at NIH for four years, the last two as

a senior investigator, before joining the faculty of the University of California, San Francisco (UCSF), in 1968 as an assistant professor of microbiology.

Dr. Harold Eliot Varmus' father was a practicing physician on Long Island, New York (5). Varmus received an AB degree in English literature at Amherst College, Amherst, Massachusetts, and spent a year in graduate study at Harvard College specializing in seventeenth century prose. Shifting to a very different course of academic study, he entered Columbia University Medical School (College of Physicians and Surgeons) in New York City in 1962 and received his medical degree in 1966. He spent four years as a research fellow working with Dr. Ira Pastan at NCI on the bacterial production of proteins. He took advantage of the scientific educational opportunities presented at NIH and enrolled in its post-graduate evening study courses expanding his scientific background (6). As his research fellowship came to a close, he decided to remain in research, but wanted to settle in the San Francisco area. While interviewing with various investigators there, he "almost accidentally ran into Mike Bishop" (6) whom he found attractive and compatible both personally and professionally. In 1970 Varmus accepted a post-doctoral fellowship at UCSF. He and Bishop began their fruitful and productive association jointly studying the mechanisms of retroviral infections, in particular their relationship to the Rous sarcoma virus. Even before he had come to San Francisco, Varmus had been particularly influenced by the idea of using reverse transcriptase as a laboratory tool in studying retroviruses. He was also interested in the new development of the isolation of temperature-sensitive viral variants in transformation studies as well as the new transformation-defective variants of Rous sarcoma.

In addition to the availability of reverse transcriptase as a tool for investigation and manipulation of retroviruses, new discoveries in the early 1970's provided additional means for studying the retroviruses. Investigators, including Martin, Duesberg and Vogt (7) discovered the transforming gene of Rous sarcoma virus. This was labeled "sarc" and was found to constitute 10-20% of the Rous sarcoma genome. It was later estimated to be about 1600 nucleotides (chromosome bases) in length. Other discoveries included the isolation of Rous sarcoma strains that were temperature-sensitive (ts) with respect to transformation and transformation-defective (td) strains that were able to reproduce themselves but not transform tissue into cancer. These "defective" strains were studied primarily by Peter Vogt (and Duesberg) (8) at the Department of Microbiology at the University of Southern California. Peter Vogt's activities were also funded by the SVCP (9)

Bishop and Varmus became interested in demonstrating what happened to the genome of the Rous sarcoma once it entered the cell. By using reverse transcriptase they made DNA copies of Rous sarcoma virus, and by hybridization techniques, they demonstrated the incorporation of the viral polymerase products into the genome of the transformed cells (10).

Another question arising in the minds of Bishop and Varmus was the origin of the Rous *sarc* gene. It was not found in other retroviruses, and they wondered when and how it appeared in the Rous virus. They decided to look for this gene in other related Rous strains and other retroviruses. They speculated whether this was an old or recent acquisition. They decided to study this issue and also to test the validity of the Huebner-Todaro oncogene theory. In order to do this they determined that they would need a viral

"probe," namely, a radioactive copy of the Rous *sarc* gene with which to explore its presence in other viral and tissue genomes. This part of the investigation was assigned to Dominique Stehelin, a French worker in their laboratory. The preparation of this probe was very labor intensive (11) and required six months of meticulous work. It involved the use of a radioactive DNA copy of a Rous sarcoma virus made by means of reverse transcriptase. This copy was hybridized or annealed with a transformation-defective Rous sarcoma strain. The mixture was subjected to fractional separation by column chromatography on hydroxyapatite, and the resultant residual fraction represented the radioactive probe labeled "cDNAsarc" (11).

The authors described the exact procedures they used in their research in the *Journal of Biochemistry* (11A): "Our procedure to isolate cDNAsarc exploits the existence of genetic variants of AS (avian sarcoma) viruses which cannot induce sarcomas in animals or transform fibroblasts in culture; they lack 10-20% of the viral genome (transformation-defective or td viruses). Results of genetic analyses indicate that the deleted nucleotide sequences include part or all of the gene (s) responsible for the oncogenesis and cellular transformation. In our procedure the genome of Prague strain (Pr-C) of ASV [avian sarcoma virus] was transcribed into complementary DNA by endogenous RNA-directed DNA polymerase [i.e. reverse transcriptase]; we then used molecular hybridization to select DNA specific for the region missing from the genome of the td deletion mutants. The preparation of cDNAsarc used was a uniformly uniform transcript from about 16% of the Pr-C ASV genome, a region equivalent in size to the entire deletion in the strain of td virus used in our experiments."

The probe worked as it was supposed to (11). It stuck to the *sarc* genes of transforming strains of avian sarcoma but not to the genomes of transformation-defective strains, avian myeloblastosis virus, Moloney mouse-leukemia virus, feline sarcoma-leucosis virus, or mouse mammary tumor virus. As a further control on the specificity of the probe, the authors decided to test the probe on normal tissue. To their astonishment, the probe detected nucleotide sequences in uninfected chicken cells. They extended their observations to other avian species including quail, turkey, duck, emu, and in each instance there was evidence of *sarc* nucleotide sequence in all these species (12A). Bishop and Varmus checked among other vertebrate species and found widespread evidence of the presence of *sarc* in most living organisms (12). It became evident that the *sarc* oncogene (later renamed *src*) was not an ancestral gene of the Rous sarcoma virus, but a component of the normal chicken genome, which had somehow been appropriated by an errant virus. The virus had incorporated the normal *src* into its genome, and, by whatever mechanism, converted the normal gene into a malignant or transforming gene. This discovery, of course, refuted the inherited origin of the oncogene as hypothesized originally by the Huebner-Todaro theory. Further observations indicated that the endogenous proviruses were much less conserved in evolution than the *sarc* gene in normal tissue, making it even clearer that there was no direct relationship between endogenous retroviral proviruses and *sarc* (19).

Over the course of several years, Bishop and Varmus constructed the evidence for their discovery before publishing a definitive report in *Nature* in 1976 (12A). They received substantial financial assistance through NIH grants and then, from about 1971, continuing contract support from the SVCP through the Viral Carcinogenesis Branch and

the Solid Tumor Segment administered by Bob Huebner. The project officer for their contract(s) was Dr. Edward M. Scolnick (13). Bishop and Varmus' work was also supported by American Cancer Society grants, and Varmus had a Career Development Award from the National Cancer Institute.

Bob Huebner was extremely impressed with Bishop, and he invited him frequently to the meetings of the PACTVIGR (Pacific Coast Virus Interest Group) to present his research to the other attendees (14). Huebner and Bishop had some vigorous debates and disagreements at these meetings (14). According to Robert A. Weinberg, Bishop was also a dramatic and persuasive speaker at these events (15): "Bishop--- was the son and son-in-law of Lutheran preachers from Gettysburg. He soon became known for his eloquence on the podium, for his wide-ranging knowledge of virology, for his love of the English language and the rich vocabulary it offered him for writing and speaking. How many Sundays, I often thought, had he spent listening to powerful sermons given by guest preachers passing on their circuits through the small Lutheran congregations of eastern Pennsylvania?"

The careers of both Bishop and Varmus flourished and advanced appropriately in recognition of their achievement. In 1989 they were awarded jointly the Nobel Prize in Medicine or Physiology for their path-breaking "discovery that the growth-regulating genes in normal cells can malfunction and initiate the abnormal growth processes of cancer." The awarding of the prize was marred by the protest of Dominique Stehelin who felt that he should have shared the prize with them since he performed the important experiments (16). The Nobel Prize Committee disagreed on the grounds that the concepts originated with Bishop and Varmus (16). Bishop is currently Chancellor of the University

of California, San Francisco. Varmus was the Director of NIH from 1993 to the end of 1999, and then he became President and CEO of the Memorial Sloan-Kettering Cancer Center in New York City.

The cellular gene precursor of the oncogene is now termed a *proto-oncogene*. The idea that a normal cellular gene that had a benign function in physiological growth regulation but could become malignant was revolutionary and it impelled molecular biologists to search for other similar genes. Within a short period up to 50 oncogenes were discovered. Subsequent studies of the relationship of these genes, suppressor genes and cellular regulating mechanisms have led to an increasing understanding of their interaction in the pathogenesis of cancer. Bob Huebner's intuition in 1969 allowed him to speculate on the reciprocal regulatory effects of oncogenes and suppressor genes (17), a relationship that is now of major importance in understanding the molecular basis of cancer origin.

On the occasion of Bob Huebner's retirement celebration, Dr. Bishop sent a note that pointed out the influence Huebner had wielded on his behalf (18): "Dear Bob, You never succeeded in converting me from chickens to mice, but you did provide vital support at a crucial time in the development of my research program, and it is also safe to say that I have spent the better part of my career chasing your ideas. I wish you all the best on this special occasion. With best wishes, Sincerely yours, Mike (J. Michael Bishop)."

1) Annual report of the activities of the National Cancer Institute 1972. Dr. Robert J. Huebner wrote the summaries of the Viral Carcinogenesis Branch and the Solid Tumor Segment. In the National Institutes of Health Library.
2) See note 3 in the chapter—The Oncogene Theory of Huebner and Todaro, etc.
3) See notes 1 and 2 in the chapter—The Oncogene Theory of Huebner and Todaro, etc.
4) Meldrum, M. 1990. J. Michael Bishop 1989. In *Nobel Laureates in Medicine or Physiology*, Eds. Fox, D.M., Meldrum, M. and Rezak, I. Garland Publishers, New York, Pp. 45-48.
5) Ibid. Meldrum, M. 1990. Harold Elliot Varmus 1989. *Nobel Laureat*es etc. Pp. 342-344
6) Interview of Dr. Harold E. Varmus, Director of the National Institutes of Health, on August 31,1995 by Dr. Carl G. Baker as part of the Oral History of the Virus Cancer Program of the National Cancer Institute. On file in the NIH Historical Office.
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10) Varmus, H.E., Stavanezer, E., Medeiros, E. and Bishop, J.M. 1975. Detection and characterization of RNA tumor virus-specific DNA in cells, *Bibliotheca Haematologica* 40: 451-461.
11) A) Stehelin, D., Guntaka, R.V., Varmus, H.E. and Bishop, J.M. 1976. Purification of DNA complimentary to nucleotide sequences required for neoplastic

transformation of fibroblasts for avian sarcoma virus. *Journal of Biological Chemistry* 101: 349-365. B) Stehelin, D., Varmus, H.E., and Bishop, J.M. 1975. Detection of nucleotide sequences associated with transformation of avian sarcoma viruses. *Bibliotheca Haematologica* 43:539-541.

12) A) Stehelin. D., Varmus, H.E., Bishop, J.M. and Vogt, P.K. 1976. DNA related to the transforming gene(s) of avian sarcoma viruses is present in normal avian DNA. *Nature* 260: 170-173. B) Bishop, J.M. et al 1978. Genesis of a virus-transforming gene. *National Cancer Institute Monograph* 48: 219-233.
13) Annual report of the National Cancer Institute. Ibid 1975. Contract number: NO1-CP3-3293. Title: Studies of the role of virus-associated DNA polymerases in malignant transformation by tumor viruses. Project Director: J. Michael Bishop. Project Officer: Edward M. Scolnick (NCI). Initiated June 2, 1971. Contract level: $200,000.
14) Personal communication—Dr. James Duff.
15) See note 24 in the chapter—Personnel Relationships, etc.
16) Marx, J.L. 1989 Cancer gene research wins Medicine Nobel—Controversy over Nobel. *Science* 246: 326-327.
17) See notes 1 and 2 in the chapter—The Oncogene Theory of Huebner and Todaro, etc.
18) Letter from Dr. J. Michael Bishop August 20, 1982 among the personal papers of Dr. Robert J. Huebner.
19) Personal communication from Douglas R. Lowy in letter sent April 26, 2002.

Chapter 22

A Second Beginning, Illness and, Decline

The years from 1973 to 1975 represented a watershed in Bob Huebner's life. It was a period of increased stress and adjustment. In 1973 he sold the Angus cattle breeding herd, his marriage to Berdi was in the final stages of disintegration, he was confronted with mounting criticism about how he was conducting his research program (which culminated with the report of the Zinder Committee), and he was about to marry Harriet, his second wife.

By this time Bob was finding it was increasingly difficult to continue the herd breeding activity. The enterprise had prospered because his children were primarily responsible for the physical labor and the bookkeeping records that were required for the meticulous operation of the breeding activity. By the early 1970's the older children had left the farm to pursue their further education and careers, leaving the younger children Ed, Lou and Danny living on the farm. By 1973 only Lou and Danny remained, and they could not by themselves perform the work that was required. According to Harriet (1), Berdi, who was a hard working, loving mother to nine children and dedicated to the concept of running family life on the farm, found it increasingly difficult to follow the stringent protocols that Bob had formulated to insure the successful breeding of prize Angus beef cattle.

Comment [PC1]:

Bob and Berdi were actually geographically and emotionally separated for many years before the divorce. Bob was extremely busy when he became actively involved

with the Special Virus Cancer Program in the 1960's, and he worked long hours in the laboratory and in professional travel. The commute from the farm to the laboratory took more than an hour on narrow two-lane country roads so he remained most of the week in Bethesda and returned to the farm on weekends, occasionally staying an extra day when he was trapped by an exceptionally heavy snowfall. The interstate highway I-270 was constructed later. At first he would sleep in the laboratory; then he would occasionally spend a night in the apartment of his laboratory technician John D. Estes. He finally rented a small apartment in Bethesda. With the physical separation, with Berdi's increasingly heavy burden of running the cattle business, with Bob's increasing prominence in virology and the growth of the children into adulthood, Bob and Berdi found that they had little in common to sustain a successful marriage.

The divorce in 1975 was amicable and the terms were generous. Bob deeded the farm to Berdi and gave her half of his salary for support. After she received the farm from Bob, Berdi formed a partnership between the farm and the children that persists to the present time (1). The size of the farm was originally 165 acres; later the partnership sold 5 acres to daughter Lou on which she built her own home. Daughter Ginny, at present, lives in the farmhouse. With rent from tenants and other revenues the farm is self-sufficient, and is still the site of family gatherings at holidays and other occasions (1).

The adult children were upset with their parents' divorce, and they harbored initial resentment against Bob and Harriet (1). This changed, however, with the onset of Bob's illness. They later accepted Harriet readily as they became aware of her deep attachment and of the important supportive role she played in caring for Bob (1). Danny was only an adolescent at the time of the divorce and was unaware of what was

happening. While he was riding a tractor on the farm, his sister Ginny informed him of the impending divorce. His feelings were “stoic and neutral.” Later, when Bob approached him to discuss the divorce, Bob became very emotional and tearful whereas Danny accepted the news “calmly” (1).

When Bob and Harriet married in 1975, they offered Danny the opportunity to live with them and to attend Georgetown Day School in Rockville in preparation for going to college. Danny, however, did not want to leave his mother alone on the farm, and he decided to remain there with Berdi (1). After Danny went to college, Berdi moved off the farm to Rockville, and she became a successful real estate agent. Sadly, Berdi developed ovarian cancer in the late 1980’s and died in 1991. Fortunately, Bob had retained her as a beneficiary on his service health insurance that helped ease her financial burden, and, through his prior connections with the National Cancer Institute, she had the benefit of expert consultations at the National Institutes of Health (1).

Danny attended parochial school until the second grade and then went to public school. His older siblings all completed parochial school education. When Bob was young he was steeped in Catholicism at home, and both parents were pious people (1). He also attended Catholic schools with the exception of several years at the University of Cincinnati. About the time that Danny’s parochial schooling stopped, Bob abandoned his Catholic faith. He remained, however, reverent and a man of inflexible moral scruples (1). The rest of the family also became less observant in the practice of the faith, and some of the members ceased regular church attendance around the same time (1).

Around this time the criticisms of the Zinder Report were also published. In spite of the accumulated stresses associated with all these events, according to Harriet (1), Bob

appeared to be more angry and annoyed than upset with the conclusions of the Zinder Report. He continued his usual activities as long as he had the resources to pursue his research goals and ongoing projects. Harriet suspected that his emotional responses around 1975 might have been blunted by the subtle changes that represented early manifestations of his Alzheimer's disease.

The major impact on Bob's life after divorce from Berdi was his marriage in 1975 to Harriet (maiden name- Harriet Lee Gutsin), his longtime secretary, administrative assistant and personal "gyroscope." Harriet is a sensitive, perceptive person who was always aware of Bob's virtues, faults and personal foibles. Harriet came from New York City and had an academic high school education and four years of college. She came to the National Institutes of Health in 1953 from the University of Chicago with her first husband, a doctor of philosophy, who had a job at NIH. She obtained a "clerk" position in the Grants Division of the Institute of Neurological Diseases and Blindness, a job that she despised. She left NIH temporarily in 1955 and took a two-year hiatus after the birth of her son. She returned to NIH in 1957. On the recommendation of a friend she went for a job interview with Bob Huebner who, impressed with her credentials, hired her on the spot. Harriet was also impressed with Bob's decisiveness in offering her the open secretarial position immediately. When Bob discovered that Harriet possessed writing ability, he gave her the responsibility of doing some writing and administrative chores for his laboratory. Harriet was somewhat frustrated at this juncture, for despite having passed the NIH test for administrative personnel, she did not receive a grade promotion, and she felt that she could not make any professional progress as a secretary. She left Bob's laboratory temporarily and went to a position as a science writer in the NIH Information

Office, a job that she did not find interesting. Shortly thereafter, Bob arranged for an administrative assistant's position opening in his laboratory, and he requested that Harriet return to work for him. Harriet did not need to spend her time with secretarial duties since Bob had several secretaries working for him. Instead, Harriet resumed her administrative duties which included writing personnel job descriptions, solving visa problems for foreign visiting scientists, and helping to prepare budgets. Harriet also did extensive library searches and increasingly helped Bob with his manuscripts and speeches. She also helped prepare annual reports for Bob's laboratory and Viral Carcinogenesis Branch. Despite initially not having a scientific background, she rapidly became familiar with the work of the laboratory and soon acquired the necessary jargon. She became very knowledgeable about Bob's expanded activities in the Special Virus Cancer Program and was familiar with the extra-mural contract projects and their directors. She kept track of Bob's complicated schedule including his appointments and out-of-town travel. She literally did everything that was needed to operate the laboratory activities efficiently except for the performance of the actual experiments. She was a source of information and a resource person for people who had professional dealings with Bob. Harriet's first marriage ended in divorce in 1975. When Bob's divorce became final, he courted Harriet, and they were married in 1975 (1).

Harriet adored Bob. Her perception of him as a person and as a husband was that "He was a sweet, gentle and generous man." She also considered him without guile, and very open in his dealings with people—his family, friends, associates and members of the scientific community. He shared his ideas and current research work without reservation. This latter trait led to several unsavory occasions when investigators came to his

laboratory by invitation, co-opted some of his data and published them as their own. He brushed these incidents aside by saying that scientists who stole others' ideas were to be pitied because they had no ideas of their own. He had amicable relations with his peers and young associates but was impatient with and intolerant of some unimaginative administrators who had jurisdiction over his activities (1).

With six talented daughters, Bob had an enlightened attitude toward the equal treatment of women in the professions and the sciences. He provided opportunities for many accomplished women in his laboratory and in his collaborative studies. At home, however, he demonstrated traits of male chauvinism. It did not occur to him that men could help out with some of the domestic chores because that was not part of his cultural background. He would do the outside work around the house and take care of the automobile since he felt that this was not women's work. He never made the coffee, primarily, because he did not know how. Despite his broad talents he was mechanically inept. If anything needed fixing around the house, Harriet would usually call a repairman. This was also the case with Bob during the years on the farm.

Harriet describes Bob as being completely driven by his research. After finishing work at the laboratory and office, Bob and Harriet would come home at varying hours and continue with unfinished tasks. One of the traits that Harriet admired in Bob was his ability to work with people of varying professional backgrounds as long as they were able to contribute help and knowledge to the work at hand. He demonstrated this in his first major accomplishment when he collaborated with Charlie Pomerantz, the pest control operator, in elucidating the etiology of rickettsialpox. Bob recognized ability in some of his trusted laboratory technicians most of whom came from limited educational

backgrounds. A prime example is Chick Turner, who was completely untutored in science when he came to work at NIH. Chick educated himself, supervised the serology laboratory for the Laboratory of Infectious Diseases in Building 7, collaborated with Bob for many years doing immunologic procedures, and moved along with Huebner to the National Cancer Institute. This attitude on Bob's part may have been a reflection of his belief, as stated to Harriet, that since he did not go to an Ivy League school, he did not know what was not supposed to be possible; so he followed his instincts and tried the "impossible," and often he turned out to be right.

While at NCI, Bob and Harriet traveled extensively overseas but usually in conjunction with scientific meetings. The U.S. Government paid virtually none of Bob's overseas travel. It was always by invitation of the host-university or government who paid Bob's way. Their own funds paid for Harriet's travel. In these situations they did not have the conventional tourists' luxury of exploring the areas that they visited. The one travel exception was a trip to Israel. The Israeli hosts paid for Bobs travel but the Huebners personally financed Harriet's travel and their vacation time spent with friends and former associates.

The onset of Bob's Alzheimer's disease began very subtly and imperceptibly. It is uncertain when the first signs became recognized. Harriet suspected (1) that the onset might have occurred around 1975 shortly after they were married. The signs began gradually with the loss of intellectual acuity and minor evidence of confusion. More overt signs became noticeable around 1978 when observers (2) noted that Bob was becoming slovenly in appearance and careless in his personal grooming. Uncharacteristic personality changes became manifest around the same time. On one memorable occasion

when Bob Chanock and Wally Rowe and their wives were dinner guests at the Huebner home, Bob Huebner suddenly developed a period of uncharacteristic confusion. As his guests were leaving, Bob got up to leave with his guests at the end of the evening, not realizing that he was in his own home. Chanock and Rowe made light of the event at the time, jokingly accusing Bob of having had too much wine. The next morning Bob Chanock called Harriet and suggested that "something serious was happening to Bob Huebner requiring further investigation." As Bob's illness developed, his scientific colleagues and laboratory associates in the Virus Cancer Program became aware that he was no longer generating the new ideas that he had usually communicated with such great regularity. As Bob had always delegated responsibility to entrusted associates, other outside investigators were unaware of Bob's failing health. His associates lead their own ongoing projects and contracts and could continue working without further direction. Some of his associates (3) noted repetition of thought, forgetfulness and hesitancy of speech. Other observers (4) noted a deterioration of Bob's participation in committee meetings. When Bob first came to NCI in 1968 (and previously) he made articulate, forceful and well reasoned presentations at meetings. As his disease progressed, his presentations were still articulate and forceful but his thoughts became confused, rambling and unfocused. People (5) began to recognize that if they needed some help from Bob's office, e.g., a manuscript review and recommendation for submission to appropriate scientific journals, they would have to deal with Harriet rather than Bob to get the help they needed.

With the further progression of Bob's illness, he became increasingly confused. At times he would have other episodes of spatial and temporal disorientation, becoming

lost in the men's toilet at work or traveling to and from his office. It was becoming difficult to mask the extent of his disability. In the late 1970's and early 1980's it could not be denied that he could no longer perform his professional duties and retirement became mandatory. His colleagues Bob Chanock, Wally Rowe, Stuart Aaronson and Jay Levy (now on the West Coast) along with Harriet, family, friends and Bob's secretarial staff organized a celebration day on, Monday October 18, 1982, that honored Huebner on the occasion of his retirement from the NIH after 40 years of incredibly productive service (1). This was to be a day of speeches and reminiscences about Bob followed by dinner. When Bob arose to speak after his introduction, he appeared alert, cheerful, jocular and unchanged (6), but, after several minutes his intellectual disability was unmistakable, and he was quickly escorted from the podium so that the rest of the program could continue. He retired with the status of "Scientist Emeritus" of the National Institutes of Health but he never returned to work.

Bob's intellectual impairment continued. In 1990 Harriet brought Bob to my office for evaluation and suggestions for further support measures. I had not seen Bob for a while. His physical appearance was unchanged; but this man who had previously projected a forceful, voluble, articulate, energetic image sat quietly in my office for one hour and not a single word escaped from his lips. There was also no sign of recognition or remembrance of our past association. That day was one of the most depressing that I experienced in my years of medical practice, and I never felt so helpless as then when I had no concrete suggestions for further therapeutic intervention or support.

Shortly after that visit Bob began to suffer from neuro-muscular impairment and loss of balance. Bob was still living at home, and Harriet had to attend to his physical

needs. Bob fell three times in rapid succession on close separate occasions. Harriet, who is a petite woman, had difficulty getting Bob back on his feet because he was still a large man who could not help himself get up. She had to call the local rescue squad for assistance. After the third call, a member of the Rescue Squad told Harriet that she could not care for Bob alone. The squad member felt that "it was only a matter of time until the doctor would break some bones." For a while Harriet tried to arrange for help at home to assist with Bob's needs but this was only a temporary expedient during which Bob's disability worsened. In 1991 Harriet arranged for Bob's admission to the Veterans Administration Hospital in Coatesville, Pennsylvania. The hospital specializes in the care of patients with chronic neurological illnesses. Harriet would visit Bob once or twice weekly. His hospital course was one of continued physical deterioration punctuated by bouts of pneumonia and urinary infections caused by prostate obstruction. Harriet would try to assess his symptoms and report changes from his baseline status to his attending physicians. On August 26, 1998, a fatal pneumonia ended his life.

1) The material in this chapter is based on the following sources: A) Conversations with the Huebner children at various times including Susie, Betty. Ginny, Danny and Jim. B) Interview of Mrs. Harriet Huebner and Dr. James Duff, previously of the National Cancer Institute, conducted by Dr. Robert Stevenson, previously of the National Cancer Institute, on July 18,1995. This interview was part of the Oral History of the Virus Cancer Program of the NCI. C) Interview of Harriet Huebner on June 12, 1998, as part of the National Cancer Institute Oral History Project. Ms. Gretchen A. Case of the History Associates, Inc. of Rockville, Maryland conducted the interview. Both these interviews are on file in the NIH Historical Office. D) Many personal conversations with Harriet Huebner from August 1998 until the most recent, April 19, 2001.
2) Personal communication—Dr. John B. Moloney.
3) Personal communication—Dr. Raymond V. Gilden.
4) Personal communication—Dr. Jack Gruber.
5) Personal communication—Dr. James Rose.
6) Personal communication—Jim Huebner.

Chapter 23

Legacy

Several days after Bob's death, I was in NIH Building 7 conducting an interview with Dr. Al Kapikian about Bob. Bob Chanock, who had an adjacent office, came in to see Al. Chanock was both irate and elated. The major local newspaper, *The Washington Post*, had printed a puny, inaccurate obituary about Bob, and the obituary editor refused to modify or expand the obituary. Chanock, with dogged persistence, contacted several newspapers and on the day of the memorial service, September 5, 1998, *The New York Times*, at Chanock's suggestion, published a detailed and appropriate obituary describing Bob Huebner's life and accomplishments (1). This obituary was published in part in other newspapers throughout the country. Dr. Vincent DeVita (1), Director of the Yale Cancer Institute and former Director of the National Cancer Institute at the time of Bob Huebner's retirement stated in the obituary, "The guy was brilliant and unconventional—he opened up the whole field of cancer viruses, looking at them in ways that people had not looked before." In another obituary, belated due to the slow arrival of notice of Bob's death, Dr. Jan Svoboda of Prague (2) paid tribute to Bob's memory by describing Bob's close collaboration with him and his students even during the occupation of Czechoslovakia by the Soviet Union. Dr. Svoboda said, "Bob Huebner's collaborators, even in this country, lost a great initiator and stimulator, sparkling with optimism and invention of unconventional ideas and concepts. In our minds Bob also lives as an extraordinary open-minded and sensitive human being, who took science as a service, not only to his own country, but to the whole world."

To recapitulate some of Bob's personal attributes, he was a physically and intellectually appealing person. In appearance he presented as a big, tall, bluff, hearty presence with a booming voice. Intellectually he had characteristics of genius. He was extremely well read and had many interests. He could expound extemporaneously on many subjects unrelated to his scientific work (3), and these subjects might include art, poetry, politics, literature, music and a host of other disciplines of the mind. He was also a marvelous raconteur. The bookshelves at the farm were stacked with hundreds of volumes including a variety of different subjects that he read voraciously (4) and that he encouraged his children to read. His children felt that they had a most unusual farm upbringing since a parade of famous scientists was entertained frequently at the farm, and many remained overnight (4).

Many of his associates marveled at his unfathomable intuition. He always seemed to be able to tell what was important before going into his next phase of scientific investigation. He had an abundance of fertile ideas, a characteristic volunteered spontaneously by many persons interviewed for information about Bob. One person described this abundance in homely terms saying that, "Ideas popped out of his head like fleas jumping off a dog" (5). He also was able to perceive relationships among disparate bits of information. Dr. Robert Stevenson (6) observed this uncanny ability on several occasions at scientific meetings when Bob synthesized the data from many presentations of seemingly unrelated material and pointed out the relationships that had been missed entirely by others. Some people accused him of stealing the ideas of others but this was an untenable notion since Bob had so many ideas of his own (7). He approached his own work with energy and enthusiasm. As mentioned previously he had the ability to inspire

and motivate young investigators. He was kind to his associates. He was generous with his ideas and shared his work freely with others. He had an outsized sense of humor; and he was self-deprecating; he did not take himself too seriously (8).

One of the remarkable aspects of Bob's career was the demonstration of progressive intellectual growth and scientific maturation. Starting with no particular formal laboratory training, he was able to progress from the relatively unsophisticated techniques of rickettsial investigation through classical virology techniques, applying these techniques to the study of tumor viruses and finally becoming familiar with the elegant methods of molecular biology. Dr. Frederick C. Robbins, Nobel Laureate (along with John Enders and Thomas Weller for growing poliomyelitis in non-neural tissue culture cells), provided an appreciation of Bob's scientific progression. Dr. Robbins (9) wrote to Bob on the occasion of Bob's retirement. Robbins was then President of the Institute of Medicine of the National Academy of Sciences. He began by saying how they both had started their early careers studying Q fever, but that Bob had "expertly elucidated the mode of transmission of Q fever, whereas we failed to do so" ---. Dr. Robbins continued, "I guess what has impressed me the most about your career is the way in which you have been able to move step by step, into more and more basic investigations. You started with rickettsias and, primarily epidemiology, moved on to respiratory agents, then on to cancer and finally to the molecular basis for oncogenesis. I will never forget the presentation you gave many years ago at one of the Perspectives in Virology where you were talking about oncogenes. This was the first time that I had heard the term, and I will admit that I listened with a certain degree of skepticism. How in the world could a rickettsiologist be dealing with such questions and making a

contribution! What you were talking about then is common knowledge with every graduate student today, and your perceptiveness has proved out."

At the time of Bob Huebner's retirement celebration October 18, 1982, there was an outpouring of testimonials from persons present and from those who could not attend but who responded with their tributes and good wishes. Among the testimonials (10) is the one from Dr. Albert Sabin, the father of the oral poliomyelitis vaccine. Sabin related an episode that occurred at a virology meeting twenty-five years before on the so-called "orphan viruses"; at the meeting when someone called for a moratorium on the search for new viruses for which there were no known diseases, Bob came right back with an answer. To make his point Bob told the story of the Scotsman, Angus, who refused to light up a lantern for the doctor who was presiding over the multiple births of Angus' offspring. Angus' reasoning was that it was the "light that attracts them (the multiple offspring)." Dr. Sabin continued, "The moral that you [Bob] drew from the story is that those who believe that one can get rid of problems by turning off the light are poor misguided souls. Bob, it can be said of you that you turned on many lights where darkness prevailed and helped to solve many problems that seemed insoluble before."

Another tribute came from Dr. S. Rasheed (11), a colleague of Dr. Murray Gardner at the University of Southern California. "The sweeping advances made under Bob Huebner's colossal interdisciplinary research program brought together the new perspectives of genetics, microbiology, and biochemistry that engendered molecular biology. Beginning with his pioneering concepts of virogenes and oncogenes that led to a diligent search of retroviruses, a revolutionary change has sparked in scientists'

understanding of genes and their products. These studies will now serve as the basis for further understanding of gene regulation, disease prevention and control.

"There was something about Bob Huebner who made us believe in him even when he sounded like a man from outer space. It was this unique characteristic that gave us strength to deal with the horrendous tasks that faced us. Bob Huebner to me has been and will always be a kind, compassionate tower of strength whose unselfish endeavors toward solving problems rather than personal gains have opened limitless opportunities for the study of cancer. With the use of these tools we and the future generations can look forward to fulfilling at least some of his dreams."

Dr. Robert C. Gallo (12), the discoverer of the human retroviruses HTLV-I and II and co-discoverer of HIV, commented: "I never had the privilege of working directly with Bob but his work, ideas, and stimulation were the chief reasons that I entered the retrovirus field (for good or for bad!). Bob has been a towering inspiration to all of us, and I just can't picture this field without him. In fact, his ideas, encouragement, his breadth of discoveries, and the stimulation he gave to us all will always be with us.

"Needless to say, viral oncology is in an enormously strong position today. It has, in many respects, paved the way in many fields. He has been central to all of this. In times when ideas and courage in cancer research were as dry as the deserts, Bob was the oasis."

These comments were representative of the many tributes received by Bob at the time of his retirement. They offer a picture of Bob and help bring his personality to life.

While it is difficult to rank Bob's discoveries in terms of importance since most of them had major impacts on virology and then viral oncology, the following provides in

short detail a list of his many accomplishments. It's a long list, attesting to the unlimited scope of Bob's genius, intuition and perseverance:

- He provided a solution to the mystery of rickettsialpox.
- He discovered the major mode of transmission of Q fever from animals to humans and made basic observations on the epidemiology and biology of the organism.
- He proved definitively the etiologic association of multiple serologic types of Group A Coxsackie viruses with the childhood febrile enanthem, herpangina.
- He confirmed definitively the etiologic association of Group B Coxsackie viruses with epidemic pleurodynia in a community setting.
- With Wally Rowe, he co-discovered and described a new respiratory viral genus, the adenovirus.
- With Bob Parrott, he described the clinical entity pharyngoconjunctival fever that was caused by adenovirus type 3. With Joe Bell and associates he described the epidemiology of adenovirus type 3 in a community outbreak.
- With Joe Bell (and associates), he set up a comprehensive longitudinal and cross-sectional study of the microbiological experience (viral and bacterial) of a nursery group in Junior Village, an orphanage in Washington, D.C. This study provided clinical, epidemiological and isolation data about known viruses and the recovery of many new serologic types of known viruses and recently recognized "new" respiratory viruses (the para-influenza viruses).
- From 1948 to 1958 Bob listed "seventy new viruses," for many of which he had played a discovery role, and he formulated a revision of Koch's postulates based on

his experience relating to the establishment of etiologic significance of newly isolated viral strains for clinical illness.

- Bob also conducted many studies in volunteers to determine the clinical characteristics of illness in adults produced by infection with newly isolated viruses, and he participated in vaccine development studies.
- He worked on studies of rubella with Dr. John Sever.
- In 1958 he began his excursion into viral oncology. He began with studies of the DNA tumor virus, polyoma, working in collaboration with Wally Rowe and Janet Hartley. He studied the incidence of infection among various strains of laboratory mice, and he then extended these studies to feral animals in both rural and urban settings. He demonstrated widespread serologic evidence of infection but rare occurrence of cancer in mice in their natural setting.
- He confirmed the oncogenic nature of adenovirus infection in rodents (hamsters) and by serologic techniques he discovered the presence of non-producing virus in the tissues of infected animals with tumors, the T or tumor antigens, the "footprints" of viral presence. These observations led to important new studies including the discovery of the group specific antigens in avian and mouse retrovirus infections.
- Bob also participated in the studies of the so-called "helper viruses," leukemia strains providing the envelope genes that enabled non-producing sarcoma strains to yield infectious virus.
- With extensive studies of antibodies and searching for evidence of viral antigens in tissue specimens, Bob was able to absolve adenoviruses as a cause of human cancers.

- He concentrated his efforts on the study of animal retroviruses as potential candidates for human cancer causation. According to Murray Gardner (13), "Based upon three of his cardinal observations (on RNA viruses), 1) the presence of group specific antigen in embryo tissues, 2) the relative immunologic tolerance of animals to their endogenous RNA tumor viruses and 3) the epidemiology of naturally occurring cancer, Bob correctly predicted the genetic transmission of RNA tumor genes . . .He repeatedly emphasized that the potential for cancer is in all of us, and whether the disease occurs depends on the interaction of oncogenes with other environmental and host genetic factors . . . Looking back fourteen years later it's clear that Bob's vision was 'right on', and that oncogenes and their activation by retroviruses and other stimuli may well prove to be a common denominator in carcinogenesis."
- Bob's extensive laboratory observations of the RNA viruses (retroviruses) led him to the concept of the oncogene (a term that he originated), a transforming factor in the development of cancer. Bob's questioning of the nature of the oncogene led him to support the studies of Bishop and Varmus who demonstrated that the precursor of the oncogene (the proto-oncogene) was a component of normal tissue (the genome) that by a variety of stimuli underwent genetic mutation to the malignant transforming gene.
- The last few years of Bob's career were spent primarily in studying the immunology of retroviral infection in mice.
- Bob Huebner was the driving force or mainspring of the Special Virus Cancer Program, later the Virus Cancer Program. As noted previously, Bob's talent in recruiting and coordinating the activities of many investigators active in the program

earned for him the title of “My General Patton” from Dr. Carl G. Baker, former Director of the National Cancer Institute. According to Dr. Ed Scolnick (14), “The genius was Huebner. He had the vision, the vision of what to do, he was not mean-spirited, he could see the role for basic research as well as targeted research, and he contracted with quality scientists. Not always traditional approaches, but quality people. That could not be said for some of the other people in charge of the program. But I think Huebner was a great man, a really great man.” Scolnick summed up the strength of Huebner’s research career, stating that (15), “Dr. Huebner clearly was a giant in the field, stimulating many ideas in oncogenesis.”

Dr. Vincent DeVita (16) who reorganized the Virus Cancer Program administratively in the early 1980’s by eliminating most contracts, firing some investigators and drastically slashing the funding, nevertheless had this to say, “The Virus Cancer Program, which history will record as a very, very important program—The Virus Cancer Program which I think is one of the greatest contributions the Cancer Institute has made to science—seven Nobel Prizes have come out of it—was considered a piece of garbage. And it didn’t deserve that criticism”.

Dr. Carl G. Baker in the epilogue to his manuscript (17) states, “The progress made in the understanding of cancer causation, especially with regard to genetics, was outstanding between 1953 and 1973. Even more impressive has been the progress after that period. Viral oncology studies contributed greatly to this progress…. The finding of oncogenes, viral and cellular, was an exceptionally important development in cancer research. This finding, along with the discovery of excision enzymes (restriction

endonucleases), and later cloning and oncogenes present in “normal” chromosomes, has led to explosive advances in cancer causation, genetics and developmental biology….. These viral oncology program components made additional contributions to the laying of groundwork for further development of molecular biology and biotechnology. The current increased rapid rate of reporting new findings is leading to greater understanding of cancer, one of mankind’s most feared enemies.”

Robert Gallo (18) credits the discoveries, reagents and techniques developed in the Virus Cancer Program retrovirus research with providing the tools and handles for studying the human retroviruses. Many of the advances in retrovirology provided a whole new generation of investigators in AIDS research the confidence to work with the techniques developed by their predecessors in the Virus Cancer Program.

Although the Program during its years of operation was disdained by many scientists unaffiliated with the Program (19), the passage of a few years and the application of historical perspective have allowed the Program to appear in a more positive light. One of the critics who maligned the (S)VCP for pouring vast sums of money into research on animal tumor retroviruses was Robert A. Weinberg (20) who, with many of his colleagues, felt that the VCP was a waste, a major boondoggle. Around 1982 they began to revise their thinking about the value of the (S)VCP. “Now we knew different. None of these retroviruses was ever found to cause a human disease but the cellular genes that they had captured from the genomes of distantly related species turned out to be enormously important for the understanding of human cancer. Now the obscure and the irrelevant suddenly became powerfully important for breaking open the human cancer problem”. From these beginnings, the first information about the many oncogenes,

suppressor genes and cellular signals emerged and has continued to provide help in understanding the origins of some human cancers.

These successes in the Virus Cancer Program, and the vast discoveries in non-tumor virology, as well as his admirable personal qualities, stand as a lasting legacy for Bob Huebner.

1) OBITUARY, ROBERT HUEBNER, 84, DIES; Found Virus-Cancer Connection. *The New York Times*, Saturday, September 5, 1998.
2) Dr. Jan Svoboda, In Memoriam Robert Huebner. 1999. *Folia Biologica* 45: 1-2. Praha (Prague). Through the courtesy of Dr. Janet W. Hartley
3) Personal communication—Harriet Huebner, Dr. Albert Z. Kapikian.
4) Personal communication—the Huebner Children.
5) Personal communication—Dr. James A. Rose
6) Personal communication—Dr. Robert Stevenson.
7) Personal communication—Dr. Carl G. Baker.
8) See note 1 in the chapter—Second Beginning, etc.
9) Letter from Dr. Frederick C. Robbins September 30, 1982 among the personal papers of Dr. Robert J. Huebner.
10) Letter from Dr. Albert B. Sabin October 1982 among the personal papers of Dr. Robert J. Huebner.
11) Letter from Dr. S. Rasheed October 18, 1982 among the personal papers of Dr. Robert J. Huebner.
12) Letter from Dr. Robert C. Gallo August 19, 1982 among the personal papers of Dr. Robert J. Huebner.

13) "Personal tribute to Bob Huebner" from Dr. Murray B. Gardner October 1982 among the personal papers of Dr. Robert J. Huebner.
14) See note 21 about Dr. E.M. Scolnick in the chapter—Personnel Relationships, etc.
15) Personal communication—Dr. Edward M. Scolnick March 20, 2001.
16) See note 21 about Dr. Vincent T. DeVita, Jr. in the chapter—Critics Anonymous, etc.
17) Baker, C.G. 2005. *An administrative History etc.*, On file in the NIH Historical Office. See previous chapters
18) Gallo, R.C. 1991. *Virus Hunting. Etc*. see previous chapters Basic Books. Pp. 92, 203.
19) See notes 8,9 and 11 in the chapter—Critics Anonymous, etc.
20) See note 24 on Weinberg, R.A. p. 195 in the chapter- Personnel Relationships, etc.

Chapter 24

Treading Water in the Secretarial Pool; Other Random Observations

At the end of his professional career, Bob Huebner received many accolades and tributes from friends and admirers, primarily from those persons who had interacted with him during the course of his many years at LID and NCI. Among these people were a succession of extremely loyal, devoted and competent secretaries. His work habits in the office were exasperating, and his personal foibles drove them to distraction. Nevertheless, several of his secretaries responded in writing to the request for testimonials at the time that Bob had his retirement party on October 18, 1982.

One respondent wrote: "I have many fond memories of my working days under your guidance, some funny (such as the call from Boston when you were supposed to be in New York City—wrong shuttle) [Bob was easily distracted and often absent-minded-EAB] but mostly I remember the feeling of competence you gave to so many of us. You assumed we were capable, and we had to live up to your expectations. Then there was the time you read me a portion of a paper you were writing on genetic predisposition to cancer and asked how it read—my answer was that if I understood it, I'd be helping to research it, not type it. You made all your staff feel an important cog in the machine—and we grew, I think, in stature because of your belief in us. Thank you and all the best for the future" (1).

Another responded in a different vein: “It is difficult to imagine NIH without you, but I hope that you will enjoy your retirement years and find new ways to utilize your many talents.

“You were Chief of LID or VCB (Viral Carcinogenesis Branch) during nearly all of my twenty years with those branches; however, I didn’t really get to know you until I left my cloistered existence on the first floor of Building 7 and moved upstairs to work directly under you. On my first day you bounded into the office and handed me the largest tube of warm blood that I have ever seen and said, “ Label it ‘Rocky. ’” I was very familiar with the thousands of samples drawn for the flu study and Junior Village so I knew that I must have more precise information for labeling. Wondering who this superhuman might be, I asked, “Rocky who?” You answered in a disgusting tone, “Rocky’s my bull! He’s covered with bovine warts.” Thus began my indoctrination into life upstairs and bovine warts.

“I have another choice memory of a day after a big snow storm when more than half of the Building 7 employees did not make it to work when you appeared around 10:30 am carrying a pillowcase full of books and papers. You had ridden a horse from the farm to wherever your car was and the pillowcase was your saddlebag. I was never sure whether true dedication or stubborn determination was responsible for your appearance on that snowy morning.

“There are also thoughts of neat stacks of data on my desk which you could reduce to rubble in less than two minutes. You never seemed to find the answers to your questions, and you usually left me with the feeling that my mind had been struck by the same whirlwind that hit my desk. I leave the accolades for your scientific achievements

for your colleagues who know best how to describe them but that does not mean that I do not appreciate your accomplishments, and I shall not forget them. I shall always remember how you came charging into Building 7 with your arms loaded with apple blossoms “for the girls.” It’s worth having your data messed up just to know a fellow like that!” (2)

In a similar vein, *Notes from a Jewish Grandmother* (portion of the logo on the stationery) read: “It is so hard to write just how I feel about knowing you. A tape would be so much easier, then we could laugh together. I want to begin by saying how in awe I was of you when we first met—the world renowned DR. ROBERT J. HUEBNER. I leaned later you were also a super boss and an understanding wonderful friend who would listen to your personal problems and feel for you. How lucky I was that Shirley Shiflett chose me for the opening way back in 1968 when she retired, and I took over. I can’t begin to tell you how frightened I was and concerned as to whether I could do the job. No matter how stupid I appeared you were there, very tolerant and patient. The first order of each day was to get your brief case. We never knew what we would find, the glasses you thought you had lost, the missing manuscript we had searched for weeks and weeks, stacks and stacks of notes. Needed a magnifying glass to decipher some of the writing. The notes were always on scraps of paper, around edges of journals, used envelopes; there were many, many times I told Jerry that one day we would find a note on T.P. [toilet paper]. Our pending basket was always full. I loved going to Frederick with you and to work with your guys, sure miss some of them, Bill, Red, Paul, Al, Bobbie. It hurt so much these last couple of years when we had to discard a lifetime of specimens—determining what to keep and what to discard. I felt so sad. The hurt was

still there when I had to start in on the files, but, Dr. Huebner, this is supposed to be a happy and memorable experience so I just want to say how glad I am that I know you, still always want to hug you. Dr. Huebner I love you" (3).

And more teasing words of friendship: " Dear Boss, For all of the years that I was your secretary, the job was always demanding, and it was a challenge to keep up with you (let alone trying to keep ahead of you) but it often had its lighter moments. My mornings in the office usually started by empting your brief case and sorting through the notes, manuscripts, protocols, letters, aspirins or even a pair of socks that somehow were overlooked the night before.

One day you called from Boston quite surprised to find yourself there. I could envision you at the Eastern Airline terminal in LaGuardia, deep in thought or writing a new protocol, and just following the crowd to a plane—in this instance one going to Boston instead of to Washington. Occasionally when John Estes was out of town, you'd spend the night in his Bethesda apartment rather than driving all the way to Ijamsville. Before John returned you would tidy up the place. You threw the secretaries into a frenzy one day when you called quite beside yourself because the vacuum cleaner wasn't working. While we pondered how to come to your rescue you called back. 'I found out what was wrong. I connected the tube to the exhaust hole. All the dust was blowing out of the bag.' Remember little *Pigpen* in the *Peanuts* comic strip? You, too, must have been surrounded by a cloud of dust. I will always remember the years that I worked with you. They were busy, exciting years made more pleasurable because you were the ideal boss—Love" (4).

The common thread that runs though these letters lends support to the affection that these women had for Bob. Very few high ranking or important men are heroes or are really beloved by their secretaries. Bob Huebner, as exemplified in these anecdotal messages, was capable of engendering loyalty and true affection in the dedicated women who worked with him and shielded him from some of his very human ineptitudes.

During Bob Huebner's 38-year professional career, he interacted with many of his peers both within and outside LID and NCI. They often had very vivid remembrances of their encounters with Bob and shared with him experiences, memorable and often humorous. These observations are related in no particular chronological order. Most of these anecdotes were written as tributes at the time of Bob's retirement.

Dr. Dorland J. Davis (5) was at the Laboratory of Infectious Diseases (then Division of Infectious Diseases) when Bob arrived there in 1944. Davis later became Chief of LID 1954-1956, and subsequently Director of NIAID from 1964 to 1975. He recalled how Bob would vividly describe his adventures in Alaska as the investigators sat around the brown bag lunch table in NIH Building 5. As Bob became integrated socially into the group, several of them, including Dorland Davis, played golf together at the old Indian Spring Country Club on the Colesville Pike [now Road] in Silver Spring, Maryland. "You [Bob] played a vigorous game, and your powerful drives put you deeper in the rough or woods than we could manage. It was fun though!" It appears that Bob played golf with more enthusiasm than skill. He did better at tennis, though, when he could compete with equivalent skill against his son Danny's high school tennis coach.

Dr. Alexis I. Shelokov (6), introduced earlier in this manuscript, humorously reminded Bob of their initial meeting: "Dear Bob, September 1949. I came to NIH to be

interviewed for a 1950 position in the Laboratory of Infectious Diseases. Arriving at the recently completed Building 7 [which at the time bore a plaque 'The Memorial Laboratory'], I was told by the receptionist that Dr. [Karl] Habel would be late for my appointment because he was at a seminar. So, Paula [Mrs. Shelokov](she came with me to see Bethesda) and I sat down in the lobby and proceeded to watch with interest the people going in and out of the building—some in white coats and others in blue coveralls. Soon I noticed two men in blue coveralls who walked up to the elevator; they were engrossed in a conversation about viruses. I listened with amazement to what they were saying, and as the elevator doors closed, I said to Paula something like 'My God, the NIH janitors not only look very intelligent, but they talk like professors.' A few minutes later, I was asked to go up to the second floor to meet Dr. Habel in his office. The man who greeted me turned out to be the skinnier of the two men in blue coveralls. In a while he took me to meet you—the legendary young rickettsiologist and virologist—still in blue coveralls." (In the mid 1950's the investigators in Building 7 discontinued wearing the blue coveralls when LID stopped working with highly virulent organisms.)

Dr. Morris Schaeffer (7), when he was with the National Center for Drugs and Biologics, Food and Drug Administration, Bethesda, Maryland, recalled his association with Bob, "Dear Bob—It has been my good fortune to have known you for a large part of your career. Our paths first crossed in 1946 in New York when you were unraveling the mystery of a new disease, rickettsialpox. You were then, as Burton Roueche′ described you in his story of that adventure 'The Alerting of Mr. Pomerantz,' a 'young, tough-minded and carefully unenthusiastic staff worker at the Institute.' Some dozen or so years later when I served as Director of New York's Public Health Laboratories, you visited the

City periodically, this time with uncurtailed enthusiasm, for your fascinating, imaginative and illuminating study [polyoma] among the rodent population and their microbes ---. [The Rouche′ article appeared in *The New Yorker Magazine*, August 1947]

"Success is judged by a variety of standards but your success is epitomized in these words of Ralph Waldo Emerson: 'To laugh often and much; to win the respect of intelligent people and the affection of children; to earn the appreciation of honest critics and endure the betrayal of false friends; to appreciate beauty; to find the best in others; to leave the world a bit better whether by a healthy child, a garden patch or a redeemed social condition; to know even one life has breathed easier because you lived. This is to have succeeded.'" Dr. Schaeffer eloquently described his extended, but infrequent, association with Bob Huebner.

Dr. Roger M. Cole (8), introduced previously, briefly summed up his own career and impression of Bob Huebner as follows: " Dear Bob, I thought that you had retired once or twice before. In retrospect knowing your boundless energy and constant spate of ideas, I should have known better—and I don't really believe it now. Having arrived at NIH in 1949—too late to be a part of your classic rickettsialpox adventure—I nevertheless profited from the subsequent association with you (and Joe Bell) in discovering and elucidating herpangina; in experiencing, in adjunct, the vagaries of Q fever at Bethesda and in the dairy study in Southern California (you recall, no doubt, the guinea pig-filled garage in Downey where I visited you and Lauri Luoto briefly in 1951); and in gaining valuable experience in administrative problems as your Assistant Chief of LID for a number of years. Since you wisely recognized that bacteriology was an area in

its own right, and later appointed me to succeed Art Saz as head of that section in ’64 and as Chief of a new Laboratory of Microbiology in ’67, our scientific paths diverged. (Now, unfortunately, bacteriology as such has become a memory in NIAID in Bethesda). However, I have not forgotten your enthusiasm for new ideas and for development of means to explore them. I must say that all this was not infrequently obscured by a deceptively unsophisticated approach: I learned better, eventually, how to read this! The first time that I appreciated the true speed of your reactions was on the occasion, when we were fishing in your creek at Ijamsville, you put your hand into the minnow bucket without looking, drew forth a water snake instead, and thereupon moved both vertically and horizontally with impassioned velocity!

“In all seriousness, however, let me say that I benefited in many ways from our early associations; that your scientific accomplishments, then and since, speak for themselves in a fashion that I cannot better; and that I wish you all that you wish for your retirement—even though I cannot imagine in your instance, the real occurrence of the latter. Sincerely.” Roger and Bob had a very cordial relationship over the years.

Dr. Stuart A. Aaronson (9), one of the organizers of the Huebner retirement event, came to NIH in 1967 as a research associate in Bob’s laboratory in NCI, and he succeeded Bob Huebner as Chief of the Laboratory of Cellular and Molecular Biology, NCI in 1982. He described his early association with Bob and an amusing anecdote in a detailed letter as follows: initially his group, consisting of new associates, was located off campus in one of the contract laboratories, and he had minimal opportunity to meet or interact with Bob. He had to become acquainted with Bob through the latter’s extensive bibliography on both the DNA and RNA tumor viruses. The first few months were spent

in the confusion of learning the arcane terminology and facts related to these viruses. On finally meeting Bob, Aaronson had "the impression of a gruff, big bear that I dare not disturb for fear that it would interrupt your [Bob's] train of thought or interfere with some momentous project. How wrong was that first impression. After gathering my courage, I took the initiative to talk with you and realized almost immediately how warm, interested in young people and their ideas, and how truly helpful you were. These attributes are the ones I cherish most, and it is this tradition I have tried hardest to emulate. You have been a bulwark of strength—supporting me, advising me, and helping me at very critical stages. For all of these things I will be forever grateful.

"Those who have worked with you have favorite anecdotes that often come to mind. My favorite is the one that demonstrates your resourcefulness in dealing with government bureaucracy, and, most importantly your concept of what the NIH is all about. The story concerns your return from a trip to East Germany with a pair of unique Graffi hamsters [named after Arnold Graffi, a German investigator], a strain of particular value for leukemia studies. The cage also contained the characteristic apple, an innovation you had introduced long before, as an efficient source of nutrients, which also maintained the animals' fluid balance. Customs was not impressed. Your importation of the hamsters violated all Public Health Service guidelines. Your response is a classic. You pulled out your PHS identity card and declared, 'I am the Public Health Service', and, with a flourish, deposited the remains of the apple in a waste-basket, a negotiated compromise that ended the matter. I daresay the custom official would be hard put to describe exactly what happened, but the hamsters were now Americans. Thinking quickly on one's feet is a very important component of any researcher's armamentarium. Dealing

with government bureaucracy of the intractable kind is an art form, and your career here at the NIH has proven that you are a Picasso. But the really important point, I think, is that besting the bureaucracy was never frivolous. You truly felt that service at the NIH was a commitment to the nation and to the public welfare, an orientation that you maintained throughout your career. You have instilled this concept in all of us who started here with you and have remained. For this and so many other things, I will always be grateful. Sincerely." This letter offers a genuine appreciation of Bob Huebner's interaction with young associates who matured under his leadership and guidance.

Dr. Jay A. Levy (10), another of the organizers of Bob's retirement party, was, at that time, Associate Professor, Department of Medicine and Research Associate, Cancer Research Institute at the University of California, San Francisco. His retirement tribute to Bob was as follows: "Dear Bob, It is a great pleasure to share in this day honoring you, one of the world's greatest scientists. I have always considered my meeting you as both a tremendous stroke of good fortune and an inevitable plan of destiny. By my path meeting yours, I was able to benefit from the tremendous insight, scientific knowledge and creative research that have highlighted your career and punctuated your brilliant achievements. I feel deeply privileged to have been associated with you and very proud to be considered one of your students.

"I often enjoy reflecting on the first time we met. It was during medical school at Columbia when I went to the Francis Delafield Hospital to hear you lecture on "Footprints of Tumor Viruses." At that time you discussed your research on adenoviruses and SV40. This discovery of yours heralded future approaches at detecting latent virus infections in many cancers. I remember questioning you about Burkitt's lymphoma, and

was impressed with your expansive knowledge of this relatively new subject, and particularly the fact that you took time to talk to me. I did not forget that experience, and knew that I had to find a position in your laboratory. During my visit to NIH in my senior year, I met you again and was further impressed by your infectious enthusiasm and drive. I was really happy to be selected to work in your laboratory.

"One of the first amusing incidences that I can recall after arriving at NIH was visiting your farm in Maryland where you introduced me to your Angus cows, whose individual numbers you were proud to identify. However, trailing behind us was Susie [Huebner's daughter], who corrected you often during our tour, much to your chagrin and my embarrassment. Then, with a quick aside from you, she disappeared!

"I also recall our tennis matches, particularly the one in which your competitive spirit was so intense that the set ended at 7-5 with me just barely eking out a victory. I had real mixed feelings about winning after your valiant performance, including a fall at the net. After that game we sat down by the side of the court and talked science, as we often did. At that moment, you were evolving your ideas on viruses in embryogenesis, and expressed your belief that you would find the viral gs antigen in early mouse embryos. Our discussions were instrumental in my own thinking about the possible role of endogenous viruses in early and late developmental processes.

"During my three years as your staff associate, I treasured our meetings when you spoke of my research. I appreciated even the critical moments when you questioned my thinking and forced me to come up with additional data to support my conclusions. Our interactions over the past fifteen years have been memorable and very helpful. Thank you for taking such an interest in me, and remaining a constant advisor. Your periodic visits

to the West Coast through the PACTVIGR meetings were exciting and creative in many ways. They certainly brought many of us together with a sincere dedication to research, and also challenged us (at least me) to prove to you that we were still thinking!

"Through all these efforts your energies never ceased to astound me. Do you recall running up 130 steps to visit me in my apartment on Telegraph Hill? Still recovering your breath, you immediately launched into some enthusiastic discussions on type C viruses with even more experiments to perform. You have an incredible ability to form new ideas, but to frustrate me with your eagerness to have the results.

"Thank you, Bob Huebner, for encouraging my curiosity, enthusiasm, and joy of scientific thought. I have sought to follow in your footsteps and be a tribute to you. You have made broad strokes in many areas of science; I had the privilege of being with you during your tumor virus days. In this field, you have paved the direction of the world toward recognizing the importance of virogenes and oncogenes. Your creative thinking, innovative scientific approaches, and ability to organize a research program helped establish the international efforts now dedicated to retroviruses.

"You are a legend in your own time, and will be long remembered for your contributions to our knowledge in many scientific fields including infectious disease, immunology, cell biology, virology and pathology. To me you are remembered as a creative scientist, a respected teacher and a sincere friend. With warmest regards." Jay Levy clearly viewed Huebner as an incomparable role model.

Helene S. Smith (11), Ph. D., Assistant Director, Peralta Cancer Research Institute, Oakland, California (one of the contract laboratories in the Virus Cancer Program) wrote the following comments to Dr. Jay A. Levy in response to requests for

Huebner tributes and anecdotes: " Dear Jay, I'm really sorry that I wont be able to be present at Bob Huebner's farewell celebration, Please extend my very best wishes to both Bob and Harriet.

" I would like to share with you one of my very special fond memories of Bob. When I was getting ready to leave George's [probably George Todaro] lab, I became pregnant just at the time for job interviews. Needless to say, at that time no one was thrilled about giving a position to any woman, let alone a pregnant one. Yet, Bob was very encouraging and said that I could go to any contract program that I wanted, and he would arrange to have money for me and my technician added to the program. When I mentioned to Harriet how grateful I was that Bob had faith in my abilities as a scientist, even if I was pregnant, she replied, 'Bob believes anyone who had worked as hard at a career as you have would not ask for a job unless she planned to work after the baby was born, so what was the problem anyway?' It's a shame that today, after 12 years there are still many men in science leadership positions who aren't as 'liberated' as Bob was then, or as kind.

"This is just one of my fond memories of Bob. He was a great boss to work for and a very fine man. Sincerely." This is a further example of Bob's well-ingrained liberal attitude toward woman investigators in the sciences.

One group of associates with whom Bob had a long-term cordial relationship was the scientists and personnel of the contract laboratories who collaborated closely in many of Bob's research projects. The following anecdotes relate some of the group's observations about Bob.

The letter written by Richard E. Kouri, Ph. D., Director of Research, Microbiological Associates, Bethesda, Maryland related the following: " Dear Bob, You have no idea how difficult it is to put to paper, some of the incredible memories that you are leaving behind. I know of no other person who has brought quite so much color into science as you have over the past years. Some of these stories are almost legends to the scientific neophyte. Examples of some of the 'legends' of which I am aware are:

- The trip from Juneau to Seattle with a boat-load of prostitutes
- The time you were locked out of your hotel room in the 'altogether' with only a morning newspaper for adornment
- Stalking wild mice in downtown Manhattan in houses of ill repute …….

"Simple conversations with you can also be an experience. I have chosen one particular conversation that stands out in my memory. I don't know if you will recall the experience, but it occurred when I encountered you and Harriet at Dulles Airport. Because of the flavor of the conversation, I have written down the actual dialogue and assigned a cast of characters. It happened like this. To set the scene: I was scheduled to go to my first Pacific Coast Virus Group (PACTVIGR) meeting in Berkeley, California. As usual, I was running late and as soon as I arrived, I dashed into the Mobile Lounge at Dulles Airport where passengers of the same destination await their flights. Among all of the people, I spied you and Harriet, and from that point it was downhill the rest of the way …… the conversation went like this:

Bob: 'Hi, Dick. Where are you going?'

(Others turn and look askance at the question, since everyone in the lounge is to go to the same destination)

Dick: 'Well, uh, Bob, I'm going to San Francisco.'

Bob: 'No kidding, so am I!'

Dick: 'I am going to the PACTVIGR meeting'.

Bob: 'Really? (Turns to Harriet). Harriet are we going to the PACTVIGR meeting too?'

Harriet: (With a sigh). 'Yes, Bob, We are going to that meeting too.'

Bob: (Turning to Dick). Yeah, Dick, we are going to that meeting too.'

Dick: 'That's good, Bob, are you giving a paper?'

Bob: (Turns to Harriet). 'Hey, Harriet, am I giving a talk?'

Harriet: (In a tired voice) Yes, Bob, you are giving a talk'.

Bob: (Turns to Dick) 'Yeah, Dick, I am giving a talk'.

Dick: (Glutton for punishment inquires): 'What's your talk about this time, Bob?'

Bob: ' Hey, Harriet, what am I talking about?'

Harriet: (In a resigned voice, tiredly repeats verbatim the exact topic of science Bob will present at the meeting)

Dick: 'Do you have your slides?'

Bob: 'Say, Harriet, do I have my slides?'

Harriet: (Serenely) 'Yes, Bob, you do have your slides.'

Bob; 'Yeah, Dick, I do have my slides,'

(At this point, all of the people in the lounge, who have been unavoidably eavesdropping on the entire conversation, begin applauding—HE DOES HAVE HIS SLIDES!)

"Bob, I know that you will go down in history as a great (if not, unusual) conversationalist! [EAB—Also, Harriet was the ultimate efficient administrative assistant.]

"I also know that I simply echo the feelings of all your colleagues when I say that we have all enjoyed working with you and have much admired your fine contributions to the scientific community. You have added a sparkle to the old stereotype of the staid, plodding scientist If anything, you have given us all a bad reputation!)

"I am delighted to be able to participate in your retirement sendoff, and I wish you many, many years of happiness. Your friend." This was an amusing and heartfelt accolade from a long-time associate.

Dr. Paul Price (13), formerly of Microbiological Associates, wrote this letter when he was at the Centers for Disease Control, Atlanta, Georgia. This letter and the following described incidents during the limited period when Bob Huebner was sporting a mustache on his upper lip. Dr. Price wrote, "Dear Bob, Congratulations on your retirement after 40 years of service at NIH. I guess my association with you goes back to about 20 of those years. During those years you were not only my project officer but also my mentor and, most important, my friend. When I was down or just plain frustrated, I knew that I could count on you for guidance and moral support. My association with you was always educational, almost always very pleasant and on, rare occasions, amusing.

"I would like to remind you of one such occasion. No one in the government could understand why you spent so much time and effort in developing a contractual research mechanism—especially your interest in and efforts to develop and extend programs at Microbiological Associates. Well, one Christmas many years ago we decided

to set the record straight. You were our honored guest at our annual Christmas party. You, of course, were introduced by our President, Mr. Schwartz, who also had (by chance) a small "Dr. Huebner look-alike" mustache. Mr. Schwartz then introduced the members of the staff who came in one by one—Aaron Freeman, Ron Wolford, me, and the rest of the guys and girls that make up your laboratory at Micro. All of us had the same Dr. Huebner mustache. Obviously you were only taking good care of all your illegitimate children.

"Again good luck on your retirement. Enjoy it in health and happiness. Love." This anecdote provides another example of imitation being the sincerest form of flattery—even if done tongue in cheek!

Finally, Aaron E. Freeman, Ph. D., who spent many years working at Microbiological Laboratories working with Bob, wrote the following: "Dear Dr. Huebner, Please don't think you can escape me by retiring. I will keep coming to you for that special insight you could always provide. You always knew the significance of my work—even when it was not accepted by others—even when I was unsure myself.

Over the years I have written a number of papers and have spoken at numerous conventions. But, there is only one special presentation that everyone remembers.

I was just out of graduate school and was working on a post-doctoral level on contract at Microbiological Associates. You were the project officer. Somehow, I was invited to speak at the PACTVIGR meeting in Los Angeles. I began my presentation with an explanation of transformation. First I described a normal culture. It was a photograph of you. Then I described a morphological alteration. It was the same photograph of you

but now sporting your brand new mustache. Finally I characterized true transformation. It was the same photograph but you had a third eye in your forehead.

I must admit that I was more than somewhat scared. First, because I am a complete introvert and the audience was quite large. I was mostly afraid of your reaction, however, because at that time I hardly knew you at all.

As I presented the slides, there was an uproar from the audience. One person in particular was laughing so loudly that it seemed he would literally roll in the aisles. I looked down and saw that it was you.

Our relationship grew from that day on. I know that I owe whatever small career in science that I have enjoyed to your support—from concept to design to interpretation to publication.

I have not been a giant myself—but I have walked with one. Sincerely." This anecdote illustrates Bob's sense of humor, self-deprecation and the ability to not take himself too seriously.

This sample of the many congratulatory notes directed to Bob at the time of his retirement paints a vivid and detailed landscape of Bob's colorful personality as experienced by his interaction with the diverse individuals who were touched by him during his active, productive working life.

Notes—Treading Water in the Secretarial Pool; and Random Observations

The letters listed below were in the personal papers of Robert J. Huebner.

1) Letter to Robert J. Huebner from Shirley “G”. October 9, 1982.
2) Letter to Robert J. Huebner from Shirley Shiflett. September 20, 1982.
3) Letter to Robert J. Huebner from Sophie. September 13, 1982.
4) Letter to Robert J. Huebner from Olga. October14, 1982.
5) Letter to Robert J. Huebner from Dorland J. Davis, M.D. September 24, 1982.
6) Letter to Robert J. Huebner from Alexis I. Shelokov, M.D. October 18, 1982.
7) Letter to Robert J. Huebner from Morris Schaeffer, M.D. October 18, 1982.
8) Letter to Robert J. Huebner from Roger M. Cole, M.D., Ph. D. September 17, 1982.
9) Letter to Robert J. Huebner from Stuart A. Aaronson, M.D. October 15, 1982.
10) Letter to Robert J. Huebner from Jay A. Levy, M.D. September 22, 1982.
11) Letter to Robert J Huebner from Helene S. Smith, Ph. D. September 8, 1982.
12) Letter to Robert J. Huebner from Richard E. Kouri, Ph. D. September 2, 1982.
13) Letter to Robert J. Huebner from Paul Price, Ph. D. August 10, 1982.
14) Letter to Robert J. Huebner from Aaron E. Freeman, Ph. D. August 4, 1982.

Appendix A:

Curriculum Vitae

CURRICULUM VITAE

Name: Robert J. Huebner

Date and Place of Birth: February 23, 1914, Cincinnati, Ohio

Date and Place of Death: August 26, 1998, V.A. Medical Center, Coatesville, Pennsylvania.

Citizenship: United States

Marital Status: Married; nine children

Education:

1932	Graduated from High School
1932-1935	Xavier University
1936-1938	University of Cincinnati
1938-1942	M.D., St. Louis University School of Medicine

Brief Chronology of Employment:

1942-1943	Internship in U.S. Public Health Service Hospital, Seattle, Washington (Assigned to U.S. Coast Guard)
1943-1944	Medical Officer in Coast Guard, Alaska Command. Transferred to U.S.P.H.S. Infirmary, Washington, D.C.
1944-1949	Commissioned Officer, USPHS Laboratory of Infectious Diseases, National Microbiological Institute, National Institutes of Health, Bethesda, MD
1949-1956	Chief, Section of Virus and Rickettsial Diseases, Laboratory of Infectious Diseases.
1956-1967	Chief, Laboratory of Infectious Diseases.
1967-1968	Chief, Laboratory of Viral Diseases. Above in National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD
1968-1977	Chief, Laboratory of Viral Carcinogenesis (RNA Tumor Viruses), National Cancer Institute, Bethesda, MD.
1977-1982	Expert, Laboratory of Cellular and Molecular Biology, National Cancer Institute, Bethesda, MD.
1982	Retired.

Societies:

Alpha Omega Alpha, Medical School Honors Society

Sigma Xi, Scientific Honorary Society

National Academy of Sciences

New York Academy of Sciences

American Epidemiological Society

The Foundation for Advanced Education in the Sciences, Inc.

International Association for Comparative Research on Leukemia
and Related Diseases.

International Union Against Cancer

American Association for the Advancement of Sciences

Federation of American Societies for Experimental Biology
(Immunology)

American Academy of Microbiology

American Association for Cancer Research, Inc.

Honors and Other Special Scientific Recognition:

Certificate of Merit for Scientific Exhibit on Rickettsialpox,
American Medical Association, 1947

Award, Biological Sciences, Washington Academy of Sciences, 1949

Bailey K. Ashford Award, American Society of Tropical Medicine, 1949

Certificate of Merit in recognition of exhibit, Epidemiology of Q Fever,

American Veterinary Medical Association, 1950

St. Louis University Medical Alumni Merit Award, 1959

Citation ("In recognition of ... accomplishments in unravelling disease causation in children..."), Variety Children's Hospital, 1960

James D. Bruce Memorial Award, American College of Physicians, 1964

Pasteur Medal, Pasteur Institute, Paris, France, 1965

Honorary Degree, Doctor of Laws, University of Cincinnati, 1965

Distinguished Service Medal, Public Health Service, 1966

Howard Taylor Ricketts Award, University of Chicago, 1968

National Medal of Science, 1969

Honorary Degree, Doctor of Science, Edgecliff College, Cincinnati, 1970

Kimble Methodology Research Award, 1970

Rockefeller Public Service Award, 1970

Honorary Degree, Doctor of Science, University of Parma, Italy, 1970

Guido Lenghi Award, National Academy of the Lincei, Rome, 1971

Honorary Degree, University of Leuven, Belgium, 1973

Founders Award in Cancer Immunology, Cancer Research Institute Inc., 1975

Honorary Lectures:

Gehrmann Lecture, University of Illinois College of Medicine, 1955

Benjamin Know Rachford Lecture, University of Cincinnati, 1956

Eli Lilly Lecture, 1957

Gudakunst Lecture, University of Michigan, 1958

Harvey Lecture, 1960

National Institutes of Health Lecture, 1960

Carl Puckett Lecture, 1960

Helen Francis McLain Lecture, 1965

Pasteur Institute Lecture, 1965

Guyiteras Lecture, 1968

Ricketts Lecture, 1968

William O'Brien Professorship Lecture (Minnesota chapter of American Cancer Society), 1972

Frontiers in Biology Lecture, Iowa State University, 1972

Southern Medical Association Lecture, 1973

Regents' Lectureship, University of California, Davis, 1974

Cesare Massari Lecture, VI Perugia International Conference on Cancer, 1977

Research Interests:

Viral Oncology, Immunology, Epidemiology, Cancer Immunoprevention and Immunotherapy, Infectious Diseases

Number of Publications: 425

Appendix B:

Bibliography

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Appendix C:

Abbreviations

AEC—Atomic Energy Commission.

AK—A genetic strain of mice.

APC—Adenoidal-Pharyngeal-Conjunctival—This was the early term used to designate adenoviruses before the formal adoption of the current nomenclature.

BALB/c—A genetic strain of mice.

C3H—A genetic strain of mice.

CCNSC—Cancer Chemotherapy National Service Center.

COFAL (test)—Complement fixation for avian leucosis.

COMUL (test)—Complement fixation for murine leukemia.

DBC—Diploid bovine conjunctiva.

ECHO (virus)—Enteric Cytopathic Human Orphan.

FA—(Immuno-) fluorescent assay.

HA—Hem-adsorption (virus).

Hep—A cell line from human cancer tissue.

HI—Hem-agglutination-inhibition.

HT-ST—High temperature, short time.

LCM—Lymphocytic choriomeningitis.

LID—Laboratory of Infectious Diseases.

LVD—Laboratory of Viral Diseases.

Mu—Millimicron.

MSV—Mouse sarcoma virus.

NACC—National Advisory Cancer Council.

NCAB—National Cancer Advisory Board.

NCI—National Cancer Institute.

NIAID—National Institute of Allergy and Infectious Diseases.

NIH—National Institutes of Health.

PCF—Pharyngoconjunctival fever.

PHS—Public Health Service.

RDE—Receptor destroying enzyme.

REO (virus)—Respiratory-Enteric-Orphan.

RIF—Resistance inducing factor (re avian leucosis).

RSV—Rous sarcoma or respiratory syncytial virus

Sarc—The transforming gene (oncogene) in avian sarcoma viruses.

cDNAsarc—The complementary DNA oncogene of avian sarcoma made by reverse transcriptase.

Src—The proto-oncogene (in normal tissue) of sarc.

STS—Solid Tumor Segment.

SG—Surgeon General.

Type B, C—Retroviruses classified according to their appearance in tissue under the electron microscope.

USC—University of Southern California.

UCSF—University of California San Francisco.

USPHS—United States Public Health Service.

VCB—Viral Carcinogenesis Branch.

VRRB—Virology Research Resources Branch.

Appendix D:

Glossary

Adenine (A)—One of the purine bases that pairs with thymine (T), found in DNA or RNA.

Adenovirus—Class of virus that infects many animals including humans and contains DNA. First described in 1953 by W.P. Rowe, Huebner, R.J., et al.

Agglutinin—An antibody that causes agglutination or clumping of specific antigens such as bacteria or (red) blood cells.

AIDS—Acquired Immunodeficiency Disease Syndrome. A disease caused by the HIV (Human Immunodeficiency Virus) retrovirus that slowly undermines the immune system by destroying helper (white blood) T-cells (T-4 or CD-4 cells).

Aliquot—A fractional part divisible into the whole without a remainder; a portion of a liquid or solid substance that represents a known quantitative relationship to the original amount.

Amniotic cavity—One that appears in the embryonic mass of the ovum; the amnion is the inner of the fetal membranes, a thin transparent sac that holds the fetus suspended in the amniotic fluid.

Annealing (Hybridizing)—The rejoining of separate strands of DNA to form a double helix.

Anorexia—The loss of appetite seen frequently with fevers and digestive disorders.

Anti-oncogene/tumor-suppressor gene—A gene that acts to suppress unwanted cell division.

Antibody—A protein of the immune system that recognizes and binds to foreign molecules (antigens).

Antigen—A molecule that causes an immune response and which is recognized and bound by an antibody.

Aortic aneurysm—An abnormal localized dilatation of the aorta due to any structural weakness of the aortic wall. The aorta is the major artery that runs in the back of the chest and abdominal wall.

Apoptosis—The programmed suicide of unwanted cells for the good of the whole animal.

Arthropod—A member of the phylum Arthropoda, invertebrate animals characterized by bilateral symmetry, chitinous skeletons, segmented bodies and jointed paired appendages. Examples include insects of all kinds, spiders, crabs, lobsters, etc.

Aseptic meningitis—A non-purulent (pus-free), inflammation of the membranes of the spinal cord and brain usually caused by viruses and not by bacterial organisms.

Ataxia—Muscular in-coordination especially manifested by attempts at muscular movements.

Avian—Pertaining to or characteristic of birds.

Bacteriophage—A term applied to a group of transmissible agents, usually viruses, which are capable of inducing lysis or dissolution of certain bacterial cells. They are widely distributed in nature and have been used in pioneering studies of bacterial genetics and molecular biology.

Base—An alkaline chemical substance, in particular, the cyclic nitrogen compounds found in DNA and RNA.
Base analog- A chemical that resembles a base of a nucleic well enough to fool a cell into using it instead.

Base pair—Two bases held together by hydrogen bonds.

Base pairing—When two complimentary bases (A with T or G with C) recognize each other and are held together with hydrogen bonds.

Biliary colic—The pain usually severe and spasmodic caused by the pressure or passing of gallstones.

Bio-safe facility—A secure physical structure designed to confine dangerous or highly communicable infectious organisms. It is usually graded from levels I to IV of safety for workers and for the confining of organisms.

Cancer—The disease due to the unplanned growth and division of mutant body cells.

Capsid—The protein shell surrounding the DNA or RNA of a virus particle.

Carcinogen—Any agent that causes cancer.

Carcinogenesis—The process of causing cancer.

CD4 protein—A protein found on the surface of many cells of the body's immune system, primarily the helper T cells.

Cell—A cell is the basic unit of life. Each cell is surrounded by a membrane and has a full set of genes that provide it with the genetic information necessary to operate.

Cell-mediated immunity—Immunity reactions due to the cells of the immune system acting as a whole as opposed to those due to antibody molecules (humoral immunity).

Central dogma—The basic plan of genetic information flow in living cells that relates genes (DNA) to message (RNA) and to proteins.

Cervical—Pertaining to the region of the neck.

Cholecystitis—An inflammation of the gallbladder, either acute or chronic, usually due to the presence of gallstones.

Column chromatography—A chemical analysis by which a mixture of substances is separated by fractional extraction or adsorption on a porous solid (column of e.g. aluminum oxide or filter paper) by means of flowing solvents.

Chromosome—A structure bearing the genes of a cell and made of DNA.

Cistron—A segment of DNA or RNA that encodes a single polypeptide chain.

Clone-a-gene—The process of obtaining the DNA making up a particular gene and putting it into a suitable vector such as a plasmid (or a virus).

Cloning- The making of an exact genetic duplicate of something, including an entire individual.

Codon—A group of 3 RNA or DNA bases that encodes a single amino acid.

Cold agglutinin—A red blood cell agglutinin that is auto-reactive and markedly increased in activity at 4C and that has little or no activity at 37C. Cold agglutinins occur as part of the disease process in some cases of viral and mycoplasmal infections.

Commensal—One of two organisms that live in an intimate, non-parasitic relationship.

Complement—It is a normal component of blood, a team of proteins, which destroys invading bacteria or foreign antigens after being alerted to their presence by the binding of antibody. It also causes lysis (hemolysis) of red blood cells sensitized by a hemolysin (an agent destructive of red blood cells).

Complement-fixation—The action of a complement, a constituent of fresh blood serum, on an antigen which in turn has been acted upon by its antibody. During the uniting of antigen, antibody and complement, the complement is rendered inactive or destroyed. This process is known as fixation of complement, and the resultant fixation prevents the hemolysis of red blood cells used as a marker for the process. This reaction forms the

basis of the Wasserman and Kolmer tests for syphilis and tests for other infectious diseases.

Complementary DNA (cDNA)—The DNA sequence complementary to an RNA sequence usually to mRNA (messenger RNA—may be generated through reverse transcriptase).

Conjugation—The transfer of genes between bacteria involving cell-to-cell contact.

Conjunctivitis—An inflammation of the conjunctiva, the mucous membrane that lines the eyelids and is reflected onto the eyeball.

Contact inhibition—This occurs when normal cells prevent their neighbors from dividing by touching them. This is used for some tissue culture cell lines.

Coryza—"Common cold" like illness". "Cold in the head". An acute catarrhal (runny nose) inflammation of the nasal mucous membranes.

Cowpox—A disease of cattle caused by a virus (vaccinia) closely related to smallpox. Cowpox causes only a very mild illness in humans but the virus is used as a vaccine to prevent smallpox (vaccination).

Coxsackie virus—A group of viruses, the first of which, was isolated in 1948 from two children in Coxsackie, NY. There are 23-24 group A viruses and 6 group B viruses (see descriptions of illnesses and virus properties in the text). They are classified among the Enteroviruses (infection of the digestive tract) and the Picornaviruses (pico = small, RNA viruses).

Cytology—The science that deals with the formation, structure and function of cells.

Cytolytic—The dissolution or destruction of living cells.

Cynomolgus monkey—A type of monkey used in research.

Cytomegalovirus—A virus that has been included in the herpes virus family. The virus is widespread but rarely produces clinically detectable disease except in young infants. It is transmitted through the placenta to the fetus from a mother with latent infection. Most infected infants are symptom-free but when the virus produces illness it takes the form of a widely disseminated infection that results in a fatal illness known as cytomegalic inclusion disease. The virus frequently produces an opportunistic infection in patients with AIDS.

Cytopathic (cytopathogenic)—A process capable of producing cellular changes in disease.

Demography—The social science of people considered collectively. It includes statistical analysis of race, residence, occupation, education, physical size, medical condition and usually economic status.

Deoxyribose—The sugar with five carbon atoms that is found in DNA.

Diploid—A cell containing two copies of each gene. It describes the somatic cells that contain twice the number of chromosomes present in the egg or the sperm.

DNA—Deoxyribosenucleic acid, the nucleic acid polymer that manufacture genes. It is a complex protein of high molecular weight consisting of deoxyribose, phosphoric acid and four bases (two purines—adenine and guanine and two pyrimidines—thymine and cytosine). These compounds are arranged as two long chains that twist around each other to form a double helix joined by bonds between the complementary components. It is a nucleic acid present in the chromosomes of the nuclei of cells, and it is considered the chemical basis of heredity and the carrier of genetic information for all organisms except the RNA viruses.

DNA fingerprint—An individually unique pattern due to multiple bands of DNA produced using restriction enzymes, separated by electrophoresis and usually visualized by Southern blotting.

Double helix—The structure in which two strands of DNA are twisted spirally around each other.

Ecology—The science of the relations and interactions of the totality of organisms to their environment including the relations and interactions of organisms to each other in that environment.

Ectoparasite—A parasite that lives on the outer surface of the body, e.g. fleas, lice ticks, mites.

Ectromelia virus—A pox-like infection latent and endemic in some stocks of mice.

Electrophoresis—The movement of charged particles in solution towards an electrode of the opposite charge; it is used to separate nucleic acids and proteins.

Encephalopathic—A term that describes any process resulting in dysfunction of the brain.

Endemic—This term describes a disease peculiar to and more or less recurring continuously in a particular locality or population, but only in a small number of cases. It is used in contrast to the terms sporadic or epidemic.

Entamoeba histolytica—A genus of ameba, a single cell, motile parasite. Specifically, it is a pathogenic form of ameba, the cause of amebic dysentery and tropical abscess.

Enteropathogenic Escherichia.coli—This term describes the capability of an organism to cause an intestinal disease. Specifically, in E. coli it describes the bacterial strain that produces hemorrhagic diarrhea, frequent rupture of red blood cells and acute kidney failure.

Enterovirus—A virus that multiplies in the human intestinal tract. The group includes Coxsackie viruses, polioviruses, ECHOviruses, rhinoviruses and others (unclassified).

Entomologist—A person who deals in the study of insects and their relationship to disease.

Enzootic—This describes the condition or infection in animals, comparable to endemic in humans.

Enzyme—A protein that carries out a chemical reaction but remains unchanged by the reaction, a catalyst.

Eosinophilic meningitis—An inflammation of the membranes of the spinal cord and brain characterized by the predominance of white blood cells with granules that stain red with the acid stain eosin.

Epidemic—The appearance of an infectious disease or a condition that attacks many people at the same time in the same geographical area.

Epidemiology—The division of medical science concerned with defining and explaining the interrelationships of the host, agent and environment in causing disease (medical ecology). Its practitioners are the epidemiologists.

Epizootiological—This term describes the epidemiology among animal infections transmitted to humans.

Erythema—This describes a form of rash showing diffuse redness over the skin or other surfaces.

Eschar—A scab or a slough especially one following a cauterization of a burn or a dried-up pustule.

Etiology—The term relates to the study of the causes of disease. Its usual usage relates to the cause of a single disease.

Eukaryotic cell—An advanced cell of higher organisms that has several chromosomes within a compartment called a nucleus.

Exacerbation—The aggravation of symptoms or increase in the severity of a disease.

Explant—A piece of living tissue removed from the body and transferred to an artificial culture medium for growth, as in tissue culture.

Expression (of a gene)—The synthesis of the protein or gene product as a result of transcription, processing and translation.

Fauces—The constricted opening leading from the mouth and oral pharynx (throat). It is bounded by the soft palate, base of the tongue and the palatine arches. The anterior pillars of the fauces are known as the glossopalatine (tongue and palate) arch and the posterior pillars as the pharyngopalatine (throat and palate) arch.

Febrile—A term for feverish or pertaining to a fever.

Feral—A term referring to existing in a wild or untamed state, especially after having reverted to such state from domestication. The term is usually used in reference to or characteristic of a wild animal.

Fibroblast—Any cell or corpuscle from which connective tissue is developed.

Fluorescence—When a molecule absorbs light of one wavelength and then emits light of another longer, lower energy wavelength. This property is used in immunology studies by combining antibodies with fluorescent compounds.

Fomite—Any substance that absorbs and transmits infectious materials.

Gametes—These are cells specialized for sexual reproduction that are haploid, i.e. they have one set of genes.

Gel—A semi-solid made by a polymer that forms a cross-linked meshwork in water.

Gel electrophoresis—The electrophoresis of charged molecules through a gel meshwork in order to sort them by size.

Gene—A unit of genetic information.

Genetic engineering—The alteration of an organism by deliberately changing its DNA.

Genome—The entire genetic information from an individual. In a virus it is the portion that contains the genes.

Genotype—The description of an organism at the genetic level.

Genus—In biologic nomenclature it is the next level of organization up from the species; a group of related species.

Germ line cells—These are reproductive cells producing eggs or sperm that take part in forming the next generation.

Guanine—One of the purine bases found in DNA or RNA and which pairs with cytosine.

Haploid—This describes cells possessing only a single copy of each gene.

HeLa cells—These are the initials of a patient with cancer whose malignant tissue gave rise to a cell line used in tissue culture.

Hemadsorption—The process of adherence of certain red blood cells to a tissue culture infected with some viruses. It is used as a screening method to determine the presence of influenza or parainfluenza viruses growing in the tissue culture.

Hemagglutination—The clumping of red blood cells.

Hemagglutination-inhibition—The prevention of the clumping of red blood cells by interaction or the blocking of the antibody or virus that otherwise causes hemagglutination.

Hemangio-epithelioma—A tumor, usually malignant, composed of a mixture of small blood vessels and epithelial cells, i.e. the layer of cells forming the epidermis of the skin or the surface layer of the mucous and serous membranes.

Hemoglobin—The oxygen carrier protein that carries oxygen in the blood.

Herpesvirus—A family of viruses of similar structure and growth characteristics including Herpes 1&2, varicella-zoster (chickenpox), Epstein-Barr virus (infectious mononucleosis, Burkitt's lymphoma) cytomegalovirus and others. At least 8 members of the group have been given numerical designations. They are DNA viruses and members have been linked to human cancers or have been responsible for opportunistic infections in immuno-compromised patients.

Herpes zoster (shingles)—An acute infection caused by the varicella-zoster (chickenpox) virus often after a prolonged latent period following childhood chickenpox. It is characterized by a painful skin eruption with chickenpox-like pustules that follow the circumscribed skin distribution of sensory nerve spinal segments. It is often associated with or predictive of cancerous illnesses.

Heterologous—A tissue, cells, blood or other material obtained from a different individual or species.

Heterozygous—The state of having two different copies or alleles of the same gene.

Histological—The term pertaining to the study of the microscopic structure of tissue.

HIV (human immuno-deficiency virus)—The causative agent of AIDS. It is a member of the retrovirus family.

Homologous—The term referring to objects similar or identical in fundamental structure and in origin but not necessarily in function.

Homozygous—The state of having two identical copies or alleles of the same gene.

Hormone—A regulatory molecule that carries commands from one tissue to another in the body fluids.

Hybrid DNA—an artificial double-stranded DNA molecule made by two single strands from two different sources.

Hybridization (annealing)—The making of an artificial double-stranded molecule from two single strands from different sources; the production of hybrids by cross breeding (offspring of parents from unlike races, cultures or species).

(Hydroxy)apatite—Apatite is a variously colored calcium fluoride phosphate Ca5F(PO4)3 with chlorine, hydroxyl (OH) or carbonate sometimes replacing the fluoride. It may be used in column chromatography as the adsorption substance.

Hyperplastic—The term for excessive proliferation of cells in the normal tissue arrangement of an organ.

Immunology—The study of immunity to disease.

Immunization—The process of preparing the immune system for future infection by treating the patient with weak or killed versions of an infectious agent.

Immunoglobulin—Another name for an antibody protein.

Infectious mononucleosis—An acute infectious disease that infects lymphoid tissue primarily. It is characterized by enlarged, often tender lymph nodes usually in the neck and an enlarged spleen with great increases of atypical or abnormal mononuclear leukocytes in the blood, frequent abnormal liver function and a white discharge on the tonsils. It is caused by the Epstein-Barr virus. It is primarily a disease of young adults and is popularly referred to as the “kissing disease”.

Influenza virus—A member of the orthomyxovirus family with eight separate ssRNA (single-stranded) molecules.

Intussusception—The slipping of one part of an intestine into another part just below it. It is noted chiefly in young children and infants causing pain and intestinal obstruction. It usually occurs in the ileocaecal region (where the small intestine joins the large intestine close to the appendix).

Karyotype—A systematic array of the chromosomes of a single cell in the metaphase stage (of mitosis = arranged in an equatorial plate). The human male consists of 22 pairs of chromosomes plus the XY pair. The female also has 22 pairs but has the XX pair in place of the XY.

Keratoconjunctivitis—The inflammation of the cornea of the eye as well as the conjunctiva.

Killer cell—A type of T-cell whose job is to kill other cells of the body that have become "foreign" due to cancer or infection.

Leucosis—A leukemia-like illness in a member of the avian species.

Leukemia—A chronic or acute disease characterized by unrestricted growth of leukocytes (white blood cells) and their precursors in tissues. Leukemia is classified according to the dominant cell type and the severity of the disease.

Leukocyte—The generic name for white blood cells.

Lymphadenopathy—The term to designate disease of the lymph nodes, generally referring to enlargement.

Lymphocytic choriomeningitis—An acute central nervous system disease caused by the virus of the same name. It is characterized by grippe-like symptoms (fever, malaise, headache) sometimes followed by acute aseptic meningitis associated with infiltration of the meninges with lymphocytes. The virus exists as an endemic or enzootic infection in some colonies of mice.

Macrophage—An immune cell that ingests and destroys invading microorganisms, and it is found mostly in solid tissues.

Malaise—A term to describe the sensation of discomfort, uneasiness or indisposition that is often indicative of infection.

Mammary tumors—In virology, the breast tumors first described in mice by Bittner.

Malignant—A term to describe a cancer with unrestrained growth that may be localized or dispersed throughout the body.

Meningoencephalitis—An inflammation of the brain and its membrane coverings (meninges).

Messenger RNA (mRNA)—The molecule that carries genetic information from the genes to the rest of the cells.

Metastasis—A spread of cancer cells from their original site to form new secondary cancers.

Microbe—A minute one-celled form of life (bacteria) or a virus not distinguishable as to its vegetable or animal nature.

Microbiology—The branch of science dealing with the biology of microbes

Micron—A thousandth part of a millimeter (millimicron) or a millionth part of a meter.

Mitochondrion—An organelle in eukaryotic cells that produce energy.

Mitosis—The division of a eukaryotic cell into two daughter cells with identical sets of chromosomes.

Molecular biology—The biology of those molecules related to genes and gene products and heredity, a.k.a. molecular genetics. (Biology = the science of life and living things).

Molecule—A combination of two or more atoms that form a specific chemical compound.

Monoclonal antibody—A pure antibody with a unique sequence that recognizes only a single antigen and which is made by a cell line derived from a single B-cell lymphocyte.

Murine—This term pertains to rats and mice (and not to the commercial eyewash).

Mutagen—This term refers to any agent that causes mutations.

Mutation—An alteration or defect in the genetic information in an organism.

Myocarditis—The inflammation of heart muscle tissue.

Myositis—The inflammation of skeletal (voluntary) muscle tissue.

Myxoviruses—The family of viruses that includes influenza, parainfluenza, mumps and Newcastle disease.

Nasopharyngeal—This term pertains to the region of the nose and throat.

Necrosis—The death of areas of tissue or bone surrounded by healthy parts; also, the unplanned death of cells as the result of injury.

Neutrophile—An immune cell mostly found in blood that ingests and destroys invading microorganisms; a leukocyte easily stained by neutral dyes.

Nosologic—A term related to nosology, the science of description or classification of disease.

Nuclear membrane—The membrane in eukaryotic cells that separates off the nucleus from the rest of the cell.

Nuclease—An enzyme that cuts nucleic acid into shorter pieces.

Nucleic acid—A polymeric molecule that carries genetic information as a sequence of bases (DNA and RNA).

Nucleolus—The structural component of the nucleus where ribosomal RNA is made.

Nucleocapsid –The innermost protein shell of the virus plus the DNA or RNA inside it.

Nucleoside—The union of a purine or pyrimidine base plus a pentose (5 carbon) sugar.

Nucleotide—A monomer or subunit of a nucleic acid, consisting of a sugar + base + phosphate.

Nucleus—The nucleus of a cell is an internal compartment surrounded by the nuclear membrane and containing the chromosomes. Only the cells of higher organisms have nuclei.

Oncogene—A mutant gene that promotes cancer.

Oncogenic—The term that describes things or processes that give rise to tumors, especially malignant tumors.

Operon—A cluster of genes transcribed together to give a single molecule of mRNA (messenger RNA).

Opportunistic infection—A disease that does not usually infect healthy people but attacks patients with immune system defects.

Orthomyxoviruses—A family of negatively stranded RNA viruses with an outer envelope surrounding the nucleocapsid that contains several pieces of ssRNA. (See myxoviruses)

Osteogenic—A term pertaining to osteogenesis, the formation and development of bone taking place in connective tissue or in cartilage.

Palpebral—A term pertaining or relating to the eyelid.

Pancreatitis—An inflammation of the pancreas.

Papillomaviruses—A family of DNA viruses that produces warts and occasionally malignant tumors

Parainfluenzaviruses—See myxoviruses and the description in the text.

Parasite—An organism that lives within, upon or at the expense of another organism known as the host without contributing to the survival of the host.

Parasitologist—A person who specializes in the study of parasites and parasitism.

Parotid gland—A salivary gland located near and in front of the ear. It usually becomes swollen in mumps.

Pathogen—A microorganism or substance capable of producing a disease.

Pathogenesis—The term describing the origin and development of a disease.

Pathogenic, pathogenetic—These are terms describing the quality of a thing or process productive of a disease.

Pharynx—A.k.a. throat. It serves as a passageway for air from the nasal cavity to the larynx (voice box = “Adam’s apple”) and food from the mouth to the esophagus (gullet); prefixes designate the anatomical regions, naso = nose, oro = mouth, hypo = below the tongue.

Phenotype—This describes the characteristics due to the expression of an individual’s genes; it usually refers to visible properties but may refer to characteristics disclosed by laboratory tests.

Phosphatase test—This test employs an enzyme that catalyzes the hydrolysis of phosphoric acid esters and is used to determine the adequacy of the pasteurization of milk.

Picornaviruses (pico = small, rna = RNA)—A member of positive-stranded RNA viruses with a single protein shell surrounding the ssRNA. Members of this family include polio-. Coxsackie-, echoviruses and rhinoviruses.

Plasmid—A circular molecule of double-stranded, helical DNA that replicates independently of the host cell’s chromosomes. Rare linear plasmids have been discovered.

Pleurodynia (epidemic)—An epidemic disease with sudden acute, intermittent severe pain in the lower chest or upper abdomen with fluctuating fever, headache, nausea and malaise associated with infection by one of the group B Coxsackie virus strains. See the description in the text.

Polymer—A long macromolecule made of similar or identical subunits linked together.

Polymerase chain reaction (PCR)—The artificial amplification of a DNA sequence by repeated cycles of replication and strand separation.

Poxviruses—A family of viruses with dsDNA (double-stranded) carrying up to 200 genes and with an outer protein layer surrounding the nucleocapsid. Members include smallpox, cowpox, and monkey pox.

Preauricular—A term describing the region in front of the ear.

Primates—An order of vertebrates (animals) belonging to the class Mammalia, sub-class Theria including lemurs, tarsars, monkeys, apes and man. This order is most highly developed with respect to the brain and nervous system.

Primer—A short segment of nucleic acid that binds to the template strand (of DNA) and allows synthesis of a new chain of DNA to get started. RNA primers are used by cells and DNA primers are used in PCR.

Prion—A distorted disease-causing form of a normal brain protein that can transmit infections such as Jakob-Creuzfeldt disease, or bovine spongiform encephalopathy (mad cow disease).

Probe (molecule)—A molecule that is tagged in some way (usually radioactive or fluorescent) and is used to bind to and detect another molecule.

Proteus X (Weil-Felix reaction)—The agglutination of certain Proteus (bacteria) organisms due to the development of Proteus antibodies in certain rickettsial diseases.

Protein—These are polymers made from amino acids; they do most of the work in the cell.

Protein kinase—An enzyme that switches other enzymes on or off by attaching a phosphate group to them. The src-oncogene encodes a protein kinase.

Proto-oncogene—The original healthy form of a gene in tissue that may give rise to an oncogene

Provirus—A form of a virus in which the viral DNA is integrated into the host chromosome.

Proximal iliac arteries—The nearest point of attachment to the abdominal aorta of the iliac arteries in the pelvis. The aorta splits at this point (bifurcation of the aorta) to give rise to the main artery that runs down each leg.

Pulmonary infarction—Necrosis of lung tissue due to obstruction of blood flow to various branches of the pulmonary arteries from narrowing or occlusion usually or often caused by a circulating blood clot (embolus).

Purine—A type of base with a double ring found in DNA and RNA.

Pyrimidine—A type of base with a single ring found in DNA and RNA.

Q fever—The febrile illness caused by the rickettsial organism Coxiella burneti. See text.

Radioactive—A description of the state of a substance or object that is emitting radiation due to unstable atoms that break down releasing alpha, beta or gamma rays.

Ras protein—A protein involved in cell proliferation that when mutated can cause cancer.

Recessive allele—The allele whose properties are not observed because they are masked by the dominant allele.

Recessive mutation—A defective copy of a gene whose properties are not observed because they are masked by a functional copy.

Recombinant—This pertains to something that has been genetically engineered; it may refer to a whole organism or to a single product.

Recombinant DNA technology—This refers to genetic engineering; it is the assembling of DNA from more than one source or organism.

Recombination—The mixing of genetic information from two chromosomes as a result of crossing over.

Regulatory protein—A protein that regulates the expression of a gene or the activity of another protein.

Remission—The lessening of severity or abatement of symptoms; also, the period during which symptoms abate.

Reoviruses—The family of viruses with two protein shells surrounding the double-stranded DNA.

Replication—The duplication of DNA prior to cell division.

Respiratory syncytial virus—A virus that induces formation of syncytial masses in infected tissue cell cultures. It is an important cause of acute respiratory disease in infants and young children. See text.

Restriction enzyme—An enzyme that binds to DNA at a specific base sequence and then cuts the DNA.

Retrovirus (RNA virus)—A type of virus that has its RNA genes in the virus particle but converts this to a DNA copy inside the host cell chromosomes by using reverse transcriptase.

Reverse transcriptase—An enzyme that starts with RNA and makes a DNA copy of the genetic material. See text.

Rhesus monkey—A type of monkey used in research.

Rhinitis—The inflammation of the nasal mucosa (lining of the nose).

Rhinoviruses—one of a subgroup of picornaviruses that causes (primarily) the common cold in man. There are approximately 100 strains, and they occur worldwide.

Rickettsia—The generic name applied to a group of microorganisms that occupy a position intermediate between viruses and bacteria. They differ from bacteria in that they are obligate parasites requiring living cells for growth, and they differ from viruses in that they are retained by the Berkefeld (bacterial) filter. They are causative agents of many diseases and are usually transmitted by arthropods (lice, ticks, fleas, mites). Diseases include the various types of typhus, and the spotted fevers. Coxiella burneti is similar to the rickettsias but it differs from the group in several ways. See text.

Rickettsialpox—An acute, febrile, self-limited disease caused by Rickettsia akari, and the mouse mite transmits it. See text.

RNA (ribonucleic acid)—The nucleic acid that differs chemically from DNA in having the sugar ribose in place of the sugar deoxyribose, the pyrimidine base uracil in place of the pyrimidine base thymine, and is single stranded whereas DNA is double stranded.

Roseola infantum (exanthem subitum)—A disease in infants and young children characterized by high fever, enlarged spleen and a rash that appears when the fever subsides. Recent studies indicate that a herpes virus causes the disease.

Rotavirus—A member of the reovirus family. It is a major cause of infant diarrhea.

Rous sarcoma virus—An avian retrovirus that causes cancer primarily in chickens.

Sarcoma—A malignancy arising from muscle, bone or other connective tissue. It may affect the bones, bladder, kidneys, liver, lungs, salivary glands, spleen and other body structures containing connective tissue.

Sedimentation constant—The rate at which small particles such as protein molecules or viruses settle when subjected to ultra-centrifugation.

Serology—The scientific study of blood serum. Serologic and serological are adjective terms relating to the study of serum.

Simian virus 40 (SV-40)—A double stranded DNA containing monkey tumor virus of the papovavirus family. This virus contaminated many monkey kidney tissue cultures used for early vaccine production.

Spasticity—The extensive contractile tension of muscles causing stiff or awkward movements, often the result of injury to the brain or spinal cord.

Src (sarc) oncogene—The oncogene originally associated with transforming Rous sarcoma virus that encodes an enzyme acting as a protein kinase.

SsDNA—The designation for single-stranded DNA.

SsRNA—The designation for single stranded RNA.

Syndrome—A group of signs and symptoms that collectively characterize or indicate a particular disease or abnormal condition; also, the sum of signs associated with any pathological process.

Temperature sensitive (ts) mutation—A mutation whose effects are harmless at one temperature but noticeable at another, e.g. ts mutant Rous sarcoma viruses. See text.

Thermistor thermometer—A non-mercury electrical apparatus for recording body temperature.

Thymic epithelioma—A cancer arising from the thymus gland.

Thymine (T)—One of the pyrimidine bases found in DNA only and that pairs with the purine adenine.

Tissue culture—The growth or maintenance of living tissue or cell types in a balanced nutrient liquid or soft gel medium *in vitro.* See text.

Titer - The standard of strength per volume of a volumetric test solution, e.g. the amount of specific antibody in an antiserum or the strength of a serum. It can also apply to the quantity of a microorganism in a given volume of tissue or solution.

Transcription - The process by which information from DNA is converted into its RNA equivalent.

Transduction—The transport of genes from one cell to another inside a virus particle.

Transfer RNA (tRNA)—These are RNA molecules that carry amino acids to a ribosome for the production of proteins.

Transformation—The conversion of a normal cell to a cancer cell.

Ultra-centrifugation—The use of a high-speed centrifuge capable of producing centrifugal forces more than 100,000 gravity; it is used in the studies of proteins and viruses.

Vaccination—The artificial induction of the immune response by injecting foreign proteins or other antigens.

Vaccinia—A member of the poxvirus family that causes cowpox. The virus is used in a weakened or attenuated form to provide immunity against smallpox.

Vector (in infections)—An animal, usually an arthropod (insect or tick), which transmits the causative organisms of disease from infected to non-infected individuals, especially one in which the organism goes through one or more stages in its life cycle.

Viral genome—The nucleic acid, either DNA or RNA, that carries the genetic information of a virus.

Virion—The term for a virus particle.

Virus—A virus is a sub-cellular parasite with genes of either DNA or RNA that replicates inside the host cell upon which it relies for energy and protein synthesis. In addition, it has an extra-cellular form in which the virus genes are contained inside a protective coat.

Zoonosis—A disease transmitted from animals to man under natural conditions.

Appendix E:

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