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Harald Norlin Johnson, M.D.

VIROLOGIST AND NATURALIST WITH THE ROCKEFELLER FOUNDATION AND
THE CALIFORNIA DEPARTMENT OF PUBLIC HEALTH

With an Introduction by
Richard W. Emmons, M.D.

Interviews Conducted by
Sally Smith Hughes, Ph.D.
in 1987 and 1988

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Harald Norlin Johnson holding a young anteater, Myrmecophaga tridactyla, at San Joaquin, Bolivia, during the study of Bolivian Hemorrhagic Fever in 1963.

Photograph by Ronald MacKenzie

Cataloging Information

JOHNSON, Harald Norlin (b. 1907)

Virologist, naturalist

Virologist and Naturalist with the Rockefeller Foundation and the California Department of Public Health, x, 391 pp., 1991.

Nebraska farm childhood; medical student, University of Nebraska, 1928-1933; intern and resident, Brigham and Children's hospitals, Boston; yellow fever laboratory, Rockefeller Institute, 1938; Cooperative Study of Rabies, Rockefeller Foundation and Alabama State Board of Health, 1938-1945; field study of vampire bat rabies, Mexico, 1944; paralysis and recovery; staff member, Rockefeller Institute, 1945-1951; scientific director, Virus Research Centre, Poona, India, 1951-1954; director, Arthropod-borne Virus Study Project, Rockefeller Foundation and California State Department of Public Health, 1954-1972; research on rabies, malaria, arboviruses, Salk polio vaccine field trial, 1954; ecological approach to viral research; natural history and field studies; memberships, awards, publications.

Introduction by Richard W. Emmons, M.D., director, Viral and Rickettsial Disease Laboratory, State of California Department of Health Services.

Interviewed 1987-88 by Sally Hughes, Ph.D. The Regional Oral History Office, The Bancroft Library, University of California, Berkeley.

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THE NEW YORK TIMES

Harald N. Johnson Dies at 89; Was an Expert on Pathogens

By KAREN FREEMAN

Dr. Harald N. Johnson, an internationally known expert on rabies and arthropod-borne viral diseases who was as much at home capturing dangerous animals in the field as working in the laboratory, died on Aug. 28 in South Shore Hospital in Weymouth, Mass. He was 89 and lived in nearby Scituate.

Not content to learn about viruses under laboratory conditions, Dr. Johnson often headed to remote, sometimes hazardous, spots to track animals harboring the pathogens.

Dr. Edwin H. Lennette, who was the director of the Viral and Rickettsial Disease Laboratory in Berkeley, Calif., during the nearly two decades Dr. Johnson worked there, described

A life dealing with rabies and other public health problems worldwide.

his colleague as a Renaissance man: "He was an accomplished pianist. He could walk into an open field and tell you the Latin and common names of the flowers, trees, shrubs, birds and other animals — besides being a superb physician and researcher."

Dr. Johnson used that knowledge of the natural world to track pathogens as they moved from one organism to another.

Working for the Rockefeller Foundation, he developed the strain of the rabies virus used in the vaccine that brought that disease under control in dogs by the late 1960's in the United States, and he dealt with rabies and other public health problems around the globe. His travels included Alabama, where he helped control a serious rabies outbreak from 1938 to 1945; Mexico, where he traced a cattle epidemic to rabid vampire bats in

1944, and India, where he set up a field station studying arthropod-borne diseases in the 1950's that later became the national virus laboratory there.

The vampire bat project led him into danger. On a foray to capture the bats in a cave, one bit him on the finger. Five months later, he became a quadriplegic. Dr. Johnson was sure that his symptoms were caused by rabies from the bite, since the vampire bats carry a different strain of rabies; his doctors thought it might be another pathogen or a reaction to his many rabies vaccinations. He weighed 185 pounds when he went to Mexico; his illness took him down to 119 pounds.

After five months at a rehabilitation center in Warm Springs, Ga., he could walk again, but with difficulty, and he returned to work. Even many years later, he used a cane, but he kept up his field studies.

An interest in biology came naturally to Harald Norlin Johnson, who was born in 1907 to immigrant farmers in Loomis, Neb. It was a time when farmers doctored their own animals, even sewing up their wounds when necessary, and he was drawn into medicine — after a detour to pursue his other interest, music, at the McPhail Conservatory of Music in Minneapolis.

He earned a bachelor's degree from the University of Nebraska at Lincoln in 1930, then graduated from the medical school at the University of Nebraska in Omaha in 1933. He trained in internal medicine, infectious diseases and pathology at Harvard, at Peter Bent Brigham and Children's Hospitals.

In 1938, he joined the Rockefeller Institute, which was testing yellow fever vaccines in the hope of protecting its field researchers. Dr. Johnson was vaccinated that year with one of the vaccines that later led to a Nobel Prize for Dr. Max Theiler.

The Rockefeller Foundation sent him to the Berkeley laboratory, part of the California Department of Health Services, in 1954 to deal with an encephalitis outbreak and work on other arthropod-borne diseases.



Wesley Swadley

Dr. Harald N. Johnson

He found that the encephalitis was being brought in by birds migrating from the North.

Dr. Johnson worked on a variety of projects that let him be a wildlife detective, Dr. Lennette said, and one pathogen he discovered was the Modoc virus, an encephalitis virus. He had to work under harsh conditions to test the mice that turned out to carry the virus.

"He caught mice in the middle of winter up in Modoc County," Dr. Lennette said, "scraping away snow and getting into the burrows to get out the mice. He wanted to know where these viruses went in the winter."

Dr. Johnson retired in 1972 but kept working in the Berkeley laboratory part time. Last year, he broke his leg in a fall, so he used his recuperation time to write his final publication, a chapter on rabies in a standard reference book, "Diagnostic Procedures for Viral, Rickettsial and Chlamydial Infections" (American Public Health Association).

Dr. Johnson's survivors include his wife of 60 years, the former Frances Maxfield Alexander of Scituate; two daughters, Marion Noble of Sebastopol, Calif., and Susan Robinson of Pittsburgh; two sons, Dr. John, of Bellevue, Wash., and Dr. Michael, of Scituate, and six grandchildren.

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INTRODUCTION by Richard W. Emmons, M.D.

Dear Reader:

If you already know Harald Johnson, you will find in these pages a delightful review, and perhaps many reminders of your own memories, of his remarkable life and thought. If you are meeting him for the first time in these memoirs, you have some pleasant hours ahead to get acquainted with a human being of many talents, with a special passion for the art of science, medicine and nature study. It will surely make you want to know him in person. Whether you have known him for fifty years or only fifty minutes, you have recognized the breadth and depth of his interests, knowledge, experience, and zest for understanding the world and its creatures -- from viruses to mankind -- and how they all interact.

I have been privileged to know Harald since 1961, when he lectured to us epidemiology students in the School of Public Health, University of California, Berkeley, about rabies and various vector-borne viruses. We felt in those lectures the authority that comes from direct experience and personal discovery. His discussion of rabies would be vividly illustrated by reaching into his briefcase to pull out weasel and skunk pelts, beautifully tanned by himself, to pass around the class so that we would really know and remember some of the important rabies hosts. I was fortunate to get much more such hands on teaching in many field trips with Harald around northern California. We live-trapped chipmunks, golden-mantled ground squirrels, *Peromyscus* mice, and many other species to get blood samples for virus testing. And we flagged acres of brush and grass in remote areas to collect ticks which we tested for Colorado tick fever virus or perhaps some new microorganism. His extensive experience and keen sense of how virus cycles operate in nature and where viruses "ought to be" have led to many discoveries of new viruses or new information about the place of microorganisms in the "economy of nature". The enthusiasm, zest, and delight with which he pursues these quests are as contagious as the "diseases in nature" he loves to study. And the energy and stamina he brings to field trips, laboratory work and teaching are all the more remarkable, considering the significant handicap he has battled to overcome after a nearly fatal paralytic illness early in his career.

Those long drives on field trips in the California mountains, and late evenings spent sorting, classifying, recording, and storing specimens for later study in the laboratory, were valuable times for earnest, far-ranging discussions about world events, history, politics, religion, anthropology, nature, ecology, medicine, music, people, bird migrations, and many other topics besides the field research at hand. Harald's intense desire to convey his enthusiasm, experience, and insights to the next generation are evident in his writings and lectures, but most especially through his personal contacts.

My trips with Harald were mostly in northern California, but the entire world has been his laboratory: an atoll in the Pacific Ocean; the far northward reaches of Alaska; the jungles of South America; the Russian countryside; the swamps of Massachusetts; the highlands of India -- as these pages tell you. In all his travels, he has pursued the same quest -- trying to unlock the secrets of how microorganisms survive in nature and interact with their natural hosts and with people.

In the laboratory, as in the field, he shows the same pride in experiments carefully designed, safe and accurate technique, scrupulous honesty, meticulous record-keeping. His voluminous field and laboratory notebooks are distinctive and especially impressive -- packed with extraordinary detail about his daily experiences and discoveries.

Throughout his career, Harald has shown a refreshing disregard for personal gain or fame. The search for truth has been his goal. You will also recognize in these pages much evidence of the invaluable contributions and support of Fran Johnson -- wife, helper and partner to the adventures described herein.

So my hope is that this book will be not only a record of his career, which is still productive and ongoing, but also a stimulus to all of us, old friends and new acquaintances, to emulate his life and carry on his search for the understanding and the enjoyment of nature's mysteries and marvels.

Richard W. Emmons, Director
Viral and Rickettsial Disease Laboratory
State of California Department of Health Service

Berkeley, California
November, 1990

INTERVIEW HISTORY

Harald Norlin Johnson's long career as a physician/virologist with the Rockefeller Foundation and the California State Department of Public Health is the subject of this oral history by the Regional Oral History Office. An authority on rabies and arthropod-borne viruses, he discourses enthusiastically on many aspects of his full and happy life--field work, laboratory research, natural history, colleagues, ornithology, music. The result is an intricate and compelling portrait of a man described as the last of the old-time doctor-scientists.

After relating his upbringing and early education in the Midwest, followed by medical training at Harvard Medical School and its affiliated hospitals, Dr. Johnson recounts the outset of his career at the Rockefeller Institute in New York City in 1938, a time when virology was beginning to be recognized as a discrete field of inquiry. He tells of his association with many of the towering figures of early American virology, who were not only studying viral diseases around the world but also struggling to define the virus as a unique infectious entity.

Dr. Johnson's first assignment at the institute to the yellow fever laboratory was soon interrupted by a call to Alabama to direct a study of rabies in Montgomery, sponsored by the Rockefeller Foundation and the Alabama State Board of Health. The assignment stimulated a life-long interest in rabies and led to a field expedition to Mexico in 1944 to study vampire bat rabies, a highly fatal paralytic disease of cattle which had prompted the Mexican authorities to ask for assistance from the Rockefeller Foundation. Dr. Johnson's description of field trips to collect infected bats in remote caves is both rich in scientific detail and human interest. In the course of one field trip, he was bitten by a bat, resulting some months later in a frightening episode of total paralysis. He tells of the slow and agonizing process to regain use of his limbs with the guidance of a physical therapist at the polio rehabilitation center in Warm Springs, Georgia.

By 1945 he had recovered sufficiently to return to the Rockefeller Institute where he studied malaria and arthropod-borne viruses (arboviruses). The latter were a major focus of postwar virological research and the impetus for the foundation of Rockefeller field stations around the world to collect information on arboviruses, the suspected cause of various infectious diseases.

The first of such laboratories was established in Poona, India, where in 1951 Dr. Johnson was named scientific director of the Virus Research Centre, a cooperative project of the Rockefeller Foundation and

the Indian Council of Medical Research. Dr. Johnson describes setting up the laboratory and a research program to study arboviruses and also how his young family of six coped with daily life in newly independent India.

One three-year tour of duty was enough, for the rigors of life in India were taking a toll on the children's health. In 1954 the family came home, eventually moving to California where Dr. Johnson became director of the Arthropod-borne Virus Study Project, yet another Rockefeller Foundation cooperation, this time with the California State Department of Public Health in Berkeley. Here, with the encouragement of Dr. Edwin H. Lennette, director of the Virus and Rickettsial Disease Laboratory at the health department, he continued the field studies which are the love of his scientific life. Products of Dr. Johnson's tireless work were the description of several new viruses and an increasing appreciation for the importance of migrating birds in the intercontinental transmission of viral diseases.

In 1972 Rockefeller Foundation funding of the California arbovirus project ceased and Dr. Johnson became a regular staff member of the Viral and Rickettsial Disease Laboratory, commonly known as "the virus lab." He "retired" in 1986 but continues to conduct occasional field studies in northern California and near the family home in Scituate, Massachusetts, where the Johnsons live six months out of the year. He also maintains his laboratory in Berkeley, meticulously updates his voluminous laboratory records, takes notes on much of what he reads, and somehow also finds time to give piano recitals with his wife Frances, also an accomplished pianist.

It is to David Lennette, Ph.D., that we are indebted for initiating this oral history and a previous one with his father, Edwin Lennette, M.D., Ph.D., who as director of the Viral and Rickettsial Disease Laboratory was in part responsible for Dr. Johnson's association with the laboratory from 1954 on. We are also indebted to David Lennett for his generous financial support, to Michael Welch Johnson, M.D., Harald Johnson's son, and to the Rockefeller Foundation. We are also indebted to David Lennette for his generous financial support, and to the Rockefeller Foundation and a long list of donors. The reader will discover a fruitful overlap of subject matter and personalities in this oral history, Dr. Edwin Lennette's, and in an earlier one with K.F. Meyer.¹

¹Edwin H. Lennette, Pioneer of Diagnostic Virology with the California Department of Public Health, an oral history interview conducted 1987-1988 by Sally Smith Hughes, Regional Oral History Office, The Bancroft Library, University of California, Berkeley, 1988, p. 201.

Karl F. Meyer, Medical Research and Public Health, an oral history interview conducted 1961-1962 by Edna Tartaul Daniel, Regional Oral History Office, The Bancroft Library, University of California, Berkeley, 1976.

Twelve interviews were conducted between January 1987 and March 1988 in Dr. Johnson's cluttered laboratory in an annex of the California State Department of Public Health in Berkeley, except for the final three, which were held in The Bancroft Library conference room. Having just completed an oral history with Dr. Lennette, the interviewer was familiar with Dr. Johnson's milieu. Hence, interviews with Dr. Johnson's colleagues were judged unnecessary, except for telephone conversations with Dr. Richard Emmons, present director of the virus lab, and Denny Constantine, who shares Dr. Johnson's interest in rabies.

Dr. Johnson, always in coat and tie, usually met me on the University of California, Berkeley, campus in his well-traveled station wagon replete with the animal traps he uses on field trips. We drove the few blocks to his laboratory and left the car in handicapped parking, to which he is entitled because of muscle weakness in the left leg which has worsened in recent years, the most obvious remnant of the paralytic disease he suffered in 1944.

Dr. Johnson, who can--and does--converse on practically every imaginable subject, always came to the interviews with a collection of supporting documents and photos from his extensive personal records.² These we discussed at length before turning on the tape recorder. He also often provided material relevant to the next interview session. Once the recorded sessions began, Dr. Johnson responded expansively, his answers a store of information both on and off the subject. Knowledgeable on any number of subjects and possessing a remarkable memory, Dr. Johnson spoke with little prompting from the interviewer.

Dr. Johnson put countless hours into editing the transcript, eliminating some of the digressions, adding facts and statistics from his notebooks, rewriting entire sections, and adding fresh material. He was determined to present a complete picture of his activities and acquaintances to the limits of his capabilities, driven perhaps by his career-long dedication to recording observations and also by his admiration for the staff and policies of the Rockefeller Foundation.

The reader will probably detect differences in tone in the transcript, the more formal representing sections that Dr. Johnson rewrote or wrote afresh, and the informal representing transcriptions from the tapes. The inserts are too numerous to indicate. Dr. Johnson completely rewrote interview twelve. Hence, this is not a true oral history in the strict sense but rather a heavily edited and, in part, completely re-written document which, nonetheless, is based closely on the verbatim transcripts.

²The Rockefeller Archives have shown interest in eventually acquiring Dr. Johnson's papers.

Because the interviews were interrupted for more than six months (between interviews eight and nine) when the Johnsons were in Massachusetts, and because the lengthy transcript was reworked piecemeal, there are many repetitions. Some of these were intentionally left because they repeat themes close to Dr. Johnson's heart: his appreciation for the freedom he experienced as a Rockefeller Foundation staff member, his interest in the source of viruses in nature, his stress on the need for impeccable virological technique, his chagrin at the declining interest in field work and natural history, and the importance of an ecological approach to research on viral diseases.

What emerges is a portrait of a naturalist whose great joy is close observation of the wonders of the natural world. He deplores the fact that: "Nowadays you don't study trees, but you study the chemistry of the leaves, or something like that, rather than looking at the large natural mystery of biology generally."

Perhaps we will one day return to Dr. Johnson's holistic approach to medicine and science. In the interim, this oral history reveals a man who has resisted the trend to high tech medicine and ultraspecialized science and has pursued his interest in natural history with little regard for monetary reward or for the prestige accompanying frequent publication and high academic position.

The Regional Oral History Office was established to tape record autobiographical interviews with persons who have contributed significantly to recent California history. The office is headed by Willa K. Baum and is under the administrative supervision of The Bancroft Library.

Sally Smith Hughes, Ph.D.
Interviewer/Editor

September 1990
Regional Oral History Office
University of California, Berkeley

CURRICULUM VITAE

NAME: HARALD NORLIN JOHNSON DATE OF BIRTH: March 31, 1907

PLACE OF BIRTH: Loomis, Nebraska

MARITAL STATUS: Married June 27, 1936 to Frances Maxfield Alexander of Scituate, Massachusetts. Four children.

CURRENT POSITION: Member of staff, Viral & Rickettsial Disease Laboratory, California Department of Health Services, 2151 Berkeley Way, Berkeley, California 94704

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High School: Loomis, Nebraska 1920-1923

Holdrege, Nebraska 1923-1924

College: McPhail Conservatory of Music, Minneapolis, Minnesota 1924-1925

University: University of Minnesota, Minneapolis, Minnesota, Premed 1925-1926

University of Nebraska, Lincoln, Nebraska, Premed 1926-1928

University of Nebraska, College of Medicine, Omaha, Nebraska 1928-1933

DEGREES:

B.Sc. University of Nebraska 8-7-30; M.A. (anatomy) 1-29-32;

M.D. 6-5-33

PROFESSIONAL TRAINING:

Harvard University, Boston, Massachusetts
House Officer in medicine, Brigham Hospital 1933-1934
Assistant Resident Physician, Contagious Disease Service, Cleveland City
Hospital March-August 1935
House Officer in pathology, Brigham Hospital 1935-1936
Assistant Resident Physician, Brigham Hospital 1937-1938
Resident Pathologist, Children's Hospital 1936-1937
Rockefeller Foundation Fellow, School of Public Health Summer 1938

EMPLOYMENT RECORD:

Staff Member, Division of Medicine and Public Health, The Rockefeller
Foundation, New York 1938-1972

ASSIGNMENTS:

1. Yellow Fever Laboratory, Rockefeller Institute, New York 1938.
2. Scientific Director, Cooperative Study of Rabies, Rockefeller Foundation and Alabama State Board of Health, Montgomery, Alabama, 1938-1945. Director of field expedition to study vampire bat rabies, Rockefeller Foundation and Department of Agricultural Sciences, Government of Mexico, 1944.
3. Staff Member, Rockefeller Foundation Laboratory, Rockefeller Institute, 1945-1951. Studies of malaria and arthropod-borne viruses.
4. Scientific Director, Virus Research Centre, Cooperative Project, Rockefeller Foundation and Indian Council of Medical Research, Poona University College of Medicine, Poona, India, 1951-1954 (Presently the National Institute of Virology, Indian Council of Medical Research, Poona, India).
5. Director, Arthropod-borne Virus Study Project, A Cooperative Project of the Rockefeller Foundation and the California State Health Department, 1954-1972.
6. Lecturer, School of Public Health, University of California, Berkeley, 1954-1984, Consultant 1985-1991.
7. Staff member, Viral and Rickettsial Disease Laboratory, California Department of Health Services, 2151 Berkeley Way, Berkeley, California, 1972-1991.
8. World Health Organization, Geneva, Switzerland, Expert Panel on Rabies, 1950-1986.

BOARD STATUS:

Diplomate of National Board of Medical Examiners
License to Practice Medicine, California and Massachusetts

HONORS:

Theta Nu, honor premed society, University of Nebraska 1928
Sigma Xi, honor society in science, University of Nebraska 1932
Alpha Omega Alpha, honor society in medicine, University of Nebraska
1933
Delta Omega, honor society in public health, University of California
1955
Distinguished Service Award, Wildlife Disease Association 1974

MEMBERSHIP IN SCHOLARLY SOCIETIES:

American Association Advancement of Science
New York Academy of Medicine
Society Experimental Biology and Medicine
American Association of Bacteriologists and Pathologists
American Association of Immunologists
American Society of Tropical Medicine and Hygiene
American Wildlife Disease Association
American Medical Association
Alameda Contra Costa Medical Society
Cooper Ornithological Society

BIOGRAPHICAL INFORMATION

(Please print or write clearly)

Your full name Harold Norlin Johnson
Date of birth 3-31-07 Place of birth Loomis, Nebraska
Father's full name Gust John Johnson
Birthplace Ryddaholm, Sweden
Occupation farmer
Mother's full name Manny Amalia Norlin Johnson
Birthplace Timmersdala, Sweden
Occupation housewife
Where did you grow up? Loomis, Phelps Co. Nebraska
Present community Albany, California
Education McPhail Conservatory of Music, U. Minnesota, U. Nebraska B.S., M.A., M.D.
Occupation(s) Physician

Special interests or activities Natural history, Church, Choral, orchestral, piano and pipeorgan music.

I FAMILY BACKGROUND AND EARLY EDUCATION

Paternal Grandparents

[Interview 1: January 9, 1987]

Hughes: Dr. Johnson, I want to start with your grandparents on both sides; could you tell me where they came from and what they did?

Johnson: All right. My paternal grandparents were from Ryddaholm in Smaland, Sweden. My grandfather's name was Gustav Johan Johansson. My grandmother's name was Stina Cajsa Anders dotter, and that could be spelled two ways. It is usually spelled Cajsa--that's a common name. Anders dotter means she was a daughter of Anders Strom. I remember that grandmother. She was born in 1826, and her father was a professional soldier. I thought she probably was an only child. Anders was away in the Swedish army for twelve years straight without a recreation period. So there were children before he went away, and then after he came back. She was born after he came back.

One of the big problems in Sweden was the compulsory military training, which is still there. One reason my grandfather wanted to come to the U.S. was for freedom of religion. The state religion there was Lutheran, but it was very much like the Catholic--very strict, and you had to have the communion at the church. The church kept all the records, which are excellent. They have the date of when parishioners were born, the date they were baptised, the witnesses, and the child's name, the mother's name, the father's name, how old the mother was when she had the baby. So the records are very good, and you could go back 200 years with little difficulty.

But the name changes. My great-grandfather was Johan--that's where Johansson or Johnson comes from. His full name was Johan Bengtson, and he was a gunsmith. The wife's name was Elin Mans dotter; that's my great-grandmother. By the way, grandfather, Gustav Johan, came to the United States in 1869. That was when a lot of Swedes came because they had heard about the ability of getting good land. At the time, southern Sweden was mostly forested, and there were small tracts, holdings of land, forest,

Johnson: and meadow. They were pretty good as far as surviving, but they wanted land, and they also wanted to get out of military training. They wanted religious freedom. That is my father's side.

Emigration to the United States

Hughes: Where did your grandfather settle in this country?

Johnson: He came first to Jamestown, New York, a short time, then to Lockport, Illinois. That was a little Swedish community. He hadn't brought his wife and children at that time. He got a job working in construction. All the Swedish immigrants were able to work with metal and wood. They could make things out of iron and build houses. So he built his own house in Lockport, Illinois. Then the rest of the family came in 1871. That included my father, Gust John Johnson, who was just age eleven. There were four children and the grandmother, Stina Cajsa. They all stayed in Lockport until 1876.

The Pawnee-Sioux war in 1873 opened the whole area of Nebraska, because the Pawnees were almost entirely killed by the Sioux. The Nebraska Territory became available for homesteads. There were about twelve families in Illinois that wanted to start a religious community of their own in Phelps County, Nebraska, where each family could obtain a homestead of 160 acres. Grandfather Gustav and his family arrived by train in April, 1876. Eighteen seventy-six was the year of Custer's massacre. This was the last major engagement between the U.S. Army and the Sioux Indians.

Hughes: Were they all Lutherans?

Johnson: In Sweden, all were officially members of the state Lutheran church. Sweden had sent missionaries to Russia, and Africa, and elsewhere outside of the state church. They called themselves the Covenant Mission Organization. The church we go to in Berkeley is called the Evangelical Covenant Church. It is patterned on the Congregational church. Each congregation is independent, but they have a national organization.

Hughes: Was Nebraska a state?

Johnson: Yes. From 1865 it was a state. 1876 was the beginning of barbed-wire fencing. The big ranching in Texas started about the same time--1875-1876. First of all, there had to be some military protection. During the Civil War, things were bad because there was no organized military presence. In 1864 cavalry companies such as the Iowa unit fought their own war with the Sioux and

Johnson: Cheyennes to keep the overland trail open from Fort Kearney, Nebraska, to Fort Laramie, Wyoming. The Sioux Indians had been raiding isolated farms, while the army was busy with the Civil War, but the large-scale Indian attacks in 1864 were soon put down in the Nebraska Territory.

Besides a homestead of 160 acres of land, more land could be purchased at fifty cents to one dollar an acre. Our family was one of three that purchased extra land on the river bottom of the Platte River, for raising range cattle. My grandfather Gustav brought lumber and other material to build a house. There were no trees on the grassland of the plateau between the Platte and the Republican rivers. Most of the settlers built sod houses in which they lived for many years. Grandfather had a little temporary one along the bank of a ravine while he was building his home during the summer of 1876. It was a square house with a chimney in the middle. That house is now at a farm near the town called Funk. It was moved three times. It is now a historic house. It's just as square as it was when he built it.

When my father married in 1887 he obtained a homestead of 160 acres adjacent to Grandfather's place. Grandfather helped him build a house and other buildings.

Hughes: Was he a farmer?

Johnson: Well, the people that came from Sweden knew all about having dairy cows and horses and farming. They all could work with tools. They could make nails, bolts, and hinges, and everything like that. That was pretty characteristic of much of Europe, I guess.

Hughes: Is that how he supported himself?

Johnson: No. Grain farming was the main source of income. We had our own little blacksmith shop; we had a little forge and could make ammunition for our guns. I learned to shoot. My grandfather brought his Swedish musket to America that he'd had in the army.

But the main thing was that 1876 was the beginning of large-scale agriculture. Within a year, they were raising crops. Very rapidly they developed good farming, and it became a very rich agricultural land. But the rainfall would vary from cycle to cycle every twenty years. When my father retired in 1924, one of my older brothers took over the farm. The big drought was in the thirties, and that's when I was away at school, and that's when the farm was lost because of failure of the crops. My father then was too old to work.

Maternal Grandparents

Johnson: My mother's family is from Timmersdala, in Sweden, near a town called Skovde, a manufacturing town and military base in central Sweden.

That grandfather took a different surname in the United States. His father was John, Johan. Many of them that came at that time were named Johnson, because that was a common Swedish surname. The oldest boy would take the same name, and the others would have a different surname. My grandfather's father was Johan, so he would be Johan's son. But there was a family name of Norlin or Norling. In Stockholm, a branch of the family spelled it Norling. My middle name is Norlin. In this country, Grandfather took the name of John Magnus Norlin, rather than Johansson.

I observed an interesting thing when I went to Sweden the first time in 1950. (I've been there various times.) I went to the graveyard in Ransberg, and the names in my mother's families were much the same as ours. Like, I had a sister, Linnea, and in the cemetery there, one of the cousins was named Linnea, and of my mother's brothers and sisters, the names were repeated in our family. I have a granddaughter Linnea. We named one of our children John Norlin Johnson. In the cemetery there was the Johan Johansson family plot. In that you'd find their children, like Carl and Johan, Linnea, Herman, Matilda, and Wilhelmina.

Grandfather, Johan Magnus Johansson, was born in Ransberg, which is an old town ten miles east of Skovde. That is where the family had lived for a long time. It was a wealthy part of Sweden where many had good jobs, and some went to Stockholm and to the military.

When Johan Magnus married and had two young children, he was appointed administrator of the crown lands, at a town called Timmersdala. It is near a lake called Langen just north of Skovde. It was a good job; they lived better than they did in the United States. He made violins and did fine cabinet work, plus having farmland for himself. He was very interested in the Pietist movement where Christians would meet in homes. They were called "readers"--"lasare" in Swedish. There was Bible reading, singing of Christian songs. But that was illegal. Mother told me that she used to be so afraid that somebody would come and raid the house and put her father in prison.

Hughes: Why did the church object?

Johnson: This was like other countries that had state religions. It was the same in England. My wife's family includes one man who came over on the Mayflower. If they wanted to have a meeting outside church, that was punishable with prison. And that was true in many other countries. Look what happened to the Huguenots.

So it was that strict in Scandinavia, too. They were apt to be thrown in jail. My grandfather would have lost his official job. So that was the reason he chose to leave Sweden, and so did many others.

There was a famous queen, Brigitta. We called her Saint Brigitta. She started an order of nuns in Sweden in the 1300s. She lived in the castle at Vadstena where they have the official records for southern Sweden; I've been there. You can stay in the nunnery, now a guest house. It was built in the 1200s. Brigitta said that the trouble with the church was that it was shiny and beautiful on the outside, but it was rotten inside. The church was part of the government. The priests were appointed by the crown.

My grandfather Norlin left Sweden for religious liberty. He was reasonably successful in the United States. He came in 1881 to a place called Saromville, Nebraska, about sixty or seventy miles from where my other grandfather settled. My paternal grandfather had a brother, Jonas, who settled at Saromville. When my grandfather John Norlin and his family came, Jonas offered them a place to stay. That was very common--"You can stay with me until you get a place." That's where my father met one of John Norlin's daughters. Manny Amalia became his wife. They were quite a cultured family in reading, writing, and interest in music and the evangelical Christian church.

Hughes: Was a cultured background atypical of the immigrants?

Johnson: Well, the ones that had government jobs really left a better background. My paternal grandfather in Rydaholm had served his time in the army and was typical of the people there. All were skilled in farming. They had to go to war when called by the crown, and that was why a lot of them wanted to leave. They still have conscription of citizens in Sweden to receive military training.

Hughes: What did the family do when many men were away for such long periods?

Johnson: There were multifamily units. They have records of the names of people who lived in each of the houses and would be carried on in that family, and then they had special living quarters for the military. The family would have a special allotment, so that would help take care of them. Then there were always children

Johnson: left, so somebody was always doing work, which was harvesting grain, and milking, and the animals were always there. So actually they could survive as in any rural community which was able to supply its own needs.

When I grew up in Nebraska, there was really very little need for money. We had all the meat, chicken, eggs, bread, and fruit that we needed. The women sewed a lot of the clothes. Grain could be ground to make our own flour.

Hughes: You were really self-sufficient.

Johnson: Yes. I saw very little cash; that was true of most of rural United States until farming became mechanized with tractors and milking machines. Even in my medical training, I had very little money. There was no salary during hospital training at Harvard in the 1930s. You got your board, room, and laundry.

Hughes: Do you want to say more about your parents?

Johnson: Oh, yes. My mother and father were married in 1887, and my mother lived to be ninety-four and my father ninety-one. They retired from the farm and went to Minneapolis in 1924. From Minneapolis, they moved to Lincoln in 1927 and to Omaha in 1929. My parents went back to the farm in 1933, tried to hang onto it. But there were no crops, and taxes and mortgages took the place. It was true of a lot of people there, particularly if you weren't young enough to do most of the work. One of my best friends stayed out there, and he said if you were there and could do the work, you'd survive and keep the land if you had not borrowed much money.

Hughes: Was there any pressure on you to do that, to leave your studies?

Johnson: No, my parents did anything they could to help us get an education.

Farming was a very healthy and good life; I'm glad I grew up there. Cattle ranching went on from, say, 1876 until about 1916. Beef cattle were raised as a major commerce, which we shipped to Kansas City. I have picture postcards from my older brothers mailed from Kansas City where they had delivered carloads of cattle. Most of my brothers were born in the 1890s; I was born in 1907. In the fall, you'd drive the cattle from the ranch land on the river up to the divide, and then certain ones were fed over the winter, but most of them were sold and shipped in carloads to Kansas City. It was always a joke--no one carried much money because they were apt to be robbed in Kansas City. So my brothers would mail a banker's check to my father.

Hughes: You didn't go along?

Johnson: No, but I saw the cattle drives going and coming. We didn't brand the cattle; we did ear-clipping. There were three families that had their own stock, so the cattle could be easily separated by the ear-clip signature.

The cattle drive was about thirty miles. The reason it stopped in 1916 was the fencing. Most of the way we had to go on regular roads. Some of the land was ploughed and planted, and the cattle would sometimes enter fields and break down fences.

So that ended in 1916. That's when the war in Europe was so heavy and they needed grain. It was actually critical. So everybody started to plant lots of wheat, oats and corn. We still had a large pasture of grassland on the main farm and raised white face Herford cattle for the market. One of the major sources of money then was cattle. We also raised hogs and plenty of chickens. You had your own horses. We had no tractor the whole time I was on the farm.

I left in 1924, and at that time everything was like the Amish in Pennsylvania--horsedrawn work, very healthy but hard work. Rural USA became rapidly mechanized during the thirties after the drought. Now that area of Nebraska is one of the richest agricultural areas in the United States.

Hughes: Do you think your grandfathers realized that when they chose to go there?

Johnson: They had no idea about weather cycles, such as the twenty-year Bergman cycle. They had good crops in the early years. Cycles of low rainfall come about every twenty years.

One relative who died recently had been farming until the present [1986]. He farmed one-and-one-half square miles all by himself and raised an enormous amount of food. When we thought we had a good crop of corn, we got thirty, thirty-five bushels an acre. He could produce two hundred bushels of corn to the acre. And now they harvest oats, wheat, and corn with special combines. The grain is stored in special wood bins for drying. These have an outer shell of galvanized iron. They use special equipment to move grain from the bottom to the top of the bins. Sensors turn on the circulation system and the gas heating units whenever the moisture reaches a certain humidity. This keeps the grain dry, and it keeps indefinitely.

Siblings

Hughes: How many brothers did you have?

Johnson: There were five brothers. One died at age seven or eight of an intestinal disease, probably typhoid fever. His name was Carl, which was a family name. The next oldest brother was Paul, and he was a farmer and did well. He rented land for farming at first. Later he farmed our place. When he left that, he went to live in Holdrege, Nebraska, and worked in the mail service.

The next older brother, Reuben, was drafted in the First World War. After the war ended, he went into railway mail service, which was a good job in those days. Later, he lived in Omaha, and he was the one that loaned me the money for tuition for medical school. He married the last year I was in medical school.

Then there was a sister, Linnea. Her husband was a successful farmer; he retired about 1940 and moved to Turlock, California. He worked in a munitions factory during the course of the war. They had no children. My father and mother left Nebraska in 1943 and came to Turlock. They lived with my sister and her husband.

There were two more brothers. I had a brother, Joseph, who was working in Omaha when I was in medical school. He married a nurse. He was trained in landscaping and horticulture. He got polio, just like President Roosevelt, about the same time, in 1935. He was severely handicapped. He came to California in 1940. He worked in an auto supply business in Patterson but later moved to Turlock. There he worked at a furniture supply company until he retired. He died at age eighty-nine in January, 1987.

Another brother, Philip, went to the University of Nebraska in Lincoln. That was one of the things that encouraged me to go on to college, I suppose. He was born in 1900, so he is seven years older than I. He went to Lincoln and then went to Minnesota for his master's in chemistry. He moved to Lincoln and taught science education at the university. In 1929 he received a fellowship at Cornell and later received a doctorate in science. He was a professor of science education at Cornell, except for a few years working for the Pentagon on science education for the military. He lives in Chapel Hill, North Carolina.

Hughes: What was your parents' education?

Johnson: They could read and write in Swedish and English, and read voraciously--everything. My father, when I was in medical school, read all my books.

One thing of interest on the ranch: we did our own veterinary work. My father was knowledgeable in this, and we sewed up animals when they were injured and took care of them. The doctors around were few and far between. Really major wounds were treated by the families themselves. And then there were

Johnson: midwives--I was the only one in my family where there was a doctor in attendance in addition to the midwife. The midwife did the delivery, probably did a better job than most doctors do today, which to me is just too much interference. So that pretty well covers my immediate family.

Hughes: Had your parents assumed that you would go on to the university?

Johnson: They were very happy by the choice of medicine. My father said he would have liked to have been a doctor.

Music was the big thing in my family; everybody played some instrument. My mother played the organ and piano. My sister gave me my first piano lesson. She was very good on the piano. Then my brother Philip played trumpet, Joseph the trombone, Reuben the violin, and Paul the mandolin. I played the piano at church and at school.

Hughes: Are you good?

Johnson: Yes. I had excellent training in pipe organ. I played in all sorts of churches through medical school and took lessons all through college and medical school. And then I got paralyzed, so I can't use my left foot properly for playing the organ.* But I still play piano.

Hughes: Did you ever consider a career in music?

Johnson: I always wanted to have it as a side interest. I made money working in popular music. I had a radio job in Omaha for three years, and I had an orchestra of my own, playing for medical school dances. And then I've done a lot of church music. I was the pianist for the children's choir at Christ Methodist Church at 60th and Park, New York City.

Grammar and High School

Hughes: Did you go to elementary school in Loomis?

Johnson: I first attended a rural one-room school. There was no government money or county money. The farmers would build a district school, where you could walk, usually a mile or a mile and a half, or ride a horse. Children didn't start school until about seven. You had to be able to walk or ride horseback.

*Dr. Johnson explains his paralysis below.

Johnson: The one-room schoolhouse had a stove in it for heating in the winter. The teacher had been through high school and a one-year normal [school] training course. Some teachers had teachers' training in high school. They were good teachers; they were dedicated.

One of the problems in winter was the sudden snowstorm. We had no weather forecasting, and I once almost froze to death. The temperature would drop fifty degrees, and then you couldn't stand the wind. You would go into a snowbank and wait for somebody to look for you.

Hughes: This was walking to and from school?

Johnson: Yes. I was very young then, probably about my second year in school.

For high school we had to go to the nearest town, which was called Loomis. They had eleven grades there. For the twelfth grade it was necessary to stay at Holdrege, ten miles away, returning on weekends.

Hughes: Was the elementary school near your home?

Johnson: Public School 62 was about one and a half miles from our home. It was a neat little white schoolhouse with seats on either side. In front there were the blackboards.

Hughes: How many families do you suppose it served?

Johnson: Five to ten families, with one to four children each. There were often fifteen to twenty students in the school. We played basketball and baseball and the usual games. We sang every day. In those days they read the Bible every day. So it was a good wholesome environment.

To get to high school in Loomis, you had to arrange a ride. Some people would come in a buggy; this was still before cars. The ones that lived in the town had it easy. Loomis High School was about three and a half miles from where I lived. That's why I had to ride horseback to school.

We had a man principal who taught physics and botany and math. We had no chemistry class, but the science courses were good. We had a basketball team, and I played on the team. We had games with similar schools in little towns, and it was really a lot of fun. We played on outdoor courts.

Hughes: Was the instruction good?

Johnson: It was excellent. These men and women who taught were good teachers. That was a real profession in those days.

Hughes: Were you showing any particular academic bent?

Johnson: Oh, yes. In high school, I was assistant in the physics class in Loomis in the eleventh grade. When I went to Holdrege High School the last year, I was assistant in chemistry and physics. I was active in music. We put on music shows. I played piano in the orchestra in high school.

Musical Interests and the McPhail Conservatory of Music, 1924-1925

Hughes: How did you come to attend the McPhail Conservatory of Music in Minneapolis, Minnesota?

Johnson: In those days, if you did church music work, you were never paid. Choral and orchestral music were things that I always enjoyed. For instance, I had a number of instruments I could play, and at this church that I went to in Minneapolis, I played the clarinet in the church orchestra. The Swedish Covenant Church was a very famous place because of the music department. The director of music, Holmgren, at this Covenant church in Minneapolis later became professor of music at UCLA--a fine tenor. The church had a male chorus that I and two brothers joined. It was called the Fellowship Glee Club. We sang for public events. The Scandinavians liked large choirs, male singing groups, and good orchestras.

The professor of music at Cal, Larry Moe, played the pipe organ at a Covenant church in Chicago. Later he studied at Harvard and in Europe. He is a distinguished organist.

I was an organist in a Covenant church in Nebraska. I did all the music, played the piano--we didn't have a pipe organ. But when I was in Holdrege High School, I learned to play the pipe organ at the city auditorium. I played for conventions. Feature musical concerts were held at the Holdrege auditorium--orchestras, soloists for voice, violin, and piano.

In Minneapolis, McPhail was a very well known conservatory. There you got a real good training in music. In Minnesota, they had one of the best public school music training programs. The name of the originator of the program was Thaddeus P. Giddings. There was rote singing in the first grade, subsequently learning to read the notes. When the kids graduated from grade school, they could sing four parts without accompaniment.

Johnson: When I was at McPhail, we would do practice teaching at the grade schools. It was beautiful the way that you could get the kids to sing. That's why I got interested in children's choirs. I loved the girls' chorus in San Francisco. Several of my students in the Trinity Methodist Church children's choir later sang in the boys' chorus in San Francisco. We did things like Amahl and the Night Visitors at the Trinity Methodist Church in Berkeley.

The director of music at that church, Dr. Earle Blakeslee, was also director of music for Berkeley High School. One of the most interesting men I worked with here in Berkeley was Garnell Copland, who at age sixteen to eighteen was our organist. He was probably one of the finest organists I've ever known. He was in high school with my kids; they were all in music at Berkeley High School. John played cello, Marion violin, Susan viola, and Michael trumpet. Marion, John, and Susan played in the Young People's Symphony. John played also in the cello club under the direction of Mrs. Rowell.

Garnell became our organist when I was working with the children's choir at Trinity. We usually had twenty-five boys and girls. He was a real Liszt. He was just an unbelievably wonderful organist. I remember one time at Cal, Hearst Hall, he played the whole hour without music. He played the classic Bach fugues. He went on to the Curtis Institute in Philadelphia for his degree. While there he was organist at a Presbyterian church. He went on to Washington, D.C. and was organist and choir director at the Church of the Epiphany, near the White House. I last saw him there in 1972. I guess he was about thirty-five. He was composing, and I consider him just as great a musician as Liszt. He wrote an elegie to his teacher, Sowerby. He used to come to the Bay Area and give concerts at Grace Cathedral and the First Presbyterian in Oakland.

The tragedy occurred about 1974. One night he was coming home from church, and his minister said, "Let me walk with you," because he'd been attacked one night and had a severe eye injury. Garnell said, "Oh, I'll be okay." On the way home that night, he was stabbed to death. He had only ten cents in his pocket. One of our greatest musicians in the United States. When he went to Philadelphia, his friends at Trinity Methodist Church helped him financially. He had done more in those years than anybody I know.

I've known a lot of very fine musicians in the Bay Area. We belong to Two Piano Club, and my wife also belongs to the Piano Club.

Hughes: Did music draw you and your wife together?

Johnson: Oh, yes. She was a nurse at the Children's Hospital in Boston, and she played piano and organ. So that was a common interest. We were married in 1936 and had four children. All our children are amateur musicians, but no one is doing it professionally.

Hughes: Did you attend McPhail University with the idea that you were going to go into premed?

Johnson: Yes. But I kept studying music. I told you I studied pipe organ all through medical school. There was a very fine organist in Omaha, Martin Bush. He had studied with the best organists in the United States. My one nephew later studied with him and now teaches music at the college at Yuba City, California. He has a master's in music.

II MEDICAL EDUCATION AND TRAINING

Decision to Go into Medicine

Hughes: When did you decide that you were going to be a doctor?

Johnson: I thought about this possibility all through high school. My dad was encouraging me in this. My brother too, and there were two neighbor boys that we knew that had gone into medicine, the Almquists. There was one other family, the Petersons, that lived ten miles north, where one of the boys became a doctor.

It was always a question, of course, whether you could qualify in this field. You had to be in the upper ten percent in high school to even get to take premed. Then when you took premed, you had to have a pretty good grade point average to get into medical school. So you had no other hope except doing well in school.

Premedical Student

University of Minnesota, 1925-1926

Hughes: In 1925 you went to the University of Minnesota. Why not the University of Nebraska?

Johnson: Well, that's where the family had gone; I was thinking that would be a good place for myself to go to school. My parents had friends there. We stayed with a family that my mother knew, until we found a place to live. Two of my brothers came to Minneapolis also. One brother was Joseph. He went to Minnehaha Academy. He was getting his degree in business administration. Philip was

Johnson: attending the University of Minnesota as a graduate student in chemistry. When Philip went back to Lincoln, he suggested we all go, and he would buy a house with two apartments.

Hughes: So all the brothers were there together.

Johnson: No, Joe was only with us in Minneapolis for two years. He came there before we did. But we had been up to Minneapolis before, visiting, so we knew the area. McPhail Conservatory was the best conservatory in central United States, as far as I was concerned. I studied with the idea of probably using music for some professional work, but not as a major money thing because in those days we never thought about getting paid for church music.

Hughes: Because you were in Minneapolis, you chose to go to the University of Minnesota?

Johnson: That was the plan. I wanted to go to the University of Minnesota. When I arrived, I was short a half unit of English. It was unbelievable, because I wasted time the last year in high school on other things. I took a course in typing, which was good. I've typed ever since. But I needed that extra credit.

Hughes: How did you work the English requirement in?

Johnson: In the summer of 1925 I attended Minnehaha Academy, a Covenant church college. Mr. Franklin there taught a wonderful course in English. You never can have too much of that.

University of Nebraska, 1926-1928

Hughes: Then the following year, 1926, you switched—still in premed—to the University of Nebraska. . .

Johnson: At Lincoln. My brother had received his master's degree in chemistry and was teaching science education at the university.

Hughes: He was the one that suggested that you make the transfer?

Johnson: Yes. It was one of those things that I wanted to do too, because he's been my mentor. We still kid about it. One of the family always seems to lead.

That was a very wonderful experience. I liked Nebraska U., everything about it. The teachers were good; we had wonderful courses. I took engineering physics and as much chemistry as I could, because I wanted to learn as much as I could about any of the [medicine-] related fields. It was all for premed.

Johnson: I learned a lot of medicine when I came back from Minnesota to be at Lincoln, Nebraska.* During three years of my premedical education, I lived at Lincoln General Hospital. By working in the hospital, I earned my tuition. I ran the office from 7-8 a.m. and from 5-10 p.m. I was on call for medical emergencies at night, an excellent training period for practical medical service. During the summers I worked longer hours at the hospital. It was a wonderful experience. In those days, tuition was thirty dollars a semester, but that would be like three hundred now. We got a dollar a day and your board, room, and laundry at the hospital, and that was all you needed. If you were really interested in medicine, you tried for those jobs. There were only three hospitals in Lincoln that employed premed students. There were few openings because those that got the jobs were apt to stay several years.

The premeds that worked in the hospitals had a kind of a club. We ran the telephone switchboards and talked about school assignments. You really were kept there, so you did study. We worked Saturdays and Sundays. We took in the money and kept track of the records. Then you would help at night to clean up the operating room or assist with the fracture cases and surgery. I learned a lot of medicine doing this work. I also helped with the autopsies.

Hughes: What was the quality of the medical care?

Johnson: Excellent. At the Lincoln General Hospital, the orthopedic section was run by Thompson and Orr. Orr was well known. He started what was called the Truda-Orr treatment for osteomyelitis. It was used in the Spanish Civil War in the 1930s. By this method you left the fracture wound open and treated it with maggots. The way Orr discovered the value of maggots was observing some farmers who had compound fractures of the tibia. When they developed osteomyelitis, the barn flies would lay eggs in the wounds and the maggots that were formed cleaned out the wound.

Later Dr. Orr raised stable flies and put the larvae into the wound. It worked. We didn't have antibiotics in those days. The usual treatment was to pack the wounds with vaseline gauze containing iodine. This was a successful treatment to some extent but chronic osteomyelitis was a common complication of compound fractures.

They had a very good surgical service. I have nothing but respect for the quality of the medicine that I saw then.

*The following two paragraphs contain sentences from an earlier discussion.

Hughes: Was the premed course at Nebraska essentially the same as that at Minnesota?

Johnson: Yes, very thorough. You had to be in the upper ten percent, or you couldn't get into medical school. That held on when you wanted to go, say, like I did, to Harvard. I had to be an honor student to even be able to take the internship exams for Harvard. I'm the only person from Nebraska I know who's gone to take the exams there. Usually you have your internships by fall of your senior year in medical school.

University of Nebraska College of Medicine, 1928-1933

Medical Student (1930-1931)

Johnson: I spent an extra year in medical school [1932-1933] as a TA [teaching assistant] in anatomy, teaching anatomy and embryology. That's where I got my Sigma Xi and my master's degree in anatomy [1932].

Hughes: Did you consider going anywhere other than the University of Nebraska for medical school?

Johnson: No. That was the only medical school I was aiming for, and if I hadn't made that, I don't know where I could have gone. It was not easy to get into medical school. At Nebraska they would admit a few from South Dakota and North Dakota, which did not have medical schools. They admitted about a hundred and fifteen first year students. But during the first term, they would drop about ten percent. It's not like today, where if you get in, you can stay.

Hughes: Were there outstanding people on the faculty?

Johnson: The University of Nebraska was a good medical school, and it still is. Nebraska has a very interesting state history, like Oregon. They do not build a building except paying for it as they go; even the state capitol was built by pay-as-you-go. The medical school was the same way. They had a tremendous campus. We had a new university hospital when I was there.

Master's Degree in Anatomy, 1932

Johnson: I had gotten my master's degree in anatomy by working in tissue culture with lymphocytes from lymph nodes and spleen tissue.

Hughes: This was in medical school?

Johnson: Yes. I was one of the first to do tissue culture. I minced spleen tissue and watched the cells grow out and stained them with Giemsa. The fibroblasts would grow out in lattice form and the lymphocytes would migrate out. I used a plasma clot, so it would contain fibrin. Multiplication of cells was identified by the mitotic figures.

Hughes: Had Leo Loeb worked most of this out?

Johnson: Yes, and so had others. The feeding material, the chemicals used, were basic, much simpler than we have now. You would make an extract of chick embryo, which would contain the food, and then serum. You can use Hank's solution as long as you add some beef serum--in fact, we use serum from colostrum-deprived calves. You can grow all sorts of cells with this medium. The blood serum has enough hemoglobin apparently; this globin or hemin is very important, and the albumin that you have in the serum, too. The blood contains the minerals that are necessary. We use ten percent serum.

Hughes: How long could you keep the cultures going?

Johnson: Oh, they grow in one cycle and survive for months. And that was true for Carrel; his cultures of chick embryo. At the time, there were only a few doing research on tissue culture, such as Alexis Carrel, W. H. Lewis, L. Loeb, and Margaret Smith. They would mince up tissues, but they would only start growing out on the glass surface if there were scratches on the glass. The connective tissue cells needed a support to grow on. The plasma clot proved to be the best material. I used this in my research in medical school. You had to bleed some animal; I used chickens. The syringe was lined with paraffin to retard clotting. The blood was expressed gently into a test tube lined with paraffin, centrifuged, and the plasma removed.

All you would have to do was to put a little bit of this plasma on the bottom of an Ehrlenmeyer flask, and as soon as you placed minced tissue, like chick or mouse embryo cells, a clot was formed. I used a drop of fluid obtained by crushing chick embryo tissue in a syringe with a sixteen to eighteen gauge needle. This would cause the plasma to clot. Unless the cells grow out, they do not begin to divide. They do show metabolic activity by

Johnson: production of acid. In plasma clots, cells immediately start forming mitotic figures, and they lay out long branching lines of cells.

I went to St. Louis and visited Loeb's lab and met Margaret Smith. It was Loeb that encouraged me to do tissue culture. I was one of the first, I guess, to see and grow heart muscle cells from chick embryos and watch them contract. At that time, they thought they had to have nerve innervation, but it's an intrinsic beat. I had all sorts of little experiments growing heart muscle cells and seeing how rapidly they developed and the timing of the beat.

Hughes: That was the basis for your master's degree?

Johnson: The thesis was on the segregation of white blood cells. Was there a stem cell and could all the cells come from the stem cell? It's only in the last fifteen years that we learned that lymphocytes are the source of antibodies and each one makes a specific antibody. That led to the hybridomas and the monoclonal antibodies.

Hughes: Your first paper is on lymphocytes, is it not?

Johnson: Yes.

Hughes: "Studies of lymphatic tissue grown in vitro with splenic extract as culture medium."*

Johnson: Fibroblasts stayed alive for a year. Certain types of cells have a longer life. The continued multiplication of epithelial-derived cells is difficult. It is the cancerous tumor growth that will continue to multiply, for example the HELA cervical cancer cell. The embryo hamster kidney cell, BHK 21, of Michael Stoker continues to multiply for many cell divisions from the frozen cell seed stock.

I didn't know anyone else in my area that was doing cell culture at that time. It was just one of the things that intrigued me. In one experiment on the origin of the heartbeat, I removed a turtle heart aseptically and suspended it in a jar in cell culture medium. It continued to beat so I brought it home to observe it. My mother was not pleased. [laughter] The heart continued to beat for several days.

* J. S. Latta, H. N. Johnson. Arch. Expr. Zellforsch. 1934, 16:221-229.

Medical Practice in Nebraska, Summer 1933

Johnson: I took a doctor's practice in Ashland, Nebraska for the summer, which was good experience. The doctor went away for a trip to Alaska, and I had his whole practice! When we graduated from medical school in Nebraska, we were supposed to be able to practice medicine. We'd done obstetrics where we'd go out with a nurse and two medical students on house calls and deliver babies. And then we had all the clinic training. We worked in the outpatient department and also rotated in the various services at the hospitals, including anesthesia. We were prepared to practice medicine. And then we could do other training when we got to the local hospitals; they would let us assist in surgery. In medical school, we were in pediatrics, medicine, surgery, and obstetrics, and then in the outpatient department.

Hughes: Did you see any interesting cases over that summer?

Johnson: Yes. Ashland was on a major highway, so you saw terrible auto accidents. They had plate glass in those days in cars, and plate glass is what we use now to cut sections for electron microscopy--it's much sharper than any [metal] knife. So wounds on those highways were just unbelievable, and that's where I had my first training in anesthesia, that you didn't [always] need it. There were cuts where the scalp was just hanging over one side. I thought from medical school you were supposed to give some novacaine. One of the old doctors said, "Oh, you don't need that!" He said, "You just sew the skin up and use the hair to tie the wound together so you don't have to use all these stitches. They won't feel pain for hours!" And it's true! We would sew them up--and there's no need [for anesthesia in these cases]. The body has natural endorphins. We got a lot of experience in sewing up wounds.

Hughes: Which you hadn't had before?

Johnson: Well, yes, we had to do all that at the medical school clinic.

Hughes: You had surgery as well?

Johnson: Yes, we had to learn to do the knots and everything else as well. That was the difference. It's something that you have to get in your residency today. In the early 1900s you trained with a practicing doctor, and you often did the amputations, everything else, and learned by doing. The Abraham Flexner Report in 1910 resulted in the licensing of medical schools.

In any small town, you observe some really amazing things. I'll just tell you one episode; there were many. When I first came to Ashland, the doctor said, "Well, I have a poker group that

Johnson: meets in the basement. You can sit in if you want." I said, "Well, I don't play poker. I don't care. Tell them to come in." There was an undertaker, an engineer, a guy who ran the pharmacy, and a couple others would sit in. They would play every week. I'd go and sit and watch them play.

I had a lot of calls when I was the doctor. The undertaker you'd get to know right away, because he's the one you used to have to call. I was asking about things going on, and who lived around there, and the doctor told me about two old maids that lived in the big house at the top of the hill in town. They were a famous family; the father had been a military surgeon. He said they were kind of reclusive; that's all I heard.

I hadn't been there more than a week, and there was a phone call after midnight, and this voice said, "Dr. Johnson, come over quick! Emergency!" And then the phone dropped to the floor. I didn't know what to do. I called the undertaker, and I said, "Will you go over with me? I don't want to go to the old maids' house alone."

So we went there and rang the bell. Nobody answered it. We said, "Let's try the door," and went in. And here was one of the gals lying on the floor cold out. I saw that she was breathing all right, so I beat it upstairs and found another one breathing, and I said, "I wonder what they've been taking?" So I looked in the bathroom and finally found an empty bottle of triple bromides. In a closet there was a box of empty bottles. The bottle had scopolamine in it, a twilight sleep type of drug that was made in a kind of cold remedy or something like that. Apparently, they were taking this as an illegal type of drug! And they actually had had a very big dose of it. I gave them caffeine as a stimulant and decided that they'd get along. One of them was the math teacher at school, and of course it led to an investigation of where they got the drugs.

In those days, there also was a morphine habit. I don't know how many people came to me, knowing I was young, and they'd feign gall bladder attacks or renal stone attacks. The old doctor said, "When they come in, look at their arms. If you see any needle marks, kick them out!" That was common in those days.

Hughes: Where were they getting it?

Johnson: Heroin and cocaine were available in the twenties. Cocaine was so bad in the 1920s that a death penalty was passed at that time. In certain minority communities, marijuana was very common.

Hughes: Were all classes using this?

Johnson: It was in the ghetto, and in the Dixieland orchestras, and so on.

Hughes: So the Scandinavian middle class wasn't doing it! [laughter]

Johnson: No, I never heard about it in Phelps County. Even smoking wasn't done in our group.

Internship and Residency

Harvard and the Brigham Hospital

Johnson: One of the reasons I got interested in Harvard was that Dr. Kirk, who was one of the instructors in medicine at the University of Nebraska, had been on the private residency service of the Peter Bent Brigham Hospital at Harvard. I'd make ward rounds with Kirk, and he'd tell me about what a wonderful place it was for medicine at Harvard. One of the other doctors from Nebraska, Ruben Schultz, went to Harvard as instructor in pathology. Dr. Perry Tolman, the pathologist at the University of Nebraska, trained at Harvard. So through them I heard about Harvard.

I should also mention that John Jay Keegan, who was doing neurosurgery at the University of Nebraska Medical School, was a house officer in pathology at Harvard for six months before entering the U.S. Army Medical Corps in 1917. He returned to Harvard in 1919 for further training in pathology, followed by training in surgery under Harvey Cushing. I believe he joined the faculty at Nebraska in 1932. He graduated from the Nebraska University Medical School in 1915.

As time went on I thought, well, why not shoot for Harvard? I knew that I'd have to go there and take a written and oral examination [for an internship at] Boston City Hospital, Massachusetts General, or Peter Bent Brigham. It was a three-day exam. Of course, money was scarce. I was working then in radio. But I decided to take a bus and go out there and take the exam during the Christmas holidays. I had no idea where I would intern if I didn't get it. I hadn't even applied for anything except that.

Hughes: How comprehensive was the exam?

Johnson: Oh, really, I think it was the best. Dr. [Henry A.] Christian, professor of medicine, and three other staff members interviewed me.

Hughes: These were oral exams?

Johnson: We also had written exams. The exam was for three hospitals having Harvard teaching services. You would give your first, second, third preference. Mine was the Brigham, which was affiliated with Harvard.

Hughes: Why did you choose the Brigham?

Johnson: Because that was the Harvard teaching service, with Dr. Christian as professor. They were all famous--[Harvey] Cushing in surgery, and [William Perry] Murphy of Murphy and [George R.] Minot--the ones that won the Nobel Prize for discovery of the liver pernicious anemia factor. I later worked in Murphy's clinic. Then there was [James P.] O'Hare in renal disease, Merrill Sosman in radiology, and Samuel Levine in cardiology.

Hughes: All these people were at the Brigham?

Johnson: Yes, and all were famous, an elite group of teachers.

Dr. [Simeon Burt] Wolbach was professor of pathology, and he came from Nebraska originally, that's one way I heard about Harvard. He was one of the great pathologists. He's the one that did original work on typhus fever in Poland--a wonderful teacher and a wonderful pathologist. That's one reason I wanted to have a house officer appointment in pathology.

Hughes: First you were a house officer in medicine [1933-1934]. Tell me how the medical service worked.

Johnson: Well, at Harvard you have a surgical service, and you have a medical service. It's not a general rotating thing; you do either one for sixteen months.

This was the problem: More than one hundred took the exams, and the Brigham only would take three medical officers for each service. You could come in at odd times because they'd start a new set every four months.

Hughes: So only eighteen out of the hundred would be selected?

Johnson: Yes. And when you got there, you were worried because they were from all over. But Dr. Christian always had the idea that he wanted some people from other parts of the country to give the group a wider range. They did not want to take just Harvard students. I started in October [1933].

House Officer in Medicine, Brigham Hospital, 1933-1934

Johnson: In Boston you had excellent training in all phases of diagnosis, particularly in medicine. I always say that the problem today, the young doctors don't look at the patients to see if they're sick or not.

In our training, you did a very complete history and it would be systemized. You went through the whole thing and asked questions about everything. You don't care who the patients were. And then we did the physical, starting with the scalp and did the whole body, and then a complete laboratory workup, complete blood and urine. Then you had a baseline, and if the patients came to the clinics, you had their whole records. You could look back and see what the diagnosis was when they were there before.

The Brigham Hospital was originally an endowed hospital where people who had money paid full rate or part rate. But the ones that didn't have money did not pay. The house staff on the wards did not know whether the patient was indigent, part, or full pay.

Hughes: So there was no discrimination.

Johnson: No discrimination.

Professor Henry Christian was a classic, a great physician. He used to say that there, but for the grace of God, is yourself in bed or somebody in your family.

Speaking about who had money: I had a patient, a girl who was about seventeen or eighteen, who was very ill with some kind of strep throat--we had no antibiotics in those days--and was getting pneumonia, and the only thing I could think of was getting some oxygen for her. So I called up the superintendent, Dr. Clay, and said, "I'd like to get a tank of oxygen here." Dr. Clay finally came by and said, "Look, this patient is not a paying patient. It's very expensive."

I said, "Well, I've been told by Dr. Christian that anything I as senior on the house staff ask for I can have, whether they pay or not." "Oh," he said, "I don't think that's possible." I said, "Well, then I'm going to call up the professor!" You didn't do that very lightly, because he was at home.

So I called up Professor Christian and said that I'd been told that I couldn't have oxygen for this patient that didn't pay. He said, "Clay told you that?!" And I said, "Yes." He said, "I'll be right over."

- Johnson: Pretty soon he came in. He was a big guy; he'd get kind of red-faced. He said to Clay, "Did you tell Johnson he couldn't have oxygen for this patient?" Clay said, "Yes, it's twenty-five dollars a day. We can't afford it." "Clay," he said, "did you ever think it might save the gal's life?!"
- Hughes: You got the oxygen.
- Johnson: Right.
- Hughes: Did Christian have a special medical interest?
- Johnson: Well, yea. Christian-Schuller's disease was named after him, but he was basically in the Osler tradition. He was trained at Hopkins. He was the first professor at this new hospital complex [Peter Bent Brigham], which had been patterned after some of the European clinics--a rather old-fashioned way where you could have patients out on a veranda in the sun when the weather was proper, and good ventilation. It was a good plan for a hospital.
- Hughes: Was the Brigham looked upon as a direct arm of Harvard?
- Johnson: It was the Harvard medical service hospital. They didn't really have a Harvard hospital. It was called Peter Bent Brigham Hospital, named for the family that endowed it.
- Hughes: Cushing was professor of surgery?
- Johnson: His whole surgical experience was at Harvard before he went to Yale. He was essentially retired when he went to Yale University. When I was in pathology, we reviewed all the Cushing patient sections, and various types of brain tumors. I had to go to the operating room, when I was the pathology officer there, and get the material and do frozen sections.
- He was very much of a taskmaster, Cushing was, but a very fine operator, and one of the first men to really tackle a lot of the brain and spinal cord tumors.
- Hughes: Is there anything more to be said about that year of medical service?
- Johnson: Today a lot of students go through medical school and really have not done much laboratory work. They may have just learned about it. But we had to spend four months doing the simple things, like blood gases and blood counts and smears and differentials, stool exams, urinalyses, and then all the special blood chemistries for nonprotein nitrogen. Dr. Christian's idea was that you would not really know how accurate these tests were unless you yourself had done them. In a red blood count or white blood count, you would never get the same number twice. But you'd get pretty close.

Johnson: You learned to recognize anything unusual in a throat smear or in a nasal smear. Then we had to do the stools--every patient had a stool analysis for the various kinds of worms and amoebae. A lot of people in those days had beef tapeworm and pork tapeworm and fish tapeworm. You did blood cultures and throat cultures all by yourself. It was a full time job to keep up with that. You worked hard day and night, and you were on call.

Hughes: That was also a service to the hospital, was it not?

Johnson: Oh, yes. We had a backup lab, but there was only one person in charge of the lab. That was a good technician, who trained us. And then the staff that had been through it could check you, especially on blood typing and pneumococcus typing.

The next four months was the time when you were a junior on the ward, and there was a similar period in surgery. When you were a junior, on every new patient that was admitted, you had to do a complete history and physical. After you completed that, the senior would go over and check it. You'd write your diagnosis, and he'd write his note. Then the assistant resident would check that, and then we had a senior resident for the whole hospital if there were any questions. They were good teachers. By the time you were assistant resident or chief resident, you really would know medicine.

Hughes: There was only one senior resident and only one assistant resident in each service?

Johnson: Yes, right.

The next four months, you worked in the outpatient department. You saw the routine type of medical cases that would come in. Then the last four months, you were a senior house officer in charge of the ward. When you were in charge, you would report to the professor of medicine every morning at seven o'clock. He'd be in his office, and you'd report how many admitted, how many discharged, how many died, autopsy permits, and all that.

Hughes: Were these general wards?

Johnson: Yes. They had some private rooms. And the private service at the hospital was where Dr. Christian and others would have their own patients. You'd work on those patients too, as a senior. You also had others that would take their residency on the private service; they would come in for training. But you would be in charge of that ward. You were responsible, and if anything went wrong, you could be sued.

Johnson: I had two episodes. One was where the nurse put a tube down the stomach to draw fluid for gastroanalysis, and the metal tip came off the tube. It had nothing to do with me. I was the senior house officer. The patient said he had a pain in the stomach, and he thought he could feel something. He knew something was wrong because the nurse said the tip was off. He insisted on going home, and he got a lawyer. He found out, of course, that there was not much chance of getting any money out of me. So he finally was readmitted. He was an alcoholic. So I went to the operating room, and Dr. Cutler operated. When he got the tip out, he dropped it in the pan and said, "There's your release." [laughs]

There was no malpractice insurance in those days, but they would still try to get money. They couldn't get the hospital; it was not liable in those days.

Hughes: You mentioned Cutler. Was that Max Cutler?

Johnson: No, this was Elliott G. Cutler. He was professor of surgery after Cushing.

Hughes: Cushing had left for Yale?

Johnson: Yes. He left after I started, that same year [1933].

Hughes: You said off tape that Christian became a friend. Did it occur at that time?

Johnson: Yes. He had no children; he married late. He would invite some of the staff over for a dinner. He was never overly friendly. Of course, I didn't get the chief residency. I knew I wouldn't when I married. But he still followed me through, because he wanted me to follow the same training. I planned to do the pathology, regardless. And then I got an assistant residency under him, too, which was all I really needed.

When I went on to the Rockefeller Foundation, Dr. Christian was very pleased because he knew Dr. [Wilbur] Sawyer, who was then director of the International Health Division. Dr. Christian recommended me for that and followed my career. One of the first things he asked me after I got to working on rabies was to write a chapter for the Oxford System of Medicine, where he was editor, which I did.*

Hughes: So you kept in close touch with him.

*"Rabies" in: Oxford Loose-Leaf Medicine, Henry A. Christian, ed. New York: Oxford University Press, 1943.

Johnson: Yes.

Hughes: We've mentioned Cushing and Christian and Cutler. Was there anybody else outstanding on the staff?

Johnson: Yes. Dr. Merrill C. Sosman was a leader in roentgenology and x-ray diagnosis. Then Dr. Murphy, who had the blood clinic, shared the Nobel Prize [with Minot and George H. Whipple in 1934]. Minot also came over for consultations. Dr. O'Hare was in the renal clinic. Dr. Samuel A. Levine was chief of cardiovascular medicine.

Hughes: Where was Minot?

Johnson: Minot was at MGH [Massachusetts General Hospital]. We used to share a lot. We had our grand rounds, and you'd go to other hospitals for those, too.

Hughes: So there wasn't competition between the hospitals?

Johnson: Well, MGH and the Brigham were always in competition, whatever line it was.

Hughes: Friendly?

Johnson: Oh, yes.

Assistant Resident Physician, Contagious Service, Cleveland City Hospital, March-August, 1935

Johnson: I haven't mentioned that after my sixteen months as a house officer was the appointment in Cleveland.

I finished my sixteen months in medicine at the Brigham Hospital, and I was going to take a month off to see my family in central United States. I stopped to visit my brother, Philip, in Ithaca, and Dr. Prexy Jackson at Cleveland City Hospital, and Dr. Ros Hildreth at the University of Michigan at Kalamazoo.

Hughes: Who was Dr. Jackson?

Johnson: He was one of the men that interned with me in medicine. He went from the Brigham to pathology at the Cleveland City Hospital. I don't know what happened to him afterward; he really was a very interesting friend. He had finished his internship four months ahead, and he'd already gone to the Cleveland City Hospital, where he was house officer in pathology. So I stopped to see him.

Johnson: It was at lunch that day when I heard that one of the residents in contagion had been forced to resign because he had been caught drinking alcohol on duty. They were looking for somebody that day.

Dr. [John A.] Toomey, chief of contagion, was there. So Jackson introduced me to Toomey, and Toomey said, "Well, can you come? Harvard! That would be great! It's yours if you want it." You were supposed to get forty dollars a month, but it was during the Depression, and they weren't paying cash; it was script. So I didn't get anything for the months in Cleveland.

Hughes: Had you gotten paid at Harvard?

Johnson: No, nothing in your first sixteen months as house officer.

Hughes: How did you live?

Johnson: You had your board, room, and laundry. You didn't spend anything. Occasionally, I would write to my brother and get a little cash, but I just didn't spend anything. Furthermore, that was your life. You were working all the time.

Hughes: There wasn't time to spend anything! [laughs]

Johnson: No. I remember one time when I was a senior [resident] that I didn't get to bed for three nights; we had a real epidemic situation. There was a similar epidemic situation at Cleveland City Hospital. Many times I never got to bed because of cases of diphtheria and cases near death from other infectious diseases.

I was supposed to start in pathology at Harvard in a month. So I telephoned Dr. Wolbach and Dr. Christian and said, "Look, here's a real good opportunity [at Cleveland City Hospital] to get some more training on infectious disease. It's for only five months. Would Dr. Wolbach wait and start me in the fall?" He said, "Oh, you must take it. That would be excellent training. I'll hold the pathology place for you." And so I was there five months, and it was extremely interesting. Imagine, 250 patients with infectious diseases--polio, measles, chicken pox--everything you could think of, and all kinds of very hot bugs. One of the interns got septicemia from a scarlet fever case and he died. We were at that time working with bacteriophage, trying to save some of our [patients with] septicemias, which we couldn't. The bacteriophage would not work in the patient; it worked fine in the test tube.

Hughes: Was the use of bacteriophage a common technique?

Johnson: Well, that was just coming in then. Bacteriophage were discovered in the 1930s. You could destroy all the bacterial cells, such as staph, in one flask in just a matter of minutes with that phage; it went so fast. In just a matter of a few hours, it would clear it. But in the body, the serum inactivates the phage.

Hughes: So bacteriophage never had a therapeutic use?

Johnson: No, not that I know of. They tried it in Russia; they made a bacteriophage for intestinal infections, but it was just a nostrum.

Hughes: Well, what did you use for these infections?

Johnson: Well, all patients that were in the hospital with measles or mumps, we would bleed them when they left. We had immune sera. It was as good as gamma globulin; nobody was making gamma globulin. So we had already available our own stock of immune sera, so if people were exposed to measles, we could give them some of the immune sera if there was a reason for it.

Hughes: You had immune sera for all the infectious diseases?

Johnson: We saved it from anybody we had, even scarlet fever and mumps and polio.

Dr. Toomey was doing some work on polio in which he was trying to show that the intestinal tract was involved [as a portal of entry for polio virus]. He couldn't get his papers published. [He was] injecting the stool material into the gut of monkeys, and certain monkeys would come down with paralysis. The reason he did it was that he felt that polio had something to do with cleanliness, because there was one town in Pennsylvania where the people that lived on the nice side got polio but practically none in the poor section. And that suggested that polio was an intestinal infection, which was later confirmed, but at that time they didn't believe it.

Hughes: How did they think it was transmitted?

Johnson: Well, at that time, they didn't know. They thought it might be transmitted through inhaling air. They later used the tannic acid treatment in the nose to try to stop it there. But the concept was like rabies, that polio and rabies were strictly neurotropic viruses and didn't involve any other organ.

Hughes: Is that why his papers were rejected?

Johnson: Yes.

Johnson: Dr. Toomey was a very fine physician, and working with him was a real thrill. We had to do our own tracheotomies for diphtheria. We had a diphtheria epidemic at that time--tragic. Residents had the possibility of doing everything that could be done, and it was a well-run service. Cleveland City Hospital was a big hospital.

Hughes: When you say Toomey was interested in polio, did he actually have a research project?

Johnson: Well, yes, but supported with funds that he got locally. There were no national funds. He had one technician, who was a Hungarian--very intelligent man. They hadn't tried inoculating specimens of feces in the brain of the monkey. There was no way to control the bacterial sepsis.

At that time, they were trying to infect monkeys in other ways. Monkeys can come down with polio just from oral exposure, and they didn't know at that time why certain monkeys would get infected if they were in the same room as those inoculated. You had to have a technique for isolating the virus from throat or stool specimens. Once they got a system for tissue culture, devised by [John H.] Enders, [Frederick C.] Robbins, and [Thomas H.] Weller, and proper antibiotics, it was possible to demonstrate the virus in throat swab and stool specimens.

Hughes: But that was later [1949].

Johnson: Yes. Virology came into being after I started working with viruses [1938]. The yellow fever lab at the Rockefeller Institute was just beginning virology as we know it today.

House Office In Pathology, Brigham Hospital, 1935-1936

Hughes: You were at Cleveland City Hospital for five months.

Johnson: Yes. Then I came back to Boston for twelve months in pathology at the Brigham, doing autopsies and clinical pathology. That was the year [1936] that I got married. When you're on pathology, you're doing autopsies day and night. They have to be done soon after death. You couldn't get away from the hospital unless the resident pathologist covered for you.

Hughes: What was the reason for doing the autopsy within an hour?

Johnson: The morticians want to take the body for embalming and the pathologist wants to minimize postmortem change.

Hughes: It really happens that fast?

Johnson: Yes. But you have to wait an hour after death before you do an autopsy. In the meantime, the body is kept in a refrigerated holding unit.

Hughes: The hour wait is a legal requirement?

Johnson: Yes. You have to get a permit to do an autopsy. It has to be [signed by] the nearest relative. The house officer does that. Being on the staff at Harvard, the only money we could make was if it was a private case and we did an autopsy somewhere else, usually in the mortuary.

Dr. Bob Gross, who became a famous surgeon and did the first blue baby operation at the [Boston] Children's Hospital, and I did an autopsy in the home of one of the leading lawyers in Boston. We did it in his bedroom. He was one of the early cases treated with intensive x-ray for prostatic cancer. So in his will he requested an autopsy. We came to the house with our equipment. It was a big house, looking down on a lake; there was a long winding staircase going up to the master bedroom, with a fire in the fireplace and big rugs. There was a private duty nurse waiting to aid us. This was where we were supposed to do the post [mortem].

It was one of those absolutely strange episodes. We always were very neat in our white uniforms and never wanted to get a drop of blood on anything. I was taking out the brain and had a basin with blood in it. The nurse came by quickly and upset the basin. Blood splashed on the rug. We knew enough to get a salt solution right away and clean up the rug. It was a job. Such are the absolutely ludicrous things that can happen. That was not all.

When we were leaving, I carried a wooden microscope case with a lock on it, with the tools in it. As we came down the staircase, they were reading the will. Guess what happened? The door came open on the case and some of the tools rattled onto the stairs. [laughter] Everybody turned to see what had happened. We quietly picked up the instruments and went out as gracefully as we could. Private autopsies were where you made some extra money.

Every organ had to be examined, in other words, a complete autopsy. That's the way we did it. In an autopsy at, say, fifty years of age, you expect to find cancer and other pathology that the person didn't know he had. A certain percentage of autopsies have tumors of the brain, some are not malignant. And that's where you really find out what the outlook is for the various diseases, which ones we have to worry about. There are such a fantastic number of types of tumors you can't see, and other causes, which are all incidental findings at the post and very interesting.

Hughes: Well, you emphasize the completeness of the autopsy. Do you mean in comparison to what other hospitals were doing then?

Johnson: Yes, it was the university teaching hospitals that did the detailed studies and kept typed records, sections of all organs, and pictures of important findings. Here [in California] we have the Oakland Regional Laboratory for doing pathology. Commercial lab autopsies are expensive. Some hospitals have a resident pathologist and a postmortem service. It is necessary to determine the cause of death in some cases. This is the job of the coroner.

Hughes: How long did a post mortem take?

Johnson: I could never do it in less than an hour, more often two or three hours, because we tied off the major blood vessels and left tubing so the morticians could embalm the bodies easily. This was the way we could get good cooperation with the morticians in Boston.

In autopsies we got terrible infections because a lot of the autopsies were septic cases in those days. I don't remember how many times I was admitted to the hospital for such infections.

Hughes: You were wearing gloves?

Johnson: Yes, but you'd cut yourself, and you were working blind in certain limited chest and abdominal examinations, and the danger was you'd get a bone spicule or something that would puncture your gloves. What we'd do is take them off, wash thoroughly, and then we'd cauterize [the wound] with phenol or nitric acid. It was not infrequent in those days for a pathologist to die of strep infections.

The last day I was on the service in June, 1936—it was one of those times with several cases lasting past midnight. One of them was a septic case, and when sewing up the body, I stuck myself in the back of one of my fingers. I took off the gloves and cauterized the puncture. I had a very short time to sleep before I had to go to where my bride-to-be was living. When I woke up that morning, I had a pain like a bee sting, and I realized that I had a serious infection. The only thing we could do in those days was to soak [the affected part] in water of 115 degrees for twenty minutes at a time and then repeat it about every eight hours. This stopped it.

On other occasions, we would have hot packs with mangesium sulfate to localize the infection. Infections were always scary. People would get erysipelas from scratches. Now we can use one of the antibiotics, but in those days it was a dangerous infection in hospital work. So that was the end of my first year path service.

Johnson: There's no other way, like Dr. Christian said, to know disease, unless you do autopsies to see why people die. You often find that a mistake has been made in diagnosis; the thing you thought killed them was not the main cause of death.

We had a big study at the Children's Hospital on infant or crib deaths, and I gave a lecture on the subject. They'd think the child choked on the bedclothes. But we had a practice there of doing a blood culture from the heart on every child that died. Some mothers at that time did not nurse their babies. It is the first nursing of colostrum which contains the protective gamma globulin. To our great surprise, we found that children were dying of just ordinary B. coli meningitis and septicemia. As far as I was concerned at that time, and I still feel that way, the majority of these crib deaths was caused by a malignant septicemia, where the blood was just loaded with bacteria, and yet there was nothing you really could see in the sections, except toxic reaction in the lymph glands and spleen.

Hughes: Why was there the reaction against breast feeding?

Johnson: The thing was concern about their figure.

Hughes: It was modern not to?

Johnson: Yes. Our children were breast-fed.

In one of my studies in which I reported on the western encephalitis vaccine, we found out that in a horse the colostrum antibody will persist in the foal for up to a year. I think this is why most children are well protected for a year after birth, if they get the colostrum.

Hughes: Was that known then?

Johnson: No. The foal of a horse that has been immunized from that first nursing has, by test, almost as much antibody in the blood as the mother, whereas it will have none if it's colostrum-deprived.

Hughes: When this fad for bottle feeding came in, it wasn't really appreciated what damage was being done?

Johnson: No.

Resident Pathologist, Boston Children's Hospital, 1936-1937

Hughes: You've mentioned Children's Hospital, but you haven't said why you were there.

Johnson: After I had my twelve months as a house officer in pathology, the next logical step was to be the resident in pathology at the Children's Hospital. You had to have the previous position or you couldn't get the job at Children's Hospital.

So the following year, I was resident of pathology at the Children's Hospital, '36-'37. Dr. [Sidney] Farber was the pathologist in chief at the Children's Hospital--a wonderful pathologist. He was trained in pathology in Germany.

Hughes: He was an American?

Johnson: Oh, yea. He also was trained in the United States, by Wolbach. He had the most advanced autopsy service. It was just like an operating room; everything was just spic and span. You did it just like you were doing surgery. All the records were kept meticulously. There are pictures of all the gross pathologies.

That's when I wrote that paper on chicken pox.* It was [based on] a child with hydrocephalus that happened to be in the neurological clinic. The child got exposed to chicken pox on the service and died of chicken pox. It was very rare that anybody would die at the height of the infection.

I found lesions, not just in the skin, but in the brain, the kidney, the ureters, the bladder, the liver, and described these. They all thought this was a specific disease of the skin. I described the visceral lesions of chicken pox.

Hughes: You were the first to describe them?

Johnson: Yes.

Hughes: It had nothing to do with the hydrocephalus?

Johnson: No. The hydrocephalus created a situation where the chicken pox was enough to kill the child.

Hughes: Was the main problem in Children's Hospital infectious disease?

Johnson: Well, we had a terrible time. The very first sulfas were coming out. That was the first break in trying to deal with things like lobar pneumonia and pneumonia in children. Many died of whooping cough pneumonia. They'd aspirate food, and then there's no way of controlling the infection.

*H. N. Johnson. Visceral lesions associated with varicella. Arch. Path. 1940, 30:292-307.

- Johnson: The death rate from measles and chicken pox pneumonia was high. But the strep infections, scarlet fever, were the top bugs, streptococcal viridans, the strep throat type of infection. So infectious disease was a major cause of death in children.
- Hughes: What vaccines were available?
- Johnson: At that time, we had a vaccine for scarlet fever--not very good. We had a vaccine for diphtheria, which was new, and whooping cough vaccine.
- Hughes: Were those routinely given?
- Johnson: No.
- Hughes: Immunization was left up to the parents? It wasn't a legal requirement?
- Johnson: No. There was no vaccine for polio or rubella or measles. If a child was exposed to measles, you could give immune serum from somebody that had had measles. Fortunately, jaundice did not seem to be a problem at that time. It was only when the yellow fever vaccine came along that we learned about serum hepatitis. There were cases of hepatitis in the army among those given the yellow fever vaccine.
- Hughes: Why was it difficult to prove that the vaccine contained hepatitis virus?
- Johnson: There were many cases of hepatitis in the army where the service men had not received the yellow fever vaccine.
- Hughes: Why do you suppose it didn't show up in these earlier vaccines?
- Johnson: The yellow fever vaccine contained human serum. It was used to keep the virus alive as you dried it. It was learned later that it was not necessary to add serum to the vaccine.

Smallpox vaccine was just the fluid from the blebs from a cow inoculated on the abdomen. The bleb material was saved in glycerol to depress bacteria. But a lot of people got bacterial infections from smallpox vaccines, too.

The Children's Hospital was a very scary place at that time, in the sense that so many children were dying, and we got all the bad cases from everywhere. I wondered if I'd ever have a normal child, because all we saw were [cases] that a lot of doctors would not see in their whole lifetimes as a pediatrician. We would have several on the ward with intestinal atresia and all kinds of strange congenital phenomena, such as valvular trouble of the heart, which you couldn't do much about in those days.

Hughes: What about staff at Children's Hospital?

Johnson: Dr. Sidney Farber was pathologist in chief, and he would be in charge of the clinical pathological conference, but I would present the cases that we autopsied, those that would be interesting for discussion. Each week, he would have that, and you would have to present what was found to the staff, what went wrong, and what we learned. It was very important. Your job was to be there seven days a week and be in charge of the pathology service.

Hughes: Was Bob Gross at Children's?

Johnson: Yes. His training was first under Cushing and later Cutler, but he was in pathology two years. He was a house officer and he was a resident. In fact, he was my resident when I was doing my house officership.

Hughes: What sort of a person was he?

Johnson: He was a very likeable guy, very easy to work with, and really a hard-working, thorough person.

Hughes: Gross is associated with the blue baby operation.

Johnson: Yes. The operation that he did was [for] patent ductus arteriosus. Now, the patent ductus can be large, and then the patients are really blue. That's the operation they started with, because it is simpler; you couldn't stop the heart [in those days]. It was the one where you tie off the patent ductus.

A similar congenital heart disease was the interventricular septal defect. The interesting thing about this clinically is the noisier they are, often the better. The main thing is, are the patients blue? You could have an interventricular septal defect about the size of a matchstick; it made a lot of noise, but the patient had perfectly good circulation. If you leave them alone, most of them will close by the time the patient is twenty-five. So you don't operate on those, even now. It's only if they have a large one, where it's probably as big as your little finger--then you're getting circulatory problems.

Hughes: Did you see any of those hearts?

Johnson: No, not patent ductus cases, but in routine autopsies you often find the scar of where there's been an intraventricular septal defect and it's healed. Sometimes patients will mention that they had a murmur in childhood.

Johnson: Nobody believed in the old days in operating on the various types of cardiac malpositions, like the tetralogy of Fallot, but now they can do the necessary surgery of the major vessels, usually a primary operation to increase the pulmonary circulation, followed by total correction later.

One of the important men when I was at the hospital was Dr. Carl Walters. He was responsible more than anyone else for the renal dialysis apparatus. He also was working on how you could maintain the heart with some kind of a heart pump, and other ways to oxygenate. They were already talking about getting a collodion film that would make it possible to oxygenate the blood outside the heart.

Hughes: When was this?

Johnson: Well, this was during the time I was there, which was until 1938. We were concerned about pulmonary emboli. They would block a part of the lung, and the patient would die of hypoxia.

Hughes: Well, open heart surgery is mainly a postwar phenomenon.

Johnson: And the same with renal dialysis. The Dutch, actually, had a lot of the early ideas of how to dialyze the serum. The first successful renal transplant was at the Brigham, done by Hartwell Harrison, whom I interned with. Dr. Quimby was the senior staff member in urology. The donor and recipient were identical twins, which was the best.

Renal transplants have gone through all sorts of variants. The big problem of using the anticell drugs, like Immuran, is that you reduce the body resistance by giving this drug to get them to not reject the renal transplants, so there is a sharp increase in infections and cancer in kidneys. But they found out that people who had had two or three transfusions tolerated the cadaver kidney much better.

Illness

[Interview 2: February 17, 1987]

Hughes: Dr. Johnson, you mentioned that you had a bout with a disease that they thought might be tuberculosis. Would you tell me about that?

Johnson: The way it happened, I was getting my assistant residency in medicine at the Peter Bent Brigham Hospital. In December of '37, I had a routine x-ray, and they found an interlobular lesion in my left chest. Of course, as many of our older people today know, tuberculosis was active around the country in those days, and you

Johnson: usually got exposed to it. I was probably infected in medical school. So I had some primary immunity from that; I had a positive skin test, whereas today my own children do not have a positive skin test. The question was when I had this lung lesion, was it an activity of tuberculosis?

They tested my sputum and could find no evidence of Mycobacterium tuberculosis organisms. We had had patients on the ward with such organisms in the sputum, so we knew we were getting exposed.

The decision then was whether I should actually take full-time off for rest, which was kind of scary at the time. Dr. Henry Christian, who was chief of the medicine department, felt that it was better if I would just go on half-time work, which I could do as an assistant resident. I could still do the clinics, make ward rounds, and write progress notes for the patients' records. As long as you had no evidence of active tuberculosis by sputum, you were in no danger. I was told to spend at least ten hours in bed each night, which reduced the stress. I did that for that whole year.

Hughes: What year was this?

Johnson: We got married in 1936, and then in December of '37, just before Christmas, I went down to look at my routine chest plate and saw there was a lung lesion. It was kind of scary because I had been years without any salary, and this was my first year with salary, which was forty dollars a month. My wife was a graduate of the Children's Hospital nurses training school.

Hughes: Were you feeling all right?

Johnson: Yes, I felt okay. I had no symptoms. Looking back on it, it's possible that this could have been a reactivation of an old primary tuberculosis, or it could have been what's now called atypical pneumonia or mycoplasma. There was no evidence that I had any increased tubercular activity, and the lung lesion gradually subsided. I had routine x-rays every year for several years, and the intralobular thickening decreased to a point that it was only a thin line. So it healed up.

Hughes: How long were you on that ten hours a day in bed?

Johnson: Until I ended my residency in medicine, which was in the spring of '38. I then began the fellowship at Harvard School of Public Health.

Hughes: Were you now beginning to think that perhaps you didn't want to go into full-time medical practice?

Johnson: That was what I was heading for. I could have had a professorship in pathology. I had two years in pathology at Harvard. And I could have had a professorship in medicine, because I'd had the whole course of training: the internship in medicine, the residency in medicine, the house officership in pathology, and the residency in pathology, and a residency in infectious diseases, which was basic training in those days. But it looked like for the time being that it would be better if I did academic work, academic epidemiology.

The Venereal Disease Clinic, Brigham Hospital

Hughes: Well, before we get into the Rockefeller story, shouldn't we talk about the venereal disease clinic at the Brigham Hospital?

Johnson: We rotated on clinics. The clinics were the cardiac clinic, allergy clinic, GI [gastrointestinal] clinic, blood clinic, renal clinic, and venereal disease clinic.

I had a special interest in the venereal disease clinic, but I also liked the blood clinic because Dr. Murphy was director. As assistant residents you would serve on these clinics, which was very good training in each field.

They had a patient load of about seven hundred at the venereal disease clinic. This was before penicillin; we had no really good treatment. Arsphenamine was the main medicine. It was a toxic medicine, and when you gave it, you had to give it intravenously. You had to be careful to have the needle completely inside the vein because the arsphenamine usually caused necrosis if it leaked into the tissue at the injection site. But it was an effective treatment in primary syphilis.

For a new case of primary syphilis, you would give a course of arsphenamine once weekly for a variable number of doses, but it usually was about four to six weekly injections. And, in the meantime, you would plan on the followup course of bismuth injections. These were two metals that were used at that time for chronic syphilis. The earliest one, of course, was mercury. Mercury had been used for a very long time, and particularly in the Scandinavian countries, where the patient was told to rub the mercury ointment into the skin in the thigh region daily. I don't know why doctors didn't think much about the mercury toxicity. The patients were told to use the mercury rub until their gums would get sore and there was salivation--symptoms of mercury poisoning.

Hughes: Had mercury just recently been dropped?

Johnson: No, mercury compounds were given by intramuscular injections weekly, for four to six weeks, until signs of toxicity appeared. It had a specific effect on the spirochetes. Bismuth compounds also were given by intramuscular injection, and this became the standard treatment after the course of arsphenamine.

Hughes: Did bismuth work by a similar method?

Johnson: Yes. It seemed as effective as mercury injections and there was less toxicity.

We did have one specific diagnostic test for early syphilis, that was the demonstration of the spirochete, Treponema pallidum, by dark-field microscopy of fluid from scrapings of the primary and secondary skin lesions. Some doctors, once they started the treatment, would tend to treat the patient until the Wassermann, a complement fixation test, became negative. If the test remained positive, a patient would develop arsenical reactions more serious than the mercury toxicity. We used the Wasserman test and also the Hinton, an antigen flocculation test similar to the Kahn and Kline tests. The cardiolipin antigen was prepared from beef heart tissue obtained from meat processing plants. Dr. [William A.] Hinton was professor of immunology at the Harvard School of Public Health.

These were nontreponemal tests like the VDRL we now use, that is, the antigen was not made from the spirochete organism. VDRL stands for Venereal Disease Research Laboratory. These tests revealed an active disease. The Wasserman and flocculation tests were also positive if the patient had malaria or some other serious infectious diseases.

Dr. Hinton was in charge of the serology laboratory at the Brigham Hospital, and I enjoyed reading the results of the complement fixation and flocculation tests. It was important to have accurate titration of the antigen and to include negative and positive control blood sera. The nontreponemal antigens failed to pick up one fourth of the early cases of syphilis, but here the dark field was always positive if there were the usual primary chancre and subsequent secondary skin lesions. These in themselves were also sufficiently characteristic so as to make a diagnosis of syphilis possible.

We now have the treponemal immobilization test (TPI) which uses a live virulent strain of the T. pallidum as an antigen. The most practical is the fluorescent treponemal antibody test (FTA). Most people have low titered antibodies to T. pallidum from exposure to nonpathogenic spirochetal organisms. The reagin (antibody-like substance) in the patient's serum is measured by the VDRL test still used for routine serology. The antigen made from killed T. pallidum is preferable to the lipoidal cardiolipin.

Johnson: Dr. Christian, when he came to Harvard in 1913, became interested in doing something to prevent chronic metal poisoning from arsphenamine, mercury, and bismuth. His decision was, if you had a patient with a history of a primary local "chancre" lesion and subsequent secondary skin lesions, he was given a primary series of arsphenamine, followed by a course of the bismuth injections. The patient would return for a blood test and physical at intervals. A woman with a positive VDRL blood test would be given a course of treatment if she became pregnant. If a baby is infected with syphilis in utero, the organism produces a chronic infection in the fetus. Congenital syphilis is associated with blindness, bone, brain, and heart disease. Often it would be so slow that their worst symptoms would come after the age of ten or twelve.

Because of the tragedy of congenital syphilis, a law was passed to require a VDRL blood test for syphilis before marriage. An adult with a positive blood test, unless there was some sign of active syphilis or a history of primary or secondary syphilis, was not treated. You would do a spinal tap to see if there was any evidence of disease in the nervous system. You would check certain bones by x-ray because syphilis tends to involve bone, especially the lower leg bones. You checked the heart for signs of valvular disease, and then you'd do an x-ray of the chest to see if the aorta was normal.

The decision not to treat people twenty years after a positive blood test and where there was no clinical evidence of syphilis led to criticism of the U.S. Public Health Service for not recommending additional treatment of a cohort of male patients after penicillin became available. There was no indication for giving the antibiotic at that time unless there was evidence of active disease in the brain, heart, or bones. The VDRL test may be nonreactive in late syphilis. Currently, if a person whose serum is positive for syphilis in the VDRL test and a clinical and epidemiological study reveals no evidence of syphilis, it is essential to do the Fluorescent Treponemal Antibody (FTA) test. If this is negative the patient can be assured that there is no evidence of syphilis. There is a stigma associated with syphilis which has legal ramifications.

We always took careful histories. You had to follow up the contacts if you saw an acute syphilis case. That was the way you would break the chain; inform people who had had sexual contact with that person and have them come in for examination and laboratory tests. The question is, what about a married couple if one of them has a positive blood test for syphilis? The male's positive and the female's negative, or the female's positive and the male's negative. When we learned from patients that they'd been married five years, and the male was negative by serologic tests for syphilis and the female was positive, she probably was

Johnson: infected more than five years before she married this person, so she was not infectious. Or vice versa. It was important to treat the woman, if she had a positive test, only during pregnancy. That was the key thing, because any persistent focus of T. pallidum infection might be activated by the stress of pregnancy, and a few spirochetes might then reach the fetus via the circulation.

Before the discovery of modern antibiotics gonorrhea could be a disastrous disease, such as acute bacteremia with Neisseria gonorrhoeae, or women with tubal abscesses, and men with acute urethritis and urinary tract obstruction. The organism could be diagnosed by the gram stain. Men learned to carry a catheter in order to empty the bladder. It was a horrible disease. It was one of the miracles of the modern antibiotics when gonorrhea was brought under control.

The problem today is that you have the resistant strains, and in certain countries of the world gonorrhea still is an acute, devastating disease with high fever and bacteremia. There are several antibiotics that are effective if the gonorrhea organism is resistant to penicillin. Penicillin remains the drug of choice for syphilis.

Another venereal disease we saw was lymphogranuloma inguinale, which is caused by an organism which belongs to the genus Chlamydia. It would cause a chronic infection of the lymph nodes around the genitalia, and there was a tendency to form strictures in the anus or the vagina, and it was a very chronic, serious problem, mostly in Haitians that we'd see in Boston. We had no specific treatment in the 1930s. Now the disease is treated with tetracycline. We did have a skin test. Fluid was collected from inflamed lymph nodes and, after processing, a formalin-treated skin test antigen was prepared. The antigen was injected intradermally, and if an inflammation response appeared, the test was recorded positive. We also saw cases of chancroid caused by the Ducrey bacillus (Hemophilus ducrey). This could be diagnosed by a gram stain. We had no drug for this infection.

At the time we were wondering, was lymphogranuloma venereum caused by a virus that we couldn't see, just like psittacosis? As soon as they got an antigen for psittacosis, we used the psittacosis antigen in a CF [complement fixation] test because lymphogranuloma venereum is caused by a chlamydial organism that gives a positive test for both strains. But there was no specific treatment for the disease in the thirties. Today chlamydial infection of the genitourinary system is one of the most common venereal diseases. It is treated with tetracycline.

Johnson: So the venereal disease clinic was a very interesting study as to when to treat and not to treat, and what you could do before we had the modern so-called miracle drugs, which have been marvelous.

Hughes: Was that a large service?

Johnson: Usually seven hundred active patients.

Hughes: How did that compare with the other services?

Johnson: Boston City Hospital would probably have more, but not many more, and then Massachusetts General would have a clinic.

Hughes: Was Murphy in charge of the venereal disease clinic?

Johnson: No. Dr. Marlow was the senior staff consultant.

Dr. [George R.] Minot and Murphy were a team originally. Minot was on the staff of the Massachusetts General and Murphy was at Brigham Hospital in charge of the blood clinic, and that was pernicious anemia primarily, and the other blood abnormalities.

Well, that assistant residenceship was meant to train you more or less in a consulting fashion. You'd been then sixteen months in medicine before, and this was your opportunity to be like a visiting physician. You'd see each new patient, who'd been worked up by the house staff, and then you'd dictate a note giving your diagnosis. At the end of your one-year service you got a typed record of all the patients that you saw, with your notes recorded in the record. So the records stated exactly what you said. Then you could find out from autopsies later, were you right or were you wrong?

Hughes: Was the Brigham unique in using that system?

Johnson: I don't think records now are as good. There was more time then. You dictated on a dictaphone, and the secretaries would type it. The same was true of autopsies, which I did during two years on the pathology service. All records were typed and bound in volumes. Our studies of the tissue sections were typed, and there were photographs of all the important gross pathology. I can go to the Children's Hospital and find the reports of the autopsies and surgical specimens I studied.

But the tragedy today is that the teaching hospitals do not do autopsies as often as we did. I think now less than fifteen percent of the patients are autopsied, where we used to do close to ninety percent. We usually had no trouble getting the permit [from the next of kin] because we would say, "This is the way we will know what [the cause of death] was, and what could be done to improve diagnosis and treatment."

Hughes: Today is it a question of lack of money?

Johnson: Well, it's a lack of money in a way because none of the insurances will pay for an autopsy. It's a big cost to maintain a pathology service with big salaries nowadays.

Hughes: Was it common practice in other residency programs at other institutions to give you the records?

Johnson: No. The medical/legal autopsies have always been done. But then it was a question of the specific cause of death. In the teaching hospitals, like Johns Hopkins or Yale or Harvard, you were expected to do a complete autopsy. You were expected to examine the entire intestinal tract, so that in someone having polyps, such as found in President Reagan, we would record how many there were in every case. A lot of hospitals and pathological laboratories today do not open the entire intestinal tract. You have to obtain special permission for a really complete study of the brain, and of course you had to have a permit for the general autopsy from the nearest relative of the deceased.

Hughes: Giving copies of the records to the resident was common practice in those days?

Johnson: In medicine, yes. But we didn't get a copy of the ones in pathology. They were filed, bound, and are still available.

Hughes: Was that true at Stanford and UC?

Johnson: I do not know.

Storage has become a problem. The records of twenty or more years are apt to be placed in storage facilities and have to be requested several days in advance. If they're bound, they keep better. The ones I have are on yellow paper, but they're holding up good because they're bound. But that's expensive today.

Rockefeller Foundation Fellow, Harvard School of Public Health,
Summer, 1938

Hughes: Let me get this straight: You were a Rockefeller Foundation fellow at the Harvard School of Public Health, but you were actually sent to the Rockefeller Institute?

Johnson: Yes. Having had a lung lesion, I was looking around for something in the line of public health which would not be so strenuous as being a professor of medicine, which I could have been. I had had some offers. Dr. John Gordon, who was at the School of Public

Johnson: Health, said, "Harald, I think that you ought to take a fellowship here at the School of Public Health and head for epidemiology. You're interested in infectious diseases, and you've been a resident in infectious diseases and medicine and pathology. We can get a fellowship for you. And then go down to the Rockefeller Institute; it's really the place to study viruses."

I was supposed to take one full year [at the Harvard School of Public Health], starting in the fall of '38, which would include statistics and epidemiology and public health administration, which I never did! [laughs] Dr. Gordon had had some experience with the Rockefeller Institute's program in viruses. He thought it would be a good idea if I would spend the summer in the yellow fever laboratory at the Rockefeller Institute in New York City to see how the modern techniques in virology were developing. It was a new field then, particularly the research on yellow fever. The upshot was, as I finished my residency in medicine, I went directly to New York to work at the Rockefeller Institute.

[Interview 3: March 3, 1987]

Hughes: Had you been interested in viruses before you got the Harvard fellowship?

Johnson: Well, sure. We knew there were viruses causing some common contagious diseases. I had been a resident physician on the contagion service in Cleveland. I saw acute cases of measles, chicken pox, mumps, and polio. So, sure, I was much involved with viruses.

The question was whether you could immunize against polio. That was tried by [Maurice] Brodie in 1934-1935, which is mentioned in the interviews with K. F. Meyer.* They had tried to make a rabies-like vaccine for polio, and, though treated with formalin, it had residual live virus in it, and it caused some cases of polio.

I was interested in viruses. After all, so was anybody that was going to go into epidemiology. Influenza A was just coming into its own. The swine flu virus had been isolated in 1931 and the first human strains of influenza A in 1933.

* Karl F. Meyer, Medical Research and Public Health, an oral history interview conducted 1961-1962 by Edna Tartaul Daniel, Regional Oral History Office, The Bancroft Library, University of California, Berkeley, 1976.

Johnson: I saw some interesting young patients in 1935 with influenza A who had renal pathology, that is, uremia and hypertension, and recovered completely. At that time, nobody would believe that uremia could be caused by influenza virus. Now we know the virus grows readily in kidney cells. Actually, the renal lesion is an important one in influenza A infections.

Hughes: You were never for any length of time at the Harvard School of Public Health?

Johnson: No.

Hughes: You went directly to the Rockefeller Institute?

Johnson: Yes. That was the summer of 1938. The school year began in the fall, so I never took any of the courses in public health. Dr. Hugo Munch was on the faculty there. He had been on the Rockefeller Foundation staff.

Hughes: Did you regret having missed out on the courses at the School of Public Health?

Johnson: No. Every seventh year, I could have gone on sabbatical for a whole year to get a master's in public health. It was the one thing I didn't care to do. I would have had to leave my [research] program. I had all the training I needed, which was very good training in internal medicine, pathology, and infectious diseases.

Hughes: What about statistics?

Johnson: Well, I learned about that later working on rabies. We used all the techniques Dr. Hugo Munch recommended, and Persis Putnam was our statistician. She analyzed all our data, and we followed her advice on how to set up experiments to get a significant result. I still have her original reviews of the dog experiments. Most people think you need only one or two controls. If you have a difference of so many percent, you may need twenty-five or fifty or a hundred controls. In human clinical studies, good controls are already unavailable because fifty percent of people will not take a medicine or vaccine just to see what happens over a year. Clinical research is hard to control.

Statistics are very important, but you have to be very careful how you plan a study. Like in dogs, you control for age and sex. They are the key factors, especially age. In human medicine we know old people will not eliminate a drug as fast as young people, so toxic reactions are common. A baby mouse, for instance, will die from a virus inoculation, but a three-week old mouse won't.

III ASSIGNMENTS

The Yellow Fever Laboratory, Rockefeller Institute, 1938

Johnson: I was very interested in the Rockefeller Institute. Who wasn't in those days? That was a very choice place.

Hughes: Why did you join the yellow fever lab?

Johnson: Well, that was a really top virus lab in the United States at the time. The International Health Division [IHD], which was a part of the Rockefeller programs, had its major laboratory at the Rockefeller Institute, so there was access to all the primary and basic studies of infectious disease. Originally, the way the Rockefeller grants were given, one was for general education; one was for basic research at the Rockefeller Institute (with a separate budget); one was for the Rockefeller Hospital, which was for clinical studies. The International Health Division was to apply what had been learned in basic studies to disease problems. Where you had enough basic knowledge, you could control the disease. You could apply this to disease control in the field, that is, where the people were ill.

In the very early days, the Rockefeller Foundation became interested in yellow fever because of the Panama Canal studies. Beginning in 1925 they started large programs around the world on malaria, hookworm, and filaria. Hookworm was the most debilitating disease of the tropics and in southern United States. The anemia caused by the intestinal parasites made the people weak and tired. Once you knew the cycle of the organism, all you had to do was to treat everyone that had the eggs of the hookworm in the stool. The reduction in the parasites would allow the bowel to heal and the blood hemoglobin to return to normal. In India, the miraculous nature of the treatment with iron sulphate, which helped to restore the hemoglobin deficiency, was regarded as the cure, rather than the worm medicine.

Hughes: What was the treatment in the South?

Johnson: Well, it was tetrachlorethylene, given in a capsule. We used this to treat hookworm in dogs in the rabies studies in Alabama.* One treatment usually was pretty effective in eliminating the hookworms. The human hookworm is Ancylostoma duodenale. The hookworm of dogs and cats, A. brasiliense, causes a creeping eruption on the legs of humans. Sanitary regulations were initiated [in the South] to stop the contamination in the soil. The eggs in the feces of infested people would hatch in the soil if intestinal excreta were left on the surface. The larvae would be liberated from the eggs and people would be infected by the larvae entering through the skin of the feet. Toilets were built over pits dug in the ground.

One virology lab at the Rockefeller Institute in 1938 was the IHD influenza lab under the direction of Frank Horsfall. The virus causing influenza A was isolated in 1933. Influenza B was isolated a few years later. There was a rabies lab in Dr. L. T. Webster's department, and then the yellow fever lab. At the time I arrived, Dr. [Johannes] Bauer was director of the yellow fever laboratories. He was trained in Sweden and had been employed by the International Red Cross. We got along fine because I could speak Swedish.

Hughes: Why did you choose yellow fever, over the other labs?

Johnson: Well, at that time the major work at the International Health Division laboratory was on the nature of viruses. There was no other lab working on yellow fever at the Rockefeller Institute. Dr. [Peter] Olitsky, one of the early virologists and bacteriologists there, had been doing studies of polio, rabies, and animal viruses. Dr. Webster was interested in Salmonella and also was doing studies on rabies. But there was very little virology at the Rockefeller Institute until the International Health Division decided to do some basic studies of these viruses.

Hughes: That was in the thirties?

Johnson: Yes.

Frank Horsfall was on the IHD staff 1937-1941. He a very well-trained technical pathologist [who had been educated] in medicine and pathology at Harvard. He was appointed a member of the staff of the Rockefeller Institute in 1942. In 1960 he became director of the Sloan Kettering Institute. [Peter] Olitsky at the Rockefeller Institute in his study of polio virus isolated rabies virus from a human being in Trinidad in 1937, before they learned that vampire bats were the cause of cases of paralysis and death

* Dr. Johnson's dog rabies studies are discussed below.

Johnson: there, in addition to poliomyelitis virus. [Leslie] Webster was studying rabies in 1938. He was more interested in the resistance of various mouse populations to viruses and bacteria.

Hughes: Were all these people in the yellow fever lab?

Johnson: No, some were members of the Rockefeller Institute. Once you became a member, you could work on anything you pleased, more or less. They could investigate a new epidemic without delay. [Hideyo] Noguchi did studies of a yellow fever epidemic in Guayaquil in 1918. Rockefeller scientists could take their equipment and go. They could study the original focus. Paul de Kruif used Dr. Peter Olitaky as a model for his book, Microbe Hunters.

Early Research on Yellow Fever

Hughes: Well, maybe the place to start is with the background of research on yellow fever. What about starting with Walter Reed and his associates at the turn of the century?

Johnson: Okay. Walter Reed was at one time an army surgeon in the Midwest where I grew up. He was stationed at Fort Laramie as a young military surgeon. He was the one that led the Panama Canal study in 1900 and 1901 on yellow fever. The Rockefeller Foundation's International Health Commission was organized in 1913 to draw up plans for controlling yellow fever by eliminating Aedes egypti mosquitoes wherever there was an outbreak of yellow fever. Major General William Crawford Gorgas, Surgeon General of the U.S. Army, retired in 1915 to accept an appointment as director of this commission. In 1916 the name was changed to the Rockefeller Foundation Yellow Fever Commission. The First World War caused General Gorgas to return to the U.S. Army as Surgeon General until 1918.

In the meantime, Dr. Hideyo Noguchi had begun his studies at Guayaquil, Ecuador, to see if he could isolate the causative organism of yellow fever. He isolated a spirochete, which he called Leptospira icteroides. He believed that this organism was the cause of yellow fever. Later it was proved that he was wrong about yellow fever being caused by a spirochete.

The mosquito control work was successful. By the end of 1924, yellow fever had been eradicated from Mexico, Central American, and Ecuador. Plans had been made to study an outbreak of yellow fever in Africa. In 1925 Dr. Henry Beeuwkes was sent to West Africa. The original staff included Dr. Adrian Stokes and Dr. Alexander F. Mahaffy. They failed to isolate Leptospira during a study of sixty-seven cases of yellow fever.

Hughes: Why were they selected?

Johnson: The Rockefeller Foundation had a board of scientific directors who were on the lookout for research and field staff for the Rockefeller studies. Dr. Adrian Stokes of the pathology department of Trinity College at Dublin had been selected in 1920 to be a member of the group to go to Africa. The death of General Gorgas, who died in London in July 1920 on his way to West Africa, delayed the opening of a field laboratory in Africa.

Hughes: Had Stokes been working on yellow fever?

Johnson: No, but he was a good internist and a pathologist. That's what they needed: What's the disease? How to identify it?

I met Dr. Mahaffy in the 1930s. He was a great brawny guy with dark hair, dark eyes, and an excellent person for field studies. He was one of those doctors who could do anything. He could travel on foot, a horse, a camel--anything.

Hughes: What was his background?

Johnson: He was a medical doctor from Canada and had been trained in pathology, more or less like Stokes. So he would investigate any report of yellow fever and get the essential epidemiological information. He drew blood specimens that later became the source of Bwamba fever virus. He was looking for yellow fever [virus]. It was not until 1946 that we started studying these new [arthropod-borne] viruses isolated incidentally during the yellow fever virus studies.*

But let's go back to Mahaffy. He was in Africa in 1927 and stationed at Accra when he heard about an outbreak of yellow fever at Kpeve. He bled two people there, and one of these was a twenty-eight-year-old man called Asibi, source of the Asibi strain, the 17D vaccine virus, which was modified by cell culture, losing its pathogenicity.

Mahaffy and Bauer inoculated this blood from Asibi into a rhesus monkey. They had just learned that Indian crown monkeys were susceptible to yellow fever. The rhesus monkey inoculated with Asibi's blood sickened on the fourth day and the next day was moribund. This virus strain was maintained by passage in rhesus monkeys by subcutaneous inoculation, using viremic blood as the inoculum.

* Discussion to follow.

Johnson: Other members of the research staff in Africa were Adrian Stokes, N. Paul Hudson, and Cornelius [Becker] Philip. Philip, who was retired from the San Francisco Museum of Entomology in Golden Gate Park, died recently. He was one of the entomologists working at the African lab.

Hughes: Had it been thought up until then that the yellow fever virus could not be transmitted to lower animals?

Johnson: At that time, there was no experimental host except man, and all the early experiments were done on human beings. They had tried all the local African monkeys, injecting them with blood from cases of yellow fever, but none became ill. They tried guinea pigs, rabbits, and other animals, but they could not reproduce the disease. We know that some strains of white laboratory mice, Mus musculus, inoculated in the brain with yellow fever virus remain well.

Hughes: Why was the Macaca rhesus susceptible?

Johnson: It is an Indian monkey. There is no yellow fever in India, so there has been no exposure of this species to yellow fever virus. [A parallel situation occurs in] the horse, which was introduced to North America by Europeans. This animal dies of American encephalitis virus, both eastern and western varieties. It is an aberrant host and has no natural resistance from long-term exposure to these viruses.

Mahaffy and Bauer had tried several species of monkeys, and it was suggested that they try some Indian rhesus monkeys that had been imported for zoos and circuses. The first ones they inoculated with yellow fever virus became ill and died. They were autopsied for special studies and that's where people got infected. The first ones to die were Stokes and Noguchi. Stokes probably got yellow fever from doing the autopsies.

Hughes: How did Noguchi get it?

Johnson: No one knows. The most likely is hand exposure; the other is from infected mosquitoes. V. I. Glasounov, who had worked with Noguchi, later came to work at the Rockefeller Institute. He was a Russian emigre. He talked to me about this episode. We know now that yellow fever is infectious via superficial excoriation of the skin, like Lassa fever virus. There is danger in doing autopsies.

I never met N. [Paul] Hudson. He went to the New York lab in 1929. He was an excellent pathologist, and he did the pathology studies of the monkeys while he was in Africa. He left the IHD to be professor of microbiology at Chicago.

Hughes: I believe Noguchi prepared a vaccine which he claimed was successful.

Johnson: Yes. It was a formalized culture of Leptospira icteroides. Most people don't get seriously ill of leptospiral infection. At that time and later on, leptospiral disease occurred in miners who worked in wet mines where they ate lunches and rats came to feed. The rats would seed the Leptospira into the water and the food. Leptospiral jaundice is a well-known disease to this day.

Hughes: So what the vaccine was successful against was the leptospiral jaundice?

Johnson: The ones that he vaccinated were mostly people that would not get exposed to that organism. There's no evidence that Noguchi's vaccine was of any value. At all events, it would not protect against yellow fever.

Hughes: How disruptive to the yellow fever research was Noguchi's claim that Leptospira caused yellow fever?

Johnson: For the time, it was. He was acclaimed for having developed a vaccine against yellow fever.

Hughes: And for discovering the cause, of course.

Johnson: And it lasted quite a while. There were two yellow fever labs in Africa. Noguchi had his own project at Accra. The International Health Division had its lab at Lagos.

Noguchi was a medical doctor and pathologist. He'd grown up as a handicapped person, from a burn on his hands.

Hughes: The burn didn't interfere with his research?

Johnson: No. But he had damage to his hands; he'd been caught in a fire as a child, as a baby, I guess. Dr. [William] Welch had heard about him when he was in Japan and suggested he come to Johns Hopkins. Dr. Bauer worked for a while in Noguchi's lab. Noguchi had a good lab at the Rockefeller Institute. He was a well-trained bacteriologist. He really developed some good information on how to grow Leptospira.

Hideyo Noguchi died of yellow fever on May 21, 1928. Dr. William Alexander Young took over Noguchi's studies and died May 19, 1928 from yellow fever. It is clear that it was dangerous to work with yellow fever virus. Young was probably infected the same way as Noguchi.

Johnson: Dr. Howard B. Cross was trained in Noguchi's lab. He went to Veracruz, Mexico, to study an outbreak of yellow fever. Cross had bled a man who had yellow fever and could have been infected from the blood of the patient or by the local mosquitoes. He died of yellow fever at Veracruz on December 26, 1921, an early martyr.

I found an interesting pathology produced by yellow fever in the kidney. There were no polymorphonuclear white blood cells or inflammatory cells, but the tubule epithelium was destroyed. I saw this pathology in 1946 at the Rockefeller Institute. They gave me the formalin material on Stokes and Noguchi, and I prepared sections. They had the characteristic central liver necrosis found in yellow fever cases. In addition, there was renal tubular necrosis. It indicated that they died of the kidney disease.

I had a similar surprise in 1957 when I studied the specimens from Kyasanur Forest disease. Telford Work sent the formalin-preserved monkey specimens to Max Theiler in New York, and Max sent this material to me in California. The lesions in the kidney were similar to those seen in the patients that died of yellow fever. The Kyasanur Forest virus is a flavivirus related to yellow fever, and it appears to produce death from the damage to the kidney.

Hughes: Had people been paying attention to the liver in yellow fever?

Johnson: Yes, because of the jaundice. Yellow fever virus is the type virus for the flavivirus group.

Hughes: You mentioned the series of deaths of yellow fever investigators that occurred between 1927 and 1930.

Johnson: That's what scared the IHD staff. They knew they'd better close the lab in Africa, and they did.

Hughes: Could you say something about the precautions that were instituted at the New York lab?

Johnson: Yes. [Wilbur] Sawyer was the director of the New York laboratories. He said that they would have to restrict the work [on yellow fever] to people who had had medical training, and then they would try to find some kind of a vaccine. At that time, they already knew that you could save the immune serum from somebody who had had measles, and you could give that to children when they were exposed, and if they would get measles, they would get a very mild case, or they wouldn't get any at all. The dosage used was thirty to forty milliliters of serum from someone that had antibodies to yellow fever.

Hughes: Why was medical training supposed to be protective?

Johnson: In the same way as when I worked in contagious diseases. You learn, first of all, what's called strict contagious precautions. You wash your hands before and after touching a patient. You wear a face mask, a gown, and you take the gown off as you leave each room that has infectious disease. It is very strict. You are required to follow these precautions.

The Staff

Johnson: But to go back to 1928: Dr. Wilbur Sawyer, who had previously been the director of the state health department in California, was a medical doctor. He became head of the yellow fever laboratory at the Rockefeller Institute. Dr. Frederick F. Russell was the head of the International Health Division, or Board at that time. They decided that they were going to work at the Rockefeller Institute lab in a special section. They would start out using only physicians. Sawyer picked Wray Lloyd, Stuart Kitchen, Martin Frobisher, and Paul Hudson. Wray Lloyd later was killed in a strange accident in South America. He was sleepwalking and fell off a building.

He and Dr. S. F. Kitchen were from the University of Western Ontario. They were trained in medicine and pathology and were willing to take risks. Now, Stuart Kitchen I met many times and I liked him very much. The last project he worked on was in Uganda in the forties. He was at the virus lab at Entebbe. He was stationed at the New York lab in the 1940s when I was there.

Hughes: The reputation of the Rockefeller was such that people would come when they were invited?

Johnson: Yes.

Hughes: The salaries were not the attractant.

Johnson: No. Even Dr. Hugh Smith, who was my boss--he's the one that did the first yellow fever vaccinations with the 17D vaccine in South America--said that he started at three thousand a year.* Well, K. F. Meyer started at four thousand in California at the head of Hooper Foundation and so did I at Rockefeller. The value of the dollar in the 1930s was ten times that of 1988.

* Hugh H. Smith. Life's a Pleasant Institution: The Peregrinations of a Rockefeller Doctor. Tucson, Arizona, 1978.

Johnson: Dr. Martin Frobisher, who had been an instructor at Johns Hopkins Medical School, joined the yellow fever lab staff in October of 1928.

Hughes: Was he a pathologist?

Johnson: He was an instructor in medicine and trained in pathology. He became a very prominent researcher later.

The first nonmedical technicians were V. I. Glasounov and George Martine. Glasounov was a Russian emigre who had worked in Lagos, Africa--a very interesting man, very intelligent. George Martine was the technician who identified a natural disease in mice. Dr. Theiler called it George's disease, GD virus.
[laughter]

Dr. Olitsky worked with the FA mouse polio strain, derived from the fecal pellets of normal mice. During passage of a virus in mice by intracerebral inoculation, the disease might change. For example, yellow fever would be weaned out, and you'd have the mouse polio virus from the mouse. This was called polio encephalomyelitis of mice. It's a natural disease in mice. At first, they couldn't figure out how it was spread. But then they found out, when they got to working with polio later, this virus spreads exactly like human polio. It's in the intestine.

I remember when I first went to Russia in 1961. They were certain that they could transmit tick-borne encephalitis virus in mice with the feces of mice that they inoculated with this tick-borne encephalitis virus. I said, "Well, there is a virus in mice that does just exactly that, and it's always in the stool. And all you have to do is to take a group of mice about two to three weeks old and collect the droppings, grind them in a mortar, add antibiotics, or filter them through a bacteria-resisting filter, and then put that intracerebrally into mice, and you get a mouse encephalitis virus." That led to some exciting protests. The Russians said, "Oh, no, this is tick-borne encephalitis."
[laughs] You have to do specificity tests.

Mr. [Thomas W.] Norton, another very good technician, was at the Rockefeller lab in 1938 and trained me in tissue culture techniques, and Miss [N. I.] Ricci, a most intelligent and skillful technician. They were key people.

I used to tell my technicians, "You keep working; you'll soon be more skillful than I. If you think you can improve the methods or safety regulations, be sure and tell me, because you're going to know more about this than anybody else if you keep working at it."

Johnson: Miss Ricci really guided all this early work on chick embryo tissue culture and methods. Others were trained in virology there, like Wray Lloyd and Hugh Smith. In three months, I learned a lot about virology.

Lyophilization

Johnson: At that time, Dr. Johannes Bauer was developing the freeze-drying setup. He was trained in Sweden. He was a good engineer and physician; he'd taken good basic sciences in physics and chemistry, and he'd been in the First World War with the Red Cross in Europe. He'd been in South Africa and came to New York to work at the Rockefeller Institute with Noguchi. He was asked to join the International Health Division staff. I used to enjoy talking to him.

Hughes: He was working on the freeze drying of yellow fever vaccine?

Johnson: Yes, and developing high-class lyophilizing equipment. It was very important.

Hughes: Was lyophilization a new technique?

Johnson: The earliest lyophilizer was where they dried viruses in a Hempel dessicator. The upper part would be sealed with vaseline to close the dessicator. You could dry the tissue virus without a vacuum pump. The glass tubes or ampoules were set on a tray on the shelf above the fluid. Concentrated sulfuric acid was added at the bottom of the dessicator to absorb the water. The dessicator was kept in an ice and salt bath. The ampoules were sealed with a torch when dry.

Bauer and [Edward C.] Pickels constructed a manifold with ports for ampoules. All joints were vacuum tight. This manifold was connected to a cold trap made of brass immersed to half its depth in alcohol and dry ice in a large thermos tank. This in turn was connected to a vacuum pump. Short pieces of hard rubber tubing were attached to the ports of the manifold, and metal plugs were inserted to close each port. When the air had been removed, ampoules of the virus suspension were quick frozen and put on the manifold. This was done by clamping the rubber tubing with a large hemostat, removing the plug, and inserting the end of the ampoule. The hemostat was then removed. Lyophilization would begin immediately and this would keep the specimen frozen. When the material was dry, the manifold was filled with nitrogen through one port, using a football bladder and a valve to regulate

Johnson: the flow of nitrogen. When the manifold was in equilibrium with the air in the room, the ampoules would be removed and the glass neck of the ampoules sealed with a torch.

The early method of sealing the necks of the ampoules after clamping necessitated filling the manifold with air to equilibrium with the barometric pressure. The air was replaced slowly and there was a trap containing phosphorous pentoxide to remove residual moisture. Later it was found preferable to fill the manifold with nitrogen.

I designed a lyophilizer which was made in Poona, India. We used a large-mouth, quart-size thermos. The five ports were attached to a unit of the brass manifold that was suspended by a flange above the thermos. There was a double chamber in the manifold so the air from the ampoules would have to travel to the bottom of the manifold on the outside next to the dry ice and alcohol and then up the inner core to the top port connected by heavy rubber tubing to the vacuum pump. We used thick pyrex test tubes for ampoules and flame sealed these under vacuum. We made dry ice from compressed carbon dioxide used to make carbonated drinks.

Hughes: Who was responsible for developing the technique?

Johnson: That was largely done under the direction of Johannes Bauer. Dr. T. P. Hughes also worked on this project. Dr. Pickels supervised the machine shop work. John P. Fox did the studies of virus stability with the nitrogen seal. He was a dear friend. He came down to help me when I was sick and paralyzed in 1944. He was professor emeritus of epidemiology at the University of Washington, Seattle, when he died in 1987.

Tissue Culture

Hughes: Was your plasma clot research at the yellow fever lab continued?

Johnson: Yes, by Dr. John Fox.

Hughes: Perhaps you want to explain.

Johnson: At the New York lab, I found that yellow fever virus multiplied better in plasma clot cultures. That was my major contribution to the tissue culture studies.

Hughes: Yet, in the case of yellow fever research, that technique was eventually dropped.

Johnson: They had already gotten what they needed with the chick embryo cell cultures.

The next step was to produce vaccine virus. This was produced using chick embryos. The seed virus was injected into chick embryos, about ten days of age. Using the periembryonic stab method of inoculation, whole embryos harvested three to four days after inoculation contained sufficient virus to vaccinate hundreds of people. The titer was not as high as obtained in infected mouse brain, but was higher than obtained in the cell cultures.

The basic problem then was preparing the chick embryo pulp and lyophilizing it properly so the virus would remain viable. A lot of the early vaccine was prepared at the lab in New York under strict supervision. Vaccine was also made in Bogota, Colombia, and in Rio de Janeiro, Brazil.

Hughes: Why was it necessary to make it at each of those places?

Johnson: It was impossible to make enough in New York to vaccinate the number of people at risk. The various countries wanted to have their own diagnostic labs, and that was like making a little Rockefeller Institute in each country.

Hughes: They had problems with the vaccine in Brazil, didn't they?

Johnson: Yes. The problem was not paying enough attention to the importance of the seed virus. They started to pass it from one batch of vaccine to another instead of keeping a single lot of seed virus. They had original dried seed virus from New York. At first they did not have a good lyophilization apparatus. As long as each vaccine lot was sterile, they used it for seed virus. Prolonged passage resulted in changes in titer and virulence for monkeys. With experience and improvement of the equipment and keeping production limited to one or two passages from the seed virus, the vaccines were usually satisfactory.

There were several possible causes for changes in the pathogenicity of the vaccine virus when subpassed in chick embryos. The viral population is not uniform, and if the majority of the viral particles are of low pathogenicity but the more virulent are also more infectious for the chick embryo, the amount of virus inoculated could affect the relative proportion of virus particles of low and high pathogenicity. The temperature of incubation has been found to favor one or the other, lower temperature selecting for low pathogenicity.

The Staff (continued)

Hughes: Is there any more to be said about the members and the projects of the yellow fever lab?

Johnson: In 1938, Dr. T. P. Hughes, physical chemist, was working on collodion membrane filtration, identifying the sizes of viruses and working with adsorption of the virus on collodion pellets for use in flocculation tests.

Hughes: Was that related to [William J.] Elford's work in England?

Johnson: Yes, Elford was the first to use collodion membranes to determine the relative size of viruses. Seitz and Berkefeld filters were used to obtain bacteria-free virus preparations.

Hughes: Was the main purpose to determine the size of the virus?

Johnson: Yes, that was the purpose of the collodion membranes. Another method was spinning a virus preparation in the ultracentrifuge.

So Hughes was working on filtration, and he determined that yellow fever virus was between seventeen and twenty-five micromu in diameter. Dr. Bauer and Dr. Pickels, who had been trained at the University of Virginia by Dr. Jesse Beam, by spinning viremic blood specimens in the ultracentrifuge, estimated that the size of yellow fever virus was about twelve micromu.

The ultracentrifuge was improved at the Rockefeller yellow fever laboratory. Dr. Pickels was an excellent engineer, and we had a German-trained machinist who could make perfectly balanced rotors. If they were not uniform, they would just take off. We had a concrete wall around the centrifuge; the wall was reinforced with steel and about two feet thick, so if they would take off, this would prevent a disaster. Dr. Pickels later joined Beckman Laboratories in California, where he continued to improve the ultracentrifuge.

Hughes: Was there one centrifuge for the whole building?

Johnson: Yes, at first it was just developing experimental models, but then finally we got some that were very good, and they progressively were better. When I was there in the middle forties, they were getting to really high speeds. When I was at the New York lab in 1946-1951, Dr. John Bugher was in charge of the electron microscope and ultracentrifuge studies.

The senior staff at the yellow fever lab in 1938 was Theiler, Bauer, Pickels, Smithburn, and Hughes. There was a malaria study section with Lowell Coggeshall and Andrew Warren as senior staff.

Johnson: There was an influenza section with Frank Horsfall in charge. Bauer headed up all the physical-chemical studies of yellow fever, lyophilization and preservation of the virus, and supervision of the lab. Kenneth Smithburn arrived in '38. He was already doing the neutralization tests. Very shortly after I left, he went to Entebbe, Uganda. And there were the usual staff people, like Ricci and Norton. Hugh Smith had gone to South America, beginning the field vaccination projects. Loring Whitman, whom I knew so well later, was in Brazil.

Max Theiler and Loring Whitman were friends in Boston and were together on a Harvard expedition to central Africa in the early thirties. Loring was the photographer for the expedition. He was a wonderful naturalist. He died in 1987. Loring took beautiful movies of the animals, reels of the hippopotomus and wildebeests. Max Theiler did blood smears on all the animals they obtained. There are two volumes describing the results of this expedition to Africa.*

Hughes: You mentioned in connection with Hughes that, aside from the collodion membranes, he was working on pellet adsorption for precipitin tests.

Johnson: Yes. The virus-coated pellet would be precipitated with immune serum. It worked to some degree but never became important. A lot of people have studied precipitin tests. The Russians particularly favored the precipitin tests. But as time went on, the complement fixation test, that Dr. [Edwin H.] Lennette worked on so much, and the serum virus neutralization tests were the standard tests for diagnosis. Tom Hughes was doing the neutralization tests in 1938.

Hughes: Were these tests being used when you came into the lab in 1938?

Johnson: Yes. But the complement fixation at that time was used primarily for the influenza work. Horsfall was working on that, and Dr. Jordi Casals in the rabies studies in Webster's lab. Hughes and Smithburn were doing the serum-virus neutralization tests in adult mice.

Hughes: You mention in your notes Smithburn's serological studies.**

* R. P. Strong et al. Harvard African Expeditions 1926-1927, New York, Greenwood Press, 1969.

** In advance of the interview on March 3, 1987, Dr. Johnson prepared and sent to the interviewer a brief account entitled, "History of research on yellow fever."

Johnson: Hughes and Smithburn were doing the serum-virus neutralization tests in mice. Kenneth Smithburn was in training to go to the yellow fever lab in Uganda. It was in 1946 that Ken was doing the neutralization tests on all the arthropod-borne viruses at the Rockefeller Institute lab.

Hughes: I read a comment about the abysmal state of the animal care facilities at the Rockefeller in the thirties. Would you agree?

Johnson: Well, there was a reason for the problem with the monkeys. When Dr. Sawyer and his group started the yellow fever study, they tried to have as clean a facility for monkeys as you can, but this is extremely difficult. Monkeys in a cage are going to throw things at you. So you had to have a cement floor so you could hose down the whole room, including the cages. From 1930 to 1946 the Rockefeller Foundation laboratory was in the old Middle Building. In 1946 we moved to the newer North Building.

The most difficult places to keep clean were the cages used for guinea pigs or rabbits because they had to have bedding. The bane of a building like that is cockroaches and mice. From the early days at the institute they had wild mice in the building. We were not allowed to use insecticides in the monkey colony because we were working with mosquitoes and insecticides would kill the mosquitoes.

In 1938 we had a mouse colony. George Martine was in charge of that, and by obtaining litters of infant mice by Caesarian section and nursing them on lactating white rats, we obtained a stock line free of the mouse polio virus.

I had to work with monkeys, and it is difficult. You have to wear heavy gloves because they bite, and they are extremely agile. I was strong in those days, but the things they could do to get away were surprising.

When we had moved to the new animal facility in the North Building in 1946, Dr. George K. Strode, director of the IHD, took some visitors on a tour of the lab. In the cage-washing room there was a spring-activated spray nozzle near a drain. When the grill side of the monkey cage was put down on this nozzle, it activated the sprayer. Strode, when making a quick move across the floor, stepped on the spray nozzle and the jet of water went up his pants. What a mess, but he was a good sport.

Strode was the editor of the IHD book on yellow fever.* He was a well-trained public health administrator, but he was not a virologist. I remember being at his retirement party at the lab. Strode was in the receiving line and one of the secretaries shook hands and then said, "I hope you'll be happy in the hereafter." Strode laughed and said, "I hope you'll be happy in the hereafter also." [laughter]

The Vaccine

Johnson: When the first yellow fever laboratory was started at the Rockefeller Institute, Dr. Frederick F. Russell was the director of the International Health Division. Dr. Russell said, "We have to have some method to protect the research staff." The nonimmune staff members were given an injection of immune serum from persons that had recovered from an attack of yellow fever. But nobody trusted that, because Hudson and Kitchen developed the disease despite a previous dose of serum. What was needed was a modified yellow fever virus that was less dangerous than the Asibi virus obtained from monkey blood serum and a method of obtaining a definite assay of the antibodies in the serum used for providing temporary protection.

Dr. Max Theiler of the department of tropical medicine at Harvard Medical School had in 1930 established a serial intracerebral passage of the French neurotropic strain of yellow fever in mice. Dr. Sawyer and his associates had discovered that monkeys given an injection of immune serum were immune to an injection of virulent yellow fever virus four to six hours later.

Hughes: What is the background of that French strain?

Johnson: The French had Pasteur institutes in their colonies, such as Mali and the French Cameroons. Cameroons was the place they had been working on yellow fever. They had injected yellow fever virus intracerebrally into monkeys. Subpassage by this route resulted in the development of a neurotropic strain which produced encephalitis. The adaption of the French neurotropic strain to mice resulted in a loss of virulence for monkeys, and by the twenty-fifth passage, the virus no longer killed rhesus monkeys inoculated im [intramuscularly] with the virus.

* G. K. Strode, ed. Yellow Fever. New York: McGraw-Hill, 1951.

Johnson: Sawyer said, "This is the one strain that we can start with, because if it's been neuroadapted, we know from rabies that the more you pass it through the brain, the safer it is." If you have a fixed incubation period, it should produce a uniform titer. It was pretty well fixed after twenty-five passes. They decided they were going to use this mouse-adapted virus as a vaccine, giving a moderate dose subcutaneously and, at the same time at another site, giving immune serum from someone who had recovered from yellow fever and had antibodies to the virus.

In Africa they'd shown that you could do a neutralization test in monkeys with the Asibi viscerotropic strain of yellow fever virus. At the New York lab, using the neurotropic mouse-adapted yellow fever virus in a neutralization test in mature mice inoculated intracerebrally, the known immune serum increased the average survival time but not all the mice died.

A new method of demonstrating the yellow fever antibodies was developed. A dose of starch solution was injected into the brain and, after that, an intraperitoneal injection of the yellow fever virus plus immune or normal serum. This test demonstrated complete protection with the serum of humans or animals that had survived natural or experimental infections with yellow fever virus. You'd have two technicians: one would first inject the mice in the brain with the starch solution, and the other would give the serum plus virus mixture intraperitoneally. By using this mouse test, they mapped the areas of Africa to show where people had antibodies to yellow fever. That was done early, and this was very important. In the meantime, it was essential to know whether a person was immune and also to test the serum before and after vaccination.

A better neutralization test was developed later. Baby mice will die of yellow fever given intraperitoneally if under ten days of age. Dr. John [Clifford] Bugher was much involved in developing that test.

But to get back to the vaccination study, Dr. D. B. Bruce Wilson was the first person to receive the new mouse-adapted yellow fever vaccine plus immune serum. He was put in the Rockefeller Hospital, which was right next to the Rockefeller Institute, and he was vaccinated on May 13th, 1931. He had no obvious reaction from the vaccination and developed antibodies to yellow fever.

Fifteen other people on the staff were then vaccinated, which included Bauer, Dr. [J. Harland] Paul, Dr. Hughes, Dr. [Porter J.] Crawford, Miss Ricci, and the other technicians. This was the immunization that stopped the deaths from yellow fever among the research staff.

Hughes: You were not vaccinated?

Johnson: Not then. We're talking about 1930 and '31. I was vaccinated with the 17D chick embryo vaccine in 1938. This was the first year this vaccine was used. We heard about the yellow fever vaccine studies when I was working in Boston in 1933 to '38. Everybody knew about these people at the Rockefeller Institute working on yellow fever.

Hughes: There were no adverse side effects from the vaccine used in 1931?

Johnson: Not serious. There was fever and some malaise. The dosage was not high. It was a dilute amount of the mouse brain virus.

Dr. Theiler was awarded the Nobel Prize in medicine for this and the subsequent 17D chick embryo-adapted vaccine. The mouse brain French neurotropic virus was soon replaced by the embryo mouse brain tissue culture virus. The Asibi strain was also adapted to monkeys by intracerebral passage to obtain a neurotropic strain of this virus at the yellow fever lab at the Rockefeller Institute. They'd passed the Asibi strain in monkeys intracerebrally to high passage. But that wasn't the one that they finally put into the chick embryos; they used the viscerotropic Asibi virus.

There was a neuroadapted strain of human polio, the old type one. It had been passed monkey to monkey intracerebrally until a fixed incubation period was obtained. Pasteur had passed rabies a hundred times intracerebrally in rabbits, and they knew that seemed to limit its power to kill, given peripherally. I later passed rabies virus intracerebrally more than a hundred times in baby chicks to get a fixed strain of the Flury strain rabies virus.

When I came on the Rockefeller fellowship in June of 1938, the yellow fever lab in New York was at the peak of the chick embryo cell culture studies. They had the viscerotropic Asibi virus in mouse embryo tissue culture, a cell culture system which I later used for cultivating rabies virus. You mince up the whole mouse embryo and transfer the tissue fragments to Tyrode's solution containing ten percent serum. They passed the Asibi virus eighteen times in this culture system. They had tried to cultivate the Asibi virus in whole chick embryo cell culture but the virus would not grow in the chick embryo cells until adapted to the mouse embryo cells. The Asibi virus was next passaged for five to eight passages in whole chick embryo cell culture. A third passage line was then begun in chick embryo cells obtained after the brain and spinal cord had been removed.

Johnson: The 160th passage in chick embryo cells, without brain or spinal cord, was designated 17D and this is the seed virus for the yellow fever vaccine. Seventeen lines of culture were tried and tested, but the 17D line proved to be the least pathogenic for rhesus monkeys. Until 1937 the human vaccine virus was the 17E mouse embryo cell culture, given with immune serum.

Hughes: Why did they stop a passage or a series of passages at a certain point?

Johnson: Selection was based on pathogenicity for rhesus monkeys. The original Asibi strain was passed fifty-three times from monkey to monkey using blood taken at the onset of illness and injecting this by subcutaneous route. At each passage the monkeys died or were killed when moribund. It was very pathogenic, as was the Asibi virus passed by the intracerebral route.

By the way, [Lowell Thelwell] Coggeshall was working in the malaria study section while I was at the IHD lab in 1938. There was a disease in the monkeys used for the virus and malaria labs. Several died, and a virus was isolated from the tissues of one monkey. It was not yellow fever virus. Dr. Joseph Smadel of the NIH [National Institutes of Health] identified it as lymphocytic choriomeningitis virus (LCM). Coggeshall, on the basis of feeding mosquitoes on the blood of monkeys inoculated with this LCM virus and testing mosquitoes, concluded that he had transmitted LCM virus using Anopheles quadrimaculatus mosquitoes.*

In 1956 I isolated LCM virus when I was testing bird blood specimens at the California State Health Department. I sent a specimen of the LCM virus to Loring Whitman at the Rockefeller lab in New York. He found that this virus did not multiply in *Culex*, *Aedes* or *Anopheles* mosquitoes. This made it evident that LCM virus was present in the guinea pig colony Coggeshall used for testing the LCM virus.

Likewise, we soon learned that LCM virus had been introduced into our mouse colony at Berkeley by house mice that had invaded the health department building. We had to destroy our mouse colony in 1956 and start another from mice obtained from the New York Rockefeller lab.

*This work was reported in Science 1939, 89:515.

George Hirst

Johnson: George Hirst was testing throat wash specimens at the New York lab in 1940 and showed the agglutination of red blood cells in the allantonic fluid liberated when he was harvesting a chick embryo infected with influenza A virus. He published a paper on this discovery.* The hemagglutination inhibition test became the standard serologic test for the diagnosis of influenza A virus infection. I used the allantonic sac inoculation of chick embryos to isolate influenza A virus on many occasions. In 1954 I isolated influenza B from my daughter Marion. In 1958 I isolated influenza A (Asian flu) from my sons John and Michael, and in 1966 I isolated influenza B virus from my son John.

George was a good flutist and in 1946, we enjoyed playing the Bach sonatas for flute and piano.

Hughes: Did Hirst stay with with IHD lab?

Johnson: No, he resigned in 1946 to take the position of director and chief, department of virology, at the Public Health Research Institute of the city of New York.

Hughes: Well, I am interested in that, because there were not, as far as I know, very many diagnostic laboratories in those days.

Johnson: There were virus labs at commercial biological companies, such as Parke-Davis, Sharp and Dohme, Lederle, Squibb, Lilly, and Gilliland, but they were mostly concerned with producing smallpox or rabies vaccine and antigens. Lederle Laboratories had the best virus research division. The National Institutes of Health of the U.S. Public Health Service was primarily a research reference laboratory. Several state health departments had virus laboratories primarily for the diagnosis of rabies. The Division of Laboratories and Research, New York State Department of Health at Albany, had a virus diagnosis laboratory.

Hughes: Was the New York Public Health Research Institute doing more or less what Dr. Lennette's laboratory was doing, namely, receiving virus specimens for diagnosis?*

* Science 1941, 94:22.

** See: Edwin H. Lennette, Pioneer of Diagnostic Virology with the California Department of Public Health, an oral history interview conducted 1982, 1983, 1986 by Sally Smith Hughes, Ph.D., Regional Oral History Office, The Bancroft Library, University of California, Berkeley, 1988.

Johnson: They studied specific diseases, such as influenza, at selected institutions, such as the Coxsackie Reformatory. Dr. Richard Taylor in '46 and '47 was getting throat wash specimens from this place. I did the pathology for this respiratory disease study.

Hirst was at the Rockefeller International Health Division lab from 1939-1946, and that's where he did most of his work on the hemagglutination test, methods, and the beginning of the neuraminidase enzyme analysis, which is the H1-N1, H2-N1, etc. There are two tests: Which neuraminidase it is, and which hemagglutinin?

Decision to Join the International Health Division

Johnson: I was about ready to go back to Harvard in the fall of 1938 when Dr. Theiler and Dr. Bauer told me they had talked to Dr. Sawyer about me. Max said, "Go over and see Wilbur Sawyer. He's interested in having you work for us." And so I did, and we talked it over. He knew Dr. Henry Christian, professor of medicine at Harvard. Sawyer said that he would talk to Christian, who felt that with my background it would be a wonderful opportunity to work on the IHD staff.

Sawyer said there were two projects where they needed senior staff. One was the yellow fever lab at Entebbe, Uganda. The other was an urgent need for somebody to work on rabies. It was becoming a big public health problem, especially in Alabama, and there were questions about both the human and dog vaccine potency. He said, "Are either of those projects interesting [to you]?"

Sawyer said he was interested in rabies. He had studied an outbreak in coyotes in California in 1915 and '16. There was a big epidemic in Modoc and Lassen counties. He said, "It's a fascinating disease, and we need somebody to restudy it, and I hope you'd be interested in doing that. I would recommend it. Of course, a lot of people don't want or dare to work with rabies, but you'll learn to do it, just like we did with yellow fever. You'll be careful, and you're a well-trained person in infectious disease. Think it over."

In the meantime I had talked to John Gordon and others, and I thought, What an opportunity! I didn't particularly want to take the public health training course. I would have been in Gordon's department at Harvard Medical School. That was not a big lab; there was not much opportunity for doing virus work there. There was none at the Brigham or Children's Hospital at that time. John Enders had not started his work on viruses.

Johnson: So that was when I chose to leave the fellowship. First I went to Montgomery, Alabama, to look over the [Rabies Study] Project. I wrote a report on it, which I still have, and what the prospects were.*

We needed to do a complete study of the pathology of rabies. There was little known about the Negri bodies, and whether there is any pathology in organs other than the central nervous system. The decision right from the beginning was to study it like yellow fever. Was there viremia? People said they were not sure that it occurred. Consequently, the virus had to enter directly into the nerves and spread to the brain. What organs were infected, and was there any pathology associated with the natural disease? I was in a position to do autopsies on people, which I did in the various hospitals where we had cases. So that was the beginning of the Rabies Study.

Confusion in Early Virus Research

Hughes: Before we get into the rabies work, could you say something about concepts of the virus in the late 1930s?

Johnson: There were so many things that caused confusion in virus research. Mostly the diseases in animals themselves.

As I told you, I wrote a paper on the visceral lesions in chicken pox. Tom Rivers and Dr. Reuben Schwenker injected chicken pox throat wash and skin lesion suspensions into rabbits. They were sure they had isolated the virus of chicken pox because they found beautiful intranuclear inclusions in the brains of inoculated rabbits. And guess what it was? It was learned later that there is a natural herpes of rabbits, and this is what produced illness and death if inoculated intracerebrally.

Then look what the problems have been with other viruses isolated during passage studies. One of the old Pasteur rabies strains, the Sassari strain, apparently was herpes of rabbits that had replaced the rabies virus. I think a lot of the rabies strains that have been used for vaccines have been hybridized with viruses present in the host system used for production of vaccine rabies virus.

* "Report on a trip of Harald N. Johnson to Montgomery, Alabama." (September 11-15, 1938)

Johnson: What was this rabies virus situation? Everyone that I spoke to, like [Peyton] Rous, Tom Rivers, Peter Olitsky, and Leslie Webster, said rabies was a strictly neurotropic virus. [They thought] it did not grow in tissue other than the nerves and the brain.

The knowledge of the natural history of viruses, even yellow fever virus, was very limited. Even today we do not know where yellow fever survives in nature.* In '54, there were big arguments that jungle yellow fever had to be only in monkeys. From 1900 to the 1940s, yellow fever was thought to be a human infection spread only by Aedes egypti mosquitoes. A sylvatic or jungle yellow fever, where people were infected by mosquitoes while felling trees, was studied by Dr. Jorge Boshell. He found the blue Hemagogus mosquitoes to be the vector of jungle yellow fever. These mosquitoes breed in tree holes where water collects from rain or dew.

Hughes: And that changed the nature of the yellow fever eradication program, didn't it?

Johnson: Yes. Then they realized the only way to deal with that was to vaccinate people wherever cases of jungle yellow fever occurred, rather than to try to eradicate the Hemagogus mosquitoes.

Hughes: So that was another impetus for the development of the vaccine?

Johnson: Yes. The IHD began developing field programs where you would study the natural ecology of the disease. Actually, in South America with yellow fever, they tested all the different animals to see if they would circulate the yellow fever virus, including bats. But the trouble was, they were using varieties of albino adult mice that would not get sick and die when inoculated intracerebrally with small doses of yellow fever virus. So they would say, these animals are not susceptible.

I've been in several of the areas of Central America and South America where there's been jungle yellow fever, also in Africa. The only small mammals that are abundant in the endemic foci of yellow fever are the fruit bats, such as Carollia perspicillata, but no one is willing to study them.

Ottis Causey began studies at Belem, Brazil in 1954. He caught all the various kinds of forest rats, using traps made out of wood. He had an alarm clock attached that would go off when the door closed so they would know the time they caught them. They would mark the animals and bleed them. The bait used was

* Dr. Johnson repeatedly returned to this subject in subsequent interviews.

Johnson: corn and bananas. They caught *Carollia* fruit bats that entered these traps at ground level. The bats were marked by toe clipping and some were recaptured two or three times. "Well, they're not susceptible," Causey said. He did not test blood or organs. The *Carollia* bats were the most abundant animals in the forest near Belem where workers slept in hammocks and contracted yellow fever in 1954.

I said, "Well, where were these people sleeping in the hammocks?" "Oh, it was right in the forest." "What are the animals here?" "Oh, there are *Orizomys* rats." "Any monkeys?" "None seen recently."

But then in the evening there were all these bats, and I said, "Where do they come from?" "Oh, there's an aqueduct nearby; it's a big brick-lined aqueduct." There were about two or three hundred thousand of these bats, and they'd be out feeding there every night where these people slept in their hammocks.

Hughes: Why are people resistant to the idea that the bats might be transmitting yellow fever?

Johnson: They say bats are not susceptible to yellow fever virus. What they mean is they do not get sick and die when inoculated with the virus. There are many *Carollia perspicillata* fruit bats in known foci of yellow fever in South America.

The Rockefeller Foundation had a field station at Villavicencio, Colombia, in the llanos east of the Andes. When I was there in 1963, I said, "Are there any caves here at Restrepo?" There had been a serious epidemic of yellow fever there in 1936 and 1937.

We were having a Coca-cola in one of the local cantinas, and there I met a local engineer. I asked where he worked and he said, "In the mine." I said, "Are there bats there?" "Oh, thousands!" [laughter] We visited the mine and there was a large population of *Carollia perspicillata* fruit bats. This animal seemed to be the common denominator for yellow fever foci.

Hughes: Getting back to the concept of the nature of viruses, in 1935 Wendell Stanley and his associates crystallized tobacco mosaic virus. That confused the conceptualization of what these filterable, in most cases invisible, infectious agents might be.

Johnson: And are they living? Do they have to have a cell to live in?

Hughes: Was there debate about the nature of viruses when you were at the Rockefeller in New York?

Johnson: Oh, yes. The big debate at that time was about the Rous sarcoma virus I had isolated in 1948 from a chicken we had purchased for bird malaria studies. I had passed the virus by intracerebral route in one-day-old chicks. I could lyophilize the virus and put the ampoule of dried virus in boiling water for thirty minutes, but it would still be infectious for chicks when rehydrated. This did not fit with the concept at that time which was that the tumor virus could not be passed unless you had some live tumor cells in the inoculum. We can understand now that cells frozen in liquid nitrogen will remain viable, but what about surviving high temperatures? It's hard to say that there aren't some living cells in the virus in frozen suspensions.

There's still an argument as to whether scrapie is caused by a virus. You can't see the scrapie virus or the kuru virus from New Guinea by electron microscopy. It's highly transmissible with very dilute ground-up suspensions of brain.

Hughes: You can't see it with electron microscopy?

Johnson: You can't see a discrete virus body.

Hughes: At the other end of the size scale of viruses are the chlamydiae. We can see inclusion bodies in cells infected with these agents and the tiny virus particles are visible with the light microscope.

Johnson: During the early studies of wildlife in South America by the IHD staff, they isolated leishmania, toxoplasma, and psittacosis-like (chlamydial) agents from small mammals and birds. The psittacosis chlamydia problem has now been solved by the discovery of tetracycline.

During World War II we were working on atypical pneumonia, now known to be caused by mycoplasma. Dr. Monroe Eaton at the [Rockefeller Foundation's] California lab was able to isolate and maintain this organism by passage in chicken embryos. He also isolated an agent from cases of respiratory disease which was called meningopneumonitis. This disease has recurred during the past ten years and the organism identified as a chlamydia. Chlamydia organisms are the cause of epidemic cervicitis and urethritis.

But what else about viruses? Are they strictly parasites of the brain, lung, kidney, or skin, or do they produce infection in many different organs? All the mosquito- or tick-borne viruses we had at the Rockefeller Institute in 1946 were high brain passage neuroadapted viruses, that is, West Nile virus, Bwamba virus, Semliki virus, Uganda S virus, Zika virus, yellow fever virus, Ilheus virus, European tick-borne encephalitis virus, eastern, western, Venezuelan, St. Louis, and Japanese B encephalitis

Johnson: viruses. Mice and hamsters inoculated intracerebrally with these viruses showed significant pathology only in the brain and spinal cord. In monkeys inoculated with the viscerotropic Asibi strain of yellow fever virus, I saw the classic central necrosis in the liver lobules and tubular degeneration in the kidneys.

During the period from 1938 to 1944, when I was at the Rabies Study in Alabama, we did complete autopsies of dogs naturally and experimentally infected with rabies virus, and on several persons that died of rabies in Alabama and Georgia. In human rabies there is no extensive degeneration of nerve cells. The large neurons are enlarged and they have Negri-type inclusion bodies but there was very little leucocytic infiltration. Salivary glands sometimes had a high titer of rabies virus, even greater than the brain, but no significant cell destruction. I have isolated rabies virus from the lungs of skunks and bats, but the lungs show no gross and little microscopic pathology. The rabies virus appears to damage the function of the nerve cells. For example, the virus interferes with the oxygen-carbon dioxide control mechanism in the brain, and death results from hypoxia, leading to muscle spasms and convulsions.

American Virologists and Microbiologists

Hughes: Well, before we move on to rabies, please say a word about a few other people at the Rockefeller. Simon Flexner.

Johnson: Simon Flexner was the first director of the Rockefeller Institute. He began his work there in 1904 when the institute was completed. He was already well known in the medical community for his development of the immune serum therapy for cerebrospinal meningitis, the discovery of the Flexner bacillus during an investigation of an outbreak of dysentery in the Philippine Islands in 1899, and the report of his study of the bubonic plague in San Francisco in 1901. From the beginning, he had his own laboratory at the institute and one assistant. In choosing staff members he looked for nonconformists who had intellectual curiosity and were interested in "finding out" answers to problems, and he let them choose what to study. He himself chose to study poliomyelitis because this seemed to be especially important at that time. This resulted in his isolation of the virus of human poliomyelitis by i.c. [intracerebral] inoculation of brain tissue from fatal cases into monkeys.* I have been on

* S. Flexner, P. A. Lewis. JAMA 1910, 54:1140.

Johnson: the lookout in hiring or recommending persons for research or teaching appointments for people who were interested in "finding out" rather than those who could memorize data.

Simon Flexner was the first editor of the Journal of Experimental Medicine. His brother Abraham Flexner wrote a book in 1910 entitled Medical Education in the United States and Canada which played an important part in the reorganization of medical schools. He later became director of the Rockefeller Foundation General Education Board. Simon Flexner had no time for idle conversation and was inconspicuous whether he was at a meeting or in the lunchroom. When he retired in 1935, Herbert S. Gasser MD became director. It was a pleasure to talk with him and visit his lab. From Gasser, Olitsky, Rous, and Rivers, I learned about the history of the Rockefeller Institute and Simon Flexner. They were all good at finding "discoverers."

Olitsky continued the studies of polio begun by Flexner, and Albert Sabin, Jerry Syverton, and Herald Cox are examples of his passing on the tradition. Tom Rivers' influence on virology in the United States is illustrated by the list of contributors to the first edition of Viral and Rickettsial Infections of Man, 1948, which he organized and edited. There were four editions, the last edited by Frank Horsfall and Igor Tamm in 1965. I wrote the rabies chapter for each edition.

Hughes: Louis Kunkel was at Princeton?

Johnson: There is also H. G. Kunkel, who studied electrophoresis and macroglobulins.

Hughes: I refer to the plant virologist.

Johnson: Yes, I met him. I visited the Rockefeller Foundation at Princeton several times. Carl TenBroeck was interested in rabies. Wendell Stanley and his associates had crystallized tobacco mosaic virus in 1935. I especially needed the advice of the veterinary staff, such as Dick Shope, regarding diseases of dogs, mice, and guinea pigs.

Hughes: Were the people in New York keeping close tabs on what was going on at Princeton?

Johnson: Yes, the Princeton staff frequently visited the Rockefeller Institute in New York City. The Rockefeller Institute at Princeton worked on animal viruses [as well as on plant viruses]. Basic studies of viruses were going on at both places. The idea was to have one group working on domestic animal diseases and the other studying human viruses. During my second assignment at the Rockefeller Institute from '46 to the end of '51, the International Health Division electron microscopy program was going strong.

Hughes: Richard Shope?

Johnson: Richard Shope was at Princeton. He was working on swine influenza A virus and would occasionally come up to New York. We would talk, and I would see him at meetings. Shope had isolated the swine type A influenza virus in 1931 and our IHD lab was working on the human influenza A virus. We were critical of the research studies he reported in 1941 identifying the swine lung worm as a reservoir host of the influenza virus.* It is of special interest that Shope was infected with EE [eastern encephalitis] virus from natural exposure. He asked to be bled. Dr. Delphine Clarke did the virus isolation at the New York lab.** He had no sequellae after the initial febrile disease.

Hughes: Albert Sabin.

Johnson: Albert Sabin was not at the institute when I was there. He did work there for a short time. He was in Cincinnati when I was working on rabies. He was interested in this disease, and we corresponded from time to time, and we would see each other at scientific meetings. I have great respect for what Albert Sabin has done in virology. He and [Robert Merritt] Chanock pioneered the use of the hemagglutination test for studying infection from arthropod viruses.***

The superiority of the Sabin polio vaccine viruses may be explained by his use of cloning of individual virus particles and so selecting out the less pathogenic clones. I used the same method for selecting low pathogenicity strains of western encephalitis and Turlock viruses.

Hughes: Jonas Salk.

Johnson: He was on the staff at Dr. Thomas Francis's lab at the University of Michigan, studying influenza virus. He visited our rabies study laboratory in Alabama to observe our methods of making and testing rabies killed virus vaccine. The Salk polio vaccine was a formalin-treated killed virus type vaccine.****

Hughes: Jerry Syverton?

* J. Exp. Med. 1941, 74:49.

** D. H. Clarke. Am. J. Trop. Med. Hyg. 1961, 10:67.

*** J. Immunol. 1953, 70:271.

**** More on this subject below.

Johnson: Jerry Syverton was not at the institute when I was there either time. He'd already gone to the University of Minnesota. He was one of the remarkable men in polio research; way ahead of the field in studying the disease and isolating the three different strains of polio virus from outbreaks. He died in the late 1950s. He was one of the greats in the polio field and laboratory research, an excellent virologist. I had many contacts with him at meetings and by correspondence.

Hughes: Herald Cox.

Johnson: Herald Cox is sort of the dean of the microbiologists I've known. I still correspond with him. He retired to live in Hamilton, Montana. In his early work on rickettsiae at Hamilton, he developed a rickettsial vaccine using infected tick suspensions. I first met him when he was director of the Lederle Viral and Rickettsial Disease Laboratory at Pearl River, New York. Typhus and Rocky Mountain spotted fever vaccines were made there from infected chick embryos.

Lederle made smallpox, influenza, and rabies vaccine, as well as many other vaccines for man and animals. It was a beautiful laboratory with strict control to prevent bacterial contamination. We followed many of their designs when we set up new quarters at the Rockefeller Institute in 1946.

I sent Herald Cox my avian-adapted Flury rabies virus in January 1946, and he supervised the development, testing, production, and licensing of this vaccine for use in dogs, cats, and cattle. Herald Cox received his D.Sc. from Johns Hopkins University. So did Calista and Ottis Causey, who were on the staff of the International Health Division for many years.

I sent the Flury avianized rabies virus to Clara Nigg, Ph.D.,* of E. R. Squibb & Sons and to Jensen Salsbury Company in the United States, and to Dr. R. Daubney, director of the Serum and Vaccine Institute at Cairo, Egypt. Herald Cox assigned Hilary Koprowski to work on the Flury avianized virus and this led to years of close cooperation. We obtained rabies vaccines from the various companies. At that time we used to deal with National Drug Company (human), Gilliland (human), Lederle (human and dog), Parke Davis (human), Ashe Lockhart (dog), and Jensen Salsbury (dog). We found the Ashe Lockhart vaccine to be the best commercial dog vaccine, and we did most of our dog studies using that vaccine.

* A rough transcript of an interview with Dr. Nigg is on deposit at The Bancroft Library.

Hughes: What about Squibb?

Johnson: We did not test their rabies vaccine. From 1937 to 1941, Clara Nigg was on the staff of the IHD influenza lab at the Minnesota State Department of Health in Minneapolis. Dr. E. R. Rickard of the IHD was in charge. Clara was a very good microbiologist. When I was at the rabies lab in Montgomery, one of her interests was in what we now call chlamydiae. She had isolated a strain from albino mice. In 1950 she did some studies of rabies vaccine production using a benzene ether calcium acetate-treated vaccine recommended by Dr. Karl Habel of the NIH. She found that this vaccine failed the antigenicity test.

The major international biological firms dealing with rabies [vaccines] were the Pasteur Institute in Paris and the affiliated Pasteur institutes in other countries, the Connaught in Toronto, and the Wellcome lab in London. The NIH was not making rabies vaccine, but several of the state health departments were.

Hughes: The liability problem hadn't arisen?

Johnson: No. Rabies vaccine was provided by city or state public health laboratories. Some purchased rabies vaccine from commercial laboratories, but the governmental institutions were responsible for the rabies vaccination complications. This was before Melvin Belli started the big liability suits [against the firms making vaccines].

Rabies vaccine was made at the state health departments in Texas, Georgia, New York, South Carolina, Michigan, and California. Miss Amy Darter, who made rabies vaccine at the Life Sciences Building on the University of California campus in the 1920s, is still alive. Dr. K. F. Meyer was in charge of this vaccine lab. He was a long-time friend of mine. We corresponded when I was working on the rabies study of the International Health Division, and we had frequent talks when I was working in California.

Scientific Director, Cooperative Study of Rabies, Rockefeller Foundation and Alabama State Board of Health, Montgomery, Alabama, 1938-1945

Foundation of the Laboratory

Hughes: Why did the Rockefeller become involved with the Alabama State Department of Health?

Johnson: The Rockefeller Foundation had done malaria and hookworm control programs in Alabama in the 1920s, so when dog rabies became an acute problem in 1936, the state health officer of Alabama, Dr. J. N. Baker, requested the assistance of the International Health Division of the Rockefeller Foundation to assist them in the control program for rabies.

Dr. Sawyer assigned Dr. Charles N. Leach to direct this study. I went to Montgomery in 1938 to visit the project. The laboratory had been built on the grounds of Kilby Prison. Leach had hired two technicians, Ellen Howell, who had been previously employed by the Alabama State Health Department, and Reginald Reagan, who had been a technician at the IHD lab in New York. Miss Howell had been doing the microscopic diagnosis [of rabies] at the health department, which was based on the demonstration of Negri bodies. We began using the mouse inoculation test for the isolation of rabies virus for comparison to the Negri body microscopic test.

Dr. Leach had begun some experimental studies of dog rabies, inoculating one milliliter of ten percent dog brain from a naturally infected dog into the masseter muscle of the head, as the method of transmitting the disease. Though the dosage was large, it did not produce a high mortality. It was equivalent to vaccinating a dog when you injected him. It seemed to me that if you wanted to simulate natural infection, give them a small dose rather than a Pasteur treatment dose of antigen.

It is pertinent to mention here why the rabies study was built at the state prison. This was at the suggestion of the state director of public health, Dr. Baker. It was a time when there was little money for research and he said that a large building facility for dogs would be a noise problem in any urban area. Few people would be interested in a job working with rabid dogs. It would be a temporary facility and it should be in an isolated place, reached by a restricted road. He recommended Kilby Prison. There were many prisoners who had special skills, so we could use them for construction work, working in the kennels, and upkeep of the laboratory buildings and grounds. Dr. Leach had worked on temporary field projects for hookworm control and he designed the building to be used for the laboratory, an excellent one.

Leach was a graduate of Stanford Medical School, a well-trained public health physician, having worked on field projects in malaria and hookworm. He had no virology training, and it was my job to run the virus research program and he was to be the administrator, i.e. public health records, finances.

Johnson: Leach had only done a few dogs. He had a few cages made. But the main thing at first was to have a diagnostic test. Some dogs that had symptoms of rabies and had bitten people did not have Negri bodies in the brain, but when the brain material was tested by the mouse inoculation test, it was positive for rabies virus and the mice would have Negri bodies in the brain when they sickened or died.

Hughes: Why was that?

Johnson: In some cases, the rabies virus can develop so rapidly in the brain of the dog, the incubation period is short and they die soon after onset of symptoms, so the classic inclusion bodies, called Negri bodies, have not yet been formed. The Negri bodies are stained with basic fuchsin and methylene blue. They are distinctive inclusions. If present, you know the biting dog had rabies. Subsequently we learned that about ten percent of the suspected rabid dogs were negative for Negri bodies.

Hughes: And then the people who had been bitten would not be treated?

Johnson: At the time, persons bitten by a dog and where the disease was known to be present in the area, the rabies vaccine treatment was begun as soon as possible. The biting dog was confined in a kennel or dog pound for ten days and if the animal did not sicken or die, the treatment was discontinued after five days. After the development of fluorescent antibody test for rabies, a specific diagnosis could be made at the onset of the disease in dogs.

Hughes: Why had the lab been set up in Montgomery?

Johnson: Dr. Baker had convinced the state legislators to pass a law that all dogs allowed at large must be vaccinated for rabies and ownerless stray dogs be killed. They wanted to know whether the human rabies vaccine prepared at the state health department would produce immunity to rabies virus and also whether the commercial rabies vaccines used by the veterinarians really immunized dogs against rabies.

Some of the people bitten by rabid dogs and given the rabies vaccine treatment within twenty-four hours after the bite nevertheless died of rabies. However, if they lived a month after the bite, then they almost always were safe. What was needed was to have immune serum to give immediately after the bite exposure to prevent growth of rabies virus until antibodies were produced by the vaccine treatment. The immediate problem was an increasing number of cases of rabies in dogs and the necessity of treating several thousand people each year.

Hughes: So Alabama approached the Rockefeller for help?

Testing Commercial Rabies Vaccines

Johnson: Yes, they wanted to know whether the dog rabies vaccine was effective. A lot of veterinarians said they believed that dogs that had been vaccinated did not get rabies. The pathologists that were doing research said, "The vaccine won't work unless there's live virus in it."

There was a good reason for that because the Japanese had developed a vaccine, probably very much like the original Pasteur vaccine, which contained live virus. The Japanese were making a vaccine using the fixed Pasteur strain of rabies. It was called the Umeno and Doi vaccine. This was a rabbit brain tissue, live virus vaccine. The rabbit brain tissue virus was emulsified in saline solution containing twenty percent glycerol and 0.5 percent phenol. They were able to eliminate dog rabies in Japan with this vaccine.

In the United States in the late twenties, this type of vaccine was produced commercially. There were a few vaccinated dogs that developed rabies from the vaccine virus. The instructions were to give the vaccine by subcutaneous injection. We know now that this type of vaccine could produce vaccine rabies in very young dogs if given into muscle tissue. On the basis of the report of suspected vaccine-produced rabies in a dog at Milton, Massachusetts, the U.S. Department of Agriculture issued an order to stop use of this vaccine. Subsequently, the American biological firms produced either the Semple type rabies vaccine, in which the vaccine virus, containing 0.5 percent phenol, was inactivated by incubation for twenty-four hours at thirty-seven degrees centigrade or the Kelser type where the vaccine virus containing one percent chloroform was inactivated at ten degrees centigrade.

The Georgia State Health Department was using a modified Hoegyes vaccine. They prepared a 1:150 dilution of rabbit brain fixed virus (Pasteur strain) in physiological saline, containing twenty percent glycol and 0.2 percent phenol. This vaccine was not exposed to incubator temperature for twenty-four hours like the Semple virus, and we found it did contain viable virus when used. More than 70,000 persons were vaccinated with this vaccine, given by subcutaneous injection, without any known deaths from the vaccine virus.

So the question was then, would a really killed virus vaccine work? The reason it didn't look like it would ever work was the study method chosen, that is, Pasteur reported he could immunize dogs against trephine (intracerebral) exposure to rabies virus. The experimental studies of dog rabies vaccine using intraocular or intracerebral inoculation of rabies virus failed to show any

Johnson: protection from vaccination. There was one study where the vaccinated dogs all died of rabies and the controls survived. The two test groups were kept in separate buildings, and the test virus must have been inactivated by sunlight during the move to the second building. They were correct in doing the controls last but still proved that the vaccine did not protect against intraocular exposure.

Our method was to challenge vaccinated and control dogs alternately, and the test virus was protected from light and heat until used. The challenge inoculations were done in the laboratory building where we had a special table for holding the dogs and could do the test inoculations correctly. First we had to study the natural disease as it recurred in dogs and do clinical and pathology studies of dogs and learn about the various diseases of this animal and the symptoms and pathology produced by the rabies infection. We also had to learn how to safely handle rabies dogs and to find a method to do vaccine potency tests in mice.

I didn't like the intracerebral challenge test from the beginning. We tried the one where we vaccinated peripherally and then challenged intracerebrally with serial tenfold dilutions of the rabies virus. We would have a ten to forty percent mortality in those given the highest doses of virus (10,000 to 100,000 MLD [mean lethal dose]). The mortality would increase to forty to sixty percent at the 1,000 MLD dose, then decrease to ten percent at the 100 LD50 dose, indicating a booster vaccination effect from the large doses of test virus. In other words, we observed a straight-line mortality in controls and a parabolic curve for the vaccinated mice. If we challenged the vaccinated mice with a medium dose of rabies street virus by intramuscular injection, we found we could demonstrate a one hundred percent protection by certain dog rabies vaccines purchased from commercial sources.

We made an agreement with Dr. Guy Phelps, DVM, in Montgomery who was vaccinating dogs as part of the enforcement of the new state vaccination law. We would supply the rabies vaccine which we had found to be antigenic in the mouse test, and as he visited his clients at their farms to vaccinate the dogs, we requested he buy any dogs they did not want to keep. He would pay for the dogs and we would reimburse him. This way we would obtain dogs for experimental studies and know the age, sex, breed, and owner.

The veterinarian made his usual fee by giving the vaccine. As a result, we eliminated dog rabies in Montgomery County in less than one year. Unwanted dogs would have been killed anyway. We would not use stray dogs picked up off the street. Those were destroyed at the city pound, usually with carbon monoxide. It is a humane method because carbon monoxide has no odor and the animals

Johnson: lose consciousness without pain. People are sometimes killed by carbon monoxide while asleep in poorly ventilated houses if they use a heating system which gives off carbon monoxide.

Cities often destroy several hundred dogs a month, some found dead and others picked up as strays and not claimed. That famous story, To Kill a Mockingbird, has a good description of a rabid dog coming down the street with glazed eyes. I have seen many rabid dogs and it is an example of an infectious insanity, an animal trying to attack and bite. Some rabid dogs appear friendly and alert but will bite anyone, even their owner, at the slightest provocation. Other dogs infected with rabies become paralyzed and die without showing any agitation or viciousness. This is called dumb rabies.

We did one field study in Birmingham, Alabama. The garbage trucks had to pick up any dead dogs left at garbage cans. We had one staff person take brain specimens on these dogs for a year. About five percent were positive for rabies, although there was no evidence of an outbreak of rabies in dogs at that time. Soon after that, there were some people bitten by rabid dogs. We had stopped the testing of the dead dogs. They reported that more than one thousand dead dogs were picked up each month, at the time some persons had to take the rabies vaccine. It is evident that a high percentage of the dead dogs died of rabies.

I decided to use rabies virus obtained from the salivary glands of rabid dogs for our test virus. We prepared a standard virus and stored this in flame-sealed glass ampoules in a dry ice chest. Having a standardized virus, we could repeat tests with the same dose of virus. By a series of studies we found we could obtain a seventy to eighty percent infection rate using a 1 to 200 dilution of dog salivary gland virus in a dose of 0.06 milliliter in each masseter muscle, near the dose that might be inflicted by a naturally infected dog.

Disease and Nutritional Problems in Dogs

Hughes: What about distemper virus as a cause of loss of dogs in your studies?

Johnson: We could vaccinate for distemper. We made our own vaccine, but on one occasion we isolated LCM [lymphocytic choriomeningitis] virus from a pool of dog spleen distemper virus sent to us to use for vaccination of dogs. I tested all viruses sent to me by intracerebral inoculation in mice. The mice inoculated with this distemper virus died in six to ten days and they showed the classic Traub's sign, which is exhibited by mice when they develop

Johnson: the LCM infection. When picked up by the tail with forceps, there is a rhythmic spastic extension of the hind legs. The dogs used as a source of this pool of dog spleen distemper virus must have been exposed to LCM virus from the urine of house mice. I did not use this dog spleen virus. Dr. Gilbert Dalldorf had a similar experience at about the same time. He was studying dog distemper virus in monkeys and he soon learned that he had both dog distemper virus and LCM virus in the monkeys.*

It was necessary to learn about the diseases of dogs and so be able to keep the animals healthy and not lose any from incidental diseases. As the dogs arrived, we would dip them in a lime sulphur bath to get rid of mange, lice, and other ectoparasites. We tested stool specimens for parasites. Almost all of the dogs had hookworm infestation. We routinely dewormed the dogs using tetrachloroethylene given in a capsule. All dogs were tested for heart worm (filaria) by examining the wet film blood specimens for microfilaria. If positive, we gave the dog an I.V. [intravenous] injection of a commercial antifilarial drug called fuadin. Not having other dogs nearby, we seldom had any recurrences. This filarial disease is transmitted by Culex pipiens mosquitoes. The stool examination would show whether the dogs had Ascaris, dog tape worms, or coccidiosis.

It was the coccidia that caused illness and death of dogs the first year I was at the laboratory. Dr. Leach had cages set in sand and that proved ideal for the development of the coccidia cysts passed by infected dogs. Dr. Leach left Montgomery early in 1941 to take charge of the IHD work in the Philippine Islands. When Luzon fell he was interned in the notorious Santa Tomas Prison Camp. This left me with the necessity of obtaining a sanitary facility for holding dogs for long-term studies, that is, about two years.

I designed a large concrete platform with drains so that the cages could be washed daily and all excreta would run out into a septic tank. Each cage unit was built to have two dogs--for example, one vaccinated and one control. There was a box open to the south and a tarpaulin cover. The grill floor of the cages eliminated the parasitic infections.

A few of the dogs were infected with strange parasites. One was the nasal worm, Linguatula serrata. Another was the esophageal cyst parasite, Spirocerca sanguinolenta. Neither of these could spread when the dogs were kept in cages. Both parasites had strange cycles involving dung beetles. The nasal

* Science 1939, 89:515.

Johnson: worm caused no health problems, but the esophageal cyst parasite damaged the aorta, and a few dogs died of ruptured aortic aneurysms. To complete the cycle, the worm had to discharge its eggs into the esophagus. The eggs would hatch in the dung and the larvae would get into a beetle. This would be eaten by a dog. The larvae would migrate through the lining of the stomach and follow the gastric artery to the aorta. It would enter the mediastinum and form a cyst and create a fistula into the esophagus to discharge the eggs.

In 1938, Dr. Leach had consulted with the University of Alabama School of Veterinary Medicine at Auburn about feeding dogs. They assured him that dogs could be maintained in good condition when fed a cereal diet composed of a grain mix. This had been initiated when I arrived. Many were ill from coccidiosis, so it was difficult to recognize the nutritional deficiencies until the dogs developed what Dr. Phelps called running fits, black tongue, and hard pad disease. In doing autopsies of the animals that died following the onset of these symptoms, I was amazed to find typical gastric and duodenal ulcers.

In the meantime, I had checked on the preparation of the dog cereal and learned that it was usually cooked at the time the prisoners arrived at the lab (trustees left the prison on the van at 6 a.m.) and they did not feed the dogs until Dr. Leach arrived, usually at 9 a.m. or later, because he went to the state health department in Montgomery first. From what I had read on nutrition, this should completely inactivate the B vitamins, thiamine and niacin, which had just been identified.

I decided we should get commercial Purina dog chow, and it was amazing how rapidly the dogs recovered. We had no further feeding problems. The running fits symptom in dogs was the result of thiamine deficiency similar to the delirium tremens or Korsakoff's syndrome observed in alcoholics, with the malnutrition that supervenes when they get most of their calories from alcohol. Black tongue and hard pad disease were the result of niacin deficiency. Subsequently, when I autopsied dogs, I saw no more gastric or duodenal ulcers, and I am convinced that deficiency of the B vitamins predisposes to the formation of ulcers. Alcoholics frequently develop ulcers, which could be expected if they are the result of vitamin deficiency. I remembered the Sippy diet given to ulcer patients when I was in the medical service at Harvard. This was a serving of cream at intervals through the day. No vitamin B in that.

Johnson: We had a similar nutrition problem with the mouse colony. Dr. Leach was using stale bread dipped in milk for mouse food. We were getting few litters, and those that were born were apt to die at birth or soon after. Having had the experience with the dog food, and having the veterinarians tell me that running fits in dogs were related to feeding them mostly stale white bread, I started feeding the mice Purina fox chow, which was better for mice than the dog chow. We used water bottles made from two-ounce prescription bottles fitted with a stopper and bent glass tubing which could be passed through the stainless steel grid in the lid of the mouse boxes.

I devised a catching pole about six feet long, with a loop to catch the dogs. A heavy cord was attached at the end of the pole and the cord was passed through a metal eye nearby. When released, this formed a loop so you could slip it over the head and tighten it, and you could lead the dog, but it could not attack. One could grab the nose with heavy leather gloves and hold the dog with one hand behind the head and one around the nose with the fingers in the slot under the jaw. The cages were made so we could open the front a couple of inches and put the loop around a dog's head. It did not hurt the dogs and they soon became used to being handled that way, knowing we would not hurt them.

For feeding, the lift gate was opened with one gloved hand enough so the scoop with Purina checkers could be used to fill a crock with the food. The other crock with water was filled by a hose when washing the cages.

Hughes: Was the design of the catching loop used anywhere else?

Johnson: It was a modification of other types of catching devices used at dog pounds. Ours had to be different because of the danger of handling rabid animals and working with a lift gate. The animal cages were our own special design so we could keep dogs off the ground and have a holding facility that was easy to clean by one man.

There were a few prisoners at Kilby Prison who were trustees and had done welding. We purchased the heavy mesh and the angle iron. Trustees also did the construction work and cement work. I had the state sanitary engineer draw up a design for the septic tank and it worked beautifully.

The Prisoners

Hughes: Was it common to use convict labor?

Johnson: Yes, for various state facilities, but we used only trustees. They were prisoners that were going to be paroled within a few years. We used trustees who seemed to enjoy the opportunity to do some interesting work. All of us had to be vaccinated with the Semple rabies vaccine, including the trustees, fourteen daily doses. All were bled before and after vaccination.

I was on good terms with the medical staff at the prison, and in 1941 Dr. [Elsmere] Rickard and I did some experimental studies of influenza vaccine in the different prisons in Alabama. (I also collected blood from some prisoners for use as normal aerum.) They received a small payment for this. It was voluntary.

Hughes: Were they sent with a guard?

Johnson: No. The trustees loved to get away from the prison. We gave them a little spending money, and they'd use it for tobacco, et cetera. When they were freed, I'd hire the best ones to do special work at the lab.

My favorite was a prisoner by the name of Barker. I trained him to do the glassware washing and instrument sterilization. He was an intelligent man. I remember his wife used to come up and see him when he was in prison. When he finally was paroled, he started to find his old friends and go drinking and get into trouble, and one day his wife called in and said, "He's been shot." I said, "Well, where is he?" She said, "He's in the hospital." So I said I'd go see him. He'd been shot in the stomach over an argument while playing cards and drinking.

I called one of the best surgeons in town and told him, "See what you can do." He opened him up, and his stomach was perforated; he sewed it up. Barker was one of the first to get penicillin. Sad to say, he developed thrombosis. The shot went through the mesentery, and he died. Such incidents were common in Montgomery. One twenty-four hour period there were thirteen such killings among the black population.

I liked most of the trustees who worked for me; some were very interesting. One man was George Brock. He worked at the lab for a couple of years. He had worked for the Henry Clay family in Georgia, the famous Henry Clay, the southern senator. George had killed somebody in an argument over fighting cocks. And guess what? George spoke French! He was in France during World War I and I enjoyed hearing about his experiences, especially about the Clay family and cock fights.

Johnson: But let me tell you examples of the things that happened. One trustee was called Preacher. He would lead the responsive singing. He'd sing the verse, and then the others would sing the chorus. One day, apparently one verse was about George Brock, and he was insulted. George took after Preacher, chasing him into one of the dog kennels.

Just as I got to the door, George tried to hit Preacher, but Preacher ducked, and George hit a two-by-four. It was almost laughable because he absolutely ruined his fist. And so I said, "George, that thing looks bad. Let's go over and see Bob Marks," who was a doctor at Kilby. We took an x-ray, and I don't know how many bones were broken from hitting that two-by-four, but his whole hand had to be splinted for a long time.

Another time one trustee went after another one with an axe. When I got there, he was chopping through the boiler room door with the axe. The warden had told me, "Don't be afraid." I went over and said, "Put down that axe. I'll have to take you to the prison. You can't do that here." No problem. We had a laugh about the scare he gave that trustee.

There were some bad prisoners we called alligators. They'd worked in the fields, and they wore striped pants. They knew that the prison had bloodhounds. If one of the prisoners would try to run away, the man in charge of the bloodhounds would be called, and he would get into his van with the dogs and find the tracks, and the dogs would soon find the prisoners. The bloodhounds would never catch the prisoners; they had an attack dog, which usually was a shepherd. Bloodhounds never hurt anybody. The bloodhound warden was Mr. Debardaleben.

One day, I heard a shot near the lab. There was a vehicle in the road. Apparently, one of the convicts had feigned illness, and as the field warden took him in his car as they were going up toward Kilby, the prisoner pulled the warden's gun and shot him.

Hughes: Killed him?

Johnson: No. The prisoner took off in the van, but they caught him. If he had killed the warden, he would have been executed. I'm against capital punishment. There should be long-term confinement for capital crimes like rape and murder. There should be gainful employment so as to earn food and quarters.

One more comment about the prisoners. The warden advised that we use only murderer trustees as they were not likely to steal. We employed seventy different trustees, all in prison for murder. They were willing to work, and we got to be friends. I got to know them and their families.

Johnson: We had a black woman named Leitha who worked for us. Her mother, Mary Jones, later took care of me when I was paralyzed.* When I came to Montgomery after five months at Warm Springs Foundation and hardly able to get around on crutches and a long leg brace, Mary appeared at the door. Her daughter had gone to New York. Mary said, "I want to help take care of you." For a time she stayed at our house. A wonderful Christian woman.

I really was all for Martin Luther King, because he said, "Love your white brother." I say, "Love your black brother." I heard Martin Luther King speak at Cal, at Wheeler Hall, in 1958, a beautiful speech. I heard him also at the Cow Palace. He had the answer. You can teach people to hate so easily it isn't even funny. But he was right. He said, "If they spit in your eye, don't strike back. That will destroy us." He was right from the beginning, and he's still right.

Hughes: Well, getting back to the rabies lab: Did you find that there was a funding problem for equipment?

Johnson: Funds were readily available for equipment, but the Rockefeller Foundation did not build large, permanent facilities. Field laboratories were supposed to be simple and serviceable.

Hughes: Did you have to have approval for expenditures before you went ahead?

Johnson: No, I could buy what was needed; I had a budget. I practically never used all of it.

As soon as we were able to control rabies locally, it became very apparent that the rabies vaccine worked. When the fox epidemic started in Georgia during 1940, I said, "Well, what about the fox hounds?" I'd vaccinate them with two doses of Kelser chloroform-treated virus. There were no cases of rabies in the vaccinated fox hounds used in the fox control program. I learned to work with all interested parties, the SPCA [Society for the Prevention of Cruelty to Animals], the veterinarians, and hunting and farm organizations, and the veterinary school. To get the application of the rabies vaccine did not cost much; show them what can be done, and almost immediately it can be applied.

I was invited to the state legislature in Alabama to speak on how the law [on dog rabies vaccination] might be phrased so that it could be effective. They passed a law, just like they did in California. In 1955 I went to Sacramento to testify about the rabies problem, because of opposition to dog vaccine legislation in California and any destruction of stray dogs.

* See below.

Testing Commercial Rabies Vaccines (continued)

Hughes: Could you summarize the work in Montgomery?

Johnson: First of all, it takes a lot of time to do a vaccine experiment. You have to hold dogs for three months to see if they're okay. We never had any cases of natural rabies in the dogs that we got locally because we quickly eliminated rabies there. But it was a long study, particularly the last one on the duration of immunity. We vaccinated fifty-two dogs and held them and fifty-two control dogs for one year before they were tested for immunity. After challenge, they were held for another three months because the incubation period was seldom longer than three months.

We had developed standard virus for challenge, and the technique of the challenge was relatively simple. The immunity study was completed in 1945 and this showed that the Semple type phenolized rabies vaccine, if properly made, would provide a high immunity in dogs after a single subcutaneous injection for at least one year. It provided the necessary evidence so that rabies control programs could be initiated on a national basis.

The same kind of vaccine would provide one hundred percent protection in dogs if they were given three weekly subcutaneous injections and challenged one month after the first dose. So finally we had proof that it was possible to make a killed virus vaccine that would produce immunity to rabies.*

In the meantime, the Flury avianized rabies vaccine virus had been tested, approved, and marketed by the Lederle Laboratories, and this is summarized in the WHO 1954 paper. The Flury vaccine virus prepared from infected chicken embryos proved to be a superior vaccine and produced one hundred percent immunity for one year. The application of a vaccination program for dogs on a national basis with this vaccine resulted in elimination by 1967 in the United States of the canine-adapted rabies transmitted from dog to dog.

Although the LEP [low egg passage] Flury live virus vaccine which we developed at the Rockefeller laboratory and which was marketed and tested by Lederle Laboratories was used in the eradication program, other types of virus vaccines were developed by commercial companies. Because cheaper vaccines could be made

* These studies were published in the Proceedings of the 49th Annual Meeting of the U.S. Livestock Sanitary Association in December, 1945, and in the Bulletin of the World Health Association 1954, 10, 725-729.

Johnson: by growing rabies virus in cell culture, Lederle Laboratories stopped production of the HEP [high egg passage] Flury vaccine virus in 1967.

To protect dogs from rabies, we need state-organized programs so that you have low-cost vaccination clinics. Then develop good pound facilities so that stray dogs can be picked up, also dogs running at large, particularly where the disease is present. In California, for example, wherever there is a case of rabies in any animal, whether it is a bat or a skunk or a cow, the county is certified as rabies infected and all dogs must be vaccinated. Certain vaccines have to be given every year; the live virus vaccines are good for three years.

Vaccines, which are like the Semple killed virus vaccines, have been developed which are approved for the three years. Because some of the companies are producing vaccines which are not as good as others and the current methods of doing potency tests are hard to interpret, we can expect that some vaccinated dogs will develop rabies from exposure to the bite of wildlife cases of rabies, mostly skunks, and this has occurred.

Once you get rid of the disease in dogs, your problem is almost finished as far as man is concerned, because there are very few people bitten by bats or skunks, and even if they are rabid, that strain is not as virulent as the dog strains. In the United States, we do not have this very virulent dog strain, but in Mexico it is still there. In Canada and Europe, there is no dog-to-dog transmission either.

Hughes: Why do you steer away from using the live virus vaccine?

Johnson: Well, that's true of any vaccine that contains a live virus. A killed virus vaccine is safer in every way. It's hard to control and maintain a live virus vaccination program.

[Interview 4: March 9, 1987]

Hughes: Please describe in more detail your work on the efficacy of rabies vaccines.

Johnson: Most public health officials believed the vaccines did not produce immunity unless they contained live rabies virus. So we tested some rabies vaccines made by the Semple method, and we found two at least that were really good for immunizing mice. Then we started experiments in dogs in which we used groups of ten vaccinated and ten controls for each set. It soon became evident that with just a single injection, and then testing the dogs a month after vaccination, the mortality of the controls would be, say, seventy or eighty percent, and the ones that were vaccinated would be ten or fifteen.

Johnson: Later we did a large study where the dogs were given a single dose of vaccine sc [subcutaneously] and tested for immunity at the end of a year. We used the Ashe Lockhard vaccine, which we had tested by the mouse potency test. We challenged the dogs with a salivary gland street virus, and only ten percent of the vaccinated dogs died and about seventy percent of the controls, a clear-cut evidence of antigenicity.

Then we did a small group of twenty-five/twenty-five vaccinated and control dogs. We showed if you gave them three weekly injections of the same vaccine, and then challenged them a month after the first dose, the vaccinated dogs lived, while the mortality in the control dogs was greater than seventy percent. You could get complete protection against this virulent dog rabies salivary gland virus.

Hughes: Was it a belief that live virus is necessary for protection?

Johnson: That was the general consensus. Dr. Rivers at the Rockefeller Institute and others had come to that conclusion, and they felt that the killed rabies vaccines were worthless. And guess why? Because the challenge dose that they were using was based on Pasteur's experiments in which he said that you could immunize dogs against intracerebral trephine injection of rabies virus. We found out that you can immunize dogs to the maximum extent of forty-five vaccine injections and they still would not withstand intracerebral exposure to rabies virus.

Hughes: Is that true in other diseases?

Johnson: Well, the intracerebral challenge with bacteria or viruses is a lethal challenge. It's one that the animal is not exposed to in nature. For instance, with yellow fever, that was one of the tests in monkeys to see if the virus had lost its pathogenicity, because if you injected the original Asibi yellow fever virus into the brain, the monkey would die. But if you injected it intramuscularly, it died also.

So the first step was that they noticed in passage that the virus had lost its pathogenicity if you gave it peripherally after high intracerebral passage in mice or just chick embryo passage. Finally they had this high passage [virus], called 17D, in chick embryo cell culture, where the monkeys would not even die of yellow fever if you injected it in the brain. But that is a challenge that we would never have naturally.

Within two years of [beginning] our work, we knew that we could eliminate rabies locally. In fact, the first thing I did when I saw that I was getting protection in the mouse test with Ashe Lockhard vaccine was to arrange a control program right where we lived in Montgomery County. With the veterinarian's assis-

Johnson: tance, we made an agreement for the Rockefeller Foundation to buy the vaccine, which was not that expensive; he would set up the clinics or vaccinate dogs on farms or at his animal hospital. He would vaccinate the dogs for a modest fee--something like fifty cents. As I said, we would buy any dogs the farmers didn't want to keep, and then we would know where the dogs came from. We would use them for experimental purposes.

So within a year after we started that program, we had no more rabies in dogs in Montgomery County where the city of Montgomery is located, and we didn't have any rabies exposures the rest of the time I was there. It made me feel safe when we started having children. Whereas, in the first month I was in Montgomery, there was a young dog that developed rabies and bit thirty children at a schoolyard. It was necessary to treat all those children with fourteen injections of vaccine. Of course, everybody that worked with animals at the lab had to have fourteen injections of the Semple type vaccine before they could work with the dogs or do laboratory work.

Hughes: Was one course sufficient?

Johnson: We would do a blood test at the end of each year and look for antibody. If negative, then you'd give one booster dose.

I had had an original course of rabies vaccine at the Rockefeller Institute in 1938. And after a couple of boosters, I was getting alarmed that I was going to get allergic encephalitis; I got pain in my back and fever and lymph node enlargement. I did not take a booster dose of vaccine when I went to Mexico because I was more worried about the allergic reaction, and I did have some antibody when tested in 1943.

Hughes: Is the reaction to the nervous material in the vaccine?

Johnson: It's called allergic encephalitis. It was known a long time ago that a certain percentage of people, about one in 5,000, particularly if they had had more than one course of vaccine, would get an onset of fever and pain in the back and develop what was called treatment paralysis, similar to the Guillain Barre syndrome. It was also called transverse myelitis or Landry's-type paralysis. It started about five days after completing a course of vaccine.

There was one patient I autopsied in Alabama that we thought had that type of ascending paralysis, also like vampire bat rabies. When I tested the brain by the mouse inoculation test, the mice were still well at three weeks. I called it treatment paralysis. Then I had to change it because at one month the mice came down with rabies.

Johnson: In some cases it is impossible clinically to diagnose rabies encephalitis. In fact, the reason probably we know so little about nonfatal rabies infection is that until recently no one has tested anything but the brain to get the virus. If you have antibodies to rabies virus in the spinal fluid after recovery from a disease, that would be accepted now as evidence of recovery from rabies.

Research on Primary Atypical Pneumonia and Tularemia

Hughes: Your laboratory outside Montgomery cooperated with the army in a study of primary atypical pneumonia.

Johnson: Yes. The Rockefeller laboratory at Montgomery was one of the few laboratories in the southern states that had facilities to test specimens from human beings for viral-type organisms. The Armed Forces Epidemiology Board asked me to help in the study of atypical pneumonia.

Hughes: There were other viral diagnosis labs in the south?

Johnson: Not used by the army. The Walter Reed Institute in Washington, D.C. was the army laboratory. The state health department in Atlanta did rabies diagnosis. But most labs had no way of doing serological diagnoses for eastern and western encephalitis virus. We could do them, so we had kind of a regional virus diagnostic facility.

Maxwell Field Air Base in Montgomery was then being inundated with the new outbreak of disease, the one called walking pneumonia, and also atypical pneumonia. Few died of the disease. The patients would cough to the point where they would spit up blood, and some of them would get true pneumonia, migrating from area to area in the lung. It would put a new recruit out for a month or a month and a half, and none of the medications, including the sulfas or penicillin, seemed to have any effect.

The organism turned out to be a mycoplasma, Eaton's agent, which was discovered at the Rockefeller laboratories in Berkeley. But we had no tetracycline at that time, and so there was no treatment for it. It was not possible to develop a specific type of infection in lab animals.

Hughes: You didn't know at the time what the causal agent was?

Johnson: No. I collected many sputum specimens from atypical pneumonia patients and put the specimens into chick embryos, baby chicks, mice, and cotton rats--Sigmodon hispidis--which Eaton also used.

Johnson: You'd get a very mild pneumonia, but you couldn't see any bacteria, and you'd pass it, and the morbidity would not increase. Eaton was able to maintain the organism in chick embryos. He used the complement fixation test to test for the organism.

Hughes: Were you in communication with Eaton at that stage?

Johnson: Oh, yes. I worked in cooperation with the Armed Forces Epidemiological Board, which was doing work at Fort Bragg, South Carolina--I went there--and certain other places, but mostly at Maxwell Field. We wanted to see if we could get [the organism] to grow in animals we had, and, undoubtedly, we had it. But there was no way we could concentrate or get enough of the disease agent. Nobody could grow the mycoplasma at that time in cell-free medium.

Hughes: People were very skeptical about Eaton's work at that stage, weren't they?

Johnson: Very much so. Eaton was trying different animals. We were able to ship him wild-caught cotton rats from Alabama. They look like a *Microtus*. Eaton was using cotton rats for his intranasal passage studies in California. Sure, you get lesions in the lung. He thought he saw some organisms, as we did too, and they were smaller even than the tularemia [organism].

I made an incidental finding of tularemia in a case at Maxwell Field. I would go there and see certain patients. I told the technician there, who had been trained in the Montgomery lab, that any time there was any patient that showed positive for tularemia, call me. Serologically it was an agglutination test for tularemia.

She called me one day, and there was this child that had been diagnosed as malaria and that had a high titer for tularemia. I called Major Little, who was head of the pediatric service, and asked what happened to the child. He said, "Oh, it was discharged on malaria therapy." I said, could they give me the address, and he did. So I went to the child's home. When I looked at the throat, there was an exudate on the tonsils. I had some media with me, and I took a swab specimen. I knew from the lab test she had had tularemia. I said, "Well, have you had any rabbits here?" "Oh, yes! We found some rabbits out at the cemetery, and we took them home." "What happened?" "They died." I said, "Well, I think she must have gotten tularemia from them. She's out of danger now, and she should be okay."

Johnson: I put the swab material into mice, and the mice died in three days from peritoneal inoculation. Bacterium tularense is a highly virulent organism.*

Hughes: How was tularemia treated in those days?

Johnson: We had no treatment for it. It was a dangerous disease and many of those that contracted tularemia from wild rabbits died of it. We had two cases of infection on the staff. I sent the organism to Dr. Horsfall at the Rockefeller Institute, because I couldn't see any bacteria, and it would kill mice in high dilution from intracutaneous or intramuscular inoculation. I saw in Giemsa stains some really fine particulate material, but it wasn't like psittacosis. Horsfall couldn't identify it, but he said it killed the guinea pigs, mice, and a monkey he injected. He said, "I wouldn't touch it. I destroyed it."

I had two technicians, and I didn't let them do any of the inoculations, but they did boil instruments that I'd used. They came down with pneumonia. We thought at first it was atypical pneumonia, and one of them, Rachel Gorrie, was very ill. The x-ray studies looked like atypical pneumonia. She later was one of the senior technicians for CDC in Atlanta. There was no specific treatment for atypical pneumonia. The other technician got over it very promptly.

In the meantime, we had done some studies in dogs and found out I could inject the agent under the skin and it would come out in the nasal secretions. We had some special tularemia media from the state health department, but I could not grow any organisms. I made some special tularemia media, using commercial Difco media powder, and then I grew the organism, and it was tularemia. So I published two papers on tularemia.** One was on the human case and the other on the dog disease.

Hughes: Did that work bring you into contact with K. F. Meyer?

* I had previously isolated B. tularense from a live ferret sent to me as a source of dog distemper virus. The spleen of this animal was saved as the source of the virus and I tested this in mice and it killed them by the ic [intracerebral] test after a short incubation period.

** H. N. Johnson. Isolation of B. tularense from the sputum of an atypical case of human tularemia. J. Lab. Clin. Med. 1944, 29:903-5. H. N. Johnson. Natural occurrence of tularemia in dogs used as a source of canine distemper virus. J. Lab. Clin. Med. 1944, 29:906-15.

Johnson: No. He was interested, but what I did was to write to Dr. Francis at NIH, and I got antigens from him. They were doing serological tests for tularemia at the Alabama State Health Department. I sent them the bloods from the human cases and, sure enough, they had a positive titer for tularemia.

Hughes: Was Meyer working on tularemia, too?

Johnson: He was interested. He knew Dr. Francis. Dr. Francis isolated B. tularensis from rabbits taken in Tulare County, California. I met Francis at his lab at NIH, and he was one of those great bacteriologists who made the study of tularemia his life work.

I made ward visits at Maxwell Field Air Base, and they had so many acute diseases, like chicken pox and mumps, anything you could think of. They'd bring in recruits, ten thousand at a time, and with a mix from all over the United States. They just got colds, colds, colds. The disease I was interested in, where they would keep coughing till they spit up blood and had terrible headaches, was what we now know as mycoplasma. It is still a problem. Mycoplasma are one of the common causes of pneumonia today.

Hughes: Were you interested in exactly what the infectious agent was?

Johnson: Yes, at that time, it was a new disease; something where you didn't have a bacteriological diagnosis.

Hughes: Were you convinced that it wasn't a virus?

Johnson: Well, at that time, we thought it was a virus because we couldn't cultivate any specific organism.

Hughes: And you couldn't see it.

Influenza Studies

Johnson: By the way, we were then doing cooperative studies of influenza A vaccination. Dr. Horsfall was making some experimental vaccines, and these were being tested at Kilby Prison where I was, and at some mental institutions. I vaccinated Pretty Boy Floyd's girlfriend at Wetumpka Prison at Wetumpka, Alabama, a prison for women. [laughter] We had programs of vaccinating prisoners and people in mental institutions that were under conditions where they needed to be immunized for flu, and we took bloods before and after vaccination. Dr. E. R. Rickard of the Rockefeller Foundation was doing field studies and I helped him.

Hughes: Was that part of the flu study that Dr. Lennette was involved in?

Johnson: Yes, in later years. Dr. Lennette had grants from the Armed Forces Epidemiological Board.* We didn't have any special grants because we had Rockefeller money, and I was able to go into and out of camps to see what they were doing.

But my primary interest was to see what I could do right there at Maxwell Field. We had these wonderful air force recruits. They didn't have a regular pathologist, so I was on call for doing autopsies, too. Didn't cost them anything. But I enjoyed my contacts there with an excellent staff of physicians.

Hughes: Well, you spoke of the diagnostic service that your laboratory provided. How big a radius were you pulling from?

Johnson: Well, this was mostly from state health departments, like they'd have some cases of lymphopathia venereum. I had psittacosis antigen from Lederle, and we could do a complement fixation test. That's a diagnostic test for psittacosis or anything suspected of being a chlamydial agent. We were interested in running tests like that; no one else was doing them.

Hughes: Did you know the agent of psittacosis was a chlamydia?

Johnson: No, but people were getting infected from pigeons. It was a pneumonia-like psittacosis and was pretty virulent. The psittacosis cases were from parrots or parakeets, usually from cleaning the cages.

Hughes: Is it true that you were more interested in treatment than in pinning down exactly what the infectious agent was?

Johnson: Yes, mostly [my work] was whatever were the current problems in [diagnosing] viruses. We included psittacosis and any [other diseases] where you needed special facilities for diagnosis.

Hughes: You weren't involved in the controversy over the exact nature of the agent of psittacosis?

Johnson: No. At that time the generic name was *Bedsoniae*.

Hughes: There was a debate about whether it was a true virus or a completely different type of infectious agent.

* See: Edwin H. Lennette, Pioneer of Diagnostic Virology with the California Department of Public Health, an oral history interview conducted in 1982, 1983, 1986 by Sally Smith Hughes, Regional Oral History Office, The Bancroft Library, University of California, Berkeley, 1988.

Johnson: Oh, we could see it; there was a stain for it. We could see the elementary bodies, which were called Levinthal-Coles bodies, with a special stain, which was a fuchsin-type stain. You could see tiny round bodies. We also were familiar with trachoma, and the best stain for that was Giemsa. When I first came to California in 1954, I used to do Giemsa stains on specimens sent over to the lab from San Francisco, because we could demonstrate these inclusion bodies.

Hughes: So you were thinking, because of the inclusion bodies, that it was a large virus?

Johnson: Well, it is a virus. It doesn't grow unless it gets into cells and can maintain itself then.

The whole field of chlamydiae now is so important; chlamydial infection is a bad venereal disease, and it's a bad pneumonia disease. I'd say in the last year, the majority of really severe human respiratory infections have been [caused by] chlamydiae. It's like mycoplasma--chronic and hard to treat. You hesitate to treat every case with erythromycin or tetracycline, unless they're more or less critically ill, because you may damage the intestinal flora and get colitis. The rest of the antibiotics are not as good. Penicillin is not effective against mycoplasma.

Hughes: The chlamydiae are classified separately among the viruses now.

Johnson: Oh, yes. This lab is called the Viral, Rickettsial, and Chlamydial Disease Laboratory. They are small agents classified as viruses because they grow only within cells.

Hughes: So when you said chlamydiae are viruses, you were using that as a diagnostic term?

Johnson: Because they tend to be intracellular in their growth. We have large viruses; we have rod-shaped, bacillary viruses; we have square, polyhedral viruses, and then round, tiny ones. The picornaviruses are so tiny that the only way you can see them is with electron microscopy. They go through any kind of filter.

Hughes: Was there any other disease that you were particularly interested in, in Montgomery?

Johnson: We were interested in polio. I was trying to isolate polio in small mammals at that time. The Armstrong strain does grow in mice. But the average type 2 strain would not paralyze white mice intracerebrally, but the occasional one would. So the problem was to find out, was it polio or was it rabies? We had no tissue culture system for polio in 1944. I was interested in whether any of the wild rodents would be susceptible to polio.

Johnson: We had a wild mammal study, in which we had nine different species of mice, including some very beautiful golden mice,* and *Microtus* and *Blarina* mice. So we had a variety of animals that we could use as test hosts. I did not isolate any polio virus from the brain specimens I tested by ic [intracerebral] inoculation of cotton rats.** Hilary Koprowski adapted type 2 polio to this animal.

Hughes: The IHD had been receptive to the expansion of your viral diagnostic work?

The Diagnostic Service

Johnson: There was no question. We had the setup to do isolation work on cases of encephalitis. We did a tremendous pathological study on an FBI man that died of encephalitis in Birmingham, and we tried to isolate the virus. We did not succeed. So any unusual brain infection that didn't have any known etiology, we would try to isolate the agent. This had been true of all the labs that I worked with, even in India. We had facilities to try to find out causes of death, where no one else could determine what the disease was.

Hughes: Did you have to increase the staff to cover this diagnostic service?

Johnson: No. A health department will notice something unusual, and it will call up and say, would you be able to test a specimen if we send it in, or would you be interested in the autopsy? This is usually the way one isolates new viruses, and always with the idea that the encephalitis case could be rabies. In fact, in most areas where they do now test for rabies, either by fluorescent antibody or by mouse inoculation, in certain countries in South America five percent of all the autopsies have been positive for rabies, without even suspecting that they had it. So having a diagnostic facility gives you an opportunity to look for new causes of disease.

Hughes: Did you expect to train technicians?

* *Peromyscus nuttalli*.

** *Sigmodon hispidis*.

Johnson: We trained the technicians at the Montgomery lab. We had people come there from all over the world. This I say: Anybody that wants to do virology will have to work somewhere where all the techniques and precautions are well worked out, before they try to work with viruses. Because the tendency is to mix up specimens and keep poor records, and that's when they get cross contamination and publish reports which turn out to be wrong.

People would come for a month or more. A doctor, Robert Navarrez, came from Ecuador to work in the lab for one month, and others came from various places in Central and South America.

Hughes: To learn the techniques?

Johnson: Yes, to learn the techniques, whether it was a serological test or a vaccine potency test.

When I was working in India, Dr. Koprowski, Dr. [Karl] Habel, and I of the WHO Expert Committee on Rabies demonstrated rabies technology to medical and veterinary doctors from sixty-four laboratories in Asia and the Far East: How to do the autopsies, collect the specimens, look for Negri bodies, inoculate the mice, and then do a vaccine potency test and the neutralization test. Everybody had to practice the tests. If you do tests once under supervision, then you can do them right. We used facilities at the Coonoor Pasteur Institute.

I had learned virus techniques at the Rockefeller Institute. You needed to see them and learn them yourself. I trained technicians who had been working at the [Alabama] state health department, like Rachel Gorrie. They had been inoculating rabbits for the vaccine, or something like that. We used more modern methods--better staining methods and pathology. You could train somebody for a specific type of virology or pathology. We picked the best technicians.

Hughes: Did you end up modifying Leach's procedures?

Johnson: Yes. All they had [when I arrived] was the intracerebral inoculation of rabbits, and he was beginning the ic diagnostic mouse test. It became very obvious that when you used large doses of dog brain from a naturally infected dog [with rabies], it was not going to be a very good challenge for inoculation, because if you give a large dose intramuscularly, you didn't get a high mortality like you would expect. In fact, you'd have a fifty percent mortality, because you were vaccinating with a natural virus at the same time you were trying to infect.

In South America, they've done that type of vaccination using cattle that were dying of rabies. They'd make up a vaccine from the brains of paralyzed animals and inject it into normal animals

Johnson: subcutaneously. The brain suspension was treated with 0.5 percent phenol and then incubated at refrigerator temperature. So you could make a vaccine right out of the natural virus and few of the vaccinated cattle died of rabies. It is difficult to infect animals with rabies by subcutaneous injections.

Hughes: What became of the lab after you left in 1944?

Johnson: Well, my interest at that time was that there were no viral diagnostic labs as such in health departments, and I thought this [facility] could be used as a regional laboratory for rabies research and viral diagnosis.

Hughes: Anywhere in the country?

Johnson: In the South. Some health departments had labs for the specific purpose of making rabies vaccine. At that time [rabies diagnosis] was just taking the brain and looking for Negri bodies. In fact, hardly anywhere in the United States were they doing any isolation in mice of rabies virus. The first really done on any scale was in the rabies study in Montgomery, and that was published.* We showed that in any dog that had rabies you could be certain that if the specimen was obtained in any decent way, that you could diagnose rabies in the mouse test a hundred percent. With the Negri bodies test, you could diagnose about ninety percent of the dogs. But in ten percent, the Negri bodies had not developed. There would be very hot strains of rabies in nature, which, if you subpassed them in dogs, they did not produce Negri bodies in the dog brains. Subpassage in mice it would produce Negri bodies.

There are wildlife strains of rabies virus--in skunks, for example--which in both the natural host and in mice inoculated with the skunk's virus are difficult to diagnose by the Negri body test. The FA [fluorescent antibody] test has been most valuable for such strains.

Hughes: What happened to the lab after you left?

Johnson: I wrote to the public health service in Atlanta and suggested that somebody take over the lab to continue the dog studies. Dr. Vernon Link and A. M. Miller, administrative officers, came over to see the lab. They had a communicable disease service center at

* H. N. Johnson, C. N. Leach. Comparative susceptibility of different strains of mice to rabies virus. Amer. J. Hyg. 1940, 32:38-45. H. N. Johnson. The significance of the Negri body in the diagnosis and epidemiology of rabies. Illinois Med. J. 1942, 81:383-388.

Johnson: that time, but no lab facility like ours. The upshot was that the CDC, Communicable Disease Center, took over that laboratory, and it became the CDC virus lab until 1960.

Hughes: Does the lab continue today?

Johnson: They developed a new facility for animals on the outskirts of Atlanta, so now the Montgomery laboratory that we developed originally is not used for any dog work, but it became the DHEW [Department of Health, Education and Welfare], PHS [Public Health Service], Southeastern Radiological Health Laboratory.

The LEP Flury vaccine was first tested in Staten Island, New York, in a special study, and then it was tested in Israel, which had good veterinary services. It was tested in Rhodesia and Malaya under WHO [World Health Organization] auspices. It was done so you could have control of whether any cases of vaccine paralysis or deaths resulted from the vaccine. It was very successful. The LEP Flury vaccine is still used in many countries.

Director, Field Expedition to Study Vampire Bat Rabies in Mexico, 1944

The Agricultural Development Program

Hughes: In 1943, the Natural Sciences Division of the Rockefeller Foundation initiated an agricultural development program for Mexico. The work was centered on plant science and domestic animal production?

Johnson: Mostly plant genetics. They were trying to develop superior varieties of wheat and corn, particularly corn. It was first called the maize research program. By the time I came down to Mexico in '44, they had already developed quantities of seed strains. When I was there later in 1963, they had large refrigerated rooms with samples of ears of corn and seeds in sealed tin cans, which would keep indefinitely, all at four degrees centigrade. They had all the data on the character of these strains. There were many varieties of corn in Mexico because a lot of them were selected because of the colors of the kernels.

Johnson: Our goal was to develop high performance seed strains of corn and wheat. Dr. George Harrar, Dr. Norman Borlaug,* and Dr. [Edwin John] Wellhausen were mostly interested in wheat and corn, but Wellhausen was also looking at what they could do to improve bean and potato yield. The thing that immediately caught their eye was the corn that was being grown at sea level and at, say, eight or nine thousand feet. Would one strain grow in one place and not in the other? It was very obvious that certain strains had naturally developed qualities which made them grow better at certain altitudes.

The main focus was diseases [such as] rust in wheat. They were growing rust-resistant strains of wheat. And then much later came research on viruses. There were viruses of corn and rice that were a real problem. They would cause streaking and yield problems.

Hughes: Where was the center for this research?

Johnson: It was very near Mexico City, at Chapingo, and still is. It's a large research facility, international now. It's supported by funds from many other countries. A rice research institute is in the Philippines, and tropical agriculture institutes are in Hyderabad, India and Ibadan, Africa, and Cali, Colombia.

Hughes: Was the staff a combination of Mexicans and Rockefeller people?

Johnson: Yes. By the time that I was there in 1944, there was a Mexican staff already involved in the program at the corn and wheat center. The department of agriculture was very interested.

Carl TenBroeck's Study, 1943

Johnson: The reason for getting into viruses was because of a disease in cattle, which was suddenly a big problem. [Mexico was] losing literally thousands of cattle to this new disease, derriengue. Dr. Harrar was head of the Rockefeller Foundation lab in Mexico City. He consulted Dr. TenBroeck, who was director of the Rockefeller Institute for Plant and Animal Studies at Princeton, New Jersey. TenBroeck went down to Mexico in 1943 and came back

* Later, Dr. Harrar became president of the Rockefeller Foundation, and Dr. Borlaug of "the Green Revolution" won a Nobel Prize for his genetic studies of wheat and corn. [note by Dr. Johnson]

Johnson: with a cow brain specimen, which he suspected was rabies, and he sent it to me. We identified it as rabies but not like epizootic canine rabies. One dog inoculated ic with the derriengue virus developed a paralytic disease and recovered. The others did not become ill.

TenBroeck went to Mexico in 1943 just to look at the situation. He went on one field trip with Dr. [A. Tellez] Giron, and then they got this cow brain specimen. That was in August, '43. When he came back, he wrote to me, and I later visited his lab, and the decision was he wanted me to work with him to see if I could pin down whether the cow virus was rabies. Dr. Tellez Giron said the disease was not rabies and there was no known association with dog rabies. Giron was making a derriengue strain vaccine, derived from a cow. They did not know about any vampire bats.

So then the Natural Science Division of the Rockefeller Foundation asked if I would be willing to go down as a virologist and see what I could do about controlling this disease. They were using a vaccine, but the disease would move around from place to place, and it seemed as soon as they started vaccinating, the disease would be somewhere else.

Hughes: Did TenBroeck have an inkling that vampire bats were involved?

Johnson: No, he was not familiar with the paralytic rabies in Brazil. It was relatively new then. At all events, he did not know there were vampire bats in Mexico. The earliest knowledge of vampire bat rabies, where it was really tied into rabies, started about 1937-38 in Trinidad. So it was going on at the time that we started our work in 1937-38 in Alabama. But the disease in Trinidad had not yet been associated with vampire bats. They had no knowledge of any host for derriengue, and they felt it was not rabies because there was no active dog or coyote rabies associated with it.

Field Equipment and Team

Hughes: Tell me about setting up to go on the expedition.

Johnson: You have to think of what clothes you're going to bring and what special equipment to take specimens. We used wide-mouthed thermos jars of quart or two-quart size, and you'd have a big cork at the top, and you'd keep things refrigerated with a small amount of ice for a week.

Johnson: We also had to consider what we were going to use to keep the ice water out of the specimens. At the lab we had rubber sleeve-capped serum vials. They were about 2 cc to 5 cc type. You'd push in this stopper, and then you could pull the sleeve over the top of the ampoule, and then if you wanted to take out fluid like blood, you could just stick a needle through the sterile top and take out some after disinfecting the cap with iodine alcohol. For rabies, we had already found out that glycerol was a wonderful preservative. It just didn't have any killing power on the virus but inhibited bacteria. You could take a specimen of human or dog brain and put it in fifty percent glycerol and saline. You could leave that at room temperature and as long as it was not exposed to heat or sun the virus would be active for months.

So I thought the best way to save these specimens--to keep them clean so that they didn't get contaminated with bacteria--was to bring fifty of these stoppered vials half full of glycerol saline. I had to have small curved scissors, forceps, and pans to boil the material. Heavier equipment we thought we could get there, like a tool to open up the heads of animals. Usually a sharp machete or something like that would work. But we had to have good knives and heavy forceps, which I had to take with me.

We finally chose a kind of gasoline torch to boil water and sterilize instruments. Primus stoves are very difficult to handle; they choke up with carbon. I found that out previously. I prefer a good acetyline torch for sealing glass ampoules. We did not need that in Mexico.

I took some Sella's stain with me, so I could stain and look for Negri bodies, and then I had chemicals to buffer the water so it wouldn't be too alkaline. [I took] slides, so I could make slide preparations, as we were ready to do autopsies. Then I always carried whatever I needed to protect myself. I knew there were a lot of ticks on the ranches, so I had some of the earliest DDT powder with me, and the sulfa drugs, at that time sulfadiazine. That was the only medication that I took, other than some aspirin. Sulfas were the first really good antibacterial we had.

Having gotten my field kit together in Montgomery, [the next question was] were there any vampire bats in Mexico? I made a special trip from Montgomery to New York City to visit the staff at the Rockefeller Institute for advice on equipment and to the Museum of Natural History to inquire about vampire bats.

Hughes: Why did you have the idea that vampire bats carried rabies?

Johnson: Because I knew that they had identified rabies in vampire bats in Trinidad in 1938. A veterinarian from Brazil named Torres had visited our lab recently. He had worked in Santa Catarina, Brazil, during an outbreak of rabies in which they diagnosed

Johnson: rabies in vampire bats. But that work was very little known; it was published only in some agricultural department journals in Brazil. We had been in correspondence with him. We had Rockefeller people working in South America on yellow fever, and they told us about the new developments in rabies there.

So I went to the Museum of Natural History and looked up whatever records they had of world distribution of bats. I found out that in 1846 the vampire bat type specimen had been sent by the German embassy from Mexico City to Berlin. This was collected in Mexico, and it was the first Desmondus rotundis murinis to be preserved there. I don't know where they derived the name murinis--it looks like a mouse, I guess. Rotundis is because when fed they are rounded, chunky, like a bag that's full of blood.

Hughes: [pointing to preserved specimen] Is that a vampire bat?

Johnson: Yes. They're bigger, of course, when they haven't been fixed so long.

So I found out that there were vampire bats in Mexico. Then I said, "Do you have any bats from Mexico in your collection?" We pulled out some specimen drawers, and there were vampire bats from the state of Jalisco. They had been collected in 1907, and the man that collected them was Dr. Batty. [laughs] Beautiful specimens. So I had a good description; I knew what they looked like. They are not a big bat. There are bats that are much more ferocious looking, like the large-toothed bats that eat fruit in Central America. Then there are flying foxes, Pteropus giganticus, which I got very familiar with in India later.

Hughes: Was there also information about the bats' habitat?

Johnson: At that time, there were just collection tags, saying collected at a certain place at a certain date. In South America, they knew a lot of them lived in hollow trees. I expected more of that habitat than caves. But we really didn't know where to look when we first went down there.

We'd written to the lab in Mexico City, saying would they please get records of wherever the disease was present. We decided we would only go to areas where they had reported deaths of cattle with derriengue within six weeks.

Hughes: How was the reporting system set up?

Johnson: They had district veterinarians in each state in Mexico.

Hughes: And the farmer would report to them?

Johnson: When animals died, the district veterinarians would usually hear about them. Particularly on the scale that derriengue was occurring, they would know. They would also vaccinate cattle. They had developed a vaccine which seemed to be efficacious. So the plan was that we would set up an expedition to go to an area where there had been recent cases.

Hughes: Who was on the expedition?

Johnson: Well, on the first trip, Dr. Tellez Giron. He was a veterinary virologist at the Instituto Pecuario.

Hughes: What does that mean?

Johnson: That's just the name they have for the animal facility in the department of agriculture in Mexico City. They have the oldest veterinary medical school in the New World.

The major diseases in cattle at that time, except for the usual things like pneumonia, were anthrax, brucellosis, bovine tuberculosis, and piroplasmiasis. They had parasitologists and bacteriologists [at the institute], and they were making vaccines for hog cholera and rabies. They used Spanish textbooks; they patterned all their teaching to the Spanish veterinary schools, which were old institutions. Giron was a very well-trained veterinarian, knowing veterinary pathology and transmission of viruses to guinea pigs.

Hughes: How about his virological techniques?

Johnson: Well, very minimal. He had done no work in serology. Negri body examination was the major thing.

They had a very good laboratory at the Institute of Hygiene in the Distrito Federal. I visited the lab where they made the human rabies vaccine, and they did the human rabies diagnosis there. They had their own stain at that time, which I didn't think was as good as the one we used, which was Seller's stain. It's a methylene blue and basic fuchsin stain in absolute methanol. It stains and fixes at the same time. They decided to use that afterwards as a diagnostic stain for the dog and human brains. Now we have the fluorescent rabies antibody test, the more accurate method of diagnosis.

The plan of the expedition was for Dr. Tellez Giron to furnish somebody from the veterinary school [Institute of Animal Pathology] that could be an assistant, and that was [A. Ladron de Guevera. He was a recently graduated veterinary medical student. He was our chauffeur for the Distrito Federal Ford truck. On much

Johnson: of the ranching region along the Pacific coast, the appearance of a Federal Government vehicle brought out some armed men. The government army stayed away from this area.

Hughes: How was your Spanish?

Johnson: Well, it was very minimal, but I had had French, and of course I grew up in a bilingual family—Swedish—and I resolved I was going to learn Spanish fast. Dr. Giron spoke English, which was very good for us, because Guevera did not.

From that time on, on each trip there was practically nothing but Spanish spoken, and I learned fast. I say, if anyone wants to learn how to hablar espanol, you do it in the field! I couldn't believe how many words would come into play rapidly once you used nothing else. None of the local people spoke English. Some of the inland Indian tribes did not even speak Spanish.

Hughes: You were going out for days at a time, so were you camping?

Johnson: No, we would stay at the local hotel or inn. In the smaller towns, these are adobe brick buildings with a dirt floor and a central open patio. The bed would be rope ties to support the bedroll, and that's all. The big problem I had with them was the kissing bugs, Triatoma, that would come out at night. I couldn't believe the size of the mouth part. If they'd bite you, you'd get a lump on your arm the size of an egg. They look like the box elder bug we had in Nebraska. The biting part is folded down along the front of the thorax.

Hughes: How many trips did you make?

Johnson: There were two major expeditions.

Hughes: Didn't you look at seven different sites?

Johnson: More than that. We spent a week and more in planning the trip.

The First Field Trip

Johnson: The first field trip was with Dr. Guevera, Dr. Giron, and myself, and we were going to go to Morelis in Michoacan. The man we were supposed to meet there was Dr. [Ruiz] Polo, who was the district veterinarian. We arrived there the fourth of April, and the first field trip was from there, looking for areas where there had been cases. Polo had contacted people at Patzcuaro.

Johnson: One of the interesting things about Morelia, which I look back on with real interest now, is a legend that Hui Shan, a white man, came from a country far away and changed the habits of the people and then went back. There is a tradition that a Chinese monk came from Kabul, Afganistan by way of China and was in Mexico in the 490s. He introduced cultural techniques, like the way they raised bamboo. The Indians had long moustaches, and the local Mexicans looked like Oriental people. The Indians who lived there on the lake used boats made of bamboo and they used bamboo for making houses. It looked like an old Chinese scroll painting.

The story was that this culture had been carried on since this person had come long ago to teach them a new way of life. And that was why Cortes was welcome when he came, because the monk said he'd come back. This is a well-known historical legend. It's in a Chinese encyclopedia which told that this monk went to a land called Fusang three thousand miles away and came back. Nehru mentions this story in his history of India.

From Morelia, we went to a place called Uruapan. There's where the new volcano Paricutin was erupting, and it had just come out of the ground in 1943. It was a spectacular volcano. It was visible from eighty miles away. We were near. It would go off about every three minutes, and it would belch up this cloud for miles, which would then drift. The drifting at that time was away from us. It was only later we found that for up to fifty, sixty, seventy miles there was colored sand on the highways in the morning.

We couldn't find any place to stay in Uruapan because there was a festival. Often, there would be some kind of a hotel where you could at least get one room for all of us. And then you could always eat at some little eating place where they served very simple foods. I can go far on tortillas and beans.

A local engineer named Murphy said he was going to help us get pyrite cord and dynamite for killing the bats in caves. We learned that was not a good idea if we wanted to go in and collect bats afterward. Nitroglycerin causes a good headache. They had a mine engineering place there. We were supposed to visit and see what Murphy thought about the use of dynamite. He said, "Well, you can't stay here tonight. Let's go see the volcano!" And so, after we'd eaten that night, we got in our truck and went up near this little town of San Joaquin. The Catholic church there had sand up to the eaves.

Hughes: From the volcano?

Johnson: Yes. Sand twenty feet deep. Then he said, "We can get some burros! Horses are no good! We can get up near the volcano." So we got on these burros, and they were comfortable riding, and off

Johnson: we went. There was light every three minutes from these brilliant explosions; it was enough light to see what we were doing.

So we went fairly close on the windward side; the sand was drifting away from where we were. The volcano had already gotten up to two thousand some feet. There were ridges forming by a buckling of the ground underneath. The lava would push up, and here and there would be steam and gases bursting out the side. You could go up near these and see sulfur crystals forming.

We proceeded along one of these ridges, which we were told was perfectly safe. We were close enough so that marble-sized pieces of round lava would come down every once in a while. I had a pith helmet, and it would beat down like rain from time to time. It was the most spectacular thing. I have a picture of it in Scituate [Massachusetts], taken by one of my friends* at another time. The major blocks of red-hot lava would go up in masses as big as houses and fall back in. Others would go over the side and roll down.

I used to argue with geologists: These round stones that they said were made by people in old times in Mexico--when I saw this, I realized that as lava rolled down these slopes, it would become just like a ball bearing, perfectly round. At a couple of villages, the walks were lined with these stones that looked like croquet balls, and some bigger. So, for the first time, I realized that some of those great stones that they'd carve faces in were really round balls of lava.

Hughes: Is that idea now credited?

Johnson: I doubt it. The books still have it the other way.

I was so amazed to see the secondary explosions. There was the big explosion, and then you would see flashes of pink. They were gas explosions--methane, ethane, and hydrogen. And these were so spectacular--and thunder and lightning. That's been well authenticated now. These are secondary electrical explosions. The sand made static electricity. As we drove the truck where the sand was coming down, we would get shocks from static electricity on the handles of the truck.

Hughes: There had been no previous volcanic activity in the state?

* Dr. Paul Mangelsdorf, a geneticist at Harvard and consultant to the Rockefeller Foundation Mexico program. [note by Dr. Johnson]

Johnson: There are two old volcanoes—Iztacihuatl and Popocatepetl. They're right near Mexico City. Popo was particularly active in sending gas smoke and steam. But there were no other active volcanoes anything like Paricutin. In the eight or nine hundreds, at the time of the Aztec power, Mexico may have had hundreds of active volcanoes. We were in Michoacan and down through Jalisco, and there was all sorts of evidence of volcanic craters of all sorts, like we have in California in Shasta County. I've been interested in volcanoes ever since, and all around the world I've studied them, especially the ones in Hawaii.

[tape interruption]

Well, the following morning after we were on the volcano, we left Uruapan for Apatzingan. There was a story of activity of this disease, derriengue, in La Estancia. They said that the disease had been present for a couple of years. Most of the people living there were Indians. Each Indian family had about seventy-five to one hundred cattle. Dr. Ruiz Polo had been notified by a local schoolteacher that there was this disease in the area. So Polo was along with us. But the man that he was going to see—with the typical political upheaval of the time—had been in a political argument and had been shot and killed the day before we arrived. Everybody there usually carried pistols on either side, and the village "presidentes" always had their "pistoles." They had taken over the American oil wells that year.

Three of the Indian rancheros came with us in the truck to a place called Los Bancos. This is an area which had caves; there were limestone ledges, and it was hard to get in these. It really was a tremendous surprise to get in that first major cave. We saw some droppings of liquid blood near the entrance, and there they were. It was my first view of a vampire bat alive. On the rocks there was a strange odor which I realized was probably from putrefying blood.

As we went into the cave, there were some crevices, and there's where we saw one scamper. They'd run almost like rats; they'd run on their thumbs, and they'd scamper around. This chamber was about twenty-four feet by forty feet. The floor was covered with big rocks that had fallen down, and we had flashlights with us, and the bats would fly by. I had a stick in my hand, and Guevera was trying to shoot one, and I said, "Look out, those big rocks will fall!" It didn't happen then, but later on I was almost killed by a rock. Guevera shot two bats with the 410 shotgun, and I got one with a stick. Three flew out of the cave; they fly like a swallow.

Hughes: But they didn't fly in the cave?

- Johnson: Oh, yes, they would fly fast. They'd go around just as fast as a swallow, right inside the cave, and we could see them with a flashlight.
- Hughes: You weren't afraid of being attacked?
- Johnson: I really wasn't, because I had my gloves on. Looking around on the floor, I found there were some dead, dried-up bats. Same species. And there were some that hadn't been dead very long, but we weren't going to bother with those. We got three, and they were all females. One had a young baby hanging on to it. So this was our first collection, and right then we knew we were in business.
- Hughes: You mean, three in addition to the dead bats?
- Johnson: We didn't bother with the ones on the floor. That young one died very shortly afterwards; we decided we wouldn't bother with it as a specimen. We wanted to keep the others alive if we could work on them. The two bats that were shot survived as well as the one I captured. They were processed later.
- Hughes: How did you feed them?
- Johnson: We knew that you could go to an abbatoir and get blood. I'd heard that you could shake the blood and defibrinate it so it would be liquid. Then I put it into a dish to see if they would feed on it, and they did. The locals made marbles of clay to play with, and we'd take a half-dozen marbles and shake the blood with them, and then it would stay liquid. One bat would drink a saucerful of blood. We processed three vampire bats. The immature bat was not tested.
- Hughes: Please describe the nets.
- Johnson: They were fish nets, but they were not the kind that bats could crawl through. We got the nets in Mexico that I used the most. One of the common foods in Mexico, minnow-sized fish, is collected from lakes or water of any kind. I'd say they were about two inches long. Rather than eat potato chips, they would fry these fish in oil, and they were just dandy. Salt them and eat the whole thing, head and all. I also had mosquito nets.
- Hughes: The Japanese also eat small fish, bones and all.
- Johnson: It's all over the world; you see it in Greece; you see it in India, and it's good! It's calcium and a wonderful source of protein.

Johnson: They use a net about ten feet in diameter with lead slugs along the edge. You throw the net out over a school of minnows, the net edges sink down to the bottom, and then you bring the edges together. It's about the only way you can get those small fish. Later I watched this in India on the Mutha Mula river near our bungalow.

Well, for bats, we bought as large a piece of netting as we could get. Then we would attach that in any way that we could to the sides of the entrance of the caves. We also used it to close the entrance to colonies of vampire bats in trees.

One net that I brought down from the United States was finer, and that worked very well in a lot of caves. It was a very loose-woven material. The bats would fly into it.

Hughes: The bats would cling to the net?

Johnson: Yes. As they hit the net, they would grab for it, then you could grab them.

Hughes: With a glove?

Johnson: Yes. And we had cloth sacks with us.

At Apatzingan there is an enormous bat cave with an estimated population of several hundred thousand bats. The caves in that region may hold millions of bats during a certain part of the year. These are almost all Tadarida brasiliensis mexicana insectivorous bats—Mexican freetail bats. And then a variety of other insectivorous bats, and, in certain parts of Mexico, fruit-eating bats, but mostly insectivorous and pollen-eating bats. There are fish-eating bats in caves along the sea that eat minnows. Those caves are noted for the guano, which is harvested and sold. It's a wonderful fertilizer, same with bird guano from Peru.

But the trouble with this big cave at Apatzingan was that something like fourteen people that had worked there that one year had all gotten some kind of pneumonia and died. So they weren't going in. We realize now it was probably histoplasmosis from the bat guano. That was one reason I didn't want to go into that cave! The flight of those bats out of the cave would go on for hours, and it would take them many hours to fly back in. So about half the time, there would be bats flying either in or out to feed.

Hughes: Cattle were their main source of blood?

Johnson: No, those were insectivorous bats. As soon as I knew they weren't vampires, I knew I was not going to bother with them. I wanted vampires.

Johnson: We processed the three bats kept alive when we got back to where we were staying at Apatzingan. I took out the brains and salivary glands, duplicate specimens for Dr. Giron and me. I'd take one salivary gland from one side for me and the other side for him, and we would pool from several bats, because we knew we wouldn't have enough vials. We took part of the brain specimen for one set, a part for the other set. We would also pool brain specimens, because the glycerol would take up the liquid, and it would be just the tissue that we would be testing.

Apatzingan was interesting medically because they had a disease there called pinta. Pinta is characterized by a violaceous butterflylike, purple-red area on the cheeks or arms caused by a spirochete, just like the one [which causes] Lyme disease and syphilis. It was a very well-known disease, and apparently was transmitted by little black flies, buffalo gnats or Simuliidae. I really was bothered by them. I had fly repellent on me, and they would bite right through the stuff. They'd draw blood right away; they were a very nasty fly. I didn't get pinta, but the fear of transmission was there.

On the 9th of April, we went to a place called Chila. That's a very interesting area, because there was an ancient culture there with a round pyramid, partly covered by a lava flow. We found some very strange figures there that looked almost like Mussolini heads. We brought some to the museum in Mexico City. We found a clay pot evidently used for keeping hot coals of charcoal to light fires. It was evidently ancient and it also was taken to the museum.

We forded the Rio Grande de Tepalcatepec. It is one of those great, wide rivers, like some in the central United States. We would take off the fan belt, grease the spark plugs and distributor, put the truck in low, and keep moving steadily but slowly.

Hughes: And the chauffeur was used to doing this?

Johnson: Well, yes. We'd take turns driving. If we got in trouble, we'd have to get out and push, sometimes up to your waist in water.

Hughes: What about gasoline?

Johnson: We had gasoline in two fifty-gallon drums because most stations were closed, and the government had taken over the refineries. We had to conserve gas. Each time we'd leave Guadalajara or Mexico City, it had to last for a round trip.

I told you I had these little containers and a thermos for the specimens, but we also had to buy ice. We had a large wooden box made that we kept in the back of the truck, and it was half

Johnson: full of sawdust. Then we would get a hundred-pound block of ice, three feet by two feet or something like that, and we covered that with sawdust, and that would keep for ten days. Then we could take from that to refill our thermos. That's how we were able to keep our specimens refrigerated.

We processed our specimens at Apatzingan and headed for a ranch called Rancho Senor Jose de Chila, where we had heard that there was a die-off of derriengue. They raised chili and there is a little joke about the ranch. They'd say, "Pura chili con agua lejos." It means, pure chili with water far away. During the wet season they would have water in their wells, and in the dry season they'd have to go to a spring about two kilometers away with burros and bring the water back. So that was the reason for the joke.

It was a 30,000-acre ranch, and they had vaqueros there with these beautiful reatas [ropes]. I grew up on a ranch, and I appreciated horses and roping. A large number of people lived on the ranch, and they had these big Orlando cattle. The military had no control over that part of Mexico, so each town had its presidente and his forces. The ranchers had to get together if there was trouble. There were bandits in the area, I'll tell you, because I got involved with them. They helped the ranchers in any fights with the government.

So we went to this Rancho Senor Jose de Chila, and then the next day, April 10, 1944, we headed for Cerro de Borrego--the hill of the goat--and there were some really big caves. Before long, we found one where we were sure there were vampires because of this bloody liquid, the excreta, in the opening.

We'd go as far as we could by horse, and then we'd have to go on foot. It was a three-hour climb to get up to these caves. We collected eleven vampires here. We always had ropes--that's another thing I had to supply, ropes for scaling and getting in and out.

Hughes: You realized this in advance?

Johnson: Oh, yes.

Hughes: What about drinking water?

Johnson: Well, that was my nemesis. I brought a can of fruit juice. But then I had this torch, and I boiled water and put it in my canteen, so I always had boiled water. We'd leave about six in the morning. About ten o'clock I'd want a drink of water, so I'd pull out my canteen. I'd say, "Gusta agua?" Tellez and the local rancher would say, "Oh! Mucho gusto." Then they'd drink some.

Johnson: and I'd have just a swallow left, and that was the end of the water. After another three or four hours, I'd open a can of juice, and it would be the same story.

All day by horse and your tongue would be black with dust. We'd meet these cowboys and ranchers who'd say, "S-yor"--they wouldn't say "Senor;" they could hardly get it out. They'd have a kerchief over their face to keep some of the dust out of their nose, and we would too. But your eyes would get full of dust, and your tongue would get covered with it too.

The only time I ever drank water that wasn't boiled was when they said, "Oh, there's good water." We'd been riding from six in the morning till about five in the afternoon. All of a sudden here was this beautiful stream. They were right off their horses and lay down and put their heads into the water to get out the dirt and then drank and drank. So what did I do? The same thing. I said, "Where are we going now?" They said, "Oh, we'll go up along the stream here. There's a village up there." So we got up there, and the villagers were washing their clothes in the stream and using it for a latrine. I got amoebic dysentery, Giardia, and bacillary dysentery. I was so sick, and it lasted almost until I got paralyzed five months later.

Hughes: Did those diseases actually hit while you were on the trip?

Johnson: Yes. In the field, we all from time to time had bacillary dysentery. We'd get a fever and be as sick as we could be. They call it turista or Montezuma's revenge, mostly bacterial toxin food poisoning. A lot of the time we would actually be living in Indian villages, either sleeping in the truck or some kind of adobe building with a mud floor. That's where we got bitten by Triatoma kissing bugs and got ticks on us. I'd have thousands of ticks on me, but the DDT usually killed all of them.

On April 11, we headed out for Aguililla. There was a village not very far from there called Coalcoman, where they'd had a terrible earthquake in 1938. We saw some of the ruins. We knew that there had been cases of derriengue at Aguililla, and they'd also had a bad earthquake that previous year, which may have been related to Paricutin erupting the same year. It was an area of corn cultivation and Mexican Orlando cattle.

We did some secondary field trips out of Aguililla, always of course on horses. We couldn't find the cow they said had been paralyzed. But the following day we found four large caves, and we went northeast through Arroyo de Rosalana. It was a beautiful arroyo, steep cliffs on either side. I had borrowed Dr. Harrar's boots that laced up to my knees, and we had also chaps, because as you're riding along on these Mexican horses, they'd bump you

Johnson: against the rocks, and they would cut you badly. It was a narrow trail along cliff faces. Those mountain horses were no trouble for me.

We finally got to the Cuevas los Monos, and there we explored these four caves. One of them was a particularly interesting one, and one of them had vampires. We did get vampires out of that. One great big cave must have been inhabited long ago. It had a pit almost like a well, that you had to get over by rope and logs to the other side, because the walls were straight, about ten, fifteen feet [high]. (Six vampires collected here.)

Hughes: Was the pit marmade?

Johnson: Well, no, I think it was a natural sinkhole. On the other side of the pit, there was a place just like a room with ledges around it. We found some objects that we brought back to Mexico City, which looked like ancient stone tools and pottery. I don't know why they call it Cuevas los Monos. I suppose there were monkeys there sometime previously. So that was one of our collecting sites.

The next day I said, "Here's this wonderful arroyo going down towards the Pacific. Let's take a trip down the arroyo and see what we can find." So we started out along horse trails running along the rocky ledges, and the ledge might be only three feet wide in places. They're natural ledge formations along the streams. We were going along, and there were some skeletons of cattle along the side. There was very little water in this stream; just here and there was a pool. And there was this Indian [Andres Chipres] who had a few words of Spanish. He'd made a pit in the stream bed and lined it with leaves, and here was this clear water. Of course, I wasn't going to drink it! This Indian immediately understood what we were talking about, this disease, derriengue. He said he had had to kill many cattle. He would dry the meat. They cut the meat in thin strips and hang in over the acacia trees to dry in the sun. That's a wonderful way to dry meat.

The Indian said that there was one cow that was paralyzed. He said, "Want to see it?" We went, and here was this cow. The eyes looked pretty good, but it was completely paralyzed. It couldn't get up. I said, "Well, would you mind killing it? We'll bleed it out, and I want some specimens." He was probably going to use it anyway, so he bled it out. I took out salivary glands and brain and took specimens for Negri bodies.

He had a young son about fourteen years old. Their home was four poles, about three, four inches in diameter, with a grass roof. It had a clay oven, and the sides were open at that time of year, because they only closed it up during the wet season. I didn't see anybody else; it was just he and his son.

Johnson: To my horror, I couldn't make him understand, when I had taken out a specimen of the brain, that it was dangerous--peligro. He took the rest of the brain with his hands and put it in a clay jar and put it on the stove! Bare-handed. What can you do?

He told us about a cave nearby, and there were some very interesting bats. We caught a couple, and one of them had a tongue that was amazingly long. It was a pollen-eating bat. Then there were insectivorous bats. He said there was another cave, Cueva Prieta, up in La Portales, way up high in the mountains. He said that it was a fearful places. "Muy peligroso." We dragged our horses up there with their packs on, and tied them to a branch near this cave. The opening of the cave went down at about a forty degree angle, and I got a rope and just let myself down. As soon as I got down halfway, the rocks were all slippery with this blood from bats.

Hughes: Don't the vampire bats live in the same caves with the other types of bats?

Johnson: They're apt to be solitary; they're not large colonies. Colonies seem to be of five to thirty.

We put up the net. Tellez Giron was with me, and I gave him my left-hand glove, and I kept the right-hand glove and we were going to grab them out of the net and stick them in these cloth bags we had. It was dusk at that time; we had to use our flashlights. As I was sticking one in the bag, it bit me on my left index finger. I didn't think anything of it until I got sick five months later. I probably sucked my finger and spit out the blood. As I remember, I was not concerned.

That night I think we got eight live bats, and then we tied the sacks to the pommels of the saddle and went back. That was the night that Tellez Giron taught me all sorts of Spanish songs. You learn everything about somebody when you travel like that, and we became very close friends. We rode for hours and there was no moon, only starlight. You could hardly see, and I had my flashlight. It was scary, but the horses went right along.

We got back to Aguililla. I imagine it was five hours by horse at night, after we got the bats. And then we had to process the bats. I remember the sun coming up when I finished. That's field work.

The next morning, we visited a local butcher, and we got some blood. He said that there had been some cattle that had paralysis, so we bled them. I had some vacuum venules, and we would bleed some of the cattle. We saw some classy roping of cattle, which I had learned to do but I didn't try there. They

Johnson: had wonderful vaqueros. They'd rope a back leg and a horn and throw a cow down fast, and they'd be on it, and I'd just bleed from the jugular, very well controlled.

On the way back we stopped at the same cave at La Estancia where we got the first vampires. To our surprise, there were still a few vampires around, and we were able to get three more. Tellez wanted to take them back alive. I bled some cows there, too. We went back to Mexico City, and then we processed the specimens. So that was the first trip.

We went back by way of Morelia, and we got to Mexico City on the 16th of April [1944]. We inoculated mice and guinea pigs; [the lab] had some white laboratory mice by that time. We tested one-half of the material. I didn't know until I got back from the next trip that two groups of mice had sickened [with derriengue], which was quite a thrill. These were from both brain and salivary pools of one group of vampires from Cueva Prieta near Las Portales.

Hughes: You were doing lab work between trips?

Johnson: Yes, in one day, you do all the specimens. We got out the containers with the glycerinated specimens and ground the specimens in mortars with saline and injected mice and guinea pigs intracerebrally. I showed Giron how we did it, but he'd already done mice inoculations, so there was nothing much to learn, except that he had never seen a vampire before. He got the best zoologist to help identify them.

The Second Field Trip

Johnson: The next trip was into different territory, going into states a little north of Michoacan. We got the virus from Michoacan state. I had a different veterinarian traveling with me, because Tellez stayed working with the specimens. The district veterinarian for Guadalajara was Ramon de Cevallos, a wonderful man. I corresponded with him at Christmas time up until a year ago. He was later the dean of the veterinary school at Guadalajara. I guess they have a medical school there, too.

We always stayed in very simple hotels. The thing I remember the most about this hotel in Guadalajara was the peace of that place. The Mexican hotels in the villages would be open in the center—a little court—and you'd eat in this court. I would meet the local presidente, and we often played dominoes to make friends. We'd play for one cent a point, or something like that. These men always had their two pistoles and their big hat, and it

Johnson: was really out of this world. They would tell me all the stories about the gringos that had been down there. [laughs] At this little hotel in Guadalajara, a man played guitar and sang these songs of the heart--de la corazon. It was just beautiful. That would be the music for the evening meal, which were mostly bacon, beans, rice, and tortillas. But good.

One of the famous places I visited in Guadalajara was the ranch of the sub-secretary of agriculture of Mexico. He had made his money originally as a bandit. He had a radio station. A local independent ruler, that's what he was. And he was taken into the government to pacify him. He had a marvelous ranch, with prize horses from Kentucky and specially bred dairy cattle and Swiss Brown and Orlando Spanish cattle for beef. He had irrigated wheat from pipes from Lake Chapala. He was very helpful. A man like that can give you a lot of background. Of course, Dr. Cevallos knew him, and he was interested in derriengue.

We were to go to Autlan de Nevarro, where derriengue had been reported. Autlan was one of the real classy cow towns in western Jalisco, a beautiful little plaza. We stayed in a hotel nearby, again it had a mud floor, but it was pretty neat, and we could eat there. In the evening, they had a band in the center of the plaza. All the young people came and walked around the square at a certain time, and Ramon said, "Let me show you how it works." The big excitement comes about eight o'clock when the girls come, usually with an older woman [as chaperone], and they walk around the square. Then the young bloods come walking up and talk to the girls. [laughs] It's a real classy way of meeting for the boys and girls. They're under escort, and lots of fun, and the band is playing, but no dancing.

Early the next morning, we took off, and as soon as we hit a town with that old federal district truck, they'd say, "What are you doing here? What do you want here?" They have no federal troops out there, and they don't want to be disturbed.

We were invited over to Senor Blake's home in Autlan. His daughter had been the bullfight queen. I got to play the piano and they sang some songs in two parts, and it was a really nice evening. He owned a big ranch.

Six o'clock the next morning, we took off in the truck for a twenty-thousand-acre ranch because there was a problem with derriengue there. The owner of the local ranch facility was Senor Felipe de Castaneda. He had lost 150 cattle and horses during the past three months--that's a lot! They had thousands of cattle. I showed you a picture of one of the horses that had a vampire bat bite on the shoulder.

Hughes: Yes, I remember seeing that.

Johnson: So I knew we were in business here. The cowboys said, "Well, there are big hollow trees down there. We've seen bats going into those trees." But they didn't know of any cave. I and a whole bunch of vaqueros, all with their pistoles, took off on horses. They wanted to do some trick shooting. I tried their pistoles, and that was one thing I was good at. It was a good way to make friends.

We finally got to one of the trees that they'd located as they were herding cattle. There was an opening in the side about four feet high and about three feet wide at the bottom, like a pyramid, and there inside was this smell and this liquid blood. They said they'd seen bats come out of there. I'd heard that you could smoke out anything, so we picked up some cow dung and I made a fire. I put the net over the opening. When the bats started to fall down, I crawled inside. I had both gloves on so I could grab them. And guess what I got out of that tree? Twenty-three bats. They'd lose their grip from the smoke and fall down. That was the biggest collection in one place, all vampire bats.

That night we got in trouble with the bandits. The road was made by the ranchers. There was a bridge made from two big tree trunks that had been flattened on one side, and then a small tree trunk on either side. You'd drive across with the wheels on the tree trunks. The road was flattened-out volcanic rock. We started back; we were really feeling good; we had all these bats. I'd sit on the front fender with my flashlight, and we'd go slowly across these tree trunks.

The Indians in this area had set fire to the forest on the sierra. They called it fuego de las montañas--fire on the mountains--and they would collect charcoal after that. So they purposely set fires. At one place there was a smoldering log in the road. So I went out there to kick this log off the road, and as I did, a stone came hurtling out of the air and hit me on the thigh. I thought I had broken my leg; I got a big hemorrhage on that side. The fire would set stones loose, and they would roll down.

Then about ten o'clock at night in the middle of the road was this whole camp. There were 100 or 150 people--guns stacked in the road, women and children. It was a local guerrilla group. Tellez said, "They won't hurt you. They just want your money." The ranchers all knew these guys, and they let them take beef, and they gave them flour too, because if they ever have any trouble with the government, these are the best fighters that they can get. The leader looked just like Pancho Villa. They had guns galore. I understood that in some of the trucks they even had machine guns. And lots of horses.

Johnson: The upshot was, how much money did I have? And how much did Tellez have? The leader said, "Oh, we'll leave you enough to get back to town." I had something like eight hundred pesos, and Tellez furnished about five hundred. So we had to get money in Autlan again. They didn't search our truck. I have been stopped by road blocks in India, but this one in Mexico was really spectacular.

When I was down in Colombia later, the bandits were even rougher. One day, on my trip in 1963, there were forty men killed when they stopped buses and broke their necks. They let the children and women go. This was on a road near San Vicente de Chucari where we spent the night.

Anyway, we got to Autlan, and I had to process those bats, which was a long job. I got two liters of blood from bleeding a cow and defibrinated it so we could feed some bats. Then I got some more ice for the sawdust. We were watching these bats feed at night where they were caged. They would come over and lap up the blood just like a cat.

On May 1, 1944, we visited Rancho La Cedra near El Chante. There were hollow trees in a corral, and we got eight bats there. We went on May 2 to El Crullo to another big ranch, belonging to Senor Jose Preciado. He had two caves. In one enormous cave, beautiful one, we got eight female and four male vampires alive.

Then we went to another town called Limon, where they had had fifty cases of derriengue the previous year but no cases in 1944. We didn't collect any vampires there.

On the 3rd, we went to Autlan, and on the 4th we went to Guadalajara, and we had a lot of tire trouble. The volcanic rock, as it breaks up, cuts through the rubber. We went by the way of Morelia to get some gas, and we went back to Mexico City on May 7. We brought back five healthy vampires that Giron could work with. We would test ones that we had processed in the field. Tellez did some physiology studies, such as feeding on goats.

During our second field trip, the mice came down [with derriengue] from the bats from the Agua Frio Cueva Prieta. That's when we knew we had the virus. I brought my set of specimens back to my lab in Alabama, and I isolated virus from the duplicate sets from the same place. There was no question of a mix-up.

There were a lot of beautiful things done for me when I was in Mexico City. The head of the state medical services, Dr. Zozaya, took me to Cuernavaca on the weekend. We went to his beautiful home there, and he introduced me to his grandmother, who was Swedish. He had a little grass-roofed place in his yard and he had a beautiful recording device, and he had these black calla

Johnson: lilies there. We were listening to Bach's B Minor Mass out there, lying in hammocks. He had musician friends and a Steinway grand piano. I met some of the best musicians in Mexico. The most famous was Reveltos. These were cultured people. Zozaya studied in Germany. He was a brilliant man.

Then, I got to know very closely a lot of their top doctors and parasitologists, which led me later to set up that expedition in 1960 to Sonora with a duplicate set of people from Mexico to study the encephalitis problem there.

Hughes: Giron went on to more closely identify the virus?

Johnson: He published on the isolation of the virus. I wanted him to, because I didn't want to take credit. Then I published my paper in the American Journal of Hygiene in 1948.*

I did all the long-term experiments. We'd immunize animals and do complement-fixation and cross-neutralization tests. It was very obvious that the virus was rabies, but serologically you could show a difference from the Pasteur strain of rabies virus. You could immunize with the Pasteur strain virus against our Mexico strain. Now, the Mexico strain didn't produce as good immunity against the adapted Pasteur strain as vice versa. That is true of a lot of the field strains of skunk virus in this country.

Hughes: Why is that?

Johnson: If you take a virus that normally is not transmitted from brain to brain, and you start putting it brain to brain, you get a neurotropic strain very fast, which probably is a good one to make the vaccine from, because after all you want to keep the virus from getting into the brain. We didn't know then that the virus would grow on anything except nervous tissue. But now we know that it's got to grow in the muscle before it can get into the nervous system.

Hughes: Both you and Giron were working simultaneously on the Mexican rabies virus?

Johnson: The main thing was for him to isolate the virus directly from bats. He was able to publish in Mexico, saying that they'd gotten the virus from vampire bats. So the thing we should do is destroy vampire colonies on the ranches. One ranch had a hollow tree in the corral, and the vampires were living right there in the corral. Of course, you could get rid of that quick.

* H. N. Johnson. Derriengue: vampire bat rabies in Mexico. Am. J. Hyg. 1948, 47:189-204.

Johnson: We assumed that these vampires were carriers. I remember seeing all those dead vampires and the disease would be in one area, and then they would die during the incubation period of the disease. They probably were all infected within a period of two or three weeks. Some of these bats would fly off to another colony, probably because they were already ill, and the disease would move from vampire bat colony to vampire bat colony. That idea developed a little bit later; we were able to see that very well when we were working in Argentina in 1967, that is, there were no healthy carriers. Those infected died. The bats died out in the infected colonies.

Paralysis

Hughes: Well, is the next step your illness?

Johnson: Well, I came back to Montgomery and went to work. I was still having trouble with my intestines. It just didn't let up.

I got back to Montgomery on May 11, 1944. One of our family jokes is that I got in at 2 a.m., and I had to take a taxi home. I had sent my wife a telegram from Mexico City. As I arrived at the house there was nobody awake; she didn't know I was coming. So I pounded on the door, and finally she woke up. I said, "Well, did you get my telegram?" She said, "No." I'd been there about a half an hour and the phone rang, and it was the telegram. [laughter] Telegrams don't go so fast sometimes.

I had this intestinal trouble and took specimens to the state health department. They found Giardia parasites, but they didn't find any amebae, which is not so easy in a chronic infestation. So, I went on, and I didn't take any treatment. I'd lost a lot of weight. I was 185 or more when I went down there, and I came back weighing 158. I was not very peppy. So they kept looking, and they finally found the cyst form. That's when I decided to take the diodaquin.

I was at a medical meeting at Sulfur Springs, New York, when I really got very weak. I was talking to some people, and I fainted. Actually, the day that I got sick, I was still on the last few pills of diodaquin, and my guts had sort of stabilized. I'm sure that the amoebic infection upset my immunity and that, if it hadn't been for this incidental disease, I wouldn't have come down with rabies.

We later studied what stress and a later severe infection will do to somebody that had been exposed but had some immunity. In fact, we can do it on purpose now. We can take animals that

Johnson: have survived the usual incubation period of rabies and treat them with cortisone, and they'll come down with rabies. We published on that experiment.*

I was playing golf one day about five months after I was bitten and I started to have this terrific headache. I was getting a little dizzy, and something was going wrong with my left hand. So I quit the golf game. (My wife and I played a lot of golf in Montgomery.) I went home and went to bed; I had the most awful vertigo.

The doctor who had taken over the practice for my friend who had gone in the military came over. Fran called my friend, Dr. Thomas Frist, from Maxwell Field. He said, "You've got to go to the hospital." So I went into St. Mary's Catholic Hospital there. I wasn't having much fever--it was about 101--but I had a lot of strange symptoms and a fast pulse. Of course, I don't remember too much of all this, but I have a pretty good record of it.

I went to the bathroom, and my left leg started to give way. I had to call for help to get back to bed. I had had no paralysis before; I had walked out of the house to the car, and this was only a couple of hours before.

So within twenty-four hours, I had ascending paralysis like Guillain Barre. Their question was whether it could have been related to rabies. I had taken a lot of vaccine [previously] and expected to be immune.

Hughes: You hadn't taken vaccine after the bat bite?

Johnson: No, I didn't expect any trouble from the bite.

Hughes: You had had the vaccine in 1938.

Johnson: Well, yes, but I had not taken a booster in 1943. I did have antibodies when I went to Mexico. I was afraid to take any more [vaccine], so I was going to be careful. I had had the original dose and two boosters.

I have my immunization records. In '42 I had a booster of live virus vaccine. My blood serum in 1943 neutralized more than 10 LD50 rabies virus. When we challenge animals [with rabies virus], some don't have much antibody, but the ones that have been immunized survive. I knew that mortality from the treatment paralysis was much more dangerous than what I expected from the bite.

* O. A. Soave, H. N. Johnson, K. Nakamura. Reactivation of rabies virus infection with adrenocorticotrophic hormones. Science 1961, 133:1360-1361.

Johnson: I had a few critical days: My pulse was running very fast, and the paralysis went on pretty rapidly, until I was quadraplegic. Both my legs and arms were gone. One eye was open and one was closed. The neck and chest muscles were involved, but the thing sort of stabilized in seven to ten days.

Hughes: You weren't on a respirator?

Johnson: No, but I had some difficulty breathing, swallowing, and eating. I was absolutely helpless.

Dr. John Fox—my friend that I worked with at the institute--was sent down by Dr. Hugh Smith and Dr. Strode, who were directors. They couldn't believe that this was rabies. They said the incubation period was too long--five months--and it must have been [caused by] these eastern and western and Venezuelan [encephalitis viruses] I was working with.

Thinking polio was most likely, John Fox brought along a thermos, and he took spool specimens on me. Then he also went with me in an ambulance over to Warm Springs, Georgia. I wasn't going to progress without physical therapy.

Hughes: There was no treatment other than that?

Johnson: No. I was completely paralyzed, and whether anything would come back was very questionable. I was there for about five months. The nursing was atrocious.

Hughes: Why?

Johnson: Well, it was primarily a physiotherapy place. There were some patients in artificial lungs. The war was on, and there was difficulty in getting a staff. There would be "push boys" to get you in the wheelchair and out of the wheelchair and in the pool and out of the pool. [laughs]

Helen Vaughn was my physical therapist, and I owe her everything I got after that! She was a Quaker and trained in physiotherapy and nursing. She later worked in the department of physiotherapy in the government in Washington. It was a matter of knowing how to train what little muscle I had left; my muscles just wasted away. I could see all the bones in my hands. I went down to 119 pounds and looked like a skeleton. In the water, I could do a lot of things, but it was always hard. When we were doing exercises, Helen would say, "Try to help me now." Then I would try to think of contracting. Then she said, "Well, you helped me a little bit." That had to be done with every muscle. It was a long process, usually a three-quarter hour or an hour each day.

Hughes: Was this therapy designed particularly for polio?

Johnson: Primarily.

The Warm Springs Foundation was President Roosevelt's place, and he had his house nearby. I met him. He'd come to special dinners, like Thanksgiving, and meet the patients there. It was just wonderful the way he reacted with the patients--jokes.

There were a lot of navy and air corps people there, some who had gotten polio in submarines and Africa. Admiral Darlan's son was there, and I used to play bridge with him. His legs were knotted up, and they had to straighten them out so he could swing them. One of the men from the Free French Flying Corps was there. These were people that needed physiotherapy.

We put on a show for President Roosevelt after he came back from Yalta. Three patients were in wheelchairs. One played Stalin, one was Roosevelt, and one Churchill. I played chords with one finger on my left hand and triads with the right, and they did a routine of songs, and President Roosevelt got such a kick out of it. He said later, "Well, we learned a lot of verses from 'Mademoiselle in Armentieres' during the war but the words were different." I was later taken to a reception at the little White House.

Hughes: Was he receiving therapy on a daily basis?

Johnson: No, he wasn't. He would come and visit on a special occasion.

Hughes: He died there?

Johnson: I had actually gone back to Montgomery when he died in '45. I was at the health department when I heard he died. When he came back from Yalta he was ill, and everybody knew it. I think he had had a small cerebral hemorrhage before he went to Yalta, and he had one afterwards.

Hughes: Was he mentally impaired?

Johnson: I doubt it at Yalta, but it isn't good to be under that stress. I think he was taken advantage of, because of that. The first time I met him, when we put on that show, he was then absolutely clear and really in command and very alert.

Hughes: What do you mean, he was taken advantage of?

Johnson: Well, if you're dealing with people and you're not feeling well, your ability to sustain the pressure of the conference decreases. Stalin got things he should never have gotten.

Hughes: What did people think you had?

Johnson: Well, [Jordi] Casals ran all my sera that I'd saved; we always saved sera. He thought he got a rise against western encephalitis virus. I don't know why that could have been, but [the rise] wasn't clear. When I got back to the lab—I had good immune sera for all the encephalitis viruses—my serum didn't neutralize eastern and Venezuelan viruses, but I did have antibodies to western encephalitis virus. In 1919 we had cases of horse encephalitis on our ranch in Nebraska and I must have been exposed at that time.

But I did have antibody to rabies. One of my notebooks has results of one of my tests from the lab, in which we ran my serum against the derriengue virus. I neutralized a hundred LD₅₀ [lethal dose for fifty percent of test animals] fine with that. In the previous tests against rabies done in '43, it protected less than 100 LD₅₀ of the virus. I had antibody after that until I got to California. When they sent my blood to Lederle, Lederle wrote back that I had antibodies to rabies before the 0.1 milliliter intradermal inoculation of HEP [high egg passage] Flury strain vaccine. I was pretty sure that rabies was what I had, but nobody would accept it at the time.

Hughes: Because of the long incubation period?

Johnson: Yes. Hugh Smith, in his book referred to previously, wrote that I had evidently had an infection with the derriengue virus.*

When I developed encephalitis and paralysis on September 2, 1944, I was certain that my illness was from the vampire bat bite because of the type of symptoms and because the early signs were related to the site of the bites on my left hand. Furthermore, a blood specimen taken in December, 1944, when I was a patient at Warm Springs Foundation Hospital, showed a high antibody titer to a strain of derriengue cattle rabies virus isolated by Dr. Carl TenBroeck of the Rockefeller Institute at Princeton, New Jersey. He collected this cow brain virus when he was in Mexico in 1943, and I had identified it as rabies virus.

The resistance of the medical consultants regarding the diagnosis of rabies was based on two factors: the almost five months incubation period and the previous history of adequate immunization against rabies and particularly because it was believed that no one recovered from rabies.

The occurrence of much physical stress during the expedition in Mexico and the repeated intestinal infections, including giardia and amebiasis mentioned previously, explains the

* P. 182.

Johnson: reactivation of the rabies virus introduced by the bat bite. We have since showed that ACTH [adrenocorticotropic hormone] will reactivate the rabies infection in animals vaccinated against rabies so that they will develop rabies as late as five and eight months after exposure.

The other factor as regards my paralytic illness was that I was infected with the vampire bat rabies, which is not the same as the canine rabies virus. We have learned from the use of monoclonal antibodies that there are different strains of rabies virus.

An example of human infection of a laboratory technician with rabies virus followed by survival is the case of Mr. Andrulonis of the New York State Health Department. He had been adequately immunized against rabies virus, with an antibody titer of 1:32, when he developed rabies after he had been exposed to an aerosol of the SAD-ERA dog vaccine strain of rabies virus.* Though he lived, he suffers from serious sequellae. We do know also from the serological studies of SAD-ERA virus that it is unique as compared with other strains of rabies virus derived from dogs, foxes, bats, and skunks. This indicates that it is probably a hybrid of the American dog strain of rabies virus from which it was derived and another virus present in the swine kidney cells used in the propagation of the virus.

I collected records of many cases of rabies. One of the patients that I consulted on was a young boy who had been bitten by a rabid dog, and he was given a very intensive course of the Hoegyes live virus vaccine. If they live past a month, you know they're safe. Three months later, this kid got lobar pneumonia, and he had a crisis. About twenty-four hours after this crisis, he started to have trouble swallowing. They were alert enough so that they did an autopsy, and found that he died of rabies. This suggested that he had the virus stabilized, but the pneumonia infection stressed his body and lowered his resistance.

Another case was a woman who had come into a clinic at night at Emory University in Atlanta. She was a syphilis patient, and she'd had a diagnosis of central nervous system syphilis. The resident noticed that she had symptoms of agitation and difficulty in swallowing, and he found she'd been bitten by a dog. She'd had the Pasteur treatment. Again, that was some months before. She went on and died, and guess what? She had an active gummatous degenerative lesion in the brain, which may have played a part. But she died of rabies. We had seen recovery [from rabies] in all kinds of animals--mice, guinea pigs, rabbits, and dogs--when we were doing experiments in Montgomery.

* [CDC] Morbidity and Mortality Weekly Report 1977, 26:182-4.

Johnson: While at the Berkeley laboratory, I ran some vaccination studies of the HEP Flury virus, using a skunk salivary gland virus for challenge. Dr. [Orland] Soave was a veterinarian in the lab, who later went to Stanford. He was interested in adrenocorticotrophic hormone [ACTH] and was using it in some monkey studies at the lab for Coxsackie and other diseases. He said, "Let me try it on some of your guinea pigs." I had done a controlled experiment in which I inoculated guinea pigs intramuscularly with the live virus vaccine, the Flury strain, and then challenged them a month later. Five controls died and seven were left of the ones that had received the virus. The vaccinated all lived. He said, "You just give me the controls to stress with ACTH."

We had been talking about my rabies infection. We waited until five months went by after the challenge, and he got out his ACTH and started giving that, and two of those remaining control guinea pigs got paralyzed. Soave isolated rabies virus from the brain; that is, he reactivated the infection five months after exposure. That was published in '61.* Everything would probably have been fine if I hadn't had the amoebic infection and other stress.

In human autopsies here in California, we've gotten secondary viruses like herpes and even rhinoviruses out of patients that have died of rabies. Both viruses were in the sputum. So I think that incidental disease plays a real part [in deaths from rabies]. Soave repeated the experiment with salivary gland skunk virus, and he was able to reactivate the virus at eight months and to isolate virus from the brain. That led us to try in every way to keep people from using cortisone in treating rabies.

One patient that Dr. [Richard] Emmons and I were involved with died over a month after onset. We'd gotten the virus out of his saliva, but particularly out of his urine and spinal fluid. He was given cortisone (betasone) because he had a swelling of the neck. He had a thrombus in his neck from having a catheter in his vena cava. So then he died. It was very clear because the virus was still active in the brain. In other words, it was reactivated by the betasone. If animals live more than five days after onset [of rabies], it is seldom possible to isolate rabies virus from the brain. This is called autosterilization.

So the betasone reactivated the rabies virus in this child who had never been treated, but he had been bitten by a rabid dog. Except for that thrombus, he may have survived. It was over a month since he became ill. He was doing fairly well until the vena cava problem. He had been on a respirator. Then the clot blocked the dural sinus and he died.

* O. A. Soave, H. N. Johnson, K. Nakamura. op. cit., p. 125.

[Interview 5: March 16, 1987]

Hughes: How was your health by the time you got to New York?

Johnson: Well, my major paralysis had cleared up in a remarkable way by the end of 1945. I'd been through the crutches, and the two canes, and the one cane, and a large brace on my left leg, locking at the knee so the leg wouldn't collapse. By the end of '45, I was doing pretty well. When I went up to the Rockefeller Institute in '45 in the later part of the year, I was still using crutches.

But the big problem for the next two years was this disease had left me with a very strange form of neuritis. At times the feeling was like a feather scratching somewhere on the body, rarely at the same place, and then little muscles would be twitching. I'd be very embarrassed because my nose on one side would be twitching, and somewhere on my face and my eyelids. The muscles would contract very readily at times. They would contract with tremendous pain, so they would bleed. It was part of the old healing or cicatrization, I guess, of the dorsal root ganglia, because I had terrible neuritis, almost what you would call radiculitis. That's inflammation of the dorsal root ganglia.

So I had a tremendous lot of pain, and this tended to be less and less as the months went on, but occasionally I would just have to get up and leave lunch or something and go somewhere else because of this pain.

Hughes: Was there any medication?

Johnson: No, I didn't take any medication. When I was first ill, I was having trouble with my bladder and bowels, and anything for pain, like morphine or codeine, would slow that up. So I made the decision to limit it to aspirin, and very little of that.

It was a type of disease which we didn't know much about. I have sections of humans and dogs that had been infected with rabies, in which the dorsal root ganglia along the spinal cord are involved. They show degeneration of the nerve cells and damage to the nerve fiber tracts.

The decision was for my wife to stay in Montgomery for a while, because she was pregnant in '45. She stayed there through May of '46. I had to live in a small room in New York City, and I ate out, and I walked to the Rockefeller Institute.

Hughes: Was the Rockefeller helping you to support your expensive medical bills?

Johnson: Well, this is very interesting, because the foundation had hospital medical insurance for us. I was sick at the end of '44, so I was in the hospital for five months, about half in one year and half in the next. This was covered by the special insurance that was started in New York City.

Hughes: Was it HIP?

Johnson: Health Insurance Plan, yes. The problem was that I had nothing beyond my salary, which the Rockefeller Institute continued to pay. The house that we were living in in Montgomery when I got paralyzed was suddenly sold, shortly after I was in the hospital. Entrepreneurs were buying houses during World War II.

My wife Frances, an RN, went with me to the Warm Springs Foundation in Georgia. She rented a small cabin near the hospital. She was my nurse for the first two months, because I was absolutely helpless. This was an extra expense. We had two children then. The children stayed with friends in Montgomery until Frances and her mother Marion Alexander came to Warm Springs. It was very difficult for Frances to get food for the family at Warm Springs because of rationing and there was only a small store there.

When we came back to Montgomery from the hospital in February 1945, we were able to get special housing because I was working for the state health department. It was a small tract house, but good enough to live in. So that's where Frances lived until she moved in May, 1946 to Scituate, Massachusetts, to be with her mother at the family home. The foundation shipped our stuff and stored it temporarily.

Hughes: You could drive?

Johnson: Oh, yes, I soon was able to drive. In New York I had a car but also rode the buses, subway, or walked.

Hughes: Did you continue with physical therapy in New York?

Johnson: I had my own. I went back to the Warm Springs Foundation several times in '45 for a checkup and also guidance on physiotherapy. I tried using swimming pools in Montgomery, which I didn't do in New York, and learning various exercises. One of the best for my hands was with the piano. For a while all I could do with my left hand was just hit one note with my index finger. But the right one came back much faster. They were in a claw shape for a while. The muscles had atrophied. You have to fight for all that you can get, after being completely paralyzed. And all these exercises-- including exercising my eyes to fuse the image (diplopia). One tended to remain open and one closed. That went away pretty well in that first year.

Staff Member, Rockefeller Foundation Laboratory, Rockefeller Institute, 1945-1951

Johnson: My wife stayed all the summer of '46 in Scituate. Come fall, I was trying to find a place to live. She was afraid I wasn't trying very hard, so she came up one week and stayed with me. By that time I was living with Loring Whitman. His father had an apartment on 10th Street. Part of the time I had the car in New York, so I could drive for weekends to Scituate that summer of '46. Loring's parents had a real nice apartment in New York City, which they seldom used during the summer. Both Loring and his parents had lovely homes in Simsbury, Connecticut.

Hughes: Was housing very tight after the war?

Johnson: Oh, yes. I could not find anything that we could afford. My salary was about five thousand.

We had special functions at the home office in Rockefeller Center. Raymond Fosdick, who was president of the Rockefeller Foundation, said, "Well Johnson, have you found a place to live yet?" I said, "No."

He said, "Well, I've got a niece, and she's got an apartment at 400 East 56th Street, and they're going to rent it furnished for a while." I said, "We'll take it!" [laughter]

Hughes: Sight unseen!

Johnson: It was \$150 a month, and I said, "Well, that would be just ideal." He said, "I don't know if they'll come back." His niece was married to Rufus Jones, of a well known Quaker family. It was absolutely perfect for us. We kept most of our furniture in storage. So that was where we lived the whole time in New York City—400 East 56th Street. It was an excellent place to live in Manhattan.

My wife Frances and the three children joined me in the fall of '46. The Rufus Jones family moved their furniture to Maine in a few months, and we kept the lease.

Hughes: Were your third and fourth children born in New York?

Johnson: Our third child, Susan, was born in Montgomery, Alabama, in February, 1946. My wife Frances went to Scituate during the summer of 1947 and stayed there so her child could be born at the Boston Lying In Hospital, and so Michael, our fourth child, was born in Boston. He is an M.D. on the faculty of Harvard Medical School and on the medical staff of Harvard Health Services.

Johnson: When we were all in New York City I could either take the bus from 56th up to 68th and walk across to the inatitute, or drive. There was a little parking place at the Rockefeller Institute, and for street parking near the apartment I knew persons who would be parking during the daytime, and they left at a regular time, and I would be there right on time to take their parking place. To pay for off-street parking even in those days was too expensive. So I parked on the street. I got to know the florist and people who worked in the little stores around. It was like a little town where everybody knows each other.

Hughes: I know from talking with Dr. Lennette that Rockefeller's salaries were not grandiose.*

Johnson: But then again nothing was in those days.

Hughes: Were they comparable, do you think?

Johnson: Yes. When I came on with the foundation, my salary was four thousand dollars a year. It bought exactly what forty thousand would do now. In Montgomery, we had the nicest home--a beautiful little one-story home and a large lot--for sixty-seven dollars a month. New cars were seven or eight hundred dollars. Today, we kid about it and say, well, you just move a decimal point. We'd write a check for fifteen dollars; it was like writing a check for \$150 now.

Hughes: So the Rockefeller wasn't trading on its prestige; it was paying the going rate.

Johnson: They had one thing that they stuck with. They did not want anybody to come and work on these projects for money. It was just like the internship I had at Harvard where you worked, say, eighteen hours a day, seven days a week, for sixteen months at no salary, only uniforms, board, and room. You never thought about stopping at any certain time; sometimes you'd be up all night long. I remember one period there was an epidemic. I never got back to bed for three days. I would take naps in my office chair.

Hughes: And the Rockefeller expected more or less the same dedication?

Johnson: That's the kind they looked for.

* See the interviews with Edwin H. Lennette, Pioneer of Diagnostic Virology with the California Department of Public Health, an oral history interview conducted 1982, 1983, and 1986, Regional Oral History Office, The Bancroft Library, University of California, Berkeley, 1988.

Hughes: Why had you decided not to continue in Montgomery?

Johnson: We had finished the big long-term experiments on rabies. I was in no position to develop the Alabama lab as a regional lab for the foundation. The rabies work was the big thing, and we had really done all we had hoped to do. We had the beginning of a live virus vaccine, which was the avianized Flury strain of rabies virus, passed 138 times by the intracerebral route in one- to two-day-old chickens, which I sent to several commercial laboratories. The only company that was interested, really, was Lederle Laboratories, and they produced the LEP (low egg passage) Flury avianized rabies vaccine virus.

Hughes: So, your health problems really had no part in your termination at Montgomery?

Johnson: No.

Laboratory Staff

Johnson: The tendency was at that time to do a lot of research at the base lab in Manhattan, and they were deciding at the time I got sick to have a better central lab. The International Health Division laboratories had one floor, primarily, of the middle building at the Rockefeller Institute. The IHD was under the umbrella of the Rockefeller Foundation.

After World War II the IHD board of scientific advisors recommended some basic studies of other major disease problems. One was typhus. John Fox and Jack Snyder were going to work on that. Elsmere R. Rickard was already doing field studies of murine typhus. Another was malaria, because the malaria problem still looked like it was going to be the number one world health problem. They wanted to learn whether any of the known drugs would cure malaria. In '43, N. H. Fairley and his group in Australia, using conscientious objectors, showed that you could let infected mosquitoes feed on a person, then take daily blood specimens, and inject others to find out when the malaria parasite was in the blood.* With blood taken up to about a half hour after the mosquito's bite, there would be an occasional infection, that is, some malaria sporozoites from the mosquitoes were still in the blood. Subsequently, the blood specimens failed to produce malaria until the eighth day. Well, where was the malaria parasite during those seven days? It was not in the red blood cells.

* N. H. Fairley, Trans. R. Soc. Trop. Med. Hyg. 1945, 38:311.

Johnson: Dr. Lowell T. Coggeshall and Dr. Andrew J. Warren were studying bird malaria at the institute when I was there in 1938. They bled canaries daily after they were either inoculated with sporozoites or fed on by infected mosquitoes. The blood was negative for two days. Therefore, the parasites had a cycle of development in tissues other than that of the blood.*

Hughes: Now, [Ronald] Ross in 1898 had shown that malaria was transmittable by mosquito. He hadn't done any microscopic work, that identified the organism?

Johnson: There was a lot of work on mosquitoes to show that the oocysts formed on the mosquito stomach and sporozoites in the salivary glands.

Hughes: He'd done that?

Johnson: Yes. It was assumed that the sporozoites infected the red blood cells directly. Prior to the antibiotic drug era, if you were inoculating mosquito suspensions intravenously, there would be introduction of large numbers of bacteria. All the early studies on yellow fever were done by allowing infected mosquitoes to feed on people. They would feed mosquitoes on a person infected with yellow fever virus. They found out that there was an intrinsic period of incubation, that is, the mosquito would not transmit the virus until eight or ten days after the infective feeding. There was a similar incubation period in the mosquito cycle of the malaria transmission.

So the major priorities in IHD were the studies of typhus and malaria. Dr. R. M. Taylor had a special study of influenza and other respiratory diseases caused by viruses.

Hughes: Was each study conducted in a separate laboratory?

Johnson: Well, they were in the [IHD] unit, two floors of the new building. That was in 1946. So much of the time in '46 was spent moving equipment and getting new equipment and supervising all the construction. I had my office, a tissue culture egg room, which was sealed, and a section for pathology. We shared large animal rooms. We had a lot of facilities for chickens, we had a monkey room, and we had a mouse and a hamster colony.

Hughes: Richard M. Taylor was in charge of all this?

Johnson: Yes. He was the director of the IHD New York laboratory when I returned to work there in 1946.

* A. J. Warren, L. T. Coggeshall. Am. Jour. Hyg. 1937, 26:1.

Hughes: What was his background?

Johnson: Richard M. Taylor (1887-1981) was born in Kentucky and graduated from the University of Michigan Medical School. After hospital training he took part in a study of influenza which was under way at the Rockefeller Institute, a clinical study associated with the Rockefeller Hospital there. This was during 1919-1920. He then received an appointment with the Hoover Relief Commission to Poland. While he was there he met a Red Cross worker by the name of Mary Stevick who later became his wife. In 1923 Dick joined the IHD of the Rockefeller Foundation. His first assignment was to Honduras where he was in charge of the hookworm eradication campaign. As with such programs, he organized a public health laboratory. During 1924-1925 he began a similar program in Costa Rica. In 1932 he was assigned to France for a study of undulant fever (brucellosis). Following the initiation of the IHD influenza project, he was assigned to Hungary to start a project on influenza. After he had trained a staff there, he was sent to Argentina to initiate influenza studies there. By 1940 he was in charge of the yellow fever lab at Rio de Janeiro, Brazil. While he was there he obtained some specimens for me of bovine rabies suspected to be from bat origin. Later that year he visited our Rabies Study in Montgomery. Before I left the Rabies Study, Dick wrote asking me to set up a pathology lab at the IHD lab at the Rockefeller Institute as a part of my assignment there.

Hughes: What was the administrative arrangement at the IHD?

Johnson: We were all nominally under Dr. Taylor, including malaria. Dr. Max Theiler was director of the malaria section, which was composed of himself, Dr. Delphine Clarke, who was a trained biochemist as well as an M.D., and Dr. Charles Anderson, who was trained in medicine and pathology and infectious disease. Dr. Anderson was doing the erythrocyte studies in malaria, using the Warburg apparatus, and trying to cultivate the parasite in red blood cells.

Dr. Loring Whitman had known Max Theiler from their Harvard days and was with him on the Harvard African expedition, 1926-1927, with Dr. Richard P. Strong, professor of tropical medicine at Harvard. Loring Whitman was the photographer for that trip.* So Loring early had been interested in all sorts of biology. He was interested in mosquitoes, and actually he had been doing a lot of the mosquito work at the yellow fever lab in South America. Loring was in charge of the mosquito studies for all the malaria

* Richard P. Strong, editor. The African Republic of Liberia and the Belgian Congo. Cambridge: Harvard University Press, 1930.

Johnson: work [in the IHD lab]. I was doing the chick embryo studies, baby chick studies, and monkey studies on malaria as well as setting up and running a pathology laboratory for the IHD lab.

Hughes: Was there any particular reason that you became part of the malaria group rather than one of the others?

Johnson: No, it was just sort of a natural. I was good at minor surgery. In my hospital training in medical school in Nebraska, we were trained to do minor surgery and assist in major surgery. And as a premed I'd worked for two years in a hospital. I was trained in pathology at Harvard. So it was just a natural for me to do the surgery on the monkeys, which was taking biopsies of liver, which really nobody else had done. They would usually kill the animals if they were going to study them at a certain time. But I wanted to do repeated and sterile biopsies.

Hughes: Did you have any choice in the matter or were you just told when you came back, go to Max Theiler's lab?

Johnson: No, it was obvious that Max was going to run it. He was my friend; I would do anything for him. I thought it would be interesting to work with malaria.

It was a big problem when I was in Montgomery. There was a lot of malaria in the South when I first came down there, and the Rockefeller Foundation had a field laboratory in Alabama to train malaria control officers.

Hughes: Was quinine still the major treatment?

Johnson: Yes. Quinine was used, and atabrine was the new drug at that time. But quinine was the major drug.

Dr. John Bugher, who had a doctorate degree in physics as well as medicine, was head of the physical sciences section, and he was carrying on the work with the ultracentrifuge and electron microscope. He was also doing studies of concentrating virus preparations.

Hughes: Had Pickels already left?

Johnson: Yes. He had gone to Spinco Associates in California. The early development of the centrifuge [at the Rockefeller] was done with Bauer as director, in the late thirties and early forties.

Then, in serology, Dr. Ken Smithburn was doing neutralization tests on all new viruses, plus the yellow fever virus strains. He had one special section, which he organized for infected animals, rooms for his mouse work. He had been in Entebbe, Uganda, for a period [1938-1946]. He came to the institute at the same time I did.

Hughes: Had he been working on yellow fever?

Johnson: He'd been working on yellow fever in Uganda at the Entebbe laboratory. That's where I almost went in '38.

Then Dr. J. Austin Kerr, who had been working on malaria in South America, was assigned to do the complement fixation tests. He had some preliminary training with Dr. Casals in Webster's lab. Dr. Delphine Clarke was helping in the biochemistry of the complement fixation test. We had probably a total of fifteen [arthropod-borne] viruses which had not been classified and which had been isolated when looking for yellow fever.

Dr. Taylor had his own lab, his own technicians. He worked on influenza A, B, C, and incidentally with Cocksackie viruses, which were isolated from specimens collected for the influenza studies.

Hughes: How tight was his directorship?

Johnson: The classic thing about all this International Health Division work, they gave you a very free hand with your particular project. I had been trained in pathology, the only one in the lab with any major training in that. So I could start from scratch and train technicians and get the equipment and get it running. Soon there was a lot of [pathology] material; they wanted to see what specific lesions were caused by the viruses.

Loring Whitman liked to work with the mosquitoes. He would get the mosquitoes infected with whatever we were going to work with, and that was primarily malaria. The reason I probably wound up with that section is that I was so fond of Dr. Theiler, and he liked me, and he asked me to work on the malaria project.

Hughes: Was there a formal system of reporting?

Johnson: Yes. The characteristic thing about the foundation's work was that you kept the director informed of everything you were doing. Our malaria section met every day at coffee to discuss our projects, and that's where you really learned. Everybody was getting pieces of information and some of them fitted together. Then again, I was meeting with both sections [malaria and arboviruses], because I would meet with Dr. Taylor regularly on the work I was doing with him. I was interested in the arboviruses, because I'd worked with eastern, western, and Venezuelan virus in the lab in Alabama, so that was already a big interest with me.

Johnson: I also tried to see whether some of these viruses would actually get into malaria parasites in chicks to see whether the virus could survive in the parasite and be transmitted from parasite to parasite. This did not turn out to be the case, but it was a good logical possibility.

Hughes: Tell me about Max Theiler.

Johnson: Well, as I say, he was a good friend. We had a wonderful relationship. When I was alone in New York and could not go to Scituate on the weekends, he'd invite me out to his house, and I'd play his piano, and we used to talk. He loved to read Arnold Toynbee's histories of civilizations, and we would talk about philosophy and civilization and of course about the natural history of viruses. Then at the lab we had a very close relationship with the five of us working together. We had a really good time.

Hughes: His father [Arnold Theiler] had a reputation for being quite an autocrat.

Johnson: Not so with Max. He was a good listener, and he also kept up with the literature completely. He did not do any of the lab work himself, but he was very interested in what everybody was doing and gave them suggestions.

Hughes: Why didn't he do lab work?

Johnson: I don't know. He was interested in the basic processes. He and Dr. Delphine Clarke would plan the drug studies using chickens and monkeys. Loring Whitman was responsible for infecting mosquitoes and providing sporozoites. Loring developed the ten percent red blood cell extract in saline solution that made it possible to keep the sporozoites alive. Delphine did the chicken and monkey inoculations and the drug treatments. There were several excellent technicians who did most of the lab work.

Malaria Research

Johnson: I was doing similar studies of avian malaria, but using chick embryos. I soon developed techniques so that we could inoculate the various stages of Plasmodium gallinaceum malaria in chick embryos. We had very small needles and injected the parasites intravenously into the chorioallantoic blood vessels. So we could inject sporozoites, exoerythrocytic forms, or the blood trophozoites.

Johnson: I kept a serial passage of the tissue exoerythrocytic merozoites on the chorioallantoic membrane of chick embryos. Those exos would go to the liver and brain. The trophozoite stage was passed intravenously, embryo to embryo, more than a hundred passages. I had two technicians whom I trained for this work.

Hughes: Were you originating these techniques?

Johnson: Well, the chick embryo work was essentially new. Dr. Will Downs had started work with Plasmodium gallinaceum malaria in chicken embryos but had not tried drug tests in this system. The technique at that time was putting tissue suspensions of exoerythrocytic bird malaria parasites obtained from the liver or the brain onto the chorioallantoic membrane. Then you'd find the organisms in the blood, liver, and brain. The brain parasites would be like sausages along the blood vessels, big exoerythrocytic forms.

Hughes: Were you trying to cultivate the plasmodium in noncellular media?

Johnson: We did a little of that, and that was done at other labs. But at the time it did not seem to be a suitable system for testing drugs. It was very obvious that you needed to do that in an animal. The two hosts were the avian host and the monkey. In the tests of antimalarial drugs in chick embryos, the drugs were given by inoculation into the yolk sac and the parasite was inoculated intravenously. We used Macaca rhesus monkeys for the study of monkey malaria, called Plasmodium cynomolgi malaria. The cynomolgus monkey was the original source of it. We didn't know it at the time, but that strain will infect man. It was several years later before lab infections occurred from mosquitoes that got loose in the laboratory. But we did not have any lab infections.

Hughes: There wasn't a vaccine?

Johnson: No, and I've never taken prophylactic antimalarials. I want to know when I get malaria. I'd rather have a chill first and then look at the blood. I always had the Giemsa stain in India so we could stain blood smears.

Hughes: Why were you reluctant to be vaccinated?

Johnson: There was no vaccine for malaria. There still is not.

Hughes: Is that because the immunity is so temporary?

Johnson: The problem is to obtain a potent antigen, and there are many antigen fractions. If you have immunity to the blood trophozoites, you can get reinfected, because the sporozoite will multiply in the liver. I proved that by the monkey experiments.

Johnson: We tested monkeys that had survived blood-induced malaria. We injected sporozoites intravenously into them later, and we found the exoerythrocytic forms in the liver just like the ones observed in the regular sporozoite-induced infection. This showed that this is an entirely different immunity, that is, the tissue form versus the blood form.

The whole idea with working with malaria was to find out what is the nature of the disease and the various forms of the parasite, and whether a drug was effective against one or the other forms.

Hughes: How were the drugs being chosen?

Johnson: Well, there were new ones being developed by Dr. L. F. Fieser at Harvard, a biochemist, and others were working on modifications of drugs like atabrine (quinacrine), a 4-aminoquinoline. The British were testing a drug called paludrine (chloroguanide), which turned out to be a pretty good drug for treatment of malaria.

Hughes: That didn't develop during the war, did it?

Johnson: The primary antimalarial drugs during World War II were quinine and atabrine. Atabrine was dropped when they got chloroquine, another 4-aminoquinoline. The interesting thing about atabrine, it turned out to be one of the best drugs for treating fish tapeworm infestation and Giardia parasites.

I had atabrine in my medical kit when I was in Montgomery. I kept it in the top drawer of my dresser, and my daughter Marion one day when she was three years old found the medicines and tried to eat them. My wife called me and said, "Come home quick! Marion's gotten into your atabrine! She's got a whole bunch of pills in her mouth!" So I dashed home, and we got her to vomit. Atabrine is a very bitter drug. You just can't understand why children will try to eat such medicines. At all events, Marion had no ill effects and I was more careful about where I kept the medicines after that. She became a public health nurse.

The new antimalarial drug developed by Dr. L. F. Fieser was a hydroxynaphthoquinone. The material we tested was M 2279. This turned out to be very effective against the sporozoite and exoerythrocytic stages, but it was never tested in humans because it was too toxic. The choice after atabrine was chloroquine and a combination of this drug plus primaquine, an 8-aminoquinoline.

This was the system whereby we could test drugs against plasmodia, avian and the monkey, and find which ones worked the best. Chloroquine was not available at that time. We tested a great variety of chemicals such as paludrine, daraprin, sulfadiazine, atabrine, and quinine.

Hughes: Does it matter in a practical sense that a drug is only effective on one phase of the malarial cycle?

Johnson: It surely does, because the tissue form persists in vivax malaria. People will be in Asia, then they come home, and the malaria recurs. There was one person in California who had been infected in the Orient and spent a weekend at a resort area. He was not obviously ill but had enough parasites in the blood to infect some local mosquitoes. Of course, it took a couple of weeks for them to become infectious, so it was difficult to explain the origin as well as the diagnosis of the few cases of malaria that later developed in the campers. The problem was to find out who in the area had recently been infected with malaria. The exoerythrocytic phase survives in the tissues, in the liver.

Hughes: And it doesn't necessarily express itself in the blood?

Johnson: No, it persists in liver cells. That's one of the things we learned in the monkey studies, and chickens too. They'd have no parasites in the blood, but we'd do a blood passage to see if they were cured. These monkeys looked perfectly healthy, but there usually would be a few parasites in the blood. You'd sometimes inject 10 cc. of blood, and the inoculated monkey would develop a parasitemia. I did splenectomies on some monkeys that had recovered from a trophozoite blood-induced malaria. They would usually develop a more marked parasitemia than after the original exposure.

Our final decision in all the studies was that the drugs alone would not cure malaria. It's a matter of assisting your body to reach a point where the organism would die off. Immunity of the host has to play a part. I believe that's true today, too.

Hughes: Did you work out a specific course of therapy?

Johnson: Well, in the Second World War, they would take atabrine as a prophylactic drug in areas where there was endemic malaria. Then, on the way home, they'd get primaquine, which had some effect on the tissue phase. My contribution was to confirm the nature of the parasitic involvement in the liver cells as the source of relapses. We reported that atabrine, quinine, and primaquine were the best drugs at the time. If you have no host resistance--say, if you were gamma globulin deficient--malaria would kill you. You have to develop your own immunity.

Hughes: Then, with the drugs, you'll have a cure?

Johnson: Yea. At the time of our study, primaquine helped to kill off the exos in the liver and so prevent relapse.

Hughes: But neither is a cure.

Johnson: No. There are three types of malaria parasites. One of the problems with P. falciparum malaria is that you can carry that in the blood for twenty years because the trophozoite stage persists in some persons. Nobody would know it until you are a donor for blood transfusion or you are a drug addict and share needles. Vivax malaria tends to relapse, and there is recurrent clinical disease. In falciparum, you are more apt to die in that early phase of black water fever or brain involvement. You have to have prompt treatment with chloroquine. Now the big problem is that the parasite is getting resistant to some of these drugs, even chloroquine. Quinine is still a good drug.

Hughes: Was anybody trying to make a vaccine?

Johnson: No, not at that time. We were trying to get systems of culturing the malarial parasite outside the body as a source of antigen. Dr. Delphine Clarke and Dr. Charles Anderson were both working on that. What were the necessary metabolic factors that you'd have to supply for the parasite to keep it alive?

One of the strange things was that the parasite did fine without any oxygen. In fact, my big trouble in storing my blood-passage gallinacean malaria [parasites] was the presence of oxygen. I'd just freeze them quick, and I'd be sure that they would be sealed. Just by mistake, later I found out that some of them that were cork-sealed survived. Finally I came to gassing all my specimens with carbon dioxide. They were the ones that did the best; the parasite just hibernated frozen. So a lot of things happen by chance.

Hughes: You mentioned in passing your work with immunity. Do you want to go into greater detail? There was one experiment in the 1950s when you were testing the persistence of the exoerythrocytic cycle.

Johnson: Oh, yes. That was to determine whether the exoerythrocytic form would be there for one month. The original studies suggested that it would appear in the liver for one cycle and then would be gone. But the evidence we got was that you could find that parasite in the liver some months later. We showed that you could have a monkey that had completely recovered from blood-induced malaria, and then when you injected the same number of sporozoites you would do in a primary injection, they would get as many of the exoerythrocytic forms in the liver as if they had never had malaria. So there is immunity against the blood phase, and there is immunity against the tissue phase.

The people that live in highly malarious areas of the world probably get the tissue infection many times by sporozoites, and they are immune to it finally. That's why they've studied sporozoite vaccines. One of the first ideas was to grow the

Johnson: sporozoite in mosquitoes and then make a vaccine out of the sporozoites. There is a stage that seems to be an addition to what you have in the trophozoite. That's the merozoite or tissue form.

Hughes: What is the mechanism for those two forms of immunity?

Johnson: Well, there's a disease in California called relapsing fever. The reason you relapse is that your immunity comes up against the major borrelia organism; there is antigenic variance among the parasite population, which you [also] find in viruses. Then your body develops antibody to the dominant variant, then another one that has been a minority grows, and then you have to develop an immunity to that. That leaves another one still left that's going to grow. It is uncommon to have more than two relapses.

The organisms have an innate variability. The best example of genetic resistance is in flies. You kill them with an insecticide and pretty soon you have flies that are resistant to that insecticide. So there are these great genetic variables in all living organisms.

Hughes: Well, shall we get into the controversy that arose between Dr. [Clay G.] Huff, and you, Colonel [H. E.] Shortt, and Dr. [P. C. C.] Garnham?

Johnson: Well, it wasn't between us, and Shortt and Garnham.

Hughes: I'm putting Dr. Huff in one camp and the three of you in the other camp.

Johnson: Very nice studies were made by Dr. Huff and [Frederick] Coulston on bird malaria. This was the great advantage in having both systems going in our lab. There was a tissue phase, which had been pretty well demonstrated in the canary work in the thirties. Lowell Coggeshall and Dr. Andrew Warren in our lab knew that there was a negative phase in the blood in malaria, in man and even in canaries.

Hughes: Negative in the sense that you could not see it?

Johnson: You could not subpass it from blood.

Hughes: And you couldn't see it either?

Johnson: Well, you couldn't find it anywhere. Drs. Huff and Coulston were working primarily with skin sections taken at the site of sporozoite exposure. They described vascular endothelial cells with parasites in them that looked like toxoplasma.

Hughes: Endothelial cells in the liver?

Johnson: No, in the blood vessels.

Hughes: Anywhere?

Johnson: Well, in the skin. They were working with skin.

Hughes: So it's not specific to the liver at that stage?

Johnson: Well, this is the difference between the avian and the monkey malaria. They had described these exoerythrocytic forms that were similar in appearance to a *Toxoplasma*, which is quite a different organism. Coulston's conclusion was that the bird malaria tissue schizonts of the liver were in the reticuloendothelial cells. In other words, there was an exoerythrocytic form which was in a vessel lining, either in the sinusoid or in the blood vessel, but not in parenchymal liver cells.

Professor Garnham and Colonel Shortt were working at the London School of Hygiene, and both of them had worked on malaria and other parasites in Africa and India. Dr. Garnham had found a liver parasite in African monkeys, which he called *Plasmodium kochi*, which formed large cystic schizonts in the liver. Shortt and Garnham started the study of *Plasmodium cynomolgi* malaria, which is very similar to *P. vivax* malaria in man, to determine whether this parasite infested the liver. If each schizont in the liver had to come from one sporozoite, they needed to feed an enormous number of mosquitoes on a monkey or inject the sporozoites intramuscularly. Otherwise, it would be difficult to find a schizont in the liver sections. They were the first to show that there were these parasites in liver cells.

When I met them at the International Congress of Tropical Medicine and Malaria at Washington, D.C., in 1948, they had concluded that the *P. cynomolgi* sporozoite entered parenchymal liver cells and the exoerythrocytic stage developed there. It seemed important to try to confirm this work and we were equipped to do so. We did most of our work in 1949 and 1950. That's when I really got the techniques worked out. Dr. Whitman had developed a method of keeping the sporozoites alive. He developed an erythrocyte extract to preserve the sporozoites. We used fifty percent normal monkey serum in saline to dilute the sporozoite suspension before inoculation intravenously.

Hughes: What had people been doing previously?

Johnson: They would tease out salivary glands of the infected mosquitoes on a glass slide using a few drops of saline solution and draw up the sporozoites with a syringe and needle and inoculate this into canaries or baby chicks into the skin. The sporozoites were not viable for more than a few minutes. It would take only one viable sporozoite to produce infection. We wanted 100,000 or more viable

Johnson: sporozoites in the inoculum. The technique that Dr. Whitman developed was to dissect out the infected salivary glands of individual mosquitoes in hemoglobin saline, tease them to one side, put a cover slip on, and gently crush them. Then you'd rinse off the cover slip and tissue with hemoglobin saline into a sterile beaker. We also used sterile instruments and cover slips.

We found that the serum tended to agglutinate the sporozoites. The final method was to use about a ten percent extract of red blood cells in saline solution, which we called hemoglobin saline. Hemin is the important component. I used that in tissue culture later. The red cell extract was good for growing the fibroblasts.

So there was a lot of technique to be developed. This was '46-49; it took about three years to work out all these techniques before we could really do good experiments in chick embryos, baby chicks, and monkeys. I had to do some studies of monkeys to brush up on my technique of opening up a monkey and sewing him up properly. The liver is very friable; you had to take very delicate silk stitches to hold the liver together when you took out a little V-shaped piece. The monkeys were anesthetized with sodium pentobarbital given intravenously in the leg vein. I had no mortality or bacterial infection in the monkeys. After closing the abdominal incision, I put antibiotic ointment over the wound, then a gauze pad and three-inch adhesive tape all around the body. The fur was cut and the skin shaved all the way around the body so the tape was attached to the skin. The monkey could not remove it.

Hughes: Were these rhesus monkeys?

Johnson: Yes.

Hughes: What was wrong with using ether?

Johnson: Well, it's hard to maintain good anesthesia, and there is the danger of explosion working with ether where there are electrical devices.

Hughes: Where were the mosquitoes coming from?

Johnson: Dr. Loring Whitman had an insectory where he raised mosquitoes, Aedes aegypti for P. gallinaceum [malaria] and Anopheles quadrimaculatus for P. cynomolgi [malaria]. The intrinsic period was eight to ten days.

Hughes: What do you mean by the intrinsic period?

Johnson: Well, the mosquito takes up the blood containing the gametocytes from the parasitized bird or monkey. The ookinete formed by fertilization in the mosquito stomach enters a stomach cell to

Johnson: form the oocyst. These can be seen in large numbers on the outer surface of the mosquito stomach. When these are mature at eight to ten days, they each release hundreds of sporozoites. They swim around in the body cavity of the mosquito but soon migrate to the salivary glands where they line up in pockets in the gland cells.

One of the things I've seen, which I have sections of but nobody else has reported, they also like to go to the oviduct and pack into the oviduct cells, showing the parasites have alternate routes of infection. Malaria has never had to use the one to go through the oviduct into the egg, but coccidia parasites develop in intestinal epithelial cells.

Hughes: So they are selecting these particular organs?

Johnson: There's a chemotropic factor, some enzyme system, whereby the sporozoites which are released in the body cavity of the mosquito migrate to the salivary gland and then pack themselves in these salivary glands. I have pictures; you can see them in the salivary gland. And then as the mosquito feeds, it sort of pumps up and down, and these actually go into the blood of the host they're feeding on.

Hughes: In these massive numbers, the sporozoites don't interfere with the mosquito's metabolism?

Johnson: Apparently, very heavy malaria infections in mosquitoes probably don't help them any. They can get infected again, as far as that's concerned.

There is another interesting thing we learned about mosquitoes. They have a symbiote organism in the body cavity, which looks like a rickettsial organism. But you really have no trouble with them, because we harvested the sporozoites from the salivary glands, and used antibiotics in the diluent to prevent bacterial infection.

Hughes: Well, now, getting into the problem with Dr. Huff. Didn't staining--?

Johnson: This is very similar to what my experience was with rabies. The early tissue studies with rabies were done with hematoxylin and eosin stain on formalin-fixed material. The staining technique, by the way, is really a histochemical method. The wonderful thing about the giemsa stain, which is azur A and azur B and then methylene blue and eosin, is its ability to stain histochemically DNA and RNA. Actually, Dr. Hans Ris was one of my friends working on DNA and RNA at the Rockefeller Institute, with Dr. [Oswald T.] Avery and his associates. The Feulgen stain is a histochemical stain for DNA, and Ris was studying rickettsiae with this stain to demonstrate the presence of DNA in that organism.

Johnson: The staining techniques are histochemical tests. Dr. Huff and Coulston were using a type of stain that just does not stain the tissue forms or even the malaria parasite very well. Like in rabies, the new stain that was developed to replace H and E [hematoxylin and eosin] was the basic fuchsin methylene blue stain. Basic fuchsin stains the Negri body in rabies very well, and its inner structure is shown by methylene blue. Psittacosis and other agents in the chlamydia group can be stained with special stains, like the giemsa stain and basic fuchsin and methylene blue.

So the trouble with Huff and Coulston, they got a sort of vague color effect on this schizont they saw in endothelial cells. They were very poorly shown.

Hughes: Using the same staining technique?

Johnson: They were using the formalin-fixed, and then staining with hematoxylin-eosin.

Hughes: Huff mentioned, in the letter you gave me, the Maximow method.*

Johnson: Maximow stain is a hematoxylin-eosin-azur stain. Using H and E or Maximow's stain, you would not see the malaria parasite in the blood cells. You would see some pigment. The key of the Shortt-Garnham technique was fixation in Carnoy's fixative (glacial acetic acid, chloroform and absolute alcohol, 10-30-60), staining with giemsa, and decolorization in acetone. That keeps the color of your DNA and RNA, the same as with the methyl alcohol-fixed blood smears stained with giemsa.

So what you see in the section then is exactly the same thing you see in the smears of tissue schizonts stained with giemsa. You would make a smear of the brain, and there would be those beautiful exoerythrocytic forms. The blue would be the RNA and the magenta would be the DNA.

Hughes: The crux of the controversy between the two groups, as I understand it, was that Huff was maintaining that the exoerythrocytic stage only appeared in the endothelial cells?

Johnson: Right.

Hughes: Was he saying that simply because the staining technique didn't allow him to see the exoerythrocytic stage elsewhere?

* Letters to the editor. Tropical Medicine News 1950, 7:22-23.

Johnson: Yes. He couldn't see them in the liver. I saw them in the liver in the chick embryos and in the baby chicks. But you have to have very good sectioning technique, and you have to fix it so that the staining characteristics are the same as for the blood parasite.

Hughes: And he wasn't doing that.

Johnson: No. He didn't see them in the liver cells. He said they were just in the reticular cells lining the liver sinusoids.

Hughes: By saying that they were in the reticular cells, he meant that they were phagocytized?

Johnson: Yes. They would be phagocytized by the endothelial lining cells of the sinusoids in the liver and the blood vessels of the brain. It's true that the P. gallinaceum parasite will form big schizonts in the brain in the lining of the blood vessels. That parasite has developed a different ability than the mammalian plasmodium. However, the avian sporozoite also invades liver hepatic cells.

Hughes: Aside from the staining technique being different, there was also the difference that Huff was working on avian malaria. In that letter, he mentions just avian malaria.

Johnson: Yes. Their primary studies were done in avian malaria.

Hughes: Would that have thrown him off?

Johnson: Oh, sure. The malaria of primates is different. And the thing about the Garnham-Shortt studies is that they had chosen to look for a liver parasite, because the Plasmodium kochi of monkeys in Africa developed a primary schizont in the liver parenchyma. They knew from subinoculation studies that the parasite was not in the blood for six, seven, or eight days.

I injected the P. cynomolgi sporozoites into the skin of a monkey. I also injected some directly in the liver. I marked the site with a silk suture so I could go back and take the section out of where I put it in the liver. At forty-eight hours I found no parasites in the skin. But in the sixth day biopsy of the liver, I did. But I also found parasites in the other lobe of the liver as well as in the one where I had marked it. Having injected several hundred thousand sporozoites intravenously, it was easy to find the large schizonts at six to eight days.

But the real big controversy at that time was that Dr. Coulston received slides from both Dr. Shortt and me, and he said that the only thing he could find was clumps of blood platelets. That was the reason why Dr. Shortt and I both went to Cincinnati to show Dr. Coulston our liver sections stained to show the parasite in liver cells.

- Johnson: I put one of my sections on the microscope in his lab showing a schizont in a liver cell with a vacuole in the parasite and the cell and nucleus enlarged. He said, "If that parasite develops first in the liver, we are wrong in what we've done." I said, "Well, they are not blood platelets."
- Hughes: Did you show the slides to Dr. Huff?
- Johnson: Dr. Huff had seen our sections, and he said he didn't accept Shortt's or my interpretation.
- Hughes: How was it resolved?
- Johnson: Well, Huff sort of dropped out of the malaria work. We had finished ours in the latter part of my time at the New York lab, when I was doing more with arboviruses. We got the information we wanted out of the malaria studies. Our confirmation of the findings of Shortt and Garnham appeared in the 1949 annual report of the International Health Division of the Rockefeller Foundation. Shortt and Garnham later described the liver schizonts of P. vivax and P. falciparum malaria of man.
- Hughes: You showed me the photographs and description of your malaria research that you produced in 1954 for the American Society of Tropical Medicine and Hygiene.* But I didn't notice anything else that you published on malaria.
- Johnson: On malaria, no. My function in pathology was to feed in diagnoses and slides for them to see. In the annual report of the Rockefeller Foundation, every year there is something about each section--what we did. I'd write a report for that. So that is published, in a way, but not under my name. In other words, that is from the section. In the reports from the International Health Division, every year they would have the work that was going on in the different places, what were the major developments at that time.

They had worldwide work on other diseases, like field malaria control, hookworm control, and all sorts of parasite control work. I mentioned before that ninety-nine percent of lab research is more or less routine work. Only once in a while does something come up that really is a new development or an incidental finding and that is reported. Otherwise the field work is described in the annual report.

* H. N. Johnson. Description of the developmental stages of avian and simian malaria. Proc. Am. Soc. Trop. Med. Hyg. 1954, 4-5.

Hughes: Compared to institutions nowadays, it sounds to me as though the Rockefeller Institute was fairly undemanding as far as publications were concerned. That wasn't the way you got ahead?

Johnson: No. The [point] was to answer a question and provide information for disease control.

Hughes: And they could care less whether you published?

Johnson: Right. That was not a big thing. Look at the few papers that Dr. Theiler published. During the malaria studies, he published one. And Loring Whitman published one on the viability of mosquito sporozoites, and Anderson published none. I gave a paper at the Federated Societies* meetings on the pathology of the new viruses in chick embryos. I said there was nothing there that deserves a paper, because all you find, when you put the viruses in the brain, is encephalitis. There was no specific pathology in the other organs.

To go back to Huff and Coulston: They had done very useful work on malaria. The Plasmodium gallinaceum was a good model for malaria research. In our work, I was able to see that, in addition to being in the endothelial cells in the brain, the parasite could be found in endothelial and parenchymal cells in the liver. There were schizonts of avian malaria in the lung in groups of endothelial and alveolar cells. But in monkey malaria, the primary tissue form that would survive and serve as the source of the first erythrocyte infection was the large schizont in the liver. They would develop from a small one, one third of the size of the liver cell. The earliest I found was five days. Dr. Shortt and Garnham had some they thought were three to four days. The schizont, as it enlarges in the liver cell, forms almost like an oocyst. It will probably have a thousand merozoites, so that when the one ruptures in the liver, it will infect a thousand red blood cells, and then you know that in a very short time, millions of red blood cells will have trophozoites in them.

The P. cynomolgi malaria parasite is not very pathogenic for rhesus monkeys. In fact, my biggest problem with monkeys was when I did splenectomies. We know that is a way to reduce the immunity of the host. If you want to find red blood cell parasites called theileria in cattle, you take out the spleen. Then the parasites appear in the blood.

So one of the things I did was to take monkeys that had had a sporozoite infection, and then months later I would take a piece of the liver and take out the spleen too. The splenectomized animal would have a higher parasitemia than it got during the primary exposure. So there is an immunity factor in malaria.

*Federation of American Societies for Experimental Biology.

Hughes: You were activating a latent infection?

Johnson: Yes, it increased the number of malarial parasites.

Hughes: You were simply removing some of the cells that made antibodies?

Johnson: Yes. Actually, when making monoclonal antibodies, we use splenocytes. That's the source of lymphocytes. Once activated by the antigen of a parasite, they continue to produce antibodies to that parasite. To make monoclonal antibodies, you immunize mice by repeated doses of tissue material containing the parasite antigen. Then you take the spleen and prepare a suspension of the lymphocyte-like cells. Then you fuse them with the mouse myeloma cells by the use of polyethylene or diethylene glycol, which is actually Prestone. It's fifty percent; you would think it would kill the cells, but it doesn't. But it makes the cells lose their membrane, and some will fuse together. Some of these cells will have part of the lymphocyte from the immunized mice and part from the tumor cell, and as the hybrid tumor cells grow, they produce the antibody previously made in the spleen of the immunized mouse.

We have the B-lymphocytes and T-lymphocytes. They're the two types. In the spleen, you have a large lymphocyte population. So the spleen has a real job to do in immunology.

Hughes: In the late forties and fifties when you were doing this work, practically nothing was known about the different subtypes of lymphocytes, was it?

Johnson: No. Of course, I had been working with lymphocytes for my master's degree in the twenties and tried to grow them and see whether they could go on to develop macrophages or other cell types.

Hughes: Were they subdivided into types in the twenties?

Johnson: Well, the lymphocytes I worked with were from lymph nodes, to see how they would migrate out from them. The cells that migrated out looked like macrophages or monocytes and some contained large granules. Another type were the fibroblasts.

During 1950, I finished my malaria studies. I had isolated a sarcoma virus from one of the chickens kept for malarial studies and carried out an investigation of this virus and erythroblastoma virus of chickens, obtained from the Bureau of Animal Industry. I had been doing the pathology studies of the viruses isolated by the Rockefeller Foundation field staff in Africa and South America. This was presented as part of the serological studies at

Johnson: the Federated Societies meeting in 1951.* I had worked with eastern, western, and Venezuelan encephalitis viruses in the Alabama Rabies Study, and Dr. Taylor and I often discussed the need of global studies of wildlife viruses.

Starting in 1950, Dr. Taylor organized some field work in Florida. He wanted to see if he could get a good way to control the bacteria in the mosquito suspensions processed for testing. We had worked out the amount of streptomycin and penicillin needed to inhibit the bacteria in the mosquito without toxic damage to the baby mice inoculated intracerebrally. We also had to have serum or bovalbumin to protect the virus. The minimum amount of fraction V bovalbumin was 0.75 percent in phosphate buffered saline. Streptomycin at 2 mg/ml and penicillin 1000 mg/ml was needed for processing mosquitoes. It is also preferable to use ten percent normal serum in the diluent. For preparing tissue suspensions we used half as much antibiotics and only the 0.75 percent bovalbumin diluent. Dr. Taylor used one- to two-day-old mice in his studies of throat swab specimens from his respiratory virus study at Coxsackie, New York. He isolated some Coxsackie viruses and I studied the pathology produced in the infant mice. The muscle lesions developed rapidly, and calcium was deposited in the degenerating muscle within twenty-four hours of the onset. It is clear that the Coxsackie enteroviruses produce a systemic disease. So before the field work started, we had a good technique for testing mosquitoes in newborn mice.

Two factors precipitated the change in the virus program in 1951. The first was the retirement of Dr. Taylor; the other was the award of the Nobel Prize to Dr. Theiler. Dr. Theiler became director of the New York laboratory, and Dr. Taylor went to the U.S. Navy Medical Research Unit, NAMRU 3, to develop a virus program there. Dr. Taylor had advanced a global program for studying arthropod-borne viruses, and Dr. Hugh H. Smith and Dr. Theiler had visited potential sites for field laboratories. The International Health Division was incorporated into the newly reorganized Division of Medical and Natural Sciences. A decision was made to establish field investigation units in representational zoogeographical areas of the world. The first of those was Poona, India, a cooperative project of the Rockefeller Foundation and the Indian Council for Medical Research.

*A systematic study of certain viruses isolated in the tropics of Africa and South America. Proc. Fed. Am. Soc. Exp. Biol. 1951, 10:44.

Tissue Tropism of Viruses*

Johnson: When I got to India, and also later at the California lab, the first thing I tackled is what I called the tissue tropism of viruses. What organs do viruses like? Well, they like the salivary glands; they like the pancreas; they like the lung; they like the kidney. And why are kidney cells so good for growing viruses? Because that's the normal place they go to. The target organ for some viruses is the brain, that is the encephalitis viruses, but that's secondary. Ordinarily, viruses don't produce encephalitis and kill the natural host. The target organ in the dog strain of rabies is the salivary gland. But if you get into the natural host, like the skunks, the lung plays a big part.

So all the viruses like certain organs to grow in. I did not see that in the pathology, because everything was injected in the brain, and that was the place where the virus developed. One virus that likes the salivary gland is mumps virus, a human salivary gland virus. It sometimes invades the central nervous system. Herpes type I is primarily a salivary gland virus. Viruses like the kidney. They also like the mammary gland, so there's a lot of transmission in young animals in natural history where the virus actually goes through the milk.

Hughes: What is there about these regions of the body that is conducive to viral growth?

Johnson: I think it's the high metabolism of the epithelial cells in these organs. Polio virus likes the intestine. You're always shedding millions of cells off the intestinal wall every day, so they're growing fast; the virus likes that. The intestinal viruses, like the Coxsackie viruses and polio viruses, produce their primary infection in the intestine. Then you have respiratory viruses, the flu viruses. Well, alveolar cells in your lungs are wonderful targets; they're right there on the surface.

The trouble when I first started working on rabies was that everybody thought that rabies and herpes and polio were strictly neurotropic viruses; they would only grow on nervous tissue. I really didn't get complete information on the target organs of rabies virus until I came to work in California with skunk and bat rabies.

Hughes: So you came away from the Rockefeller still believing the myth that rabies was a strictly neurotropic virus?

* One of Dr. Johnson's prime interests, he returned repeatedly to this subject.

Johnson: Well, no, because I had already shown in rabies that the salivary gland had more virus than the brain in the natural disease and also that rabies in dogs would get into the pancreas and the kidney and the mammary gland. In California, I got the rabies virus out of the mammary gland of a spotted skunk. Then I went from that directly to tissue culture and to inoculating other spotted skunks I captured, then subpassed the virus by intramuscular inoculation. This showed that the mammary gland probably is a way where the mother skunk can transmit rabies to the young. We've always wondered why, if a dog comes down with rabies and has very young pups or is giving birth to pups, almost every pup gets rabies. With any other animal, the young will almost always come down. They get infected right after birth from either the saliva or the milk of the mother.

Hughes: Can you trace that neurotropic belief in rabies back to Pasteur?

Johnson: Yes, the original idea of the trephine was to be able to pass the virus of rabies and get a consistent take. [Emile] Roux was the first to get that idea. He'd actually make a trephine hole with a sharpened awl or something in the skull of the rabbit and then put the needle through there and injected the rabies virus directly into the brain. Then they would get a consistent infection and a high titer of virus. They later used that method of inoculation but used the spinal cord as a means of getting attenuation by drying the cord in a jar containing potassium or sodium hydroxide. They knew the saliva was infectious as early as 1804 in experiments based on allowing rabid dogs to bite normal dogs. But the general idea I think all the time was that, sure, it was in the saliva, but once they found that they could not get it out except out of the brain, they concluded the virus must be leaking out of the nerves.

When I was working on infectious diseases at Cleveland City Hospital, Dr. Toomey was trying to prove that you could infect monkeys by injecting polio virus into the intestine and, as I said, they would not let him publish his paper. The reason he started to use this technique, he said, "Why was it that in Amsterdam, Pennsylvania, when they had an outbreak of polio, the people who lived on the one side of the tracks in nice, clean homes got it, and the people that lived on the other side of the tracks, who were not careful about personal hygiene, didn't get it?" It seemed like the ones that had poor hygiene were protected. Well, they were the ones that got infected, early in life probably, when they still had immunity from the mother. That was true all over the world. The earliest polio epidemics that were described were in Sweden, a country known for personal cleanliness.

Hughes: The Rockefeller was one of the strongholds of the neurotropic theory?

Johnson: Yes. Dr. Tom Rivers was the authority on the nature of neurotropic viruses and that was his belief.

Dr. [Ernest W.] Goodpasture, professor of pathology at Vanderbilt University in Nashville, Tennessee, worked with rabies virus, and Dr. J. R. Dawson worked with Webster on rabies and then went to the department of pathology at Vanderbilt. I discussed the role of the salivary gland in rabies with Dr. Goodpasture. I said, "Look, I can weigh the salivary gland of a dog, and I can get more virus by weight out of the salivary gland than I can out of the brain." He said, "Oh, it's in the central nervous system, and it just leaks out of the nerve endings." All three of these virologists also worked on herpes simplex virus and considered it to be a strictly neurotropic virus. Well, I could show sections where there was acinar degeneration and infiltration. But actually, rabies doesn't damage the tissues very much. Even the brain cells look pretty good when a rabid animal dies of rabies. So looking for the lesion is not the key.

Actually, what we know now is that you can have absolutely normal looking kidney cells, and you can be excreting cytomegalovirus in the urine at a high titer. But those cells are still functioning, and they are intact. Most of the viruses we deal with today apparently do infect the kidney, even in influenza A. (Apparently, it also causes kidney disease during epidemics.) I saw that when I was in the Brigham Hospital in Boston during the 1933 epidemic. We had a typical influenza infection in a young male on my service when I was house officer at Harvard. This young man developed a shutdown of his kidneys and almost died of hypertension and anuria, but he recovered. It was just clearly tied in with the influenza infection which he had.

All the hamsters and mice sent to me [in the pathology section at the IHD] that had been inoculated by people like Smithburn with these various viruses had no specific pathology in the salivary glands, liver, lung, or kidney. But when the virus had been inoculated in the brain, there would be evidence of encephalitis. There was really no new information here. It did fit in with the old neurotropic idea. Characteristically, when you first put it into the brain, the virus immediately is selected for the virus particles that like the brain.

Let me digress and tell about the Modoc virus I isolated in California in 1958. In the first passage of this virus in newborn mice, inoculated intracerebrally, the baby mice would get sick on about the sixth to tenth day. If you did not kill them, they would recover. The ones that were a little sick when examined, would show polys [polymorphonucleocytes] and acute degeneration in the brain. In the second passage the virus would kill both adult and baby mice through 10⁻⁸, one to a billion dilution; a fantastic amount of virus. This shows the selection of a virus population

Johnson: that prefers to grow in the brain. But in the [natural] cycle the virus population prefers the lung or kidney. I think it's true of all the viruses. That's the reason I've tried to get non-neuroadapted strains of all the viruses that I've worked with, not to pass them in the brain at all, and so keep the natural tropism.

It was the opposite from what I was trying to do with the rabies virus in the avian host in Alabama. The reason I used intracerebral injection of baby chicks was that I had found out that the Pasteur strain did not get into the salivary glands. I could inoculate twenty-five mice intramuscularly with the Pasteur-fixed virus, and as they became ill, I took out the salivary glands. I got mouse herpes virus out of some of them, but I did not isolate rabies virus.

I wanted to have a [rabies] vaccine strain that did not get into the salivary glands. Apparently the brain-fixed strains do not invade the lungs, kidney, or salivary glands. They like the brain. So I decided to pass the Flury strain virus in the brain of chicks, and I did it just like Pasteur passed rabies virus in rabbits. At first, the chicks wouldn't sicken until twenty-five days after inoculation. If you waited until they sickened, then there was usually no active virus in the brain. So I decided to pass the virus at ten days. The virus would be already there in ten days, and when they sickened at ten days, I shifted to eight days, and then six days, and finally I had a fixed virus. That was the virus which did not seem to be pathogenic for dogs when inoculated intramuscularly. So when I was leaving Alabama, I reported that I believed this would be a good vaccine virus. You can use it either live or killed. I had made a good killed virus vaccine from chick brain. It was a good antigen, and it was an American virus, not French, like the Pasteur virus, and it did not invade the salivary glands.

I did not do extensive studies of the commercial Flury vaccine strain, but others have. In no instance did they get the Flury virus out of the salivary glands of vaccinated dogs.

Again to digress and mention an incident that might have been regarded as proof for the Flury avianized rabies virus being able to invade the salivary glands. In 1956 Dr. Orland Soave decided to test some commercial Flury LEP rabies vaccine. He injected the mice intramuscularly, and when they developed paralysis he harvested the salivary glands and injected a suspension of the salivary glands into adult mice intracerebrally. He called me later and said, "I've got it. It's in the salivary glands! Your vaccine virus!" I said, "I'll be right over." I looked in the box, and here were these mice with typical Traub's sign, a spastic convulsion with extension of the legs. I said this had to be LCM virus. People will do something like that, and they say, well, that's it, and they won't even do a specificity test. But that

Johnson: could have been published saying Flury LEP virus was in the salivary glands. Orland was satisfied with my conclusion and we later identified LCM virus in the mouse colony.

The Pathology Section

Hughes: Why don't you tell me about setting up the pathology section at the New York laboratory?

Johnson: First, let me say something about the typhus study section at the New York lab in 1946. Dr. Fox had a good offer as professor of epidemiology at Columbia, and Dr. Jack Snyder was offered the deanship of the Harvard School of Public Health. They decided they were leaving the foundation, and that left the typhus lab space for another project. It became Dr. Smithburn's serology department, a big lab. He had the biggest staff, because they had to run large numbers of neutralization tests, using mice. They used them by the hundreds every day.

I didn't mention the fact that we had to have a mouse colony. The Rockefeller Foundation mouse colony was one of the best, because they had gotten rid of the mouse polio virus. The study of this virus provided basic information on the nature of human polio virus.* Several scientists worked with Dr. Theiler as a major study on this disease. So we had to have a good mouse colony, which meant that we had to get rid of all the [extraneous] viruses we could find.

The thing that I've got to mention, because it never was published, is that when you have a big research building, you can be sure you have house mice somewhere. That was true of the Rockefeller Institute in Princeton; that was true of the Rockefeller Institute in New York; it was true in almost all big biological laboratories around the world. You have animals, you have animal food, you have a house, and you have house mice.
[laughs]

I had this unit at the New York lab built almost germ-free. I had a special egg room, where there was a tight seal for the door; it had a ventilator in the ceiling. I had ultraviolet light there so I could sterilize the room, and it was just a wonderful place to work with tissue culture and chick embryos. Well, one

* M. Theiler. Spontaneous encephalomyelitis of mice, a new virus disease. Science 1934, 80:122.

Johnson: day after we had had that lab going about eight months, I pulled open a drawer and there was a mouse with baby mice. [laughs] It had come through a very narrow place in the vent in the ceiling.

At that time, I did not know the source of the epidemic of lymphocytic choriomeningitis in monkeys at the lab in 1938 when I was there. We knew it was lymphocytic choriomeningitis, and Dr. Lowell Coggeshall claimed he transmitted it by mosquitoes. But what he really had was lymphocytic choriomeningitis virus [LCM] in the guinea pigs that he was using for his research. It was published in Science magazine.* We know now that LCM virus does not multiply in mosquitoes.

Hughes: What did you do?

Johnson: We did not test the mouse I found in my lab. We did close all possible entries in our lab for mice.

Well, another thing that happened at that time, we had a woman, Mrs. Compton, who was caretaker for the monkeys, and she came to me one day and she had what looked like a boil on her neck. I made a smear of it, and it didn't look like it was a staphylococcus infection. A rickettsial disease had been discovered that was called Queen's fever. Dr. George Hirst, director of labs at the New York City research lab at that time, was working with this rickettsia of Queen's fever, which is called rickettsialpox. Mrs. Compton must have been bitten by mites, because the blood specimens we took were tested by Dr. Hirst and they showed she had been infected with the rickettsia of Queen's fever. It turned out that this rickettsia is transmitted by the mites parasitizing mice; it's a mite-borne rickettsia. This is other evidence of the presence of house mice at the Rockefeller Institute.

Hughes: Well, let's get back to the pathology section. You mentioned that you weren't working just with Max Theiler and his group but also with Dr. Taylor.

Johnson: Yes, I was the pathologist for the New York laboratory.

Hughes: Anybody who needed pathology would--?

Johnson: Would bring me the specimen and we would process it, and then I would have my technician cut it. I'd look at it and give my opinion, and they could have the sections. I still have lots of these slides, which I've got to throw away.

* L. T. Coggeshall. Transmission of lymphocytic choriomeningitis by mosquitoes. Science 1939; 89:515.

Hughes: Do you want to mention the work you did with Noguchi's and Stokes' tissues?

Johnson: Yes. One day Dr. Theiler brought me two bottles with formalin-fixed tissues, and they had been taken at Dr. Adrian Stokes' autopsy in 1927 and Hideyo Noguchi's in 1928. I imagine that Hudson probably made some [slides] in the Africa lab. But all that was there at the institute were these formalin-fixed tissues. I processed this material. They both had characteristic yellow fever lesions. Also, people sent me liver specimens to be examined for diagnosis of yellow fever.

The lesion in the liver in yellow fever is so characteristic that it is almost diagnostic. Dr. Elsmere Rickard was the first to develop a viscerotome, which was used by public health officials in South America if there was a suspected case of yellow fever. This was a little gadget which was originally used for sampling coffee beans from a bag of coffee. You'd make a hole, the point would just go through, and it would fill in afterwards. You'd turn a handle and you'd cut out a little block [of tissue] about the size of a coffee bean or two.

Hughes: Right through the skin of a dead person?

Johnson: Yes. It would not make an incision, so they wouldn't call it an autopsy. They would put the specimen of liver in formalin. Formalin is okay for that. They developed diagnostic laboratories, and the main one was at Bogota, Colombia. They would get large numbers of specimens every year, and then they would report on whether they were positive for yellow fever. It was like the Negri body test for rabies. The characteristic lesion was, the liver cells would degenerate around the central vein, a midzonal necrosis. There was sort of a degenerate type of cell, called a Councilman body, which was just acute hydroptic or acidophilic degeneration of a liver cell. Then there was also an intranuclear inclusion in liver cells. It is different from the intranuclear inclusion formed by herpes simplex and varicella viruses.

The pathology service ended when I left for Poona. Dr. Jordi Casals, who had worked with Webster, joined the IHD staff in 1951. He ran the complement fixation tests. When I was in India, Smithburn was still at the central lab. Blood serum specimens were sent to him for tests for antibodies to the viruses already isolated, and he would send a report, usually within two months, on which human sera neutralized any of the viruses available at the New York lab.

The pathology lab was important. For instance, I described the lesions of influenza virus in all the different host systems, particularly in the chick embryo studies for Dr. Taylor, including the newly discovered influenza C.

Rockefeller Personalities

Hughes: Well, before we leave the Rockefeller, would you like to say something about personalities?

Johnson: Dr. Herbert S. Gasser was the director of the institute the whole time I was there. Dr. Gasser was a physiologist and a very intelligent person. He had a wide interest in all biology. He was a good director. He was a good man to get the right people and was kind of a recluse. He continued to do research in his own laboratory after his retirement in 1954.

You asked about Dr. Thomas Rivers. I used to have lunch with him frequently. He was director of the Rockefeller Hospital. We talked a lot about rabies. I wrote the rabies chapter for each of the editions for his book, Viral and Rickettsial Infections of Man. Dr. Rivers died in 1962, and then Dr. Frank Horsfall and Dr. Igor Tamm were the editors. That was the last edition. That still is a good book, and if you look at the names who wrote chapters for this and previous editions, you will note that they were the leading virologists of that period. A very interesting group of people.

Dr. Rivers was a very stimulating person, and he would guide a lot of the people there in the research that they were doing. Dr. Rivers was a very good pathologist and physician. His chapter on general aspects of viral and rickettsial infections is in the first edition. You will note his extensive knowledge of the nature of this group of diseases.

Hughes: A very strong-minded man.

Johnson: Oh, very strong opinions, but very fair, and I enjoyed him in every way. We've talked about K. F. Meyer; Rivers was that type of person. He had a wide-ranging mind, very fertile mind. He was a wonderful one to guide people or pick people to work on projects.

Dr. Rivers' first really big viral project was chicken pox, and he wound up isolating a rabbit herpes virus and thinking it was the cause of chicken pox, but that did not prove out. That was many years before anybody was able to grow chicken pox virus in tissue culture, like Dr. Tom Weller [eventually] did.

Herpes viruses in general are very slow-growing in tissue culture. They form syncytia, and you have to wait sometimes for two or three weeks before you see these little groups of nuclei, a syncytium. Now we have fluorescent antibody, and you can see the virus-infected cells. In tissue culture of influenza virus, the cells may appear normal but if you add a suspension of guinea pig

Johnson: red blood cells you find that there is hemadsorption to infected cells. The specificity test is staining the cells with fluorescent antibody to influenza A or B.

Hughes: You mentioned that you had lunch at the institute each day.

Johnson: The Rockefeller Institute was a wonderful place to work because there was a decision on the part of the Rockefeller Institute and the foundation that they would subsidize the lunches to get people to eat there. So you would pay a very modest amount for your lunch. They had one of the best medical libraries in the country near the lunch room.

Hughes: To get you to eat there so you would then talk to the people around you?

Johnson: Right. So everybody had lunch there. It was a very wide range of people at the time working on different biological problems.

There was Dr. Oswald T. Avery and Dr. Maclyn McCarty. They were among the earliest to work on the RNA and the DNA extracts from the pneumococcus--basic work which led to a lot of subsequent work on DNA. Dr. Avery was a very wonderful, intellectual person. He had been recruited for the Rockefeller Institute staff in 1913 by [Simon Flexner] at the suggestion of Dr. Rufus Cole of the Rockefeller Hospital.

Dr. Peter Olitsky was one of the great biologists of the United States. His range of interests was everything from polio to rabies. He was one of the first to isolate rabies virus from the patients in Trinidad that were infected by vampire bats. He received a spinal cord specimen from a person that had died of a strange type of polio and injected the specimen intracerebrally in monkeys and it was rabies. They said it couldn't be; they hadn't had any dog rabies in Trinidad for fourteen years. But he was the one that made the diagnosis.

A similar specimen had been sent to the Lister Institute in London. They also isolated rabies virus. That was the beginning of the study of vampire bat rabies in Trinidad. The cases were like my own illness--an ascending paralysis with no evidence of hydrophobia or anything like classical rabies.

Hughes: You mentioned Peyton Rous and Rene Dubos.

Johnson: Rene (Ronnie) J. Dubos and Peyton Rous became friends [of mine]. I remember writing to Dr. Rous after he got the Nobel Prize. He had been working with chicken sarcoma in 1911, 1912, and I was so glad that he got recognition, because it was deserved. It is a cancer virus.

Hughes: At the time when he was doing that work, it wasn't the thing to talk about the viral cause of cancer.

Johnson: No.

This chicken sarcoma virus I isolated at the Rockefeller Institute was a secondary problem which Max Theiler was interested in letting me play with. We clearly showed that this virus, which we now know is like AIDS, is a retrovirus. It causes sarcoma in 100 percent of chickens. I developed an intracerebral neurotropic passage of the sarcoma virus in one- to two-day chickens. They develop a rapidly fatal encephalitis without tumor formation. The AIDS virus produces a similar encephalitis in babies infected by the mother.

One of the things I showed is how resistant the sarcoma retrovirus was to heat. I would seal the lyophilized virus in nitrogen in a tube, and I could boil that tube for an hour, and it would still be alive. I had some of that lyophilized and kept it in the refrigerator for twenty-some years, and it was still viable.

Hughes: Is that true of the AIDS virus?

Johnson: I imagine so, but I don't know. Retroviruses are pretty resistant agents. So in making vaccines, they're going to have to be very careful about inactivation of the virus.

Hughes: Who was Eugene Opie?

Johnson: He was one of the original members of the Rockefeller Institute in 1904, together with Samuel Melzer, Phoebus Levine, Hideyo Noguchi, Joshua Sivert, and Jacques Loeb. Simon Flexner was the director and continued to occupy this office until he retired in 1935. All of these men were devoted to research and "to apply knowledge to the prevention and alleviation of disease."

Dr. Opie worked with malaria in the 1890s and was first to show the fertilization of the ookinete in the stomach of mosquitoes. When I was at the institute, he was interested in the chronic diseases of chickens, like Marek's disease, which turned out to be herpes virus of chickens. He was interested in lymphomatosis of chickens. I was too, because in the work with malaria, I got involved with the avian sarcoma virus and also erythroblastosis virus. The latter virus, when injected intravenously in the chicken, caused death from erythroblastosis, like leukemia, within a month.

Several studies were made at that time at Sloan-Kettering and Lederle Laboratories, using these two viruses. If you injected the flavivirus, Russian spring-summer encephalitis, after

Johnson: the avian viruses, it would save all the chicks from death. We still don't know exactly how this process works, but it was the erythroblastosis virus of chickens and a sarcoma tumor of chickens which were prevented by inoculation of a flavivirus.

That led to other studies at Sloan-Kettering, where they would inject certain of the arboviruses into the tumors of people with cancer to see if the virus would stop the growth of the cancer. There was no curative effect.

So Dr. Opie was interested in leukemia and lymphoma of chickens. We had no concept at that time that there would be something like EB [Epstein-Barr] virus, herpes, which would cause lymphoma in people and lymphatic leukemia of children. Dr. Sidney Farber, my chief when I was resident pathologist at the Children's Hospital, was professor of pathology at Harvard. He was testing methotrexate for the treatment of leukemia in children. It would knock down the total population of the lymphocytes in the blood and produce a remission.

You mentioned Ronnie Dubos. Well, everybody enjoyed talking to Ronnie. He was studying the action of streptomycin and other antibiotics on the tuberculosis organism and how the bacterium developed resistance to the drug. I enjoyed talking with his assistant, Cynthia Pierce. She had had polio and was handicapped. She did all her work in a wheelchair. They were working on cultivation of low-virulent strains of tuberculosis, swine tuberculosis, bovine tuberculosis, and human tuberculosis.

Ronnie Dubos was interested in antibiotics. He had developed the antibiotics gramicidin and tyrothricin, the early antibiotics. They did not turn out to be safe for use in man, but it was the beginning of a study of all kinds of bacteria and fungi which produce antibiotics.

Hughes: That was post-penicillin?

Johnson: Yes, but I do not remember the dates. His gramicidin came about the same time as streptomycin. I did some studies during the forties of organisms from the floor of the lab and, just for fun, to test to see which ones would have an antibiotic effect on cultures of staphylococcus organisms.

An interesting thing is where some of the major antibiotics have come from. I've been in the cemetery in Missouri where they got the original strain of fungus that produces aureomycin. One of the ideas was to look for undisturbed natural soils to get antibiotics.

Johnson: Streptomycin was the antibiotic that we were most interested in at the time I was in New York, which was '46 to the end of '51. We used penicillin and streptomycin to prevent bacterial contamination in our studies. But Ronnie Dubos said that he felt, even at that early day, that although this antibiotic was an effective drug for tuberculosis, TB was a cultural disease problem, a combination of stress, crowding, and diet--the lack of vitamin C and D, because you can't encapsulate the tubercle bacillus unless you can lay down collagen around it. If you don't have vitamin C, that bacillus is going to spread. It's as simple as that. It wasn't only the sunlight [that aided recovery]--sending TB patients down to Arizona--but also the fruit and other foods. If you get in a poverty-stricken area where people live on nothing but bread and meat, well, they're going to die of tuberculosis.

Some of the early work of [William G.] MacCallum, who traveled around the country on a presidential request, proclaimed that meat and potatoes are not enough to maintain health. You have to eat vegetables. And, of course, a lot of the rural people do. Even rutabaga, potatoes, cabbage, and onions contain vitamin C.

Another factor [in tuberculosis] was getting enough calcium. With low calcium, they won't calcify the lesions. If you don't have enough vitamin C, you don't lay down collagen. If you don't have A or D, you can't have the healing effect of the lung. Tuberculosis reduction around the world, as with leprosy, has been largely due to a cultural change. And I think it's the same way with stroke. People are not getting enough calcium, and their blood vessels change largely from that.

The food in the United States is the best in the world. We have fresh vegetables and frozen vegetables any time of the year; anybody can have them. The problem is what food and drink is consumed. Chronic calcium and magnesium deficiency seem to be the major cause for arteriosclerosis, that is, secondary hyperparathyroidism, with temporary deposition of calcium in the walls of blood vessels and in muscle tissue.

Howard Schneider, who worked with Webster on nutrition, became one of my good sources of information on diet for animals. He showed that you could keep mice alive and well if you fed them only whole wheat grain. It is the processing of cereals that destroys the vitamin B.

So Ronnie Dubos was a thinker. We could talk philosophy, and we'd have long conversations. He was a very stimulating person, and of course Max Theiler was always good to talk to. We often had lunch together.

Hughes: Did you tend to sit at the same table with the same people?

Johnson: I was apt to eat lunch late. Max and Loring Whitman were old friends, and they always ate early, and they left early in the afternoon. They lived on Hastings-on-Hudson, and they'd come in early. They were always heading for the subway about four o'clock. So they would eat lunch promptly at twelve. I was apt to eat after twelve-thirty. It was a sit-down meal in a lovely dining room, looking down on the East River.

I got to talking with the people who were working on the biochemistry of viruses. I was very interested in Dr. Alfred Mirsky's study of ribosomes. And then the people working on the flu viruses. There were a lot of visitors and temporary staff. It was a very stimulating place.

Hughes: Was Delphine Clarke in the biochemistry group?

Johnson: No. Delphine Clarke went to medical school at New York University. She had been a chemical technician in biochemistry. That's where she made her money to finish school. She had some friends who later became very well known researchers--Dr. [Charlotte] Friend, who died recently, one of the staff at Sloan-Kettering working on the leukemia viruses.

Delphine Clarke was an excellent biochemist, as good as they come. Besides working on inoculating the chicks, she was an M.D. She and I were the only ones that were licensed to practice medicine at the lab. I had a New York medical license. So we would give vaccines. When we were working with some of the virulent viruses, before we started this [arbo]virus program, we had to be immunized against the most dangerous viruses, such as eastern, western, Venezuelan, Russian spring-summer, and Japanese B encephalitis virus.

Del and I vaccinated the whole staff because we were licensed physicians and insured. There were five vaccines. We decided to combine them and give a half cc of each [laughs]. Nobody got a serious reaction other than George Martine and me. About thirty-five hours after taking the first dose of the vaccine, I remember waking up shaking with a temperature of 103.5. And George Martine had the same experience.

Well, I didn't think about it until after the serological studies came through. We all had high antibodies to Venezuelan encephalitis virus. So we were infected with this virus that was supposed to be dead. The vaccines we used were made at Walter Reed Medical Research Laboratories, and formalin had been used to inactivate the virus. It seems that in mixing the vaccines some of the virus was reactivated, similar to what happened in mixing types 1, 2, and 3 of the Salk polio vaccines.*

* See discussion below.

Johnson: So Del Clarke was an all-around person. She was a good M.D., had good hospital training. She was the one that did all the highly technical chemistry of developing reagents for tissue culture and producing high quality antigens for complement fixation and hemagglutination tests. She is no longer alive.

Then there was Dr. Charles Anderson. He didn't have a New York license, but he was trained in medicine and pathology. We had a similar background. He was born in Kansas; I was born in Nebraska. We had had about the same training. He followed me in India. He was director of the lab in Poona for many years, a good friend and now dead.

Hughes: What was Loring Whitman's background?

Johnson: Well, Dr. Loring Whitman graduated from Harvard Medical School in 1930. He was a friend of Max Theiler from the time they were members of the Harvard African expedition to Liberia and the Belgian Congo, 1926-1927, headed by Professor Richard P. Strong. Max did the studies of protozoa and plasmodia. Loring was the photographer. As I said, we worked together on malaria and he furnished me with the sporozoites. Loring died last year.

I had known Dr. Richard Taylor earlier, because he used to visit us in Alabama. Socially, we were very friendly, and same with Dr. Kenneth Smithburn. Smithburn worked at the lab, so I knew him from '38. We were good friends. He had no children and his wife was an artist. When they were in Africa, she did some marvelous jungle paintings.

Dr. J. Austin Kerr was doing the complement fixation tests of the neurotropic viruses. He had conducted public health mosquito eradication campaigns, particularly of the Anopheles gambia in South America, which had been introduced from Africa. He and Fred L. Soper had been working on field malaria control. He was an excellent administrator on field projects. So when we finally went to India together, he was the administrator and I was scientific director in charge of virology.

Dr. John Bugher, I mentioned previously, had been working in South America at Bogota and Villavicencio. He came during the time that I was in New York to run the electron microscopy section. I used to cut sections for him to look at in electron microscopy. We were trying to grow things on little disks so that he could look at them under the electron microscope. The microscope had just come in then, in the fifties.

Hughes: Was he working on the improvement of the 'scope?

- Johnson: No. He was assigned to examine new viruses to determine size and morphology. He also was improving the ultracentrifuge. He was interested in physical science. After the war, he was very much involved with the results of the atomic bomb tests. He spent a lot of time going to Japan and the islands affected by the Bikini tests. He was in charge, the last few years until he retired, of the atomic center in Puerto Rico.
- Hughes: When Pickels went to the Spinco division, did the fact that he was now in the commercial world break his ties to the Rockefeller?
- Johnson: Oh, no. He used to visit, and I used to see him when I came out here in 1954, because he was one of the old group.
- Hughes: What I was meaning by breaking ties, was because he was interested in the commercial aspects, was he reluctant to share his improvements of the ultracentrifuge?
- Johnson: No, the Spinco products were the best. The foundation often was the stepping stone for anybody in high tech and virology to go to professorships or public health. He went commercial, rather than to a professorship. He could have been a professor in electrical engineering at almost any university. He was constantly improving the ultracentrifuge, because the shafts used to break, even the commercial ones. So he was constantly improving the quality of this machine that had to spin at such a fantastic rate and for so long.
- Hughes: Why did he go to California?
- Johnson: He went because the Beckmann Company was there that was going to build the ultracentrifuge. He also had a cattle ranch there.
- Hughes: Had Beckmann been making centrifuge equipment?
- Johnson: Oh, yes. And they made all kinds of spectrosopes and lab equipment.

All these [technologies] come out of basic research, like freeze-drying and diagnostic equipment. We made the early freeze-dryers; you couldn't get them made. When I went to India, I made my own there. I told Darshani what I wanted and worked with him, and he later became the engineer for the lab. Bugher had one man working with him when I was there at the institute, and he worked with Pickels too. He was a machinist from Germany. He could machine parts to perfect balance—a really good machinist. Working with metal is like the finest cabinetmaking. They have to be able to work with metal like you'd work with wood.

Johnson: What Bugher was doing at the Rockefeller Institute when I was there in the fifties was improving the way you use an electron microscope and handle the specimens, and the little membranes that you put your specimens on, and growing cells on the membrane. There was a cytology lab at the Rockefeller Institute. Dr. Keith R. Porter [and his group] were doing the finest of the electron microscopy of cells. I enjoyed talking to them.

After mentioning Beckmann Instruments and the Spinco division, I would like to comment on the importance of commercial biological laboratories. Some have excellent bacterial and virus laboratories and they are the ones that can test and market the vaccines developed in basic research studies. Lederle Viral and Rickettsial Disease Laboratory was very good.

The event that hurt the Lederle virology program was the federal suit stating that they and other companies had conspired to fix the price of tetracycline. The companies spent millions fighting this suit. Well, they lost. These companies had to pay out four hundred million dollars, I think it was. The government rented a building in San Francisco and paid out this money. Anybody who had purchased tetracycline, if it was under \$100, could just make a claim. All the young kids in the country sent in claims and got paid, and all that money was practically thrown away. Few if any doctors sent in claims. That four hundred million was just thrown away. A tube of acromycin ointment is now eight dollars. I used to buy it for sixty cents before that suit.

Hughes: So the public pays in the end.

Johnson: Yes. It concerned me because Lederle Lab quit making the rabies vaccine that was developed from the Flury LEP (low egg passage) seed virus I supplied. This vaccine was of high quality and could be depended on to produce immunity to rabies. The financial loss from the federal suit and the competition, which had begun production of cheaper tissue culture vaccine, caused Lederle to stop making rabies vaccine. Fortunately, dog rabies was eliminated in the United States by 1967. So they gave up a majority of their vaccines, but their polio live virus vaccines have been used on a large scale.

I think in a way it was too bad when the Rockefeller Institute became a part of the university system. Probably it was an economic [decision]. The cost of running an institute in New York City became so high. That's why it had to become the Rockefeller University. But once it becomes a university system, then you're bound by the state, a bunch of regulations. It's still a good postgraduate school, but its work now is more clinical medicine [rather than basic science].

Polio

Hughes: Dr. Olitsky was one of those, if I remember correctly, that believed in the nonintestinal route of polio infection.

Johnson: He was the one that showed it in the mouse polio for sure in Theiler's disease. T-O was his strain, which they isolated from the intestinal contents of mice, and then showed that this was a polio virus of mice which was transmitted through the feces. It would be a latent infection of the colony, and it could be there and not a single animal would be sick that you could see, but probably five, ten, fifteen, twenty percent of the mice would be infected with the virus. That was probably true of many of our laboratory mouse colonies in the United States during the early thirties. When we finally got a technique for getting polio virus out of sewage by testing it in cell cultures, you could get virus from clear water in New York City sewage mains. But you had to have a technique for concentrating it, either by ultracentrifuge or by suspending some cotton material in the sewer line that would adsorb it.

When Enders showed that polio virus would grow in cell cultures of skin, then people realized it would grow in something other than brain. The next step was to show it would grow in monkey kidney cells and then later HeLa cells. HeLa cells were one of the standard tools used for a long time for culture. [They came originally] from a black woman in John Hopkins Hospital. Her name was Helen something.

Hughes: Lane.

Johnson: Thanks. The Rockefeller Foundation had been supporting the biology department at Tuskegee Institute in Alabama. They were given the grant for making HeLa cells. HeLa cells from Tuskegee were shipped to all the labs that wanted to work with polio for isolation of virus. The National Polio Foundation* supported the Tuskegee cell culture project. So the way that they found out that polio had a viscerotropic character came out of the tissue culture research.

Now, we already knew that rabies grew in the salivary gland. Robert E. Kissling in 1958 was able to get the Pasteur strain of rabies virus to grow in hamster kidney cells. Most hamsters must have myxovirus or paramyxovirus in the kidneys, because the Kissling strain of the Pasteur virus is different by the monoclonal antibody test from other strains of the Pasteur fixed rabies virus. It also proved to be less antigenic than other

* National Foundation for Infantile Paralysis.

Johnson: strains of this virus. I suspect that the Kissling strain had hybridized with a hamster paramyxovirus.

Remember what happened to the polio vaccine? The SV40 virus is a cancer virus. It produces 100 percent cancer in hamsters, and it was in some early Salk polio vaccines. Although they treated the material with formalin, they didn't inactivate all the SV40 virus. It's a papovavirus of the SV40 polyoma subgroup. There has been no evidence of disease related to this virus among those vaccinated with Salk vaccine containing this virus.* They had no idea in the early days of the Salk vaccine that it had another virus in it. The SV40 virus did not produce any cytopathic effect in rhesus monkey kidney cells.

Hughes: When did they find the SV40 virus?

Johnson: See Shah and Nathanson's article. SV40 virus was one of many viruses isolated from primary cultures of the rhesus monkey. Others were parainfluenza viruses, lymphocytic choriomeningitis virus, measles virus, adenoviruses, and monkey B herpes virus. They would screen these cultures, and you were not allowed to use any for passing that had evidence of a second viral agent in the culture.

Hughes: They were aware of the problem?

Johnson: Well, they did waste a lot of monkeys. Now we can store rhesus monkey kidney cells and start with one kidney and produce millions and millions of cells by freezing down one-hundredth of an aliquot of that kidney and then always go back to the original each time. So if you had a clean one, you can continue to use that source.

Hughes: I'm not quite clear. In those original trials of the Salk vaccine--

Johnson: It had SV40 virus in some lots of vaccine.

Hughes: Did they know that?

Johnson: They didn't know that during the early vaccination campaign.

Hughes: Had they screened them?

Johnson: The big problem was live polio virus in the Salk vaccine. There were more than seventy people that contracted poliomyelitis from the Salk vaccine.

* K. V. Shah, I. V. Nathanson. Human exposure to SV40. Review and comment. Am. J. Epidemiol. 1976; 103:1-12.

Scientific Director, Virus Research Centre, Poona University
College of Medicine, Poona, India, 1951-1954

Genesis of the Rockefeller Foundation's Arthropod-borne Virus Program

[Interview 6: April 7, 1987]

Hughes: Dr. Johnson, I thought we should start with the genesis of the arthropod-borne virus program. Could you tell me how the idea arose?

Johnson: Dr. Smithburn and Dr. Kerr were doing serological studies of viruses which had been isolated looking for yellow fever. The [arboviruses] obtained from human blood specimens were Bwamba virus and West Nile viruses from Africa. Semliki Forest, Bunyamwera, Ntaya, Zika, Uganda S, and Kumba viruses were obtained from mosquitoes in Africa. Mengo virus was isolated from the spinal cord of a rhesus monkey in Uganda. The Ilheus, anopheles A, anopheles B, Wyeomyia, haemagogus A, haemagogus B, leucocelanus, and sabethes viruses from South America were obtained from mosquitoes.

So, what are they? Are they really important? Two of them had been isolated from people that were ill, one a febrile patient bled by Dr. Mahaffy at Bwamba in 1937, the other from a febrile patient bled by Dr. A. W. Burke at West Nile District, Uganda in 1937. That is a very difficult way to find a virus, by the way, because we have learned that the virus will be in the blood only two or three days, possibly four. And by the time that they're really sick, the blood is negative. But that's beside the point.

Hughes: Was each virus associated with a disease?

Johnson: This was not certain at the time.

We had seventeen viruses, and I helped with that, because I was sectioning the infected laboratory animals, including the chick embryos, that were inoculated with these viruses, to study the pathology. The viruses were all neuroadapted, and so the organs showed little pathology except for the brain where the virus was inoculated. So the pathology studies did not help much in classifying the viruses.*

* H. N. Johnson. Histopathological lesions produced in chick embryos and laboratory animals. Proc. Fed. Soc. Exp. Biol. 1951, 10:44. R. M. Taylor. J. Immunol. 1952, 68:473.

Johnson: Dr. Austin Kerr was responsible for the complement fixation (CF) tests. That was a serological test used for viruses such as polio, rabies, and influenza. Dr. Casals, working in Dr. Webster's lab, had been doing CF tests. Dr. Delphine Clarke in our lab was interested in the biochemistry of the antigens used in the complement fixation tests. So new antigens were prepared for each virus included in the complement fixation tests. That was Dr. Kerr's job. He prepared his antigens from infected mouse brain. Lipids were extracted by the acetone-ether method of Casals and Clarke.*

Dr. Smithburn prepared immune sera for all the viruses, mostly in monkeys. He would inoculate a virus subcutaneously or intramuscularly and bleed the animals every day for one to five days to find out how long the virus was in the blood. He did a very wise thing: He would inoculate one monkey with a minimal dose, about one thousand LD50 mouse doses, and another monkey with about a hundred thousand or a million LD50. He found that the one that got a small dose would develop neutralizing antibody but with a low titer of antibody, and after six months, you could inoculate the virus again and it might produce a low titer viremia. We had no idea at that time that lymphocytes were the source of the antibodies formed in the monkey, and the titer of antibodies produced depended on the number of lymphocytes receiving the viral antigen.

He prepared immune sera against each of the new viruses. We had immune sera for yellow fever virus. Then the thing was to test the seventeen viruses to see if there was any grouping. That led to the discovery of the yellow fever group, which we called arthropod-borne viruses group B, and later called flaviviruses, which means yellow, from yellow fever, and alpha virus, group A of the genus Togavirus, signifying viruses with a lipid coat.

By the two methods, the serum-virus neutralization tests in mice and complement fixation tests, some viruses were not of the arthropod-borne variety. Kerr found that some of them were mouse viruses, such as mouse polio picked up from the test animal. These were the haemagogus A, haemagogus B, leucocelamus, and sabethes viruses obtained in South America. The Mengo virus from Uganda was found to be an enterovirus of the encephalomyocarditis virus (EMC) group.**

Hughes: What about Coxsackie virus?

* J. A. Kerr. *J. Immunol.* 1952, 68:461.

** K. C. Smithburn. *J. Immunol.* 1952, 68:441.

Johnson: We isolated Coxsackie virus during Taylor's studies at Coxsackie Reformatory in New York. However, the EMC virus is a picornavirus, similar to the Coxsackie B group viruses. It seems to have a worldwide distribution.

The group B viruses, besides yellow fever, turned out to be West Nile, Ntaya and Zika viruses of Africa, and Ilheus virus from South America.

Last night, I was at the Cooper Ornithological Society lecture on birds of the Cameroons, and one of the strains of group A viruses is from the Cameroons. It was called Kumba virus. This was one of the viruses included in our study. That turned out to be Semliki Forest virus. There are other strains in Africa of the Semliki Forest virus. That is a group A virus, like our eastern encephalitis, western encephalitis, and Venezuelan encephalitis viruses. We had a number of viruses; some group B, one group A. Bwamba virus turned out to be another group, and that is a member of the big Bunyamwera group, which is one of the largest groups of insect-borne viruses.

Hughes: Is that group C?

Johnson: No. Group C is a subgroup of the Bunyavirus group and Bunyamwera virus was the first one isolated. We don't have group C viruses, as far as I know, in the USA. The Bunyavirus group contains group C, Bunyamwera, Bwamba, California, Guama, sandfly fever, Simbu, Congo hemorrhagic fever, Nairobi sheep disease, Thogoto, and Rift Valley Fever viruses.

Hughes: These viruses are immunologically related, is that the common denominator?

Johnson: Yes, with group B, between the various viruses there is a serological relationship by the CF, HI [hemagglutination inhibition], or virus neutralization test. When we tested yellow fever immune serum against St. Louis [encephalitis] virus, we obtain a little over one log neutralization, and a relationship shown by the CF and HI tests. This is a similar finding with the group A viruses.

One of the things that fascinated me is, the Sindbis virus that we isolated from white wagtail birds in India when I was there is closer to western encephalitis in California than to eastern encephalitis virus. We've now found out that Sindbis virus is in Sweden, Norway, Finland, Czechoslovakia, Russia, and Saudia Arabia. I was suspicious of that because the birds that I studied in India, the Motacilla alba wagtails, were migrating in from the far north in great numbers during October.

Johnson: Dr. Hugh Smith, who did a lot of the basic field work in the early days of yellow fever research, and Dr. Max Theiler of the New York base laboratory looked for suitable sites for field laboratories and where governments were interested in a virus laboratory. The Rockefeller Foundation had conducted malaria control studies in Brazil, Egypt, and India, so we did have good contacts there.

Hughes: What was the primary purpose of the foreign labs?

Johnson: This program was to see, in representative areas of the world, what arboviruses were present, and in what mosquitoes, and what were their cycles of transmission.

Hughes: Where was the malaria control study in India?

Johnson: Dr. Paul F. Russell of the Rockefeller Foundation was in India from 1934 to 1940. Dr. W. C. Sweet had conducted malaria surveys and control projects in India beginning in 1928. The malaria work was mostly in the State of Mysore and Maharashtra (Bombay) State. Dr. Sweet had a field lab at Sawantwadi, north of Goa and near the old coastal village of Vengurla, Bombay State. He also had a study site near Cochin, Travancore State, for research on filariasis. So it wasn't just malaria, but also filariasis and hookworm. When I arrived in India the Rockefeller Foundation malaria project was situated at Bangalore, with a field lab at Sakleshpur near Corg, Western Ghats.

Leishmaniasis, also called kala azar, is transmitted by Phlebotomus papatasi sandflies. The British researcher, Sir S. Richard Christophers, discovered the leishmania parasite of this disease in India in 1904.* I have a copy of this publication. I met him and we talked about India. He was in charge of malaria control in India (1901-1932). He published a book on Aedes egypti in 1960 at the age of 87. He died in 1978, age 104.

Hughes: Was he a bacteriologist?

Johnson: He was a medical officer (M.B., Liverpool, 1896) and except for the hospital training and an expedition to the Amazon River, he made his career in tropical medicine in India.

* S. R. Christophers. A preliminary report on a parasite found in persons suffering from enlargement of the spleen in India. Scientific Memoirs of Officers of the Medical and Sanitary Departments, Government of India, No. 8, Calcutta Gov. Printing, India, 1904.

The Rockefeller Field Stations

Johnson: In order, the Rockefeller field stations were: Poona, India (1952); Port of Spain, Trinidad (1953); Johannesburg, South Africa (1953); Belem, Brazil (1954); and the cooperative project of the Rockefeller Foundation and the California Department of Health, Berkeley (1954). So that was the beginning of the worldwide study of arboviruses.

Dr. Taylor, when he retired in 1951 as head of the lab at the Rockefeller Institute, was assigned to the Navy Medical Research Unit (NAMRU) in Cairo to help set up a virus lab there and organize a survey of the Nile delta. As a preliminary for that, he had completed a study in 1951 in Florida, testing out methods of collecting mosquitoes and using baby mice for test animals. Dick Taylor was on a temporary assignment to Egypt to help NAMRU 3 develop a virus laboratory.

Hughes: With the Egyptian government or with the navy?

Johnson: Well, both. NAMRU 3 had been there for some time. Dr. Harry Hoogstraal, who died in 1986, was the director. The navy unit had a cooperative infectious disease study with the University of Cairo and the Egyptian Ministry of Health. President Nasser was familiar with the public health work and favored continuation of such projects.

Hughes: Was Taylor still a Rockefeller person?

Johnson: Yes. He was continued on the Rockefeller Foundation staff for 1952-1954. Dr. Telford Work of the Rockefeller Foundation staff later joined him to help with the field studies in Egypt through 1954. Dr. Taylor had full access to our New York laboratories, to which he sent blood specimens for serology and viruses for identification.

The Trinidad lab was in Port of Spain, at the University of the West Indies. Dr. Wilbur G. Downs was director from 1953 to 1961, when he was assigned to the New York office. Dr. C. R. Anderson was in charge of virology from 1954-1956, when he went to the India lab. Dr. C. Brooke Worth moved from the South Africa project in 1960. Dr. Thomas H. G. Aitken was in charge of the entomology studies. Dr. Elisha Tikasingh of the University of the West Indies was on assignment for study leave in Berkeley in 1964-1965. He is still active at the Trinidad lab.

When I was in Trinidad in 1963, I spent a night at the Bush Bush field station in Nariva Swamp. Fran and I set up a bird net on the catwalk running out into the swamp. We netted seven

Johnson: species of bats, including vampires. Dr. Leslie Spence, M.D., of the University of the West Indies was in charge of virology after Anderson left in 1956. Leslie was trained at the New York lab.

Dr. Kenneth Smithburn went to the South African Institute of Medical Research (SAIMR) in Johannesburg in 1953, to direct the Rockefeller Foundation project there. Dr. James Gear was the director of SAIMR and was working on poliomyelitis vaccine and enteroviruses. Dr. Smithburn set up special lab facilities for the arbovirus studies and trained local staff. Dr. Robert H. Kokernot and Dr. C. Brooke Worth of the Rockefeller Foundation field staff, and Dr. B. de Meillon, Dr. M. Paul Weinbren and Dr. Bruce M. McIntosh of the SAIMR staff were able to carry out a remarkable series of field studies in South Africa and Mozambique.

Then in South America there had been a lot of work in Rio and Buenos Aires on urban yellow fever, but now we needed a field station near a big river system, and Belem (which means Bethlehem) at the mouth of the Amazon River became the main arbovirus study area in South America. This Rockefeller Foundation project opened in 1954 in cooperation with the Brazilian government. It is now operated by the Brazilian Health Services.

They were the five major labs, at the beginning. I have done field studies at the other four labs, and the staff of those labs has visited the California lab and field study sites.

Hughes: I saw reference to the laboratory at Ibadan in Theiler and Downs.*

Johnson: In 1963 Ottis Causey went to Ibadan, Nigeria, to organize the virus project there in the microbiology department of the University of Ibadan. He was director of the Belem lab from 1954 to 1963. He and his wife Calista Causey, also a D.Sc. from Johns Hopkins University, were an ideal combination, Ottis for entomology and field studies, and Calista for running the virology lab. She was a volunteer, not regular paid staff. Dr. Robert E. Shope was at Belem from 1958 to 1964, when he moved to New Haven to the new Yale Arbovirus Research Unit (YARU). Graham Kemp, DVM, who worked on my WE vaccine project here, joined the staff of the IHD and was assigned to Ibadan.

Hughes: Theiler and Downs also list Cali, Columbia.

* M. Theiler, W. G. Downs. The Arthropod-borne Viruses of Vertebrates. An Account of the Rockefeller Foundation Virus Program 1951-1970. New Haven: Yale University Press, 1973.

Johnson: The Cali lab was begun in 1962. The Rockefeller Foundation was financing the new medical school there. Dr. Carlos San Martin, professor of microbiology at the Universidad del Valle, had been trained at the New York lab and also visited the California Arthropod-borne Virus Study Project. Dr. Robert H. Kokernot established a field laboratory at Rio Raposo on the west coast. Dr. Vernon H. Lee, an entomologist, was on the staff there from 1962-1966.

The cooperative project in South Africa ended in 1960 because Dr. Smithburn became ill and had to retire in 1958. Kokernot was subsequently assigned to the Cali project and Brooke Worth to the Trinidad lab. There had been a Rockefeller project in Bogota; a yellow fever laboratory started in 1938. Marston Bates and John Bugher had done some really great work there on jungle yellow fever at Villavicencio field station. The program continued after that initial field study and later on help was given in training staff. They'd come to the Rockefeller Institute in New York and to regional labs and then go back and do arbovirus studies where they were originally.

Hughes: The Rockefeller Foundation virus lab in New York was the coordinating body?

Johnson: That was the base for advanced training. Government labs in other countries could send viruses there and blood sera for antibody studies. That was the way we operated in India. We did not bring any viruses with us, so that there wouldn't be any possibility of cross infection. As we isolated a virus, we did some basic studies at the field lab. I lypholized two virus strains before I left in 1954. Dr. Kerr later sent these to the New York lab. One turned out to be Sindbis virus, which we had obtained from birds, mites, and mosquitoes.*

The other virus isolated from the brain of a fledgling koel, Eudynamis scolopaceus, turned out to be Newcastle (Ranikhet) disease of birds, a virus never before adapted to mammals, in this case, baby mice.**

Hughes: Was it a new strain?

* K. V. Shah, H. N. Johnson, T. R. Rajagopalan, B. S. Lamba. Isolation of five strains of Sindbis virus in India. Indian J. Med. Research 1960, 48:300-308.

** K. V. Shah, H. N. Johnson. Isolation of Ranikhet (Newcastle) virus from a (Linnaeus) fledgling koel, Eudynamis scolopaceus. Indian J. Med. Research 1959, 47:604-608.

Johnson: It is a natural strain, from a wild bird. By passing it in mice, it lost its pathogenicity for chickens. They did try to use that as a live virus vaccine.

Hughes: The New York lab was not depending on the field labs for virus shipments?

Johnson: No. The New York lab was doing basic studies with electron microscopy, ultracentrifugation, and chemistry, and improving techniques. This made it possible to find out quickly whether the viruses isolated at the field labs were related to known viruses or new to science.

The other thing the New York lab pioneered was the HI [hemagglutination inhibition] test. We had the HI test for influenza in 1941. When we found out that several arboviruses would hemagglutinate red cells of various species at a certain pH and temperature, then that became better than the complement fixation test for identification and grouping the newly isolated virus, also for testing for antibodies. The basic chemistry was done by Dr. Delphine Clarke, to make it more accurate. Dr. Albert Sabin was the first to use the red bloodcell inhibition test for arthropod-borne viruses.*

Hughes: He was actually working on the hemagglutination inhibition test?

Johnson: Yes, Sabin had discovered that Japanese B encephalitis virus would agglutinate red blood cells. But the development of this test as a major method of grouping arthropod-borne viruses was done at the Rockefeller lab at the Rockefeller Institute.

Hughes: Maybe we should emphasize the common principle that underlay all these Rockefeller labs abroad. You mentioned the training and the supply of equipment, but I understand that the actual choice of personnel was left up to the native government.

Johnson: No, the directors of the field labs selected the people to be trained.

Hughes: I was actually thinking of the field station employees.

Johnson: Yes. We had to interview those recommended. The idea was, you'd first train people locally. Those that showed special aptitude would be recommended for extra training, probably a year in a school of public health in the United States. Some came to the UC School of Public Health in Berkeley and the wildlife virus lab at the California Department of Health.

* A. B. Sabin, E. L. Buescher. Proc. Soc. Exp. Biol. & Med. 1950, 74:222.

- Hughes: You made a comment off-tape that Hugh Smith wanted to send you to India for your health.
- Johnson: Well, I think the big reason was that I was trained in pathology and internal medicine, much like he had been at Johns Hopkins University, and I had a special interest in the source of human diseases in wildlife. However, he no doubt thought India would be good for me, and also the program in New York was tapering down. He knew my interest in field work and that I had had experience in field work, rabies particularly. But also I had isolated eastern encephalitis virus in Alabama during 1945 and had done studies of eastern, western, and Venezuelan encephalitis virus.
- Hughes: It seems strange to send you to recuperate in a country which is not healthy for the western human being.
- Johnson: Well, by 1951 I had recovered from the paralytic disease I developed in September 1944 and was able to do anything necessary in working with animals at the lab and could do collecting and processing of field specimens.

Choosing the Location for the Laboratory

- Hughes: Well, why don't you tell me about choosing the location for the laboratory?
- Johnson: During 1951, the staff at the New York lab at the Rockefeller Institute (Theiler, Taylor, Whitman, Smithburn, Anderson, and I) spent many hours discussing the prospects of the projected world-wide study of the natural history of animal viruses transmitted by insects. The decision was made, I think wisely, by Max Theiler and Hugh Smith, who knew a lot about field work, to have the virus laboratory at Poona (now Pune), 100 miles inland from Bombay. Coonoor, a hill station in the Nilgiri hills of south India, had been recommended because they already had the Pasteur Institute there. The basic criteria were: It had to have a good electrical and water supply. It had to be not too hot or too cold, near a major port, with good transportation. We wanted a fairly decent place to have the lab, and a good modern community, with a good hospital and medical school.

There were several small colleges, Poona University, and B. J. Medical College at Poona. The medical school was one of the seven upgraded medical schools which taught in English, and they were getting assistance from the British Commonwealth, particularly, and also from the United States. So they were building a new hospital and medical school. There was a good clinical facility and an autopsy service. The professor of

Johnson: pathology, Dr. P. G. Gollerkiri, I found to be a good friend. The B. J. Medical School was named for Byram Jijibhoi, a Parsi who financed the school. The Sassoon Hospital was endowed by a Jew, Sir Philip Sassoon, who had been knighted by England for his philanthropic projects.

At Poona there was a big military base called Kirkee, an engineering and munitions center. Khadakwasla, a kind of West Point, was inland from Poona. The British had established an officers' club in Poona called the Poona Club, where government officials and British army officers stayed. One of these, Major Cook, was there when we came and he gave me a lot of help. Houses had been built for British families around the central cantonment of Poona. It had all the things that made life a bit easier. Delhi didn't look like a good place. It was too dry and hot. The government wanted the laboratory in Delhi, where they later developed the All India Science Institute.

Poona turned out to be a good choice. But the political concern in India at that time was, Gandhi had been killed by an assassin from a Hindu sect at Poona. This was in 1948, just after the terrible partition riots in which about six to seven million had been killed. We knew this was going to affect us when we got there. The British were all being forced to leave. The police inspector of Poona had expected to stay another two years but was given notice to leave shortly after we arrived. Major Cook, one of the last of the British army officers, also left within two months. We stayed at the Poona Club until we found a house to rent.

Hughes: So the Indians distinguished between the Americans and British?

Johnson: At the time, the British were treated well because they had given India its independence. There were no anti-USA protests in Poona. They knew from the beginning we were just there to help build up a facility, and they wanted a facility for identifying yellow fever. In fact, that was their major concern. There were three very famous Indian physicians in 1952. Those were Dr. Raja, Dr. V. R. Kanolkar, and Dr. C. G. Pandit. Dr. Pandit is still alive. He was head of the Indian Council of Medical Research and was looking for ways to improve medical training. They were really wise men. Kanolkar was head of the Bombay Cancer Institute. They wanted the laboratory. It later became the National Institute of Virology, for India.

Planning the Trip to India

Hughes: Why don't you tell me about the research you did before you left the country? You visited the Museum of Natural History in New York.

Johnson: Before you go to another country to do natural history studies, it is a good idea to research what is known about the wildlife. I knew a lot about the animals in the United States; I grew up in a rural area. I was always interested in birds and animals of all kinds; I was trained in shooting and trapped fur-bearing animals as a teenager.

I went to the Natural History Museum in New York City, which has wonderful collections of small mammals and birds from all over the world. Another place I visited was the Harvard department of zoology. They have excellent collections from Africa and India.

The other thing I wanted to do was to learn as much as I could about entomologic methods. I knew a good deal [already] about mosquitoes, working in our lab with the three genera of mosquitoes. I was anxious to learn about mites. The consensus of opinion in 1951 was that bird mites were the source of some arthropod-borne viruses. This was based on work in St. Louis by Dr. Margaret Smith, who said that St. Louis [encephalitis] virus was present in one particular colony of chicken mites. Any time that she would test a group of them, she'd get St. Louis virus. So that was one place to visit.

Then there was Dr. W. C. Reeves and his staff in California, who had isolated western encephalitis virus from mites collected from bird nests but had been unable to demonstrate transovarial passage of western encephalitis virus in bird mites. Dr. Roy W. Chamberlain, working at the CDC lab in Montgomery, Alabama (formerly the Rockefeller Rabies Study Field Lab), had been unable to demonstrate transovarial passage of eastern encephalitis virus in bird mites. Dr. J. Allen Scott was working on mites as a host for filaria organisms at the University of Texas School of Medicine at Galveston.

Hughes: Did you actually visit all these places?

Johnson: Yes. In October and early November 1951 I visited Chamberlain, Scott, Reeves, Lennette, and Margaret Smith. I spent several days at each lab to learn how they were collecting and processing the mites and I reviewed their lab procedures. Dr. Albert Miller, who had worked on mosquito transmission of St. Louis virus and also on chicken mites, Dermanyssus gallinae, was now at Tulane University in New Orleans, Louisiana, so I visited his lab. Also John Fox, previously on the Rockefeller Foundation staff, was professor of epidemiology there.

Johnson: In my visit to California I included the State Health Department. There was a meeting of the American Public Health Association in San Francisco October 29 to November 3. This was a good opportunity to talk to a lot of people about what was known about arboviruses and bird mites. The prevalent opinion was that bird mites were involved in the epidemiology of western and St. Louis encephalitis viruses. In the USSR today, they still think that arboviruses have a primary host in arthropods. I don't like to call them arthropod-borne viruses; I now believe they're all either mammalian or avian viruses maintained in such hosts, and that the insects are only secondary and are involved only during epizootics in aberrant hosts. When I left for India I was prepared to collect bird mites from nests and knew how to identify them. I brought mounted specimens of the various bird mites to India.

I had already decided, before I went to India, that rabies in dogs seemed to be an aberrant parasitism because it killed the population so rapidly that it could not have a long-term host system in dogs. Also, dog rabies was relatively unknown until the 1700s, when the dog population of cities became large. Wolf and jackal rabies was known since the time of Aristotle, so wild canids were believed to be the reservoir host of rabies. The Russians, even the last time I was in Russia, still feel that the fox is the natural host of rabies in Russia just because so many of them were found infected with rabies during the recent epizootic in that country. It is the very high mortality observed in fox rabies outbreaks that makes me believe that they are an aberrant host.

Hughes: Did you do research on rabies while you were in India?

Johnson: I was interested in all virus diseases and I did isolate rabies virus from dogs and a horse.

Dr. Austin Kerr was in charge of administration of the Poona laboratory, and, though he had his ideas of what equipment to take, I was going to be the one to run the scientific program. So, what equipment did we need? We wanted refrigerators, incubators, and a complete pathology laboratory; all the instruments that we were going to use for processing specimens, and a variety of glassware.

Hughes: I understand that you had to buy certain items from the Commonwealth countries.

Johnson: Well, India was in the Commonwealth, and I thought that we would not be restricted on Pyrex ware. (We got in trouble with that, because anything that was made in the Commonwealth countries had to be purchased in England.) We bought some material from England that we would otherwise have obtained from the United States. But

Johnson: for equipment such as cars, refrigerators, and incubators, they didn't make any rules. When it came to instruments, some of the best, like scissors, come from Germany, so that was no problem. I had to decide what was the total amount of equipment that we needed to start working. We needed so much for the field work, but we sure needed some good vehicles. We had to have good trucks.

When I came to California, I helped design a truck that was built in San Francisco to send to India. This vehicle was like a covered wagon. It had a four-wheel-drive chassis with a big cab built on it so you could carry a lot of equipment, water and gas, and you could live on top of it in a fold-down Conistoga-like tent. You could set up a clinic under a tent awning to bleed people. That was used in India when Dr. Charles Anderson was there. But I didn't get to use it. I got my plans from Dr. [Edward Sherman] Ross of the California Academy of Sciences in Golden Gate Park. He had traveled all over Africa and Australia in one of those vehicles. It was largely his design. He was an entomologist.

For personal use I wanted to have a car that was rugged and a right-hand drive. So I ordered a Plymouth station wagon, built for foreign service. We had to get collecting equipment--traps, guns, and ammunition. We knew that one of the quickest ways to collect animals was like shooting rabbits at night on the roads, when they come out and feed. You can get samples faster, and with birds similarly in daytime. A lot of birds you could never trap or net; you would have to see what's there, and what's most abundant, which one is migrating, and then bleed them. I would get blood smears on every one shot, and then collect all the ticks and fleas and any [other] insect on them.

The most important factor was my family. What would we have to be immunized with? Dr. Delphine Clarke and I used to do a lot of the vaccinations at the Rockefeller Foundation. So she had to vaccinate all six of us for plague, cholera, typhus, and smallpox. There were also boosters for tetanus, diphtheria, and whooping cough. I brought with me human and dog rabies vaccine and additional cholera vaccine. That had to be refrigerated on the ship. I brought medications, like chloroquine, sulfadiazine, aureomycin, penicillin, chloromycetin (for typhoid and rickettsia), and antibiotic ointment, as well as a variety of nonprescription type drugs.

Hughes: The Rockefeller with all its foreign programs didn't have a protocol for setting up a foreign laboratory?

Johnson: There was plenty of information from the International Health Division. We had a booklet on health hints for living in the tropics. Our hospital experience gave us a good idea of what was

Johnson: needed for the tropics. The most important thing was to boil the drinking water and eat only cooked fruit, or papaya, mangos, bananas, and sweet limes that could be peeled. Milk obtained fresh (usually water buffalo milk) was heated to near boiling and then cooled. Some of this was fermented with a yeast culture to a jell and was called "curds."

Dr. Kerr and I had a lot of discussions about what we were going to feed the mice. We were going to start a mouse colony. We had to get clean mice from the Rockefeller Foundation lab in the United States. We had to be sure we had a place where the wild mice and mosquitoes couldn't get in the facilities. The building we were going to use had no screens, so we had to make arrangements to get screening for the rooms. We planned to import the Rockefeller stock of Swiss mice that had been brought from Switzerland by Clara Lynch in the twenties. They were inbred, and that would be our mouse colony. That was at least as free of viruses as any we could get.

Hughes: You made some reference in something I read to problems with the chick embryos.

Johnson: Yes, that was a problem. At that time it was difficult to find a stock of chickens free of egg-transmitted Pasteurella, Salmonella, and viruses of chickens. We built housing for the white leghorn chickens. They were several feet off the ground with a hardware quarter-inch screen for the floor. On the sides we used ordinary chicken-wire screening. We obtained fertile eggs and incubated these in a commercial chicken egg incubator. We did have an occasional hen infected with Pasteurella, evidently brought into the pen by sparrows. Some of the chicken embryos were infected through the eggs. I isolated Pasteurella organism from the peritoneal cavity of one of the hens.

One of our decisions on what to feed the mice was based on nutritional studies conducted by Dr. Howard Schneider, who worked in Dr. Webster's lab at the Rockefeller Institute. He provided water and fed them whole wheat as their only food, and that seemed sufficient. There was wheat and rice in India, and so which one were we going to feed them? Well, we decided to feed them wheat. How were we going to process it and keep it clean and sterile? In India, the wheat is threshed on the ground. The wheat we used for cooking had to be washed, dried, and checked for stones. We used the "Poona diet" here in California. We had a special mechanical grinder. Part was ground fine so when cooked it would jell. Part was a coarse grind to give the mice something to nibble on. The proportion was one pound of the mixture (ground wheat, two parts, and whole powdered milk, one part) to four pounds of water, containing one percent salt. The problem was to sterilize it without destroying the vitamin B.

Johnson: I had had that problem in my mouse colony in Alabama, because we could not feed the mice bread and water and get them to produce healthy litters of mice. We would grind the wheat the same day, then heat it to a boil and simmer for five minutes. The mash was poured into large stainless steel pans set up on little blocks of wood and cooled rapidly with an electric fan. After it cooled, it jelled and we put paraffin paper over the top. The jelled cereal was cut in blocks. That would be the food for the day, and it would not spoil. The mice would get all their water from that, and we did not need water bottles. We used shredded paper for bedding.

En Route to India

Johnson: The British colonial medical service was based in England. So on my way to India, I tried to learn as much as I could about India. Where's the place to go? The London School of Hygiene and Tropical Medicine. Also, I planned to visit Michael Stoker, who developed Stoker's BHK baby hamster kidney cell line. He was at Cambridge University as professor of pathology, so I went there with my family. He had lived in Poona.

Then on the boat going over, we spent hours with Dr. Paul Russell of the Rockefeller Foundation staff. He'd been in India previous to World War II. He and I enjoyed meeting and talking with British scientists, such as H. E. Shortt and Gordon Covell, who had served in the colonial medicine service in India.

Hughes: Was it just chance that Russell was on board?

Johnson: It was definitely my good fortune. I had been in communication with him and we had met previously but here was a chance to spend about five or six days together.

Hughes: Where was Dr. Russell going?

Johnson: He was going to visit the Rockefeller Foundation office in Paris.

In London, I talked with Dr. Spooner, Dr. P. C. C. Garnham, Dr. H. E. Shortt, and Dr. S. R. Christophers. I visited the Colindale Diagnostic Laboratories to talk to Dr. R. W. McCollum. Dr. Osler Peterson of the Rockefeller Foundation was in London where he was observing the British medical services, so he helped to arrange appointments.

In 1951, Rockefeller Foundation staff going to England, Europe, the Middle East, and India traveled on Cunard Line ships to England and then took the P and O [Pacific and Orient Lines]

Johnson: boat to India. This was our plan. The foundation always shipped furniture and supplies by sea transport. We wanted to have our piano with us, as well as other furniture.

We left New York City on the Cunard S. S. Mauritania on the 8th of December of 1951 to go to Southhampton. On December 23 we went to Tilbury Docks to board the Pacific & Orient S. S. Strathmore for Bombay. We docked at Algiers and Aden. We arrived in Bombay January 5, 1952.

Hughes: Did the laboratory equipment go separately?

Johnson: That came later, because it had to come in large shipping containers. It takes a long time after you order it before it gets there. The trucks had been ordered some months previously. We ordered two Chevrolet carry-alls and a personal car for Dr. Kerr and one for me.

When we arrived in Bombay, we had to get through customs. I sent my wife and children in a taxi to the Taj Mahal Hotel, where we stayed. That is an experience, because every room has a chokadar that sits outside your door to protect you, so to speak. They have many adept pickpockets in India so you have to keep alert. We did have things stolen at railway stations. Dr. Kerr came down to meet us in Bombay. I had guns and special equipment and all my medical supplies; everything had to be declared. Later on, I had to get permits for the guns. They kept the ammunition at the police station in Poona. You could only charge out so many rounds at a time.

We were in customs for a couple of hours. Then we had to make arrangements for a truck from the lab to take up some of this equipment. The next morning, we took the train from Bombay to Poona; it's about 125 miles.

Settling In

Johnson: We did not plan to take the car back to the United States. Dr. Marshall Balfour of the Rockefeller Foundation staff in Delhi wanted my car when we left India. He assumed the monthly payments left on the purchase cost. There was no profit for me. It made it possible to have a car you could use both officially and privately. Trucks, of course, were different. They were laboratory trucks, which became permanent transport for the laboratory.

Johnson: We were inside the tropics—Poona is inside seventeen degrees—and so we got ten percent more salary for being in the tropics. But our salaries in those days weren't the attraction for the job. We were reimbursed for part of our cost of living.

One of the big problems was that food was in short supply. When the Indian government took charge, they rationed rice and sugar. We expected to get wonderful rice in India. It turned out that rationed rice was of poor quality. The good rice went to the black market. We were instructed to never buy black market rice because we might be arrested and jailed. Wheat was not rationed at that time, so we were able to buy that grain. We had it washed and dried before having it ground.

It was wonderful to be able to stay at the Poona Club for the first three months. Dr. Kerr had made a reservation for us. There at the club, it was all organized. It was like a motel. We had to learn to say certain phrases in Hindi as none of the servants spoke English—"Bring hot water," or "Bring tea," etc. I was concerned for exposure to infectious diseases. The man that had been making up our beds didn't appear one day, and we found out he had died of tuberculosis. So I was very conscious of the hazards. The children wore shoes when outdoors because there might be hookworm eggs in the dirt.

We ate at the mess at the Poona Club. That was a wonderful thing to have, because then you didn't have to scramble. You couldn't get beef, of course. There would be lamb or goat meat, not much chicken, and then fish once a week. You could get good rice at the club. And the club obtained cows' milk from a government dairy. Mr. L. E. Walsh was the manager of the Poona Club.

Hughes: Were you a member of the Poona Club?

Johnson: Yes, we lived there for three months. There were tennis courts there and they had a swimming pool, which Dr. Kerr and I sort of upgraded because we wanted to have a proper amount of chlorine. So Austin ordered some chlorine-testing equipment, and we tested it to make it safe. His daughter Sally, age eleven, was there for a while, but she went away to Kodi Kanal, an English missionary school.

Hughes: How old were your children?

Johnson: The children were Michael, four; Susan, five; John, nine; and Marion, eleven. That's a critical age, but it's also a good one. I'll say this: I do recommend having children when you go to a foreign country. You meet people through your children. If you take part in the community—the church, the school—you get to know the people and learn the language and customs easier. Our

Johnson: children were enrolled in St. Mary's and Bishop's schools run by the Anglican church of India, Burma, and Ceylon. These were English language schools, but Hindi and Marathi were also required. We had a Muslim teacher, Munshi Mohammed Khan, come to the house to teach Indian history, customs, and language.

Hughes: Was there much of a foreign crowd in Poona?

Johnson: Not in Poona at that time. When we arrived, Dr. Kerr was there. There was the National Chemical Laboratory, which had been started in Poona for basic research in chemistry. Professor Finch from England was the director. He had tried to climb Everest and reached within a thousand feet from the top. We were in India when [Edmund Percival] Hillary climbed Mount Everest. Professor Finch had shortwave contact with Hillary. He came over and told me that Hillary reached the top. Professor Finch also did some hunting with me. I was one of forty-three civilian people in Bombay State that had licenses for guns. Ordinary citizens in India were not allowed to have guns.

The deputy director of the National Chemical Lab was Dr. Sully Marsdon of Stanford University. He was trained in chemistry and his job was to supervise the chemical equipment and train people in chemical work at the laboratory. As far as other foreigners, the last of the British were just leaving. There was only one English person staying at the Poona Club, and that was Major "Cookie" Cook, and he left within six months. There was Inspector Crone, Chief Inspector of Police, and he expected to stay another couple of years but had to leave. There were very few foreigners left. The people that were in the positions of leadership were mostly Anglo-Indians, which were outcastes to the British, as you saw in The Jewel in the Crown (by Paul Scott) TV series.

Mr. Walsh, secretary of the Poona Club, was my guide and advisor. He was a most delightful person. He'd been in India for most of his life as a noncommissioned officer and had married an Anglo-Indian and raised two sons in India. One went to Kenya and the other to Australia. We last saw him at Adelaide, Australia, in 1966 where he had gone to live with his son.

Dr. Kerr had already rented a house in a suburb of Poona. But I didn't like his setup, because I have always wanted to be close to the laboratory. I wanted to get to know the people in a special way. So I asked Mr. Walsh what to do. He knew how to live in India. He said, "Don't get your servants from the same caste. The important one is the bearer. He is the number one next to you and your wife."

Johnson: The way I got our bearer is really funny. I was in my office when a tall man appeared. His head looked like it had just been shaved. Shankar was a typical Marathi, a low caste native of Bombay State with dravidian skin and straight black hair. He handed me a note from Kerr that read, "Harald, here's a Swede for you." I said, "Do you speak Swedish?" and he said in English, "A little." I said, "Where did you learn Swedish?" He said, "Well, I worked for a time for a Swedish missionary." That seemed good, so I said, "Would you like to be our bearer at the Poona Club?" He said he had hoped to get a job at the lab, but there was no work for him there at the time. He decided to take the bearer job and Fran later taught him to cook.

We had to have an ayah (nurse) to look after the children and to wash the stone floors. Then we had to have somebody that took care of the yard (the mali), another to do the laundry (dhobi). The mali was the wife of the dhudwala. He brought the buffalos to our back porch and milked them there. His wife's name was Sully. We were invited to the wedding of his nine-year-old daughter. She had never seen her bridegroom who came from the Pathan region of north India. She went with the groom for a visit but did not return to her husband until she was twelve.

Hughes: Do you remember what you paid all these people?

Johnson: We decided that we would pay the going rate, following Mr. Walsh's advice. For example, the bearer received thirty rupees a month and had his own quarters in the "godown" building behind the bungalow. A chauffeur for a local maharaja and his wife were living in one section of the servants' quarters at the house we rented. The chauffeur's wife agreed to take the job as ayah. Her name was Bobee and she was an excellent ayah. We found a classic old Indian bungalow, mud brick walls two feet thick and the center divided in half with wood screens. There were large porches in front and at the rear. We learned that no one had lived there for years. The roof leaked. We liked it. Mr. Walsh said, "Don't move in until the workmen have it all finished." The owners were Muslims, the Mootha family.

They had the roof fixed and the place cleaned. We had an American-style toilet and a shower in the house, and an Indian toilet, which is level to the floor, for the servants in the washroom off the rear porch. We had to have the windows secured so that thieves couldn't get in. There were bars and blinds that could be bolted inside and expanded metal screens to prevent opening of the blinds. The double doors on all four sides were locked with padlocks on the door bars inside. The tile roof leaked. After the very long and hot dry season it was not unpleasant to hear the rain water falling into the tin buckets we set out, real music to our ears.

Hughes: Was thievery a constant problem?

Johnson: Yes. For the people that live there, too. Some foreigners hired chokadars that had been policemen. Mr. Walsh said not to have a chokadar. He said they would help the robbers and expect a bribe. This really happened to Mr. Schubatis from US AID who came to Poona when we were there. One night he lost cases of supplies. He had to borrow some clothes from me. The chokadar slept!

There was a robbery at our "godown." Shankar caught him. I had a double barrel Winchester 12 gauge shotgun and two pistols, one 22 bore, the other 32 bore. This was known because I would arrive with game and hunters would bring specimens. You can collect all kinds of small mammals, birds, and lizards with 22 shot cartridges without damaging the body very much, like a small shotgun. The 32 was for personal protection and for large game animals at close range. The shotgun with slugs or buckshot was the best for panther, pie dogs, or large snakes. The main use was for collecting birds and small animals.

Poisonous snakes were a real problem. We had an episode of cobra snakes in our compound and one day a Russell's viper was found in a classroom at St. Mary's. I had a snake treatment kit with a supply of lyophilized antisnake-poison globulin from horses, plus ampoules of distilled water, sterile needles, and syringes.

Whenever we were doing field work, we ate some of the birds we shot, after getting blood smears and tissue. Our favorite game birds for food were the Imperial grouse and green grouse, but also doves, the Imperial pigeon, and jungle fowl. The famous coturnia gray quail came into India by the millions in the fall. In fact, they'd sell them in the market. Native hunters would collect them at night under the desert brush using lights. The best wild game was cheetal (large deer), small "barking" deer, and porcupine.

Hughes: What about illness in the family?

Johnson: The most serious was Susan. She became ill with hepatitis in April, 1952. It was evidently hepatitis A virus because I had antibodies to that virus after I returned to the United States. The rest of the family had symptoms at the same time that indicated liver damage, but Susan developed severe jaundice and had a long illness. Marion and John had fever of unknown origin for about two months, so they were kept from school. We had attacks of bacillary dysentery, and when this was associated with fever I treated this with sulfadiazine. There were frequent skin infections which I treated with antibiotic ointment. We had cases of scabies and hair lice which I could treat promptly. When I was on a long field trip in 1952 I contracted a very severe intestinal infection with high fever when staying at the tea planters' club at

Johnson: Sakleshpur. That time I took chloromycetin, and when the fever began to subside I went to Bangalore and checked into the West End Hotel and called Dr. Robert Watson, head of the malaria project there. He came to the hotel with a thermos full of hot beef boullion and some saltine crackers. What a wonderful treatment! I suspected I had typhoid and continued the medication three days after the fever subsided. The other severe illness was Marion having amebiasis, which was treated with aureomycin.

There were several Rockefeller Foundation staff in Bangalore, among them Joe Carter and Fred Knipe, engineers on the malaria control team directed by Watson. Brooke Worth was doing the mosquito studies at Sakleshpur. He was a great naturalist and gave me an interesting introduction to the natural history of the tropical forest on the Western Ghats during the monsoon. There were many different species of leeches, and though I took the usual precautions of treating my boots, I had two leeches that had fed on me and were full of blood before I noticed them.

I must mention also that Dr. Richmond K. Anderson was at Bangalore doing public health surveys in nutrition and anemia. I had met him in Mexico in 1944 where he was doing nutrition studies. He was born in India of missionary parents and was a great help to me in planning my field studies.

Hughes: You had a problem getting enough calcium into your children. They weren't eating enough curds?

Johnson: They wouldn't eat enough, and there was no milk to drink. Powdered milk was not available in the local market and no cow's milk. We did have plenty of bananas and sweet limes. When I noticed small pale areas (opacities) on Marion's teeth, we began taking calcium pills. The Indians at the end of the major meal eat a tasty pahn (pan) leaf covered with a natural lime paste. They have beautiful teeth.

We did have a good source of bread. There was a Goanese woman by the name of Mrs. Fernandez who had a bakery. When we discovered her, which was pretty soon after we got out of the Poona Club, we bought bread from her. I'd go down there and get five loaves at a time. I'd put these loaves, still hot, in a big tin with a tight lid and then keep it sealed. We made chapatis from wheat we had ground and also wheat cereal.

We could get corn flakes imported from England and also Grape Nuts. Now, Grape Nuts are part barley, and of course barley is a very good cereal. When I traveled I could often obtain barley cereal. Indian sugar, called jagari, looked like yellow erasers. It would come in big chunks and you'd break it up. It was made by boiling sugar cane juice. Indian tea and coffee were excellent. Condensed canned milk was added to the tea.

Johnson: I am convinced that the cause of one of the major diseases I saw was fungus contamination of a millet called bajra. I hadn't been there long before they had an epidemic that was called "the thirst." The disease is associated with eating moldy rice, wheat, and corn. Those affected would drink a lot of water and pass a lot of water and die, clinically diabetes insipidus. They had a mobile hospital which I visited at Bota, near Nasik north of Poona. Some of the patients were taken to B. J. Medical School in Poona and they recovered.

There was no hospital food service for routine patients. Families brought food for patients. The patients' families cooked the food outside and brought it in, and they stayed with the patient. It is a country where each caste prepares its own food and stays with members of its own caste.

I traveled all over India accompanied by a cook and driver. I usually stayed in district or inspection bungalows, which are for officials of the government. I had special identification as a government official. There are western type hotels in Bombay, Madras, Delhi, Calcutta, and a few other cities. But otherwise, there were no hotels. In Tanjore, population of a couple of million I guess, there was not a hotel. You could stay in the railroad station; they have four or five rooms for railroad employees, and I usually could get a room there.

Hughes: Did you take food along on your field trips?

Johnson: Yes. I'd carry boxes of Grape Nuts, sugar, tea, fruit, bread, jam, and cheese. You had to boil your water. You did not need to let it settle; you used it with everything in it because it was sterile. I drank a lot of tea. You always could get coconuts, which I opened with a kukeree (scimitar). This is a curved knife that cuts through a two- to three-inch tree limb.

When we would get to a village, we'd go down to the market, and they would have hot boiled rice. You'd have your own container, and they'd just scoop up a serving of rice and dump it into your container.

Hughes: Well, the first general election was in 1952, very soon after you arrived.

Johnson: Most of the people couldn't read, so they had signs with figures for the different parties. The Congress Party dominated. It was really a one-party system but there were many small local parties.

Hughes: The Muslim party by then had pretty much disbanded?

Johnson: Yes. Jinnah had organized an Islamic state, Pakistan. He would not cooperate with Gandhi; they offered him the premiership. He was going to have a religious state, and he got it. There's been nothing but trouble ever since. He died in 1947, shortly after the partition riots, which were terrible. There would be occasional Muslim-Hindu riots in India, with burning of houses, and they'd say a Muslim had killed a cow or offended the Hindus in some other way.

Hughes: In your area?

Johnson: Not in Poona, but such riots did occur in Bombay.

Hughes: Well, getting back to the Virus Research Centre, I understand it was a cooperative project between the Rockefeller Foundation and the Indian Council of Medical Research.

Johnson: Right.

Hughes: What did the medical research council do?

Johnson: It was responsible for medical education, research, and medical care.

Seven medical schools had been selected for training in modern medicine. These were English taught, as against what they called ayurvedic, which was the old Hindu medicine. It's like homeopathy. They did a lot of things which didn't do so much harm, and they recited mantras (poems) to relieve pain. But they could handle fractures and minor surgery, and they were like the barefoot doctors of China. They could do basic care and used antibiotics and modern nonprescription medications, if available. The thing that absolutely baffled me in India is how awfully people could be injured there, and with just simple leaf poultices with the mucus from cow dung as a dressing, they would recover.

The big problem in the hospital was how do you handle the laundry? They did the laundry in the river and then dried it on the ground. There are tetanus spores in the soil and tetanus was a common complication in the hospital. Another was staph infection.

Hughes: Did the populace hesitate to come to the hospital?

Johnson: Yes. The Sassoon Hospital where we worked was sometimes referred to as the Die Soon Hospital, because so many people died of bacterial infection, especially enteritis (cholera, salmonella, typhoid), pneumonia, tetanus, and tuberculosis. There was a special hospital for leprosy.

Johnson: One day two men appeared outside my residence. One of them had been gashed by a wild boar from the ankle up to the knee. You could see the bone. The doctor at the hospital had insisted on taking his leg off, and he had said, no! So they said, there's a doctor here who has some special medicine; you might go and see him. I had antibiotic ointment and a lot of three-inch adhesive tape. My bearer Shankar talked to the man. He was a member of a hunting tribe. They killed the panthers that would come into villages around Poona and kill people and animals, particularly buffalo and cows. They would receive a bounty of a hundred rupees for every one they killed. They were not allowed to have guns. They were not even allowed to have a spear blade longer than five inches. Shortly after we arrived a panther was killed after attacking a policeman in Poona. So they suffered a lot of casualties.

This man was a handsome person and I was interested in making contact with these rustic people [for field work]. I cleaned out the wound and decided not to suture it. I wanted it to heal from the bottom. There was no broken bone; it was just ripped down to the bone. It was pretty dirty, so I cleaned it out with sterile salt solution and rinsed it really thoroughly, sponged the wound dry, and covered the wound with antibiotic ointment, and closed the wound with three-inch tape, bringing the margins together from top to bottom. I told him not to open it. (They walk in mud and water in crossing water courses.) He came back a week later and it looked great. I cleaned and bandaged it twice more, and the wound healed up. That led to a friendship with the hunters. I learned so much from them about wild game animals and how they collected them with snares and traps.

The other way we met interesting people was through our interest in music. I went by a store one day with a sign in the window, "Poona Music Society." I asked who ran it, and they said it was Mr. Adi Framji, and that he worked at the Bank of India. I went to see him and learned that he was the local impresario for obtaining artists to perform in Poona. So within a week we were going to his concerts. He had a lot of records, and he had made a hi-fi with several speakers he had obtained from army surplus stores. He was able to schedule concerts by two internationally known pianists, Dr. Thornton Lofthouse of London on November 9, 1952, and Richard Farrell of New Zealand on December 13, 1952. He used our piano and had specially trained men from Bombay carry the piano to St. Vincent's School auditorium. They were accompanied by a piano tuner from Bombay who tuned the piano before the concert and again after it was returned to our bungalow. So he became a good friend.

Fran played the flute in the Poona Music Society orchestra. The director of the orchestra was the son of the man who had been director of the Bombay Symphony, Mr. Schmuck, and his sister

Johnson: taught music, and she taught Marion piano. Her mother was a musician. It was a thrill to hear this eighty-year-old woman play music by Bach on the piano.

The best field experience in 1952 was going with Dr. T. Ramachandra Rao and other health officers, visiting the malaria areas. It was thus possible to see ordinary homes and learn about the Anopheles mosquito that spread the malaria parasite. I was preparing an ecological survey of India, including the flora, arthropods, birds, and mammals of India. I selected Devimane Ghat as a study site in the tropical evergreen forest of Kanara District, Maharashtra (Bombay) State.

Hughes: What were the health officers patrolling?

Johnson: The big problem was malaria. At that time, they had begun to use DDT. I later got them to spray my house. We didn't have screens, so we slept under bed netting.

I loved the gekkos. I did everything I could to keep these little salamanders happy. Another thing I had in the house were praying mantises. I had one that had a leg off, and he'd get on my shoulder as I would brush against a curtain. I had one family of shrews, Suncus murinus, which would patrol the house for cockroaches at night. They would shoot across the floor, and there would be a little click, and they'd have a cockroach. [laughs] You learned to use local [insect] control.

Hughes: Did Austin Kerr stay in Poona throughout your term?

Johnson: No. He returned to the USA for two months leave while I was there. He left Poona in 1956, when he was assigned to the Pan American Health Organization in Washington, D.C. We were the two [representatives of the Rockefeller Foundation]. There was a Rockefeller Foundation office in Delhi with Marshall C. Balfour, M.D., in charge of a study on population control. The Far Eastern headquarters of the Rockefeller Foundation was in Bangalore, Madras State, where a new project on malaria control had been started in 1949. As I said, Robert B. Watson, M.D., was in charge of this project. Richmond K. Anderson, M.D., was deputy director and Joseph C. Carter and Frederick W. Knipe were engineers to supervise mosquito control operations.

Hughes: Well, getting back to Austin Kerr, once the lab was set up and people hired, what did he do?

Johnson: Well, the administration was complex, with responsibilities and reporting to both the Rockefeller Foundation and the government of India. We had a separate staff for the Rockefeller Foundation and G. S. Chari was the executive secretary. Letters had to be written in Hindi, English, or Marathi, the language spoken in

Johnson: Bombay State. The constant problems of electric and water supplies, management of a considerable number of employees, transport, salaries, bookkeeping, ordering and paying for supplies--that was taxing, as I found out when Austin was away. Mr. Chari later transferred to the Rockefeller Foundation Indian agricultural program and remained a Rockefeller Foundation employee until he retired in 1971 with twenty-nine years on the Rockefeller staff. We were friends and he was another person that guided me.

When I was away on field trips, Austin was in charge of the virology program. I knew I had a limited time. I felt I wasn't going to stay in India more than one term. I said, I should be able to train people in that time; the main thing is to find the right people to train. The three senior staff I trained were Baldev Singh Lamba, zoology, Pylore Krishmair Rajagopalan, entomology, and Keerti Vandarvan Shah, virology. Lamba had a M.Sc. from Punjab University at Hoshiarpur. Rajagopalan had an M.Sc. from Benares Hindu University, and Shah had an M.D. from B. J. Medical School, Poona.

Lamba later became curator of the zoological survey of India. Rajagopalan and Shah later came to the United States for further education in 1958-1959. Both returned to India. Rajagopalan later obtained a Ph.D. in entomology in India. Shah left India in 1962 to take a professorship at Johns Hopkins University. Dr. T. R. Rao joined the staff of the Virus Research Centre in March, 1953, and we worked intensively together in the field program until I left in 1954. He returned to his previous job as director of malaria control in Bombay State until 1962 when he became director of the Virus Research Centre. He visited the United States in 1963. We kept in touch by regular correspondence until he died in 1984. We shared a wide interest in wildlife and had a close friendship.

The technicians I trained were Madhav Narayin Patil in virology and Miss Vasundhare S. Chandekar for pathology, that is, cutting and staining tissue sections. The basic training was strict virus technique, always working on trays covered with paraffin paper and paper towel and handling mice with sterile forceps (boiled in water). The mouse colony was started in December, 1952, and a white leghorn flock of chickens to provide fertile eggs for chicken embryo studies. The papers from the work trays were put in heavy paper bags which were burned in an incinerator. Glassware was sterilized in an electric oven or in large pressure cookers.

Hughes: I read that on November 12, 1952, the Bombay State PWD, which I presume is the Public Works Department, handed over the laboratory building, which had been remodeled at the expense of the Rockefeller Foundation. Could you explain how that happened?

Johnson: Yes, and the formal opening of the Virus Research Centre took place on February 4, 1953. The Honorable Rajkumari Amrit Kaur, minister of health, government of India, presided at this function.

Hughes: How did you set the salaries?

Johnson: They were set according to a standard scale by C. G. Pandit, director of the Indian Council of Medical Research, and the Indian staff received their pay from the government of India. It was a place where people wanted to come if they were good, and that's how we got the best. Dr. Kerr as director had the authority of hiring and also of discharging employees. The senior secretary and some staff salaries were paid by the Rockefeller Foundation because this was Rockefeller business.

Dr. Kerr made all the contacts with the government and visited Bangalore and Delhi for conferences. He and I had to go to Bombay to clear shipments through customs. It was a job Austin was particularly good at. He had studied public health administration and had conducted disease control operations in Egypt and South America, and we had a good working relationship.

We didn't have a big staff. Dr. Telford H. Work, who became director in 1956, increased the staff because of the epidemic of Kyasanur Forest disease. When it came to animal care work, you had to have people that meticulously washed their hands and handled the mice with forceps. Everything had to be sterile. Then we had sterile food. I believe they still use the same mouse diet that we prepared.

Hughes: How many technical staff positions were there?

Johnson: The five I mentioned previously, Lamba, Rajagopalan, Shah, Chandekar, and Patil. When Rao joined the staff in March 1953, he gave additional training to Rajagopalan, plus training additional staff for entomology. Kerr and I were both involved in training the animal care staff. Mr. Diwan was put in charge of the mouse colony. There was office staff besides Mr. Chari--the storeroom clerk, drivers, carpenter--a staff of forty-eight besides Kerr and me.

We trained technicians how to work at a lab bench, how to harvest mouse brains, bleed and autopsy birds and animals, and how to inoculate mice and chick embryos. You picked people who wrote well and seemed intelligent. You could train them fast. They were not supposed to leave any virus on the table; we worked on trays covered with paper which could be burned.

Hughes: Were these techniques that you had evolved?

Johnson: The methods were developed at the Rockefeller Institute, and while studying rabies and yellow fever. These were hot viruses, and you worried about them. A lot of people that are trained in ordinary diagnostic laboratories may be careless. My idea was to make sure that you would never get virus on your hands. You wouldn't need gloves; you just never contaminate your hands. You'd use gloves when you did autopsies or handled birds or animals. I had heavy leather and rubber gloves. When I inoculated mice, I would say, "Don't cross hands." You have a syringe and needle in one hand, and don't cross the other hand, because you're liable to stick yourself. The mice were handled with forceps.

I always told the people that worked for me, if you see any way you could make procedures safer, tell me and we'll discuss it. Because you never can be absolutely safe. And if you spill something, be sure and tell me right away.

Hughes: And they were good about that?

Johnson: Yes, they couldn't stay if they broke the rules. Four died in two years in the yellow fever work, remember?

Nobody ever died from infections contracted in my lab. They certainly got infected. In Alabama, two of the technicians got tularemia at the rabies study, but that was from an aerosol that they made filling and emptying syringes in water before boiling them. At the time we did not know we had isolated a tularemia organism from a dog distemper specimen. It was tularemia killing the mice, not dog distemper virus.

Hughes: Did anybody get sick at the Virus Research Centre in Poona?

Johnson: Not when I was there.

You learn to trust people. When you live in a foreign country, you sometimes develop a closer friendship to people than you do in your own country. Your life depends on whose advice you follow. I was also so fond of Mr. Walsh. A few M.D.s in Poona became close friends.

There was a good physician in Poona, Dr. Grant, who had been trained in the United States, and I trusted him. Austin and I would rely on him if we needed an x-ray or other tests. But our advantage was that we had quick access to all the biologics from the United States. The foundation could fly them out in two days.

Hughes: They weren't available in India?

Johnson: Not in Poona. I still think that sulfadiazine is one of the excellent medicines. It would prevent infection with cholera and inhibit multiplication of the organism. It had some effect on

Johnson: malaria; it was effective for treating salmonellas, and it was the only medication generally available at the time for typhoid fever. As I said, I'm pretty sure I had typhoid fever on one of my field trips; you can get it even if you've been immunized. I'd say typhoid fever in India was killing more people than any other infection, particularly children. They drank water from really highly contaminated rivers and ponds, and surface water was drunk without boiling.

Cholera came every year. The Hindus would have a big puja or prayer ceremony at a certain time, and they would come hundreds of miles to Poona where two rivers, the Mutha and the Moola, join. They'd all walk out into the river at the appointed time and take a ceremonial bath and take the water in their hands and drink it. Within a few days, many were dying of cholera. That's something to see, the bodies burning on the places along the river.

I would see cholera cases at the infectious disease hospital, rows of patients on mats on the floor. They would dehydrate from vomiting and diarrhea and die within twenty-four hours. During the 1952 outbreak I said, "Don't you have sterile saline for intravenous injection?" They said, "We ordered it by government lorry." Then I asked, "How many days?" They said, "Two days." I said, "I'll drive down there and get it today." "Oh, no, it has to come by government lorry." It did not come for ten days. It was reported in the Times of India.

We made distilled water in our lab, and after adding sodium chloride and boiling for five minutes in a pyrex flask, it was okay for intravenous administration. The Coaji Parsi Hospital made physiologic saline solution, but large amounts were needed. It took ten days to bring intravenous salt solution to Poona. You can safely treat cholera with just salt solution. The victims just dehydrate. Patients with normal skin turgor will hardly be recognized twelve hours later. A fold of skin will not retract.

Hughes: The ten-day delivery period was due to government red tape?

Johnson: Yes, but supplies were available the next year.

Hughes: Was there any other viral work going on in India?

Johnson: Yes, polio and rabies. There was the Pasteur Institute in Kasauli, North India, and the Coonoor Pasteur Institute in South India. The one in Coonoor was where I and other members of the WHO rabies committee in July 1952 to conduct a rabies laboratory training course.* Coonoor was well known for making rabies

* See below.

Johnson: vaccine. The ICMR [Indian Council for Medical Research] was planning to produce the live polio virus (Sabin) vaccine at Coonoor. Grant Medical College in Bombay had a good lab for doing research on polio, and Dr. C. G. S. Iyer was isolating polio virus from patients. There was a virus section in the school of public health at Calcutta and another at the Hafkine Institute in Bombay, which was originally set up to make plague vaccine.

Hughes: Did these places have foreign staff?

Johnson: No. They had been started in conjunction with the British medical services and had well-trained Indian doctors and technicians. I saw people who had been paralyzed in 1952, whom I suspected had been infected with Japanese encephalitis virus. The cases were mostly in children. Those I saw in Dohad, Northern Bombay State, had been tested by C. G. Iyer for polio virus and were negative. This was a local epidemic. There were many cases of dengue in India. Hookworm and malaria had been reduced.

The worst disease problem in 1952 was enteric fever, caused by Salmonella and typhoid bacilli, and oh, how the children died of that. It would be so disconcerting to come out early in the morning to the river on field work to collect mosquitoes or birds, and you'd see women putting a little one-year-old or younger infants under the rocks. A regular Hindu cremation would cost too much. They couldn't afford to burn the bodies and conduct a ceremony. I am sure these deaths did not appear in the mortality statistics. When I was at Vellore Christian Medical College I saw several young children who had been left by the parents at a place where the Vellore ambulance would stop. They were expected to die. Almost all of these children had typhoid fever and most of them recovered if treated with sulfadiazine and fluids.

The Work of the Virus Research Centre

Hughes: What was the primary purpose of the Virus Research Centre?

Johnson: Well, the primary purpose was to study arthropod-borne viruses spread by mosquitoes, ticks, and mites, the last suspected to be an important reservoir host. The human blood serum survey done in 1952 before the laboratory facilities were ready provided valuable information as to the areas where West Nile and Japanese encephalitis had occurred, as shown by the presence of antibodies in the blood of the people. This required training in record keeping, laboratory methods and, for field studies, how to trap and shoot birds and animals and process them. Very little was known about the birds and animals of India. There had been some

Johnson: studies in north India at the Benares Hindu University, but that was just a checklist of the small mammals in that region. This was probably a by-product of plague control operations.

Hughes: When you say not much was known about the birds and mammals, do you mean in the sense of being disease vectors?

Johnson: No, just in description and taxonomy. British scientists of the India colonial staff helped form the Bombay Natural History Society and trained Indian and Anglo-Indian scientists, especially Muslims, such as Salim Ali who wrote the book Birds of India. Studies of birds were done by several colonial officers in India, Burma, and Malaya. We began a scientific collection of birds and mammals when I was there.

Every animal we collected was examined for blood parasites, and they had to be measured and identified so we knew where they nested and migrated. The big skill, of course, was virology and how to run animal colonies, and you had to have culture media so you could identify bacteria, and media for processing tissues, such as bovalbumin fraction V (0.75 percent) in physiological saline.

Hughes: Were you doing most of the bacterial and viral identifications?

Johnson: Yes. Actually, no lab was diagnosing typhoid in Poona when I was there. I brought out Difco bacteriological media and could identify B. coli and typhoid organisms. If you go to the cemeteries of the British colonial churches in India, there are many children buried there: "Died of enteric." More British soldiers died of cholera or bacterial enteritis than from military operations. Lacking proper sanitary facilities, the servants and produce handlers were apt to be carriers of intestinal parasites and pathogenic bacteria and viruses. They didn't have flush toilets, and it was hand contamination that spread the infections. We experienced this in certain respiratory virus infections where children get viruses on their hands from each other and from play equipment and then rub their noses or put their fingers in the mouth. The best example is wart viruses.

Susan contracted hepatitis and Marion amoebic dysentery. Both were very serious infections. Susan had scabies and Marion, head lice, which I treated promptly. Bed bugs were common but we had our house treated with DDT.

We had trouble diagnosing amoebic infection because bacillary dysentery was common and probably provided an entry for the amoebae. In chronic tissue infection it is unlikely to find amoebae in the stool. I was away from Poona when Marion began to have loose stools and fever after a bout of dysentery. Fran mentioned it to a missionary woman and she said to go to the Coaji

Johnson: Clinic (Parsi) Lab in Poona and have them check a stool specimen. They found some amoeba cysts. Fran then contacted Dr. Leo Krainer of the Indian Armed Forces Medical Pathology Lab and he said to start aureomycin treatment.

Laboratory Staff

Hughes: How was laboratory staff selected?

Johnson: Dr. Kerr and I selected them. The technical positions available were listed by the government at the colleges, and the letters of applicants were reviewed, and some were chosen for interviews. Other jobs were posted locally. The big problem was to find those that could work with birds and animals, because Hindus, if they are very conservative in their religion, will not kill birds or animals, even an insect. Ornithology in India was done by Muslims, like Salim Ali, or by Anglo-Indians. There had been extensive entomology research. Then there were the Indian scientists who had worked in the British medical services in malaria and public health. Who do you trust when you go to a country to ask questions? The people who have been trained in modern sciences. It's very important.

Hughes: Had most of these people been trained by the British?

Johnson: Yes. There was a long tradition of Anglican and Catholic schools, also American missions. In the colleges and universities English was the official language.

Hughes: Were these sought-after jobs?

Johnson: Yes, both for the technical and clerical staff. They all had wonderful letters of recommendation. The college transcripts helped in selecting technical staff. Local staff [selection] depended on interviews. Dr. Kerr took care of that job and he had long experience in selecting clerical and field staff.

Having my office in the pathology department of the medical school (next to the morgue), I soon began visiting the pediatric ward, looking for cases of acute fever. It is there where I met Keerti V. Shah. He was very helpful and interested in what we were planning for the Virus Research Centre. I said, "Why don't you come over to the lab," which he did. He would not graduate until June, 1953, and the research medical officer job would not be open until then. By that time he had decided to apply. He was chosen for the medical research officer position and proved to be very good.

Hughes: So you were interviewing prospective staff members?

Johnson: Yes. Dr. Kerr and I both interviewed technical staff candidates. I would take them into the lab and show them the chick embryos and mosquitoes. I would say, "Well, this is where you will have to chloroform the mosquitoes, and then you have to harvest the chick embryos and take out mouse brain specimens." [laughs] That would quickly rule out most of the candidates. The medical staff was required to take blood specimens and assist with post mortem examinations. Field staff were told they would have to be willing to trap and bleed and kill birds and animals and also to learn to use guns for collecting wildlife. Medical students trained at the modern medical schools, such as B. J. Medical College in Poona, had to practice their diagnostic and treatment skills in the same manner as those taught in the USA or Great Britain.

The 1952 Serum Survey

Hughes: Were you sending any material back to the Rockefeller laboratory in New York?

Johnson: The first field project was Austin Kerr's responsibility.* To determine which of the known arthropod-borne viruses were active in India, we had to get human sera and see what antibodies they contained. The man selected by the Indian Council of Medical Research to do the bleedings was Dr. P. B. Gatne, a relatively young [Indian] state health officer. On March 25, 1952, we went over the map of south India to select representative ecologic places to sample the immune status of the population, including various age groups. This work was completed within two months.

Dr. Kerr had obtained some horse sera from a military post 300 miles north of Poona. This was near Dohad where there had been a small epidemic of encephalitis in horses. When Gatne had collected the blood specimens, he would return to Poona in the lab truck with the bloods, and Kerr and I would take off the sera and ship them to the New York laboratory. As Dr. Smithburn finished the tests, he would send us the reports.

The first report came on June 26, 1952. Nine of ten horse sera from Deolali horse farm were positive for West Nile antibodies. Having only single specimens taken after the

* J. A. Kerr, P. B. Gatne. Reconnaissance of immunity to six viruses in southern India. J. Med. Res. 1954, 42:319.

Johnson: outbreak, we could not be certain whether the horses had been infected with West Nile virus or another in group B (flaviviruses), such as Japanese B virus.

Dr. Kerr had run serum surveys for yellow fever and had developed an easy and practical way of processing the bloods. They were kept on ice until returned to the lab. The vacuum venules had the needle set into the rubber stopper at an angle. The needle was inside a glass sleeve sealed at the end. This was broken easily. The needle was inserted into a vein and, when the angle of the needle holder was increased, the vacuum drew in the blood. This was a 20 ml. collection tube. The vacuum could be checked with an ultraviolet light. The needle was broken off at the base. The rubber valve sealed the tube.

At the lab, the tubes were shaken to loosen the clot, then the tubes were put in racks and these put in the refrigerator overnight. The next day the stopper was heated in the flame of a Bunsen burner and twisted off with heavy scissors which had been sterilized in the flame. After the top of the tube had cooled, the serum was drawn off with a 15 ml. vacuum venule. The needles were broken off with the scissors and the tubes were ready to ship.

Each tube had a label with the name, age, sex, date, and location. At the New York lab, the sera were tested without heat inactivation against 200 LD₅₀ of each of six viruses. A total of 576 sera from thirty-seven places were tested, all from within 500 miles of Poona.

Hughes: He didn't have to pay the donors?

Johnson: No. Gatne was an official public health officer and he explained to the chief of the village what they wanted the bloods for.

Hughes: Is that what you mean by a collecting station?

Johnson: Yes. A collecting station refers to the source of specimens, such as human bloods, mosquitoes, ticks, birds, and animals.

Hughes: That was a designated area?

Johnson: Yes, a blood collection representing a village site. These included coastal plain (60-160 ft.), eastern ghats (400-1600 ft.), East Khandesh (700-900 ft.), Deccan Trap (1600-2000 ft.), Saurashtra-Porbander northwest coastal, Nagpur-Ramtek (1000 ft.), and Hyderabad (1600 ft.) in the central plains, Mysore southwest coastal and Malnad to 2800 ft. Shimoga and Holehour were in the area I was doing field studies from our forest camp twenty-three miles west of Sirsi, in Kanara District. This is where an epidemic of Kyasanur Forest disease began in the 1956-57 fall-

Johnson: winter season. This was a tick-borne disease closely related to Russian spring-summer encephalitis (RSSE) virus. Austin Kerr picked the Porbander collecting site because sailing ships (dhows) arrived there from Africa, and he believed that this would be the most likely place for the introduction of yellow fever virus.

All the sera were negative for yellow fever, Bunyamwera, and Bwamba virus antibodies. There were people with antibodies to RSSE virus at Porbander, Kingaun, to the east of Poona, and in a few people in south India on the plateau. There was no evidence of antibodies to Semliki Forest virus, the group A virus from Africa. There were people with antibodies to West Nile virus at Ahmedabad, northwest India, and also at East Khandesh, central India. Some of the sera with West Nile virus antibodies also neutralized Japanese B encephalitis virus. Some of the sera from Nagpur and Chingleput in Mysore had antibodies to Japanese B virus.

Hughes: So the first year you were mainly doing the serological surveys?

Johnson: Yes, but there was much to do to learn about the ecology of India. My first major field trip to south India began July 4, 1952. I traveled in a Chevrolet carry-all, accompanied by a driver and cook-bearer. We arrived at Bangalore, Mysore State, on July 6, where I visited Robert W. Watson, Richmond K. Anderson, C. Brooke Worth, Joseph C. Carter, and R. Lyman of the Rockefeller Foundation staff. This was during the monsoon and I was anxious to confer with Joe Carter, the engineer, about roads in the western ghats. He proved to be a source of valuable road information.

I was on my way to Coonoor to take part in the World Health Organization rabies laboratory methods course for East Asia, July 14-27. From July 7-10 I visited the Sakleshpur malaria field station 130 miles west of Bangalore. Brooke Worth was with me. This is in a high rainfall area, and it was a test of our ability to observe the flora and fauna during the southwest monsoon. We spent several hours one night riding the front fenders of the truck observing the wildlife that was active.

Between heavy showers the moon was out and I collected a couple of flying foxes. Bats follow a pattern and if you see one you stop the car and wait. You have to shoot so they will fall in the road clearing. There were tigers in this district and we would occasionally hear them. Brooke wrote a poem about that night study. Tippu Sultan, the Muslim ruler of south India in the latter part of the eighteenth century, had a fort at Manjarabad on the Sakleshpur road. From the ruins we could see the west coast.

Johnson: On July 12, I stopped at Seeringapatam near Samanthpur to see the Tippu Sultan palace. Tippu was killed here when the British army took the fort. I was shown a prison wall where a British officer had been chained for three years before he was rescued. On July 12 and 13 I visited the Krishnarajsagar dam and the Somanathapur Sandal Forest Preserve. The forestry officer was my guide.

During breaks at the conference at Coonoor I visited a malaria control project at Kallar and Mettupalaiyam below the Nilgiri ghats south of Coonoor and the Moyar dam and Gundalur forest station below the Ootacamond ghat on the north side of the Nilgiri Hills. Here the forestry officer took me on an elephant trip to the dam. On July 28-29 I was in Cochin and on July 30-31 in Trivandrum and Cape Cormorin. On the trip to the cape I was accompanied by a local ornithologist from the museum. Here I learned of the gerbil, Meriones hurrinae jerd., which is like the Meriones species in the plague focus in Iran.

August 2 I visited the Edapalayam preserve at Thekkady where there are large numbers of cape buffalo and elephants. The forestry officer took me on a motorboat trip on Lake Periyar to have a good view of the elephants. August 4 I visited Madurai and Tanjore, primarily to see the malaria lab at Pattukkotai near Tanjore. August 5-8 I was in Madras visiting the Guindi Institute and health department. I was particularly interested in the malaria, filariasis, and kala azar disease studies there. On August 9-10 I was at Christian Medical College and Hospital at Vellore, which later served as a field station for the Virus Research Centre.

From August 10-15 I made an intensive survey of the western ghats starting at Sakleshpur via Belur, Chikmagalur, Aldur (Devanda State Forest) across the Bhadra River on a dugout boat ferry, via Koppa, Tirthahalli, across the Tunga River on another ferry, via Kavala, Durga, Nagar, Hosanagara, Sagar, across the Sharawati River on a ferry to Gersoppa (Jog Falls, 830 feet high), the site of the Mysore-Bombay hydroelectric power station. From Jog Falls forest bungalow I went to the Sirsi forest bungalow via Marigundi, Siddapur, Manchikeri, and Yellapur. It was on the Sirsi-Kumta road that I found an ecological succession that seemed ideal for study, and I returned for a more intensive survey October 3-8, and in February 1953 from the 21st to the 26th covered the entire region about Devimani ghat where we established a field camp in October 1953.

Humayan Abdul Ali, brother of Salim Ali, joined me here October 20-23 and we did both day and night hunting and collected a representative collection of the birds of the area. In November and December Lamba and Rajagopalan were hunting and trapping at the field station, and in December I stopped there for three days to help collect birds and mammals. It was on that visit that I

Johnson: collected chicken mites December 22 at Ragihosali, twenty-two miles west of Sirsi Forest bungalow. This collection yielded Sindbis virus.

A pool of *Culex* mosquitoes of five species collected at the Benihole station one mile east of Ragihosali on December 18 also yielded Sindbis virus.*

T. R. Rao met me at Cochin on December 22, 1953, and we collected mosquitoes at Shertallai, a focus of filariasis. On December 29 I visited Chandi Pylie at Alwaye. He collected fledgling "talking" mynas, Gracula religiosa, in the forest each fall. I learned about this in July 1952. That morning they had collected 400 of these myna fledglings. I pulled out four of the youngest and brought them back to Poona.

The birds were fed and protected from insects. B 571 was dead in the cage January 6, and B 572 died later that day. A virus later identified as Sindbis virus was isolated from the spleen tissue of both birds. The blood smear of B 571 showed Hemoproteus, filaria, and leucocytozoan parasites, and the smear of B 572 showed Hemoproteus, filaria, and trypanosome parasites. The amount and variety of blood parasites illustrate the high exposure to insect feeding these birds had endured in the tropical forest.

During the monsoon season in 1952 we collected 572 birds. In the Poona District we isolated a virus from the spleen of B 125, a common house crow, Corvus splendens, collected moribund July 3, 1953. This virus was not lyophilized before I left, and the mouse brain specimens held in glycerol saline were not passaged until a year later, and the virus was no longer viable. The course of the disease in mice was similar to that produced by West Nile virus. A virus later identified as Sindbis virus was isolated from a spleen pool of three white wagtails, Motacilla alba, collected at Pashan tank seven miles west of Poona on October 13, 1953.

Hughes: How did you trap birds?

Johnson: We did not have any nets for collecting birds in India. We collected nestling birds by hand, and we could obtain fledgling crows to an age of ten to twenty days. We collected 101 nestling crows, fifty common mynas, eleven weaver birds, eight sparrows, and sixteen of other species. The studies of birds collected by shooting were mentioned previously.

* See Devimane ghat field station description. [Undated report by Dr. Johnson, written in India, and on deposit at The Bancroft Library.]

Johnson: We had "Hav-A-Heart" live traps for small mammals. We did very little testing of small mammals when I was in India, preferring to study abundant local and migratory birds, of the latter primarily the white wagtail.

Hughes: How did you get them identified?

Johnson: We were only interested in common birds that were numerous. These could be identified from Salim Ali's book, Birds of India. Any doubtful identifications were checked at the Bombay Natural History Museum. Lamba prepared museum specimens of some of the birds and mammals. Dr. T. Ramachandra Rao later made collections of insects—the different ticks, mites, mosquitoes, and flies. The Poona entomology laboratory now has an excellent entomology museum. I took some mounted specimens to the United States for final identification.

Hughes: You mentioned funnels for collecting mites.

Johnson: We used a large, metal funnel, about twelve inches in diameter at the top, above this a seventy-five-watt electric light bulb. The bird nest contents were put into the funnel. There is a screen at the base of the funnel; the heat from the light drives the mites down into a glass jar. We obtained ten thousand mites out of some crows' nests. In the process, you find other insects such as *Culicoides* flies. We did test many pools of bird mites from crows' nests, but these were negative for viruses. We also used traps for collecting mosquitoes. Because of our primary interest in human virus diseases we usually collected mosquitoes from the exposed backs of children by flashlight using a mouth suction tube. Single feeding or fed mosquitoes can be collected by placing the open end of the test tube over the mosquito and stoppering with cotton. This makes it easy to examine the mosquito.

We knew there was a limited time period for the virus program. It was a continuation of a series of specific disease studies [by the Rockefeller Foundation], the hookworm, malaria, yellow fever, and rabies studies.

Hughes: When did the Rockefeller Foundation stop supporting viral research?

Johnson: The Rockefeller program on the viruses officially ended in 1970. My project [supported by the foundation] at the California lab continued until '72. The Rockefeller Foundation decided that research was now needed in plant diseases, tropical agriculture, and in genetic studies of corn, maize, wheat, and other crops, particularly cow peas and cassava. So they have agricultural research in India, Africa, the Philippine Islands, Mexico, and South America.

Johnson: I feel it was too bad that the foundation didn't keep one or more virus programs, because there are still plenty of problems with viruses. It was the ecological approach that interested me. The various organisms that cause disease in people have their natural host system in wildlife.

The virus program of the Rockefeller Foundation was characterized by field teams directed by physicians, with the technical assistance of veterinarians, entomologists, and zoologists. Over thirty million dollars were spent on the virus program from 1950-1970. It left a legacy of knowledge that will not be lost. The studies continue in the public health laboratories where the Rockefeller Foundation conducted field operations. The Virus Research Centre in Poona is now the National Institute of Virology of India.

Return to the United States

Johnson: In November, 1953, I wrote to Hugh Smith, director of the virus program, telling him that I planned to take my accumulated leave beginning in March, 1954. Austin Kerr was on leave in the United States and was due to return to India in mid-January. I asked about possible assignments after returning to the United States and indicated my interest to return to the Rockefeller Foundation lab at the Rockefeller Institute. Hugh Smith replied that they had been negotiating with the State Health Department in California about a cooperative program on arthropod-borne encephalitis along the lines of the previous program on influenza (1939-1945). This would be a field study project like [those at] the four labs already developed at Poona, India, Johannesburg, South Africa, Port of Spain, Trinidad, and Belem, Brazil.

There had been more than 700 human cases of encephalitis in California during 1952. Only fifty percent of these were caused by known viruses. Dr. Lennette was anxious to have help in determining the etiology of the unknowns and to initiate a field study of human cases of encephalitis and investigate the wildlife aspects of the arthropod-borne virus diseases. I was familiar with the research studies on encephalitis by Dr. K. F. Meyer and his associates, William M. Hammon, Beatrice Howitt, and William C. Reeves at the Hooper Foundation at U.C. San Francisco, and also with the studies underway at the virus lab in the California Department of Health at Berkeley under the direction of Dr. Lennette.

I replied saying I would be delighted to take this assignment. A new building was under construction for the State Health Department and also a new building for the University of

Johnson: California School of Public Health. Dr. Charles E. Smith, dean, promised to provide a faculty appointment for me. I requested permission to travel by sea to the United States by way of Japan. I wanted to visit the National Institute of Health of Japan in Tokyo and the 406 General Hospital of the U.S. Army where there was a special field study of Japanese B encephalitis. Oliver McCoy of the Rockefeller Foundation staff was in charge of the office in Tokyo and made the arrangements for my visit.

We moved to the Poona Club about two weeks before we were to leave India because I had to crate all our belongings and pack the personal baggage we were to take with us. There are the usual invitations to dinner and farewells which are always a sad occasion after establishing some warm friendships. However, the big event was the ceremony at the Virus Research Centre. We have a large photograph of our family with the entire staff of the VRC, at that time forty-eight persons. Many came to see us off at the Poona Railway Station.

We left India on the Italian Line S. S. Asia on February 14, 1954. Keerti Shah and his wife Usha, Raffat and Kishan Kishindas, and Captain Nagesh C. Gupta of the Indian army came to the boat to see us off. Raffat, daughter of the aide to the Ali Khan who was in charge of the Agha Khan's palace and properties at Poona, and her husband Kishan had built a new bungalow next to ours. Raffat and Fran became good friends, and she invited Fran to attend a sewing class. These women made Fran's stay in Poona a very pleasant one. Nagesh Gupta, whom I met shortly after arrival, introduced us to several fellow officers, especially Madan Arora of the air force. They enjoyed helping collect birds. It's hard to find anyone interested in taking off at 4 a.m. so as to reach places where water birds rest during migration, and of course I could not go by myself. Nagesh introduced me to the sport of gliders, launched by a winch. Keerti introduced us to political action in India, and he was surely a wonderful associate during the development of the virology studies. We all went on early morning picnics and special excursions.

We traveled on the S. S. Asia to Hong Kong, stopping at Ceylon and Singapore. From Hong Kong we sailed on the S. S. President Cleveland, stopping at Kobe, Yokohama, Honolulu, and San Francisco. We reached San Francisco on March 18, 1954, and on the 22nd I visited Dr. Malcolm Merrill, state health officer, Dr. E. H. Lennette, director of the virus lab, and Dr. W. C. Reeves, who was in charge of the university's field study lab at Bakersfield, California, to make tentative plans for the Rockefeller Foundation project. I and my family went on by train to Boston.

On March 29 I went to New York City to report to Hugh Smith and to visit the Rockefeller Foundation lab at the Rockefeller Institute. On March 31 I had lunch with George Harrar, director

Johnson: of the agriculture program, in company with Hugh Smith, to discuss my experience in India.

I continued to work at the offices of the Rockefeller Foundation at Rockefeller Center until May 4, 1954. I had to complete my chapter on rabies for the third edition of Viral and Rickettsial Infections of Man, edited by Frank Horsfall and Igor Tamm. I also had to complete my paper on arthropod-borne virus diseases, which I presented at the Industrial Council for Tropical Health at Harvard School of Public Health April 22, 1954. I spent as much time as possible at the Rockefeller Foundation lab. The requests from the field studies around the world were coming in, and Max Theiler, Loring Whitman, and I were most excited about the amount of new information [about arboviruses] that was beginning to accumulate.

[Interview 7: April 21, 1987]

Director, Arthropod-borne Virus Study Project, Rockefeller Foundation and California State Health Department, 1954-1972

The Rockefeller Foundation's Arbovirus Research Program

Hughes: Dr. Johnson, according to the annual report of the Rockefeller Foundation for 1958, the virus research program of the Rockefeller Foundation was concerned with the study of arthropod-borne virus infections of man and domestic animals around the world. The virus lab in New York City was chiefly concerned with basic research on the major viruses borne by arthropods. This work was complemented by field stations in different parts of the world. How was the laboratory here in Berkeley seen to complement the general arthropod virus research program?

Johnson: It was number five of the Rockefeller Foundation field labs. Subsequently field stations were developed at Cali, Columbia (1962) and Ibadan, Nigeria (1963). The first part of the program was to work up [the arthropod-borne] viruses in New York.

We talked a lot about why these viruses were present in certain areas and not in others. The general conception of arboviruses was that they were in the tropical forests and that they were transported to the temperate zones by people or by migrating birds. The obvious course was to sample tropical environments where there was a lot of malaria, filaria and unexplained fevers, also known mosquito-borne viruses, like dengue and yellow fever. The selection [of viruses to study] was made largely in cooperation with laboratories already in existence.

Johnson: The base lab at the Rockefeller Institute in New York was to remain the same. Certain field laboratories, like the one in Entebbe, Uganda, had been taken over by the British Colonial Medical Service. It had been a joint venture with the Rockefeller Foundation until 1949, when we withdrew in keeping with the IHD's policy of turning field projects over to local governments. The Entebbe lab had originally been built with Rockefeller money and supplied with equipment. It was an excellent lab, and the British doctors assigned there were very good—people like David Gillette and Alexander J. Haddow.

As I said, beginning in 1951, the idea was to start some new field projects [supported by the Rockefeller Foundation], and the Poona, India, Virus Research Centre, opened in 1952, was the first. The plan was to study the natural environment of India and search for arboviruses there.

The Arbovirus Program in California

Johnson: There was really no major thrust in arbovirus research in California until I came in 1954.

Hughes: Why did the Rockefeller Foundation choose the California State Department of Public Health?

Johnson: Well, just look at the virus diseases here--St. Louis and western [encephalitis]--real big problems.

Hughes: How was the California lab conceived as fitting in with the arthropod-borne virus program?

Johnson: The Rockefeller Foundation needed a field laboratory in North America to complement its studies in South America. We knew yellow fever epidemics had occurred during the 1800s in the United States. They began in southern or southeastern seaports, and the public health authorities assumed the disease was introduced by people infected in Central or South America. Prior to the elimination of urban yellow fever by vaccination, beginning in 1938, yellow fever was believed to be maintained in the human population. The identification of sporadic cases of yellow fever using viscerotome specimens led to the identification of jungle yellow fever. The source of the disease in wildlife remains unknown.

One reason for having a field laboratory in the United States was to study the natural history of the western and St. Louis encephalitis viruses. California has [always had] one of the best

- Johnson: microbiology public health laboratories. After Dr. Lennette came on [as director of the virus laboratory], it became an equivalent to the Rockefeller IHD lab in New York City, which 1964 moved to Yale. There were only a few state laboratories which were really equipped for research in viruses. The major ones were California, Massachusetts, Georgia, Texas, New York, Michigan, and Minnesota.
- Hughes: To summarize, there were really two reasons for choosing California. First, the number of viruses in the area, and secondly the prestige of the laboratory.
- Johnson: Yes. The other thing which interested me to come here was that California had the major ecological environments, from the alpine to the desert. The latter interested me very much after I had studied the natural history of India. I then realized that the desert scrub is teeming with animals and birds, whereas the beautiful agricultural land in the central United States with the big fields has small populations of wild animals. Even the harvest mouse is almost gone.

Here in California there is a big river system. The Sacramento River is a highway for birds and bats, like the Nile River in Egypt. You find the viruses in mosquitoes in marshlands along the Sacramento River. The best collecting stations are under bridges and in chicken houses near the river. The birds roost in the big trees along the river. If you're at the U.S. Wildlife Refuge at Willows near the Sacramento River or at Blythe near the Colorado River, an isolated willow near a marsh will be used as a roosting tree. That's where you find viruses in the mosquitoes, using a Chamberlain mosquito trap. Open marsh with tule reeds and fragmites reeds are used by red winged blackbirds, and a large tract of marsh of this type may have more than a million blackbirds roosting in it during the fall migration.

Then we come back to your question--why California? Well, there had been studies of St. Louis encephalitis virus in St. Louis, Missouri in 1933. There was a similar epidemic in '32 in Paris, Illinois, which is on the Wabash river system northeast of St. Louis. There had been an extensive study of western encephalitis going on since the thirties here in California at the Hooper Foundation. The state of California was interested in the Rockefeller Foundation coming in to see what the nature of the wildlife reservoir was, because epidemics of encephalitis would come just once in a while.

There was a big epidemic that Bill Hammon and Bill Reeves studied in Yakima, Washington, in '41. It was at Yakima that the mosquito vector of western and St. Louis virus was discovered, but field studies were not continued there. The Sacramento and the

Johnson: Colorado river basins with large tracts under irrigation were like tropical marshland and it seemed to be a logical place to see whether western and St. Louis viruses persisted there.

Hughes: Wasn't it 1930 when Dr. K. F. Meyer first isolated the western encephalitis virus?

Johnson: Yes, with Dr. Beatrice Howitt, who was a virologist, and Dr. William McDowell Hammon, a top physician and epidemiologist. He joined the staff of the Hooper Foundation later.

Hughes: Because the Hooper had been working on encephalitis in California, how receptive was it to the state lab coming into the same field?

Johnson: Well, they were very cooperative. Virology was limited to rabies at the state lab until the Rockefeller Foundation set up a lab here in 1939 for the study of influenza.

In the thirties, the Hooper Foundation staff found that both man and horses were being infected with western encephalitis. The mosquito-control work appeared to have eliminated the encephalitis viruses, so further research did not seem urgent until 1952 when there was a terrible encephalitis epidemic.

Hughes: Was it the 1952 epidemic that precipitated the Rockefeller involvement in California?

Johnson: Yes, it was. Dr. Lennette, who had worked for the Rockefeller Foundation (1939-1946), became director of the Viral and Rickettsial Disease Laboratory at Berkeley in November of 1947. He was, of course, familiar with the world program of the Rockefeller Foundation, and that's why he asked Dr. Hugh Smith of our New York staff if the Rockefeller Foundation was interested in a cooperative arbovirus program in California. Hugh Smith wrote to me in 1953, asking if I was interested in being director of a cooperative study in California. I replied in the affirmative.

Hughes: Why were you asked to come?

Johnson: Well, it was probably for two reasons. One, I had been in India, I had trained the local staff, and the program was going well. There were other staff personnel that could follow me there. Two, I wanted to come back to the United States because of the illness of two of my children. It was a difficult time to live in India as far as food and everything else were concerned.

The California assignment seemed ideal. Dr. Lennette was here, and I knew Dr. Meyer, Dr. Howitt, and Dr. Reeves, and it would be a good cooperative place to work. But most of all, my mother and father had moved to California from Nebraska in 1943, and a brother and sister were living in Turlock, California.

Johnson: My father died January 26, 1952, aged ninety-one, soon after I arrived in India. I had visited him at Turlock shortly before we left for India and he was able to be up during the day and take short walks. He died while resting, so we had no warning. This reminds me of the death of my brother-in-law, Alfred Mattson, at Turlock, October 17, 1953. I was in the field camp at Sirsi, India, when Fran sent a telegram to me. It was a strange thing to get this message there. A runner had come many miles to deliver the telegram and bring my reply to my wife. This is when we realize we are far from home.

When we returned from India, I had accumulated leave of three months. It looked as if there was going to be another [encephalitis] epidemic in California because cases were already occurring in the Bakersfield area in May, 1954. I had just had a month to write up some reports at the New York office and write a paper that was to be given at an international conference on tropical health at the Harvard School of Public Health. It seemed to me that if I waited to have my three months leave, then I would have to miss the opportunity to study the epidemic of encephalitis. [So I decided to go immediately to California.] That was a good choice.

When I arrived in Bakersfield in June, 1954, I immediately began to work at the Kern General Hospital. Dr. Reeves and Dr. Lennette were very pleased to have me doing the hospital work, because I'm an M.D. I saw all the encephalitis cases admitted to the hospital and arranged for blood specimens and throat and rectal swabs so we could test for viruses and do follow-up bleedings for serology. I studied seventy patients with encephalitis, sixty-four of whom were admitted to the Kern General Hospital.

Hughes: Did you move your family to Bakersfield?

Johnson: No, they stayed at Scituate, Massachusetts, until the end of summer. In the meantime, I rented a house from one of the U.C. Berkeley faculty who was going to Norway for a year.

Hughes: So you were commuting.

Johnson: I would go to Bakersfield early Monday morning and work the whole week, bringing back the specimens on dry ice on Saturday. That was a very intense study.

Dr. R. E. Bellamy was in charge of the Bakersfield laboratory. He was most cordial and helpful in teaching me to identify the local mosquitoes. He was employed by the U.S. Public Health Service and assigned to Dr. Reeves' field station. He was doing large-scale mosquito studies. Glenn A. Hutson, also

Johnson: employed by the U.S. Public Health Service, was a valuable source of information on bird trapping and banding. He had previously worked at the U.S.P.H.S. field station in Colorado.

Hughes: How long had the state lab been involved in encephalitis?

Johnson: Dr. Lennette had started studies of encephalitis virus while assigned to the Rockefeller Foundation respiratory disease project in 1944. After he resigned from the Rockefeller in 1946, Dr. Gordon Meiklejohn continued the study of diagnostic methods. During the summer of 1947 a survey of the incidence of encephalitis viruses in mosquitoes was carried out at the state virus laboratory using infant mice as test animals. The field team under the direction of Richard Dow included Willis Wirth, Jules Fine, Don Grant, and Ernest Meyers. This work was supported by a grant from the U.S.P.H.S. These entomologists concentrated on mosquito collections near homes where people had developed encephalitis. Fifty-six virus strains were isolated, fifty-one of western encephalitis virus and four of St. Louis virus. One other virus had been isolated but it had not been identified or characterized.

The Hooper Foundation was not involved in testing mosquitoes at that time. Dr. Reeves had left the Hooper Foundation and was professor of epidemiology at the U.C. School of Public Health and had developed an encephalitis project with a field station at Bakersfield.

Hughes: But the Hooper was still interested in encephalitis?

Johnson: No, not in 1954. Dr. Meyer's program consisted mostly of bubonic plague. Then there was the United States Public Health Service plague lab in San Francisco. Dr. Meyer was interested also in brucellosis, Q fever, and psittacosis.

The Salk Vaccine Field Trial

Hughes: The Salk vaccine trial came along in the mid-fifties. How did this fit in, if at all, to what you were doing?

Johnson: I had no direct interest in polio at the time. We did isolate type 1 and type 3 polio viruses, Coxsackie type A and B virus, and rabies virus from specimens I collected in Kern County, looking for encephalitis. I was very interested for the sake of my own four children who were at a very vulnerable age. My own decision was not to use the Salk vaccine, mostly because the Mahoney strain used in the production of this vaccine I knew was a very highly

Johnson: virulent virus for monkeys. The plan was that the virus would be killed in the Salk vaccine, but I was concerned that if it wasn't killed, it would be a very dangerous virus.

Dr. Lennette was doing serologic studies on people given the Salk vaccine. The results of the antibody tests didn't appear satisfactory for this killed-virus vaccine.

Hughes: You mean it was not very antigenic.

Johnson: It was not producing a satisfactory level of antibody. Some people were developing very high antibodies to the type 1 virus, which was the virulent one. The other two were the MEF (type 2) strain and type 3 Saukett strain. These did not produce satisfactory antibodies.

Hughes: Why did Salk choose the Mahoney strain?

Johnson: Because he felt he got better antibody with this virus strain. When he was immunizing people and according to his tests, he had inactivated the virus with formalin treatment. That's why he didn't want to use another strain, although others around the world selected the high brain passage strains which were of low pathogenicity for monkeys.

Hughes: Why did Salk insist on using that strain?

Johnson: Well, because it did so well in his serological tests. We noticed in California that when the first tests came out, the type 1 antibodies just shot up, whereas the type 2 and 3 were low. It was the Mahoney strain, type 1, that was dangerous. So that was one problem. That's why the vaccination program was stopped. When it was resumed after changes were made to insure complete inactivation of the vaccine virus, the serological tests did not show a good immunity response.

It was not known until some time afterwards that probably over a million people had received some batches which also had SV40, a simian virus, which was present in the kidney cells which were used. This virus produces cancer in hamsters. There is no evidence that there has been an increase in cancer in those vaccinated with such lots of vaccine. Dr. Robbins, who with Enders and Weller won the Nobel Prize, made a special study of this cohort of vaccinees.

Hughes: Was the danger of the Mahoney strain ever brought out in the discussions before the decision to conduct the trials?

Johnson: The original field trials conducted by the National Polio Foundation were to test the effectiveness of pooled gamma globulin from the general population for the control of outbreaks of polio.

Johnson: There were two trials to see if they could use it as a preventive in a field situation where they were having a few cases. Both of those trials showed that it was not effective. Since by the time they got around to giving the serum there were so many exposed, the morbidity and mortality were the same in the ones that had the gamma globulin as compared to those that had just the placebo.

Hughes: Was this project sponsored by the March of Dimes?

Johnson: Yes. That was the National Polio Foundation [NPF]. The money was used very wisely at first. For example, the foundation provided grants for monkey studies to see if there were more than one type of polio virus for tissue culture studies for growing and doing antibody studies. Vaccine potency tests were done using rhesus monkeys.

I attended the original NPF conference at Hershey, Pennsylvania in 1951* because we had shown you could make an effective killed-virus vaccine for rabies. The new discovery was that Drs. Enders, Weller, and Frederick C. Robbins had shown that polio virus was not strictly neurotropic but did grow in [non-neural] cell cultures. Cell culture was a new method of getting a lot of virus, much better than using brain tissue virus from an animal like a monkey, which would be practically impossible to use on a large scale.

Various cell lines were susceptible, but they chose the rhesus monkey kidney as a source for making the vaccine. This led to a really serious complication, because these were all wild-caught Indian monkeys. These monkeys were brought into the United States. You'd take one kidney at a time and you'd make up a lot of cultures, and then you'd have to take the other kidney, so one monkey would [only] last for two big batches. There was a lot of concern about the number of monkeys that would have to be used. They were overpopulated in certain areas of India, and it was possible to get them. So the monkey kidney was the source of the cells for growing the virus.

Dr. Jonas Salk worked out the details for getting a lot of virus. He had high-titer viruses, all three strains, and then he treated each virus pool with formalin. To make a single injection, you'd pool the three antigens, type 1, type 2, type 3, in equal amounts. That would be given to children as a single dose.

* Proceedings, Round Table Conference on Immunization in Poliomyelitis, March 15, 16, 17, 1951, Hershey Hotel, Hershey, Pennsylvania. Kenneth F. Maxy, Johns Hopkins University, Baltimore, chairman. Basil O'Connor, president, National Foundation for Infantile Paralysis, sponsor.

Johnson: We'd had the same concern with other killed virus vaccines. In fact, at the Rockefeller Institute in the forties, we were involved with vaccinating our staff with killed virus vaccines for eastern, western, Venezuelan, Japanese B, and Russian spring-summer encephalitis, which we were working with in the lab. These were supposed to be killed vaccines.

Hughes: Did you speak up about this potential problem in regard to the Salk vaccine?

Johnson: There wasn't much that I could say about the Salk vaccine, which was getting so much publicity. It was going to solve the polio problem [so some thought].

There were two things that we knew epidemiologically: One, that polio would come and there would be a big epidemic; then there might be many years before we had another one. It comes in cycles. You have to build up a susceptible population. The other thing we knew, and that was [learned] when I was working at the Cleveland City Hospital as a resident on the contagious [disease] service, was that you could have a town, like Dr. John Toomey studied in Pennsylvania, where the people in the low economic area had no cases of polio, and the ones in the nicer homes on the other side of the tracks had polio. That was true of polio in Sweden in the early days when it was noted that all ages were susceptible and there was a high morbidity and a fairly high mortality. It had been observed that almost all the Eskimo populations were susceptible to polio.

Hughes: Simply because the children hadn't built up resistance?

Johnson: They had not been exposed to polio before. If you're living in an area where the virus is present endemically, then with poor hygiene you will become immune during the first year of life when you still have some immunity from your mother.

Hughes: You spoke up about your fears about the use of gamma globulin for passive immunization against polio?

Johnson: I said that I didn't think that the gamma globulin project as designed would be successful. Dr. Hammon was in charge of the field study, and because he was an expert virologist and epidemiologist, he was willing to test the gamma globulin. They were going to wait until there were at least five cases of clinical polio in a town of about 100,000 or more people, and then they would give gamma globulin and a placebo, alternately. The placebo was made to look like the material that had the gamma globulin. They did it twice.

Hughes: Do you remember the year?

Johnson: 1951.*

The meeting was held in Hershey, Pennsylvania. I said then that the reason I was against the gamma globulin trial was that, knowing the general epidemiology of viruses, when you'd had five cases of polio in any community, particularly a relatively small one, the population had been widely exposed. Everything pointed to the fact that only a small percentage of the total population would get paralyzed. We have paralytic and nonparalytic polio. Whenever there was an epidemic of polio, like the one I saw in Cleveland in 1935, a lot of people just had a stiff neck and a fever and little or no evidence of paralysis. Then you'd have those that were completely paralyzed. There was much to suggest that the total virus exposure must have something to do with it, for example, ingestion of highly contaminated water or hand contamination in a nursery when changing diapers.

At that time they didn't believe that the virus was transmitted through the intestinal tract. They thought it was respiratory-borne. It was thought that the virus had to get into a nerve, usually through the nose or throat. There was a big field trial using tannic acid, a spray in the nose, to prevent infection of polio. It was not possible to determine whether this was effective in preventing infection.

Hughes: The second time you spoke up in regard to using the Mahoney strain was later at the same meeting?

Johnson: Yes. We discussed the entire possibilities of a vaccine. I said I was pretty sure you could make a killed virus vaccine with any virus, but you had to be concerned with several things. Will the antigen stand the treatment that you are giving it? In my experience with rabies, formalin was not a satisfactory inactivating agent for use in making a rabies vaccine. Phenol had a double value because it would control the bacteria. We had no antibiotics in those days. Furthermore, it inactivated the tissue enzymes.

Hughes: At the time of this meeting, was Salk already launched on making the polio vaccine?

* Proceedings, Round Table Conference on Immunization in Poliomyelitis, March, 1951, National Foundation for Infantile Paralysis.

- Johnson: No, Salk had been working on flu with Dr. Francis at Ann Arbor, Michigan, and Francis had helped him get a grant for trying to develop a killed virus vaccine using tissue culture as a source of the virus.
- Hughes: You spoke out against the use of the Mahoney strain at the Hershey meeting?
- Johnson: Dr. Isabel Morgan-Mountain had done some studies of a monkey brain vaccine, using formalin inactivation.* As with rabies potency studies, the intracerebral challenge test in monkeys was evidently too severe, but serology studies showed production of antibodies.
- Hughes: Did anybody listen to you at this meeting?
- Johnson: What I spoke up about was what strain of polio virus they were going to use.
- Hughes: You didn't talk about phenol inactivation?
- Johnson: No, they had already decided to use formalin inactivation. Salk had already done some studies in which he felt that he was getting good antibody and that formalin inactivated the polio virus satisfactorily.
- Hughes: Did you believe that?
- Johnson: I believed it was possible to get antibody with a formalin-inactivated vaccine. After all, there are a lot of formalin-treated vaccines. There was a formalin vaccine for cholera. So that wasn't my concern. My concern was that of the three strains of virus he'd chosen to use, the one I was most concerned about was type 1, the Mahoney strain.

Dr. Mahoney of the U.S. Public Health Service had tried to get an intramuscular test system which would paralyze monkeys pretty consistently without inoculating them in the brain, just like I had worked on a system to infect dogs with rabies virus by intramuscular inoculation. Dr. Mahoney had passed a type 1 polio virus intramuscularly. Each time he passed it, it seemed to become a little bit more infectious and virulent, until about seven out of ten monkeys would get paralyzed if he injected the virus in the muscle, whereas with the other field strains you could expect none or only one out of a hundred monkeys to get paralyzed. But they would get infected, and they would have antibodies.

* I. M. Morgan. Am. J. Hyg. 1948, 48:394.

Johnson: Dr. Mahoney and others had shown that the Mahoney strain virus came out of the intestinal contents of experimentally infected monkeys. That was very interesting to me because, as I have said, in 1935 Dr. John Toomey, clinical physician on the contagious service at Cleveland City Hospital, believed, based on the epidemiology of the disease, that infection occurred from ingestion of the virus.

Hughes: But nobody paid attention to Toomey's studies?

Johnson: He could not get his papers published. He finally paid with his own money to get a small article published. But his setup was such that you couldn't make much of his results. He was unable to test stool specimens for virus.

Hughes: Why were people still wedded to the idea of the respiratory route of polio infection?

Johnson: Let's go back to herpes, polio, and rabies. At the time I began working on rabies, these were considered to be strictly neurotropic viruses. [It was believed that] they only infected through nerve endings, either in the nose or in the mouth or in the fingers or wherever the virus entered. It was known you could infect animals with rabies by intranasal inoculation. It was possible to infect monkeys by dropping polio virus into the nose.

The reason that Dr. Salk wanted to continue with the Mahoney strain was that he had done some human studies, including on his own children, and he thought he got much better antibodies against type 1 polio with the Mahoney strain than any other strain he tested. In retrospect, this was due to the presence of residual live virus in the vaccine. Scientists in England and South Africa, where they were testing polio vaccine, were going to use their own virus strains, high brain passage strains. It so happened that in Europe, England, and South Africa, there were no cases of polio from the vaccines.

Hughes: They were explicitly fearful of the Mahoney strain?

Johnson: I don't know. They had their own [strain]; they knew about the Mahoney strain.

Hughes: But it wasn't just a matter of having their own; it was the fact that they feared the consequences of using the Mahoney strain of polio virus in a vaccine?

Johnson: I had said at the meeting that my concern was that, if there was any live virus left, it would be likely to produce paralysis. It was like using street rabies virus for making a killed virus vaccine.

Hughes: What was the reaction when you spoke up against the Mahoney virus?

Johnson: It's in the report.* As I remember, nobody said anything. Dr. Basil O'Connor's concern was when I spoke against his big trials with the immune sera. My fears turned out to be true.

Hughes: Was O'Connor's concern not to stop the momentum of the polio campaign?

Johnson: Yes. O'Connor made an announcement that injection of gamma globulin would stop an epidemic of polio. When the final results were summarized, it was obvious that it had been given too late to have much value.

The tragedy of the Salk polio vaccine was the seventy cases of polio that occurred in 1955 from the use of Salk vaccine containing residual live virus.

Hughes: Using the Cutter vaccine?

Johnson: Yes. They were nearly 100 percent from Cutter vaccine, as far as I know. Cutter vaccine was a very well-made vaccine, in the sense that it had a very high titer of virus, which you wanted. But the problem was, was it antigenic and also was it noninfectious?

The U.S. government stopped the use of vaccine.** Changes in production were made to be sure it was safe. When they did that, the antibody titers in children were low or negative.

The biggest problem in assessing antibodies was, if they were using monkey kidney cell cultures for testing the serum of the children after vaccination, you were also immunizing against monkey kidney cells as well as the virus. Dr. John Fox said, "I am concerned that this is a nonspecific antibody test." If there was acid produced in the culture, the cells were growing. If there was no acid, the virus had killed the cells. The fluid would remain pink.

There was the high avidity test and the low avidity test. If you read the cytopathic effect as positive, the virus was not neutralized. They tested the serum after vaccination, looking for cytopathic effect rather than just color change. It didn't show

* Proceedings, Round Table Conference on Immunization in Poliomyelitis, March, 1951, National Foundation for Infantile Paralysis.

** For more on the decision to stop the Salk vaccine trial, see Dr. Lennette's oral history.

Johnson: much neutralization of the virus. What they were actually doing was immunizing people against monkey kidney, so that you would inhibit the growth of the monkey kidney cells. That led to reassessment of that vaccine's effect.

The other finding was that we were getting too many cases of paralysis in children who had had two, three, four doses of the vaccine. That was my greatest concern, especially after learning that there were cases of polio type 1 related to the vaccine.

Hughes: When people were talking about initiating these trials, did you have any opinion then about the timing?

Johnson: My concern was for my own children. I didn't want my children vaccinated with the Salk vaccine because of the use of the Mahoney strain. All the classmates of my children were being vaccinated. My youngest came home from school crying because he was the only one in his class that had not been vaccinated. That was when I decided I'd better do something.

Dr. Hilary Koprowski of Lederle Laboratories had the Lederle strains which were developed by both Dr. Herald Cox and Dr. Koprowski. They had selected strains based on pathogenicity tests in monkeys. They were high intracerebral passage strains. Hilary had done large-scale studies in countries like the Congo and Yugoslavia, showing the vaccine was safe for oral immunization. Dr. Albert Sabin was testing attenuated polio virus strains of type 1, 2, and 3 that he had developed. Ninety million people were vaccinated with Sabin live virus vaccine in Russia later, but this was in 1960. Field trials of the live virus polio vaccines were allowed in this country, but these vaccines were not licensed. That's what Dr. Koprowski and Dr. Cox were doing, and also Dr. Sabin.

Hughes: You got some of the Koprowski vaccine?

Johnson: I called Hilary Koprowski in February of 1960, and he sent me on dry ice the three live virus vaccine strains. I had watched him do the live virus vaccine studies at Sonoma State Hospital in California. Dr. Cox and Dr. Koprowski had tested and marketed the Flury avianized live virus rabies vaccine I had developed, so we had cooperated in previous studies. Beginning on February 16, 1960, we took the type 1, then types 2 and 3, at three week intervals. I felt more secure about my children, my wife, and me after we had taken the vaccine. The secondary cases of polio in adults whose children had taken the Salk vaccine were unusually severe.

Hughes: Koprowski's was a live vaccine?

Johnson: That was a live virus vaccine, given by mouth. I diluted the vaccine in about an ounce of milk for each dose. Dr. Koprowski had done the same with his children. Dr. Sabin's studies were similar; parents had to sign willingness to have their children immunized with the live vaccine.

Dr. Koprowski and his staff at Lederle were doing feeding experiments at that time at Sonoma State Hospital. The studies were done on mentally retarded children. They would recover the virus from the stool, culture it, and feed it to another child who had no antibodies to see if it would revert to virulence, which was a very important thing. The vaccinated children remained well. Specimens of the feces were taken at weekly intervals and tested for polio virus to find out how long the virus was excreted, usually several weeks.

Dr. Sabin was working in Cincinnati on a larger scale, and he did one thing which was different from the Lederle group. He started doing cloning in tissue culture and picking colonies cloned from a single virus particle. He would test several clones by intracerebral inoculation in monkeys to see if the plaques were different. He got some evidence of a difference and then selected the clone which did not produce disease in the monkeys. He finally had types 1, 2 and 3 live virus that did not kill the monkeys inoculated intracerebrally. Hilary and Herald Cox used the same intracerebral tests in monkeys in selecting their vaccine strains.

Hughes: Why, despite Sabin's good results, was there such initial resistance to the use of the live vaccine?

Johnson: The U.S. Public Health Service would not allow a live virus vaccine for vaccination against polio.

Hughes: Why?

Johnson: Well, they felt it was dangerous. [laughs]

The reason that Sabin's strains were licensed [eventually in the U.S.] was the large field study in Russia. Dr. Sabin was born in Russia. Dr. [Mikhail P.] Chumakov, chief of the poliomyelitis institute in Moscow, and Dr. Anatoli Smorodintsev of Leningrad were most interested, and they proceeded to vaccinate ninety million people in Russia. There was no evidence of the vaccine virus strains producing disease. The Russian scientists incorporated the polio vaccine virus in candy.

Hughes: It was only then that the United States said, all right, go ahead with live virus vaccination?

Johnson: The NIH set up a study to test the live virus vaccine strains, and the Sabin strains were chosen for licensing in the United States. The occurrence of paralytic polio from the Salk vaccine had stopped production of the vaccine. After the development of more stringent requirements to make sure that there was no live virus in the vaccine, production was approved. It was evident that children with multiple doses of [Salk] vaccine by injection still were coming down with polio. That's what led to the final confrontation and the approval of the live virus vaccine. The California Medical Association sponsored free clinics for polio vaccination at public schools. Twenty-seven million doses [of Sabin vaccine] were dispensed: type 1 was given September 1962, type 2 November 1962, and type 3 in March 1963. All of our family took the live virus vaccine again at that time.

Hughes: Say something, please, about fecal transmission in relation to the Salk vaccine.

Johnson: There were two things that came out in research about the Salk vaccine that were kind of a surprise. Monkeys vaccinated with the Salk vaccine and then challenged by feeding live polio virus excreted the virus in the feces. That is, they developed an intestinal infection. Whereas, if you fed the live virus to monkeys or people, the next time you fed them a medium dose, there would be no virus in the stool. The big problem in polio is that the virus persists in the intestinal tract and is excreted for up to two months or more. There is very high excretion of virus for the first several weeks, enough to infect anybody that gets exposed to the natural virus.

Hughes: And that was actually what was happening with the Salk vaccine?

Johnson: We did know from the monkey studies that the Salk vaccine did not prevent intestinal infections, whereas when you'd give the live virus strain, it could be demonstrated in the sewage. All we had to do to vaccinate people was to place a drop of the vaccine virus on a sugar cube. The only cost was whatever anybody wanted to give. Somebody suggested fifty cents. Some would pay a dollar; some would pay nothing. When the Sabin trial was finished in 1963 in California, and we had paid for all the vaccine used, there was another seventy thousand dollars left over. It was a voluntary campaign, which is wonderful. This money was donated to public health agencies.

The press was very influential in saying, "Get your children immunized." Dr. [Carl M.] Eklund of Hamilton, Montana was attacked. They tried to keep it quiet that he had gotten live virus out of the [Salk] vaccine right off the shelves.

Hughes: Those results of Dr. Eklund's were suppressed?

Johnson: They were published.* But there was an effort to negate his results.

Hughes: Dr. Eklund did these experiments at the same time as the Salk trial?

Johnson: Yes. He was a good virologist. He was a friend of mine; he's dead some years. He was medical director of the U.S.P.H.S. Rocky Mountain Lab at Hamilton, Montana. He was not that interested in polio; he was there to do the studies as a physician and virologist, a very good one. Dr. Syverton and his associates also reported finding live polio virus in the Salk vaccine.**

The problems faced by scientists studying the value of vaccines and drugs in the control of infectious diseases are summarized in John Ramon Wilson's book Margin of Safety*** He tells about the Maurice Brodie-William H. Park killed virus vaccine for polio and John A. Kolmer's live virus polio vaccine, both introduced in 1934. In 1935 both of these vaccines were found to have produced poliomyelitis in vaccinated children. The vaccine virus in the Brodie-Park vaccine had not been completely killed and the polio virus in Kolmer's vaccine had not been attenuated.

In a way, this set back the development of killed virus vaccine, and when I was able to show that a killed virus vaccine would produce immunity to challenge with rabies virus, it was difficult to convince some of the leading virologists in the United States of this fact.

The second problem was the national press and how exaggerated claims of new breakthroughs have led to excessive demands on public health authorities to approve certain vaccines or drugs. For example, the Salk vaccine, like the earlier polio vaccines, was found to contain live virus and caused poliomyelitis in some of the vaccinated children. One of the vaccine strains, Mahoney type 1, was evidently more virulent than the natural virus. A second complication, which we discussed earlier, was that the Salk vaccine also contained another live virus, SV40, a monkey

* C. M. Eklund, E. J. Bell and W. J. Hadlow. Detection of live virus in certain lots of poliomyelitis vaccine by inoculation of monkeys. *Am. J. Hyg.* 1956, 64:85.

** Jerome T. Syverton, et al. Recovery of viable virus from poliomyelitis vaccine by use of monkeys pretreated with cortisone and x radiation. *Am. J. Hyg.* 1956, 64:74.

*** New York: Doubleday, 1963.

Johnson: papovavirus. Subsequently, it was found that it is possible to produce immunity against poliomyelitis with a killed virus vaccine but it is not effective in preventing intestinal infection and excretion of the polio virus encountered naturally. The Sabin strains of types 1, 2, or 3 polio virus proved to be best for controlling outbreaks of poliomyelitis because the spread of the natural strains of type 1, 2, or 3 polio virus could be stopped promptly. So there is a place for both types of vaccines.

All vaccines and drugs can produce disease and death under some circumstances. Live virus vaccines should not be used in persons known to be immunocompromised by gamma globulin deficiency, immunorepressive drugs, or adrenocorticotrophic hormones, such as cortisone. The natural deficiency of gamma globulin was first noted in children vaccinated with smallpox vaccine (vaccinia virus) where the vaccine virus continued to spread in the vaccinated arm and subsequently into the rest of the body, resulting in death. This condition is rare.

We know also that natural stress will be effective in causing an increase in corticotrophic hormone release from the adrenals, resulting in activation of latent virus infection, for example cold sores with type 1 herpes simplex virus and shingles with herpes varicella virus. An allergic encephalitis or transverse myelitis may occur as the result of postexposure treatment with rabies vaccine prepared from animal brain tissue virus. The incidence of this complication is very rare unless the person has one or more previous courses of rabies vaccine.

A similar allergic problem occurs following immunization with bacterial and rickettsial vaccines following repeated vaccination with the same antigen, that is, immediate allergic reactions such as pruritis, hives, joint pain, and fever. Allergy to synthetic drugs occurs in a similar manner, also to antibiotics. The choice of treatment is based on the hazards of the disease versus the possible complications encountered if we give synthetic drugs, antibiotics, and vaccines.

Encephalitis Research in California

Hughes: Well, let's get back to California. We've mentioned the encephalitis research, but we really haven't gone through it in a systematic way. Do you want to tell me how it proceeded?

Johnson: Okay. My way of working with the viruses was patterned after the original studies in New York, where we decided to use one- to two-day-old mice. In the main lab in Berkeley, when I came in '54, they were still using weaned mice, about two to three weeks old,

Johnson: and chick embryos for their virus isolation studies. I wanted to use baby mice. So I started a special colony of breeding mice from a Rockefeller Foundation strain of white mice that had been originally brought from Switzerland in the twenties by Clara Lynch of the Rockefeller Institute.

Hughes: Why did you decide to use baby mice?

Johnson: The main lab bought its mice from commercial breeding establishments. It had no mouse colony when I came.

So we started a mouse colony in this compound where we're having our discussion [Acton Street, Berkeley]. As that started to produce, then we used the baby mice. It took a while, but not very long.

Hughes: What was the advantage of the baby mice over the two- or three-week-old mice?

Johnson: Well, Dr. Taylor had shown in 1952 in our studies that Coxsackie virus that would not kill a three-week-old mouse injected in the brain would kill a one- or two-day-old mouse. The baby mouse is a highly susceptible host. That was our test system for viruses in India. My contribution was getting a mouse colony started, and then we used chick embryos also.

My personal staff in 1954 was a secretary, a technician, Wesley K. Ota, B.A., and field and laboratory assistants. These were housed in the Viral and Rickettsial Disease Lab. I had a separate Rockefeller Foundation budget. The salaries were paid by the health department and reimbursed by the Rockefeller Foundation. Wes Ota tested the specimens that I was collecting for arbovirus studies. The state mosquito surveillance specimens would go into the regular virus laboratory to be tested in chick embryos and three-week-old mice, and then serology. The main lab would also test for Coxsackie and polio viruses.

Hughes: Was Dr. Lennette directly involved?

Johnson: Yes. He was director of the Viral and Rickettsial Disease Laboratory and I was in charge of the Rockefeller Foundation project on arboviruses.

I was running a special field project on encephalitis cases and was the field man in the Bakersfield area in '54 to collect specimens from the patients that came in and to evaluate them for any evidence of neurological disease. I'd do physical examinations. All the patients were on the contagious service, with standard contagious precautions. I could take blood specimens or throat swabs and rectal swabs, and get material at the autopsies.

Johnson: Also, any case I had I would try to see the area where the patient came from, see how they were probably exposed, take pictures of it. All the serological specimens would go into the main lab to the serology division. We tested for antibodies to the western and St. Louis viruses. Also I collected specimens from any horses I knew died of encephalitis. I'd take the brain out, and that would come into my unit to be tested.

Hughes: How was Dr. Reeves fitting into this program?

Johnson: His mosquito specimens went to the [U.C.] School of Public Health lab. The regular surveillance for the virus in mosquitoes was done at the state lab. The mosquitoes from Bakersfield were collected, sorted, and sealed in glass tubes by Ernest Meyers of the State Bureau of Vector Control and shipped on dry ice to Berkeley. Only fifty mosquitoes were saved from each of the state light traps. I was collecting mosquitoes by suction tube at Rio Bravo School and Hart Park, and when Ernie had some extra mosquitoes from those places, I processed those for my unit. It was a very cooperative program. The mosquito control work was done by the mosquito abatement district. The Kern County Health Department submitted the serological specimens to the state lab. The hospital was purely a clinical care facility. But it was very valuable for me to see how they had learned to take care of encephalitis cases. It was an intensive learning process. I visited all the local state mosquito collection stations.

When the brain stops functioning properly in encephalitis, you have to be sure that the patients do not die of hypoxia. What started saving lives was doing a tracheotomy and putting the comatose patient on a respirator. The first really good respirators were being produced in '54. A characteristic of the patients that had western encephalitis was that they would go to sleep when you were talking to them. They would be somnolent. You'd ask the patient to count to ten. They'd start out, "One, two, three . . ." and they would just go to sleep. That showed that the brain was not functioning well. If children had convulsions, you knew they had hypoxia. It became very apparent that you needed to give them extra oxygen and protect them against pneumonia, because of secondary infection.

Hughes: How did you do that?

Johnson: Penicillin or sulfadiazine. After 1954 very few patients died. It's just amazing. The autopsies were mostly on people who had been infected in 1952, particularly as babies, where the mother was infected by mosquitoes, and the virus got from the mother's blood into the baby. Some of these children would deteriorate over a period of a year or two or three and die.

- Hughes: Was the underlying reason for the Rockefeller's involvement fear of another human epidemic of encephalitis?
- Johnson: I'd say that the Rockefeller Foundation's main interest was what was the nature of the source of arboviruses around the world.
- Hughes: What was your feeling in the fifties, when you were first working on the problem of encephalitis, about the natural cycle of encephalitis?
- Johnson: Well, my concern was that a lot had been left out of studying the reservoir of this virus, to see where it came from. The idea was birds were the only reservoir.
- Hughes: Who had worked that out?
- Johnson: The bird host was very obvious in the early days. Western encephalitis virus was isolated from a prairie chicken, shot in 1941 by Dr. Cox in Montana. We knew that baby chicks were susceptible to peripheral inoculation of the virus; they died of it. The eastern encephalitis virus is very pathogenic for pheasants and baby chicks. They raise pheasants in pens in Massachusetts, and they would have die-offs caused by eastern encephalitis virus. Pigeons also get sick and die of it. In later years, it was found that very young sparrows would sicken and die of eastern encephalitis virus. Dr. C. A. Anderson of the Yale Arbovirus Unit tested some sick sparrows from farms along the Connecticut River in 1973 and isolated this virus from them.
- Hughes: What did you think at that time about the bird hypothesis?
- Johnson: It seemed certain that they were involved in the epidemiology of some of the arboviruses. I decided to test sparrows, which were the abundant nestling bird in any area where Dr. Reeves' group and the state lab were getting the western encephalitis virus from mosquitoes. The Rio Bravo School was one of the study areas which was being tested regularly, once a week, for western virus and St. Louis by testing Culex tarsalis mosquitoes.

I had purchased a three-section ladder that would reach thirty feet in the eucalyptus trees. I began looking for sick birds and on August 10, 1954, I collected B4, a sick fledgling sparrow, in a nest twelve feet above the ground at Rio Bravo School. This was the fourth nestling bird that I collected in California for virus testing. Wes Ota isolated western encephalitis virus from both the blood and spleen of this bird.

It was in 1956 when I hired Don R. Roberts, a zoology graduate student at the University of California, to help me collect nestling birds, and we demonstrated that nestling sparrows were important amplifying hosts for western encephalitis virus.

- Hughes: Were the children at Rio Bravo School getting encephalitis?
- Johnson: No, nobody at the school got it, not a single case, and yet there was virus there in the daytime.
- Hughes: How do you explain that?
- Johnson: Well, when the kids were at school, the mosquitoes had been fed, and they were pretty dormant during the day. There was really no [human] activity at the school at night. I think that's the reason. The people that lived in the vicinity had screened houses.

In fact, I think the reason that there were so many cases in the epidemic of 1952 was that they had that terrible earthquake. That was definitely the major reason for the number of cases, particularly in children. It was very hot, and people didn't dare to sleep in the house; they'd sleep outdoors. Anyway, it was an occasion where there was more exposure of people outdoors in '52. There were tremors over a period of a week.

- Hughes: What was the impetus for the epidemic in 1930?
- Johnson: It was the result of large-scale irrigation and the development of high populations of mosquitoes.

Where I grew up on a ranch in Nebraska, there were outbreaks of the Kansas-Nebraska horse plague. The years when that disease was present were years of high rainfall. The ranchers knew that every ten or fifteen years, during the times when all the ponds filled up, they would have this disease. But they didn't know at that time that it was mosquito-borne. My father thought it was related to flies, and he did everything he could to reduce the fly population in the barn. The horses that were kept in the barn were not affected, but a few that were out in the pasture, particularly where they were around natural water sources, developed encephalitis. We had horses that developed encephalitis in 1919. I remember very well these cases. Then it came around again in the middle twenties, and '37-'38, and then 1941 was the big epidemic in the central U.S., with many human cases. When I came to Bakersfield the horse encephalitis cases were similar.

- Hughes: In a paper published in 1963, it was speculated that in the temperate zone the breeding period of birds was just not long enough to sustain a virus.* I'm not sure that the study concerned encephalitis. Would it apply to encephalitis?

* D. D. Stamm, R. J. Newman. Proceed. Seventh Int. Congresses Trop. Med. and Malaria, Rio de Janeiro, Sept. 6, 1963, Anal. de Microbiol. 11:123-133, Part A.

Johnson: Yes. Birds breed once a year.

I isolated virus from barn swallow nestlings in 1955, and they are the ones that migrate to South America. The evidence, as it accumulated in our labs and at CDC, was that the bird infections increased in the fall in Alabama near the coast. Don Stamm isolated eastern encephalitis or western encephalitis virus in nine percent of the 649 birds that he bled in September through October as they were leaving for South America. So I said, "Well, it looks to me like the virus is coming from North America and going to South America, and not from the tropical forest to North America."

A factor about the role of blackbirds in spreading encephalitis virus is that many millions of red-winged blackbirds and crows breed in Canada, and when I lived in Nebraska these birds migrated south in the fall at the time the horses became infected with encephalitis. That is, these birds were the most likely hosts for introducing the encephalitis virus.

Hughes: What was the incidence of St. Louis vis-a-vis western?

Johnson: During the epidemic of '52 there were 370 western and forty-four St. Louis cases. But in '54, there were ninety-six St. Louis cases and twenty-two western type. By 1954, the clinicians had become accustomed to taking spinal fluid and bloods on all persons with encephalitis. The St. Louis cases were mostly in their fifties or sixties. They were people that worked in the fields picking cotton, where they were exposed to a lot of mosquitoes. So if anybody came in with a little paralysis of the face and a little mental aberration, the physicians would do a spinal puncture. We would test the spinal fluid for virus and the serum for antibodies.

To our surprise, we'd bleed these patients that looked like they'd had a stroke and the first blood wouldn't have any antibodies to St. Louis, but the second one would and they had St. Louis encephalitis.

Hughes: Why was that?

Johnson: Well, the specific diagnosis is where you could show a rise in antibodies to a virus from the time they come in to three weeks later. Then you know that that virus is causing the encephalitis. They didn't have antibodies when they came in.

Hughes: Why were the patients older?

Johnson: The migrant laborers that came in to pick cotton were mostly older people and not local residents, so were not immune. I did see one child of about two or three who had St. Louis encephalitis. You

- Johnson: couldn't tell it clinically, but she showed a rise against St. Louis rather than western. I went out to the place where she lived, and there was a big earth-banked water pond for irrigation right next to the house. She had been bitten by a lot of mosquitoes because she had been put out on the porch to cool off in the evenings. So her exposure was on this heavily irrigated cotton farm.
- Hughes: You couldn't differentiate between St. Louis and western until you'd done the lab tests?
- Johnson: Cases in children were similar. The St. Louis cases I think would be more liable to have some paralysis, where the western would be more brain involvement with drowsiness.
- Hughes: The treatment was basically the same?
- Johnson: The reason for the respirator and antibodies for the western was that these patients became comatose, whereas usually you didn't have to do that for the St. Louis cases. They would recover pretty quickly.
- Hughes: Did you give sulfa drugs as a matter of course in both diseases?
- Johnson: No. You would only give them if there was evidence of pneumonia. The St. Louis cases usually recovered. I don't remember any of them dying, even that child. The fatalities came to an end after 1954 when we got better respirators and better antibiotics. It was rare if anybody died of the disease, but there were emotional and behavioral changes.
- Hughes: Dr. Lennette feels that it is a bad idea to name a virus after a geographical area. He maintains that as soon as you name a virus after a specific place, it immediately begins to appear somewhere else.* You don't have any strong feeling about that?
- Johnson: No. The Rockefeller Foundation preferred to use geographical locations rather than people's names. I'm interested in where the first case [of a disease] occurred. Then one can do annual surveys to see whether the virus will be found again at the same place and time of the year. Then you say, well that might be where it started. My interest now is, we know certain areas where a virus is found over a long period.

* Edwin H. Lennette, Pioneer of Diagnostic Virology with the California Department of Public Health, an oral history interview conducted 1982, 1983, 1986, Regional Oral History Office, The Bancroft Library, University of California, Berkeley, 1988.

Johnson: Every summer, I study a swamp in Massachusetts where both eastern and western encephalitis viruses are present year after year, usually late in the summer. From the end of the first week to the middle of July, you might get the western encephalitis virus. The eastern encephalitis virus appears in August. There's no virus in the mosquitoes during the nesting season.

The epidemiology of the encephalitis virus is different in Bakersfield. In 1954, there was virus in the mosquitoes in May and in the nestling birds in June. The following year the western virus appeared later. In 1956 the western type again appeared in May. This makes me believe that in this place the virus is able to overwinter under conditions where the bird-mosquito-bird cycle can be maintained. This requires a mild winter and plenty of susceptible birds. At Rio Bravo School we removed the sparrow population during the winter of 1956-1957. It was very interesting, because birds, like finches and doves, that had not been seen around the school, started to nest there. The sparrows had monopolized the area.

In '56 I felt I had my answer. Everything pointed to the fact that encephalitis was coming in from somewhere else. There are periodic outbreaks of encephalitis in northern Montana and Canada. So I said, "Let's look at northern California." So that's when I decided to do serum surveys in horses. The first study was bleeding a bunch of horses up in Modoc County on the plateau. They'd had horse encephalitis; that was well-known. So George Humphrey and I bled horses in Modoc County at an elevation of five thousand feet. The horses had antibodies, and about half of them were western and almost as many were St. Louis.

So then the question was, are small mammals involved in the maintenance of western encephalitis virus? That's when I got into Colorado tick fever, testing small mammals from Modoc County. There was one small mammal definitely involved in the epidemiology of western encephalitis. That was the tree squirrel. The first positive squirrel was a young Sciurus griseus found paralyzed at Doyle Park, Santa Rosa, Sonoma County, August 17, 1955. It was negative for rabies, but mice inoculated with the specimen produced a fatal encephalitis. I was asked to check the slides to see if I could find any Negri bodies. There were none, so the specimen was checked for encephalitis virus and was found to be western encephalitis virus.

Subsequently, western encephalitis virus was obtained from tree squirrels at the same place August 15, 1956, and August 12, 1958. Western encephalitis virus was obtained from two tree squirrels killed when paralyzed in Marin County, August 21, 1956 and July 15, 1958, and at Glen Ellen, Sonoma County, August 12, 1958. It seemed likely that the western encephalitis virus was introduced by birds.

The Arthropod-borne Virus Information Exchange

Hughes: I saw a reference to the Arthropod-borne Virus Information Exchange, which is put out by the CDC in Atlanta. Tell me please the history of that program.

Johnson: Yes. In 1958, an informal meeting of arbovirologists was held at the International Congress of Tropical Medicine and Malaria at Lisbon, Portugal. Plans were then formulated for an international arthropod-borne information exchange and a catalogue of viruses. A working catalogue of viruses was begun in 1961.

Hughes: What year did the newsletter appear?

Johnson: The first newsletter, Info Exchange, was sent out in April, 1960 from CDC, edited by Telford Work. There are two mailings each year. There is no peer review; the reports are reproduced from the typed manuscripts as submitted. Dr. Charles H. Calisher is now editor and the Info Exchange is produced at the CDC lab at Fort Collins, Colorado.

Dr. Emmons helped Dr. Taylor in getting the first edition of the International Catalogue of Arboviruses ready. Dr. Taylor shared an office in the Acton Street laboratory, after he moved from the Yale Arbovirus Research Unit in 1960. The first edition appeared in 1962 and the second edition in 1975.

The Info Exchange has been a most successful project. I prepared the California reports for Ed Lennette until Dick Emmons took over that job. Previously, we had annual reports put out by the Rockefeller Foundation field labs. These were also sent to arbo labs that were doing cooperative field studies.

Hughes: You were trying to encourage communication with non-Rockefeller labs?

Johnson: Yes. We like to know about the current prevalence of arboviruses in different states and also other countries.

Dr. Taylor was the real architect of the Info Exchange; [he determined] what information was wanted. He had a tremendous career in the field, and he was very good at organizing data. First of all, the punch card and a loose-leaf notebook, and finally the catalogues.

Hughes: What are the groups participating in the Info Exchange?

Johnson: The major institutes of health of forty-five countries and about 100 separate laboratories in the United States. There are state laboratories in several countries or regional labs run by the government. I imagine there are two or three hundred places cooperating with the Arthropod-borne Virus Information Exchange.

Hughes: Was the rabies exchange also mediated through CDC?

Johnson: Yes, by George Baer of the veterinary division of CDC in Atlanta. It is patterned after the arbovirus information exchange. Here again is an opportunity to present studies that will not be published in regular scientific journals.

Hughes: Were you involved with starting that?

Johnson: No, [but] Dr. Emmons and I have contributed to it.

Hughes: Is there a peer review system?

Johnson: No, they'll take what you send. That's the beauty of it. Of course, some of it is probably not suitable for regular journals.

More on Encephalitis

Hughes: In 1957, you reported the isolation of twenty-three strains of western equine encephalitis virus from wild birds.* Did you actually perform all those isolations?

Johnson: Those were all done in my section.

I was thinking about that first bird, B4, in '54. I bled the bird but also saved the spleen. At that time, if you tested anything other than blood, you tested the spleen as a source of virus. It stays there a little longer. I had the spleen in glycerol, like we used to do for rabies. We isolated western encephalitis virus from the blood, and a few months later I took that little tiny spleen and gave it to Wesley Ota to test and the western encephalitis virus was isolated from the spleen. So the virus had survived very well in fifty percent glycerol in saline at refrigerator temperature.

* The ecology of western equine virus in California, USA: the virus in resident and migratory birds. Proceedings, Ninth Pacific Science Congress 1957, 17:99.

Johnson: But to get back to the twenty-three isolations of western encephalitis virus from birds, after I had those, there was some pressure on me from the Rockefeller Foundation to keep on isolating viruses from birds. I said, "Well, I think we have the answer." The year that we had the epidemic, there was an amplification system mostly in sparrows. Any place where you got rid of the sparrows, the mosquitoes were negative for virus the following year, or there were only sporadic isolations late in the summer.

The evidence favored introduction of western encephalitis virus from the north. It's got to come from some other host system. We're looking in the wrong place, because in all the places I worked, and I've gone on field studies all over the world, group A viruses, particularly western encephalitis, have to have fresh-water swamps or irrigation. You can have active virus and no evidence of bird involvement, at least during the time you're getting virus from mosquitoes. In Massachusetts, by the time that you get the virus out of the Culiseta melanura mosquitoes in July or August, there are few birds seen.

Hughes: Where do the birds come from?

Johnson: They roost in the forested swamps during the northward or southward migration. In April, May, and into June there's no virus in the mosquitoes. What host do the mosquitoes feed on when the birds are scarce or absent?

There are two vertebrate populations that are very high in those swamps. It's the same in Canada. We know that western encephalitis occurs in horses and people in Canada, right up to the dense northern forest. And what do you have up there? You have enormous populations of shrews, also Microtus, red backed voles, Clethrionomys, and Peromyscus mice. The animals that I'm interested in are: the short-tailed shrew, Blarina brevicauda, and the Sorex palustris, the water shrew. In the northern fresh-water swamps in North America, you have very large populations of shrews. The black, velvety Blarina, the short-tailed shrew, lives near the swamp and is not out in the swamp. It feeds mostly on mature and early stage insects, worms, frogs, and salamanders.

I've been on expeditions in the Canadian forest with Keith Murray. We trapped extensively there in '63. There is an enormous number of pupae in the forest leaf-bedding. Both types of shrews can eat up to 700 of these pupae a day, and when the insect populations are high, the shrews increase in number. They have a very high metabolism; they have to eat a lot. The whole winter long, under the snow, they're feeding on pupae and worms. Then when the spring comes and they breed, they have many young. They are an insectivore. The populations are in millions. The population may go down in the winter, but they do very well under the snow.

Hughes: Your feeling then is that these small mammals are the natural reservoir of western encephalitis virus?

Johnson: They could be. They are susceptible. I told the people in Russia and Czechoslovakia to be on the lookout for the overwintering of Sindbis virus in shrews, and actually they have gotten one isolate of the Sindbis virus in Czechoslovakia from shrews. But no one so far is interested in working on it in the US.

Water shrews are very difficult to study. They have the same distribution as western encephalitis, while the Blarina shrew has the distribution of eastern encephalitis virus. My feeling is that if you were to look at any vertebrate in this northern forest area, you should study the shrews.

Now in the last five years, something has happened that absolutely intrigues me. We isolated Sindbis virus in India from migratory wagtails, which breed in the far north. Swedish arbovirologists have found this virus in northern Sweden. The white wagtail, Motacilla alba dukensis, migrates from Sweden to India. This is an example of the complexity encountered in a study of the natural reservoir hosts of an insect-borne virus.

For a long time, it was believed that the eastern and western virus came north during the spring bird migration, until Dr. Donald Stamm did some tremendous studies on the coast of Alabama, in which he bled birds going over the Caribbean in the fall. He had done studies in the spring, too, when they move north. The ones coming from South America were negative, but as they were leaving the United States going to South America, up to two percent of the birds he bled would have eastern or western encephalitis virus in the blood. The virus is in the blood only for three or four days. They can fly across the Caribbean in one night. Birds can fly very rapidly. He said the birds would wait in the thickets where he set up his nets. He ran twenty-eight nets, twelve by twenty-seven feet. He'd watch the birds and when the wind was in the right direction—it was from the north, a good strong wind—they would often take off at night in enormous numbers.

One of my hobbies is to be in an area where there's either spring or fall migration. So I've visited the Boca Chica Flats of Texas in the spring, and it's absolutely thrilling when you see flock after flock of 250, 300 swallows coming in. And other birds. Birds you rarely see, like the scissor-tailed flycatcher. You'll see 200 of them. Elsewhere in Texas you see only one pair.

One of my friends, Carlos Lehman, an ornithologist in South America, now dead, sent me a letter from Colombia, and he said: "Well, some of your barn swallows arrived." He put a tail feather from one of these swallows in the letter. The white bar on the tail is diagnostic.

Johnson: The studies now show that there are probably one hundred million birds that migrate from North America to South America and vice versa every year. The black and white warbler, which I like, goes to Brazil. I've seen them in Brazil, coming in there. They find the same environment there in winter as they find here in North America during the summer.

The swallows in California nesting at a certain latitude probably go to the same latitude in South America. There are barn swallows that go to Alaska, and they have found barn swallows in Patagonia. So some of them make a long trip. The red knot that breeds in the arctic goes all the way to Argentina. Swainson's hawk migrates to South America. I have counted hundreds in Texas in the fall at Santa Maria Wildlife Study Area during migration.

So, to summarize: The evidence now indicates that the encephalitis viruses pass through the Amazon Forest in our fall season. You find the virus in Belem if birds are netted in the tops of the trees on their way through. They put up nets at seventy-five feet and isolated the western and eastern virus from birds netted there.

Hughes: So your opinion is that it's the birds that are bringing the virus south?

Johnson: Yes, the birds are bringing it south rather than bringing it north. I've been interested in looking for these viruses in the northern part of the United States and in Canada, and it's there. Eastern and western encephalitis and St. Louis occur in Canada. Up to the edge of the forest there are horse and human cases of western encephalitis. In 1975, St. Louis appeared in Ontario and Illinois at the same time. That suggests that the Canada geese and snow geese should be included in the bird studies. They do have antibodies to St. Louis encephalitis virus.

Hughes: Why don't you tell me how you developed the live virus vaccine for western encephalitis.

Johnson: Whatever disease we worked with, the Rockefeller Foundation wanted a practical control method for the disease. When I came here, they had no really good killed virus vaccine [for encephalitis]. There was a formalin vaccine which had been used, and I still don't think the one they're using is very good. I wanted to develop a live virus vaccine for both western and St. Louis. My idea in working with these viruses was not to neuroadapt them, because I was pretty sure once you started passing something in the brain, you selected a population of virus particles which will grow in brain, whereas in nature the virus is not very neurotropic.

Johnson: Figuring the same thing with the western encephalitis virus, I decided to start with a non-neuroadapted strain which had never been injected into the brain of an animal and then cultivate it in various systems to see if we could get one that wouldn't kill mice when inoculated into the brain. We tried long-term cultivation in chick embryos.

However, long-term passage in chick embryos did not reduce the pathogenicity. So then I decided to test individual virus particles by cloning. I started with a western encephalitis virus I isolated from a nestling sparrow, B628, collected at Knowles Ranch, Oildale, California, July 29, 1957. I was buying hamster kidney cell cultures from a commercial laboratory and was doing the tissue culture myself.

This bird western encephalitis virus was isolated directly in hamster kidney cells. There was 4+ cytopathic effect in three days and the titer of the virus in passage one was greater than 10^{-4} when tested in primary hamster cell culture. The hamster kidney passage four was passed in the chick embryo tissue culture, and tenfold dilutions were tested in monolayer cultures of chick embryos. After incubation for one hour, the cell sheet was overlaid with agar. Four days later, the agar sheet was overlaid with an additional layer of agar containing neutral red. In the dilution containing one to one hundred tissue culture infecting doses plaques were produced. The western encephalitis virus in each of these plaques was derived from a single virus particle. In the first cloning, one of the twelve plaques tested produced progeny which were relatively nonpathogenic for young adult mice when inoculated intracerebrally. Successive cloning, based on the incubation period, morbidity, and mortality of the infection in adult and infant mice inoculated by the intracerebral and intraperitoneal routes, was continued for fifteen passages.

I selected the fifteenth clone for doing vaccine studies. We prepared a stock of lyophilized H4, CE15, B628 western encephalitis virus. This strain was supplied to reputable commercial laboratories engaged in producing biologics and to various government agencies. Lederle Laboratories conducted extensive studies of the B628 strain, testing this in horses and monkeys. We conducted field trials in horses in California using vaccine prepared by Lederle Laboratories.

We couldn't get a permit from the USDA to do anything but the experimental work. We finally vaccinated more than two thousand horses and showed that the vaccine was not pathogenic for horses. The USDA would not license the vaccine. The vaccine strain is still available. If another epidemic of human and horse encephalitis occurs like the one in 1941, there may be a demand

Johnson: for the vaccine.* This work was finishing up by 1968. In the meantime, I had developed a St. Louis virus strain which is not pathogenic for adult mice.**

Hughes: Was either of these vaccines made commercially?

Johnson: Lederle made up a large lot of western encephalitis vaccine for testing in horses but was not able to get a permit to sell it.

Hughes: So that was the end of that?

Johnson: Yes. They also made a production lot for test in humans. That's when the litigation problem became too formidable. The Rockefeller Foundation didn't want to patent the vaccine viruses. They are still available.

In the meantime, as a result of the entomological studies, we have excellent control of the vector mosquitoes. As long as you can stop the breeding of Culex tarsalis in California, you can protect the horse and the human population. But there may come a time when there will be so much virus that there will be disease in horses and people.

My feeling is that the same is true for the high-passage Flury rabies vaccine, which was used intramuscularly in some ten thousand people as a live virus vaccine. We have rabies viruses now in nature. One is the cave Tadarida rabies which can infect people via the respiratory tract. If that ever got loose in the human population, we would need something that would immunize quickly, and that's when we could use the live LEP Flury rabies strain of vaccine.

I think our knowledge of viruses now is such that we can make good killed virus vaccines from tissue culture virus. I'm not for live virus vaccine for dogs in the United States unless the dog street rabies virus becomes established here again.

* H. N. Johnson. Selection of a variant of western encephalitis virus of low pathogenicity for study as a live virus vaccine. *Am. J. Trop. Med. Hygiene* 1963, 12:604-610. M. Roca-Garcia, E. L. Jungherr, H. N. Johnson, H. R. Cox. An attenuated strain of western equine encephalitis virus as a possible live immunizing agent. *Proc. 68th Annual Meeting, U.S. Livestock Sanitary Assn.*, October 1964, pp. 24-40.

** H. N. Johnson. Selection of seed virus for the preparation of non-neuroadapted strains of St. Louis encephalitis (SLE) virus and western equine encephalitis. *Arthropod-borne Virus Information Exchange*, CDC Atlanta, Georgia, March 11, 1983, pp. 174-178.

Hughes: In 1963, you published a paper in which you seemed to urge the use of several different methods for assaying the potency of western encephalitis vaccine and, I presume, other vaccines.*

Johnson: The antigen extinction test was the main one. You can test the live virus vaccine, for instance, in mice. You inoculate tenfold dilutions of virus intracerebrally and later challenge them intracerebrally with the virulent virus.

Hughes: What about killed virus vaccines?

Johnson: This test can also be used for testing killed virus vaccines. It is possible to immunize mice against intracerebral exposure with a small amount of antigen if you give it in the brain. This is a new discovery. We know that lymphocytes are the source of antibody. If you put the antigen into the brain so that it gets to the choroid plexus, the antibody will be produced inside the brain case, because the antigen given intramuscularly does not penetrate into the spinal fluid. So a very small dose of killed virus antigen in the brain of mice will immunize them against intracerebral exposure with a big dose of rabies street virus. I'm sure this will work with other viral vaccines. So if you want a real good test of antigens for encephalitis virus, you will immunize intracerebrally with the killed virus and later give the virulent virus by the same route.**

It also opens one possibility for rabies treatment, and that is, if you were to give a very small dose of rabies antigen into the spinal fluid through lumbar puncture, that would be a possible method for immunizing people against rabies after exposure. That would immediately start the production of antibodies in the brain. Perhaps the vaccine could also immunize using intranasal inoculation.

Hughes: Is anybody receptive to the idea of immunization via lumbar puncture?

Johnson: The rabies information exchange article is available worldwide, and the test is included in my rabies chapter in the sixth edition of Diagnostic Procedures for Viral, Rickettsial, and Chlamydial Infections.***

* H. N. Johnson. Selection of a variant of western encephalitis virus of low pathogenicity for study as a live virus vaccine. Am. J. Trop. Med. Hyg. 1963, 12:604-610.

** H. N. Johnson. Studies of the immunological mechanisms for the prevention of rabies. Rabies Information Exchange, CDC, Atlanta, Georgia, no. 12, June 28, 1985.

*** New York: American Public Health Association, 1989.

Johnson: Dr. Emmons has done studies of the spinal fluid of dogs, and he found that dogs that recover from rabies have antibody to rabies in the spinal fluid. This shows that antigen must reach the brain before it can affect the course of the brain infection.

[Interview 8: May 5, 1987]

Hughes: Please tell me how you became interested in Colorado tick fever.

Johnson: In 1957 I was finishing up my studies of the encephalitis viruses at Bakersfield. We had learned that the virus was transmitted by Culex tarsalis mosquitoes. Dr. Reeves and his group studied that, and it was very well worked out. What I was doing there was trying to see whether we could get the viruses from vertebrates, rather than mosquitoes. The first thing was to go around with Dr. Reeves and Ernest Meyers to all the collecting stations where they had isolated western encephalitis and St. Louis viruses to see how and from what wildlife the mosquitoes were getting their infections.

It became obvious to me after looking at every place where there had been a case of horse or human encephalitis, and also where the mosquitoes were apt to be positive, that you had to have three factors. One was, there had to be roosting trees, big oak trees or the equivalent, where birds could come in and rest at night. My habit was to visit the virus-positive stations and spend several evenings there to see what happened. The second factor was flooding of pastures, alfalfa fields or cotton fields, to provide a high population of Culex tarsalis mosquitoes. The third factor was nestling birds to serve as amplifying hosts for local production of viremic birds.

I remember one particular place. It was a very simple environment. It had a grove of tall eucalyptus trees, and there was a house but no other buildings. It was near an irrigated cotton field. Under the trees were placed red-painted boxes, open on one side. Early in the morning one would find blood-fed Culex tarsalis resting on the ceiling and back wall of the boxes. The mosquitoes bred in pools of irrigation water. They were collected from the boxes with mouth suction tubes. I visited this place in the late afternoon and Brewer blackbirds would come about 5:30 p.m. to roost in the eucalyptus trees during May and June. In the latter part of the summer, flocks of red-winged blackbirds would also use this place for a nighttime resting place. Sometimes four or five thousand blackbirds spent the night in these trees, so the mosquitoes had a ready source of blood. Bill Reeves had used bird-baited mosquito traps and sometimes more than 100 mosquitoes would feed on a single bird.

Johnson: The Brewer blackbird seemed to be the principal vertebrate host for mosquitoes where they were found infected with the western encephalitis virus, whereas the bicolor redwing blackbirds were migrating through in the late summer and fall when St. Louis encephalitis virus was found in mosquitoes. The Brewers were the ones breeding locally during the early part of the summer, and they would nest in small trees of all kinds, but particularly the chinaberry trees.

The male Brewer blackbirds would defend a territory and try to attract a female. Sometimes one male would defend two nesting females. So roosting trees, irrigated land, and nestling birds were the key factors for introducing and amplifying the encephalitis viruses. Domestic chickens, ducks, and pigeons could also serve as amplifying hosts.

Then the question was, where was the virus all year around? Were there any small mammals involved? That was to be my major interest. What were the mammals? Well, they were the usual desert animals--the Beecheyi and Nelsoni desert ground squirrels, the grasshopper mouse, the pocket mouse, the Peromyscus and Microtus mice, kangaroo rats and bats, all in marvelous populations in the desert around Bakersfield. So we trapped small mammals and birds. We used Japanese mist nets for collecting bats. By studying the susceptibility of the small mammals, we found out that some appeared to be aberrant hosts, because kangaroo rats and Beecheyi ground squirrels, when infected with western encephalitis virus, would sometimes get sick and die, which you wouldn't expect in a carrier host. The Peromyscus mice inoculated subcutaneously did not sicken but developed antibodies to the western or St. Louis viruses. The most common bat was the Mexican freetail bat. These bats were abundant in buildings with tiled roofs.

I chose the Rio Bravo School thirteen miles west of Bakersfield as one of my study areas. Here was a large grove of eucalyptus trees used by blackbirds as a nighttime roost. It was near irrigated fields, and mosquitoes collected in the hallways of this school had been found infected with western and St. Louis viruses. There were no farm animals nearby but there was natural brush desert immediately adjacent to the school. A thorough trapping survey failed to reveal western or St. Louis viruses in heart and spleen specimens from the small desert mammals. There was a colony of Mexican freetail bats in the roof of the school. It was from these that I isolated the group B Rio Bravo flavivirus.*

* H. N. Johnson. The Rio Bravo virus: A virus identified with group B arthropod-borne viruses by hemagglutination inhibition and complement fixation tests. Proc. Ninth Pacific Science Congress, Bangkok, Thailand, 1957, 17:39. See the discussion of Rio Bravo virus below.

Hughes: Was it known at that time that nestling birds were particularly susceptible?

Johnson: Yes. Dr. Clarence A. Sooter, of the U.S. Public Health Service lab at Greeley, Colorado, isolated western virus from the blood of three nestling birds in 1950.* That stimulated my interest in working with nestling birds.

As I told you, in 1954 I obtained western encephalitis virus from a nestling sparrow at Rio Bravo School. By 1956 we had demonstrated that nestling sparrows were the amplifying host for this virus in Kern County.** There were more than 100 sparrow nests at Rio Bravo School and at Stocksdale Ranch; several thousand in the rows of palm trees. Tests of sparrow tissues at Rio Bravo School taken during the winter and spring were negative for western encephalitis virus. The resident sparrow population was trapped out. In 1957 there were only two isolations of western virus from mosquitoes at Rio Bravo School, evidently derived from migratory blackbirds in July. There were three isolations of St. Louis virus, two in August and one in September. There was no evidence that winter of persistence of the western virus in birds, other than infection of the fall migrants, such as house finches. This could maintain the virus through the winter by mosquito-bird-mosquito passage. This was indicated by the early appearance of western virus in mosquitoes in 1954 and 1956.

Dr. Reeves' group was continuing its excellent studies on the role of the mosquitoes, and I thought, where else in California should we look for the wildlife hosts of viruses?

Colorado Tick Fever [CTF]

Johnson: Miss M. Dorothy Beck, senior epidemiologist of the Bureau of Communicable Diseases, wrote a paper about cases of Colorado tick fever in Modoc County, California, during 1957.*** There were five cases of CTF in 1957 in California, two diagnosed by

* C. A. Sooter, et al. Proc. Soc. Exp. Biol. Med. 1951, 77:393-394.

** H. N. Johnson. The ecology of western equine virus in California, U.S.A.: The virus in resident and migratory birds. Proc. Ninth Pacific Science Congress, 1957, 17:99.

*** California Vector Views, August, 1958, 5:54.

Johnson: neutralization tests at the State Department of Public Health in Berkeley. The others were diagnosed at the U.S.P.H. lab at Hamilton, Montana.

The early history of Colorado tick fever in California begins in the 1930s at the California Conservation Corps Hackamore Camp, thirty-five miles northwest of Alturas. About 100 young men were working there, doing forest care. During 1933 and '34, about fifty percent of them developed a severe febrile disease called tick fever. The first isolations of Colorado tick fever virus in California were obtained in 1953.

Hughes: Do you remember who did the isolations?

Johnson: Dr. Carl M. Eklund reported this finding in 1955.* A summary was submitted to the California Health Department.** In the report by Dorothy Beck I referred to, there was one patient that became ill on April 21, 1957 near Alturas following a tick bite. A doctor sent the blood to Dr. Eklund's lab at Hamilton, Montana, and they isolated Colorado tick fever virus from this specimen.

Another person became ill on July 8, 1957, in the Alturas area. He had a history of tick bites a few days previously, followed by chills, fever, and low white blood cell count. From his blood, the Hamilton lab also isolated CTF virus. One case was diagnosed only by neutralization test at Hamilton. With this information, I decided to visit Hackamore Forest in Modoc County and collect ticks to see if I could isolate the CTF virus.

Hughes: Was this 1957?

Johnson: Yes, the cases were identified and reported in 1957 and that is when I heard about them.

[interruption]

I was looking up the first field trip I made up to Modoc County to visit that old campsite at Hackamore Station where there had been tick fever. My first field trip to Modoc County was in May, 1958. I collected ticks on May 24, 1958, and tested these using one- to two-day-old mice as test animals. There were nine

* C. M. Eklund, G. M. Kohls, J. M. Brenna. JAMA 1955, 157:335-337.

** G. M. Kohls. California Vector Views, 1955, 2:17.

Johnson: female and eight male Dermacentor andersoni ticks in AR--arthropod group--426, which was positive for CTF. Another pool, AR 427 containing three female and five male ticks collected the same day, also was positive for CTF.

I returned in June to Hackamore Station for another collection, and on June 9, 1958, I collected ticks and tested eleven female ticks, AR 470, and ten male ticks, AR 471, separately. Both were positive for CTF virus. Both male and female ticks feed on blood.

Hughes: Had there been speculation that it was just the females?

Johnson: Not that I know. It is the female mosquito that transmits western and St. Louis encephalitis viruses because it takes a blood meal, but the male mosquito does not feed on blood.

The questions were, what mammals were infected and at what stage did the ticks get the virus? You will notice that people were getting infected with Colorado tick fever in April and May; well, that's when the adult tick feeds and the female lays her eggs. The seed ticks or larvae will be feeding in May, June, and July. What will the larvae feed on? They'll feed on small animals like *Perognathus*, *Peromyscus*, and *Microtus* mice.

After the larvae have fed, they become nymphs, which feed on the larger animals, such as wood rats, ground squirrels, chipmunks, and kangaroo rats. They will have more than one feeding before they grow up to become adult ticks. It often takes two years to reach the adult stage. There will be a nymphal stage, feeding in the fall. To my surprise, the fall feedings were really good times to bleed the medium-sized animals. For instance, on September 7, 1970, I collected a wood rat, M846, and from the blood we got CTF virus, and a chipmunk, M851, collected the same day was positive. The next day, I collected two more chipmunks, M869 and M871, whose bloods were positive for CTF virus. I wondered if the virus was in the red or white blood cells or just in the serum.

In bleeding these animals, I wetted the syringe with heparin so the blood would not clot. As these blood specimens came back to the lab, I tested the serum separately from the cell fraction. To my great surprise, the red cells were loaded with virus, and there were only a few occasions where I got the virus out of the serum alone. I had one chipmunk in which I was able to get the virus from the washed red cells up to more than a month after I collected it. But in each instance, the blood serum alone would be negative for virus. That led to a thorough study by Dr. Emmons on the persistence of CTF virus in red blood cells of infected animals, and the mechanisms of overwintering of CTF virus in

Johnson: hibernating golden-mantle ground squirrels. Dr. Emmons was awarded a Ph.D. degree in epidemiology at the School of Public Health as the result of this research.*

In the meantime, we did some studies in the white laboratory mouse. If we inoculated them subcutaneously or intramuscularly with the virus, they would have CTF virus in the serum up to the eighth day, but the virus in the cells would be high for more than thirty days. That led to a lot of studies to see whether the virus was actually growing in the red cells or was only adsorbed to the cell. The final work on that was done by Dr. Emmons and Dr. Lyndon Oshiro using electron microscopy and fluorescein-tagged antibody. There would usually be small round collections of antigen and also some nonstructural antigen in the red blood cells. Later on they showed that infected people had virus in red blood cells up to more than two months after the onset of the disease. This explains the chronicity of CTF and the relapses observed in this disease.

We've been following the Hackamore focus of CTF since 1958. Almost any year, when we go up there in May, we can get virus from ticks. So the disease is established there. My own feeling is that the principal host is probably the *Perognathus* mouse, which is called the pocket mouse, because this animal is present where you find Colorado tick fever and infected ticks. Some of the other hosts get sick, for instance, the chipmunk. I've isolated CTF virus from a chipmunk that died in the trap.

It would be very difficult to prove exactly which animal is the major host, but my feeling is that it is the one that is fed on by the larvae. The larvae would get infected from some small animal, and the virus would persist and continue to multiply to the final stage. The adult tick feeds primarily on deer, but also on man. We isolated CTF virus from the M735 pocket mouse, *Perognathus parvus*, collected May 27, 1960.

Hughes: Were you the first to describe replication of the CTF virus in erythrocytes?

Johnson: Yes--Dr. Emmons, Dr. Oshiro, and myself. In the original studies, we were able to show in the lab that it was associated with the red blood cells. But the question was, was it inside the red blood cells? Dr. Emmons for his doctoral thesis did extensive

* R. W. Emmons. Colorado tick fever: Prolonged viremia in hibernating *Citellus lateralis*. *Am. J. Trop. Med. Hyg.* 1966, 15:428-433. R. W. Emmons, L. S. Oshiro, H. N. Johnson, E. H. Lennette. Intra-erythrocytic location of Colorado tick fever virus. *J. Gen. Virol.* 1972, 17:185-195.

Johnson: studies on the cell association of CTF virus. One problem was how to demonstrate the virus in the red blood cells, and that was done by fluorescent antibody microscopy. Number two, he showed by studies of the golden-mantle ground squirrel that you could feed the ticks on infected animals in the fall, and the ticks would harbor the virus through the winter, and that during hibernation the virus would stop growing in the golden-mantle ground squirrel but would be available after they came out of hibernation. So there is the means for overwintering. But I still feel that the virus has to be in some other animal for its permanent host. We have isolated the virus from five species of small mammals in Modoc County: chipmunks, golden-mantle ground squirrels, pocket mice, *Peromyscus* mice, and wood rats.

The people at Hamilton, Montana, say that the golden-mantle ground squirrel and the chipmunk are the natural hosts. Well, they are, as amplifying hosts for the nymphal stage. They do get sick and die, and they probably are just a means for making a lot of virus but probably are not the ones that keep the virus going indefinitely.

We intend to maintain a surveillance at Hackamore Camp to see if the virus persists.

Hughes: Who is "we?"

Johnson: Myself, Dr. Emmons, and Dr. Bernie Nelson of the vector control staff.

Hughes: Do you yourself go to Modoc County?

Johnson: I've gone there almost every year. I do the processing of tissue specimens and tissue culture, Dr. Nelson identifies the ticks, and Dr. Emmons arranges for the virology tests. [looking through papers] The last positive ticks were collected May 9, 1984. There were seven male and five female ticks, tested separately, and one of the female ticks had Colorado tick fever [virus] in it. The few ticks collected in 1986 and 1988 were negative for virus.

Hughes: Do you have any explanation for why the virus has been consistently found in that area?

Johnson: Only that it is maintained in small mammals, probably the pocket mouse.

Endemic Foci of Viral Diseases

Johnson: Many diseases have endemic foci. I've been interested in the endemic foci of yellow fever. Any time I'm in yellow fever areas,

Johnson: I'm apt to go and see where there have been sporadic cases of yellow fever where people who live there get ill and die of the disease. There again you observe certain things. You can trap there and observe what are the various forms of wildlife that could be the source [of the virus]. During the early field studies, it was concluded that man and Aedes egypti were the only source of yellow fever virus. Then came the discovery of jungle yellow fever, which was transmitted by the Hemogogus genus of mosquitoes. This is a forest mosquito that rests high in the trees, and when the workers felled the trees, the people working in the woods would get bitten by these mosquitoes and later come down with yellow fever. The next theory was that yellow fever is maintained by monkeys. Then, how many monkeys are there? In these endemic foci where year after year there will be one or two cases of yellow fever, usually there are very few monkeys.

In 1963, I visited Villavicencio, Colombia, where the Hemogogus vector was first identified. There is a brass plate on a tree at a rancho near Villavicencio where Dr. Jorge Boshell first identified the Hemogogus mosquito as the arthropod host of jungle yellow fever. He built platforms fifty feet up in the trees with ladders so they could collect these Hemogogus mosquitoes. I asked the mosquito collectors who had helped Jorge, "What else do you find when you go up? Are there birds or bats there in the trees?" They said, "Oh, there are lots of fruit bats." It was the Carollia perspicillata fruit bat which was abundant there. I collected a large number of these bats in Cueva Cuchilla near the old collecting station. They would roost in mines, wells, or trees. So I believe that the fruit bat is the amplifying host for yellow fever.

In each instance, you look for what is the host that might maintain the virus year after year. Like in rabies, which has been my lifework, it has finally come down to a few animals which are always the index animals--the spotted skunk, the weasel, and the mongoose. Other wild animals, like the striped skunk, fox, coyote and jackal, develop epizootic rabies. But they seem to be secondary hosts. So in the history of wildlife viruses, you tend to believe from what you see that the virus gets along very well for long intervals without producing disease. Then something happens and the disease breaks out.*

* H. N. Johnson. Sporadic cases of rabies in wildlife. Relation to rabies in domestic animals and character of virus. Proc. National Rabies Symposium, National Communicable Disease Center, Atlanta, Georgia, May 1966, pp. 25-33.

Johnson: I have visited most of the endemic foci of bubonic plague. They used to think that this disease was maintained in the cities by the Norway rat. But as the studies continued, you would find an isolated outbreak some place where they had had no bubonic plague for fifty years. The classic case was an outbreak in Iran in 1947 where there were no *Rattus* species. I visited that area in 1968. It is now believed that the host that maintains the disease there is the desert gerbil of the *Meriones* genus. Here we call them kangaroo rats or *Dipodomys* rats.

So there are certain things in epidemiology that interest me. Where is the reservoir system? We will never completely understand this, because it's hidden and the animal that carries the disease is hard to identify, but we can spot the site of sporadic cases.

Modoc virus and Rio Bravo virus are classified with the arboviruses, but there is no evidence that they are transmitted by mosquitoes or ticks. They maintain themselves in certain hosts without any illness. The Modoc virus is a group B flavivirus, like St. Louis encephalitis and yellow fever. We can collect *Peromyscus maniculatus* mice in the Modoc study area where I first isolated the virus in 1958, and almost any year, if we test tissues or cell cultures of kidney, we can isolate virus from that animal. I think that's a classic reservoir host system.*

Denny G. Constantine and Rabies

Hughes: Just a word or two about Dr. Constantine, if you don't mind.

Johnson: He began his studies of rabies with Dr. John Enright in the 1954 field study of bat rabies.

Hughes: He is a veterinarian, but does he have a particular interest in viral diseases?

Johnson: His major interest has been rabies related to bats. He's probably one of the world authorities on all the species of bats.

Hughes: When did you meet him?

* H. N. Johnson. Long-term persistence of Modoc virus in hamster kidney cells. In vivo and in vitro demonstration. *Am. J. Trop. Med. Hyg.* 1970, 19:537-539.

Johnson: I first met him when I went down to visit him in 1956 at Carlsbad, New Mexico, where he was working on bat rabies. He did some studies there and later changed to Frio Cave near Concan, Uvalda County, Texas, which was a very large bat cave. Tremendous studies were done on the airborne rabies in this cave.* There were two human rabies cases from exposure to the air in the cave. I visited Frio Cave in 1957 to search for ticks. I collected only two Ornithodoros stageri ticks, which actually fell onto my neck while I was trying to locate ticks on the floor of the cave.

The man that died of rabies in January, 1956 was George Menzies. He had been banding bats there, and they knew of no exposure by bite. The other person that died of rabies after visiting this cave was a mining engineer who specialized in finding guano for fertilizer. He was in his fifties when he entered a hospital in the Los Angeles area. The admitting doctor suspected at first that he'd had a stroke. On the basis of some symptoms and because of the history of being in a bat cave, they later suspected rabies. The autopsy showed that he died of rabies. This was in June, 1959.

Hughes: How did the men get rabies?

Johnson: Apparently from inhaling the air in Frio Cave. Up to ten million bats are in this cave in the daytime. As they fly about, there's a mist from their respiratory and urinary tracts and thousands of mites. (When I visited Denny at Carlsbad in 1956 we spent several hours in the area of the main colony of bats. I was careful to wash my hair when I showered, but after I got on the plane I felt something crawling in my ears and discovered that some of the mites were traveling with me.) The next study that was made by Dr. Constantine was to see if this liquid aerosol would infect animals.

The upshot of that study was that the bats reached a peak in the fall, and if they put coyotes or foxes in cages in the cave, with very fine cloth screening over the cages so the mites couldn't get to them, and then brought the animals back to a holding laboratory in Las Cruces, New Mexico, some of these animals would come down with rabies.** Even more frightening was that they had some unexposed animals being held in the same general room, but in separate cages, and thirty-nine of those that

* D. G. Constantine, DVM, MPH. Rabies transmission by air in bat caves (1967) U.S. Government Printing Office Publication No. 1617. USPH&W, PHS, NCDC, Atlanta, Georgia, 30333.

** See previous footnote.

Johnson: were not exposed came down with rabies.* They closed off the study, because it was very obvious that this strain of rabies virus in the freetail bats was particularly adapted to the lungs and spread via the air from one cage to another. These bats live in such intense crowding; they hang onto each other so there would often be 400 of these bats on the ceiling per square foot.

Hughes: Did the cave bats themselves get rabies?

Johnson: During the peak population in the fall, there would be die-offs. Most of the bats found freshly dead were found positive for rabies--it decimated the population. I studied Tadarida colonies in California, and when rabies appeared in an isolated colony, it usually killed them. I found that over fifty percent of the rabid bats had the virus in the lungs.

Hughes: Was it a new finding that the virus had a predilection for the lungs?

Johnson: Yes, the finding was that this particular strain of virus does not need to be transmitted by bites, and Dr. Constantine coined the term "non-bite transmission" of rabies.**

The Modoc-Rio Bravo Complex of Viruses

Johnson: Now, we probably should go on to the other group B viruses, the Modoc-Rio Bravo complex of viruses. I told you we were working on Colorado tick fever virus at Hackamore Forest in 1958. Female mice that were nursing baby mice were used as the test animals. It so happens that if you inoculate an adult white mouse intracerebrally with the Colorado tick fever virus, it doesn't become sick, but if you put it in the brain of a one- to five-day-old mouse, it will die. The suckling mouse was the best test animal until tissue culture systems were found suitable, namely, baby hamster kidney cells.

* W. G. Winkler et al. An outbreak of non-bite transmitted rabies in a laboratory animal colony. Am. J. Epidemiol. 1972, 95:267-277.

** D. G. Constantine. Rabies transmission by non-bite route. Pub. Health Rep. 1962, 77:287-289.

Johnson: We'd inoculate six or eight baby mice with a tick pool. If one or two would sicken we would harvest the brains. The others would be left in the box, and when they died the mother mouse would eat them. The tenth day after the baby mice started to die, I would bleed the mother mouse and test various organs. To my great surprise, the virus had invaded the mammary gland.

Hughes: Why did that surprise you?

Johnson: There was nothing in the early work on viruses to make you suspect any organs other than blood and brain of being the source of virus. As time went on, as I've told you, I worked on target organs for several viruses. The target organs tend to be tissues that are highly secretory or physiologically active. The polio virus group will be in the cells lining the intestinal tract. Other target organs are the brain, lungs, kidneys, and pancreas. Rio Bravo, rabies, and western encephalitis viruses will multiply in the salivary gland. The kidney is commonly infected with a great variety of viruses.

Hughes: Why?

Johnson: The blood is constantly filtered by the kidneys and thus intimately exposed to any virus that gets into the blood. Urine contamination is one way to spread the virus. The famous example is the herpes virus called cytomegalovirus, which used to be called swimming-pool disease. Now it is a problem in dialysis treatment centers. Cytomegalovirus infects the kidney and is excreted in the urine. People who have had kidney transplants will be given drugs so that they become unable to produce much antibody to viruses, and they'll excrete a large amount of this cytomegalovirus in the urine. The other example is rubella, where the baby gets infected at birth. They sometimes excrete rubella virus for months.

In 1958, I was looking for Colorado tick fever virus in the mammary glands of *Peromyscus* mice. (I might mention that at about the same time I got rabies virus, one of my type strains, out of the lactating mammary gland of a spotted skunk from Glen County, California.) We trapped a *Peromyscus maniculatus* mouse, number M544--that's our type strain--on July 9, 1958. I ground up the mammary gland and injected that into baby mice and got Modoc virus--not Colorado tick fever virus.

Hughes: You were looking for Colorado tick fever virus.

Johnson: Right. I also found out the Colorado tick fever virus could be found in the liver. I isolated the virus from the liver of a *Peromyscus* mouse in 1958. Knowing that the red blood cell is infected with CTF virus, it must be in the bone marrow. It so happens that in young mice there is erythropoiesis in the liver. I

Johnson: tend to believe now that the reason I got Colorado tick fever virus out of the liver of that one mouse was that it was growing in the erythropoiesis foci in the liver.

The important discovery was getting the type strain of Modoc virus out of the mammary gland of a nursing Peromyscus maniculatus mouse, indicating a method of transmission through the mother mouse to the baby mice, which then becomes tolerant and the virus would be able to live in the host and be excreted for an extended period. That seems to be the situation with the Rio Bravo virus also. It can be excreted from the salivary gland for months. Therefore, there must be some inability of the host to develop antibody to it.

A good example of tolerant virus infection is lymphocytic choriomeningitis [LCM] virus. Much work has been done on that, and the way that happens in the Mus musculus house mouse or white laboratory mouse is that the mice get infected in utero or at birth. The tolerant host does not recognize the virus as a foreign antigen and the virus will be in all the tissues, including the brain. The adult mouse will look healthy, and yet it will be excreting the virus in the urine and saliva. Such infected healthy lab mice, if used as test animals in attempting to isolate viruses from wildlife specimens, will have the virus in the blood. If you stick the needle of the syringe into the brain to inoculate the test material, you will contaminate that needle. If you inoculate six adult mice and any of those has a tolerant infection, those inoculated subsequently and are not already infected will sicken in six or seven days and die of lymphocytic choriomeningitis. Where LCM virus has been introduced into a breeding colony by infected wild Mus musculus house mice, usually only a few will be found to be carriers.

Dr. Emmons organized a survey of house mice in the San Francisco Bay Area in 1973-1974 because of the outbreaks of lymphocytic choriomeningitis virus in pet hamsters. One of the techniques that he used in the lab to identify LCM in wild house mice was to test a pool of the organs--salivary gland, lung, kidney and brain--by either a radioimmune assay or by a fluorescent antibody inhibition test. It seems that any organ you test of these tolerant mice will be heavily infected with LCMV. It was one quick way of demonstrating the virus. Of course, you could also take that pool and inject it into three- or four-week-old mice intracerebrally. They will die of the disease. The virus can also be isolated in vero cell tissue culture.*

* R. W. Emmons et al. A survey for lymphocytic choriomeningitis virus in the San Francisco Bay Area. Calif. Vector Views 1978, 25:21-27.

Hughes: Is excretion of the LCM virus a means of transmission to man?

Johnson: Yes. The way that man is infected from the house mice that are tolerantly infected with the lymphocytic choriomeningitis virus is from the saliva or urine. Bolivian and Argentinian hemorrhagic fever are caused by a virus related to the lymphocytic choriomeningitis virus. In field epidemics, it seems that food or water is contaminated by infected wild mice.

The reason I used the title the Modoc-Rio Bravo complex is based on cross-immunity studies with the two viruses, one which comes from the Tadarida bat and the other one from the Peromyscus wood mouse. If you immunize mice peripherally by subcutaneous inoculation with Modoc virus and challenge them in the brain with Modoc virus, they're immune. They're also immune if you challenge them with Rio Bravo virus, which shows it's really a very closely related virus. Now, if you turn that around and you immunize two groups of mice with Rio Bravo virus, they will be immune to Rio Bravo virus inoculated intracerebrally, but the immunized mice show little immunity to Modoc virus, which suggests that the Rio Bravo virus has been derived from the Modoc strain and is lacking some of the original antigen of Modoc virus.

It is possible that bats have been exposed by an arthropod host such as ticks which have fed on the Modoc carrier, the Peromyscus mouse. Then bat ticks might transmit it to other bats, or the virus is spread directly. There must be some means by which those two viruses have a common ancestor.

Hughes: I think we should say something about your interest in the chronic kidney infection cause by Modoc virus. You gave me a paper, published in 1970, entitled "The long-term persistence of Modoc virus in hamster kidney cells."*

Johnson: Okay. We inoculated some hamsters intramuscularly to obtain immune serum. About four months later, I was testing various organs of a hamster after obtaining blood, and to my surprise I found active virus in the kidney. It is hard to explain how that would be a tolerant infection because the hamster was an adult when inoculated. To look for the ability of this virus to be excreted in the urine, I took this infected kidney tissue and inoculated two hamsters intramuscularly with the kidney suspension. After that we bled them at two and three days to see if the virus would get into the blood. One of the hamsters that was inoculated did have virus in the blood. By a careful study of this hamster we were able to demonstrate the virus in the urine for five months.

* Am. J. Trop. Med. Hyg. 1970, 19:537-539.

Johnson: When I killed the animal and bled it one year after the first urine isolate from that animal, I could not find any virus by grinding up the kidney tissue and inoculating mice. We trypsinized the cells of half of the kidney and grew out the cells, and to my great surprise, it produced Modoc virus, showing true latency. That is similar to my finding Powassan virus in the kidney of a spotted skunk in California. Direct tests of the organs were negative for virus, but the kidney cells cultivated in tissue culture produced Powassan virus.*

Hughes: Both Modoc and Rio Bravo virus are not necessarily transmitted by arthropods?

Johnson: We have no evidence that they are. One patient in California whom we know was infected in nature with Modoc virus was a child that lived in Kern County. He handled some wild mice at a mountain cabin. His blood showed an antibody rise against Modoc virus.

The Rio Bravo virus encephalitis case in California I suspect was the result of a similar exposure to *Tadarida* bats at the girl's home. It was noted that there were bats at the house. Now, when it comes to the Modoc virus, it's a matter of picking up or trapping wild *Peromyscus* mice. We know now that people that have helped trap house mice have become infected with LCM.

Hughes: Both Modoc and Rio Bravo are classified as arboviruses. Does that classification bother you?

Johnson: No. The reason for that is that they are so closely related serologically to the arboviruses that are spread by either ticks or mosquitoes. Now, the St. Louis encephalitis virus, for instance, which is present in this state, is transmitted by *Culex tarsalis* and *Culex quinquefasciatus* mosquitoes. You can show that this virus infects ticks. So it could be tick-borne. For instance, the *Tadarida mexicana* bat, the so-called freetail bat, is parasitized by *Ornithodoros stageri* ticks. I've only collected a very few of these ticks. According to Denny Constantine, they are abundant on the ceiling of Frio Cave. They feed on bats there. So someone should do a special study of that tick.

Studies should be made to determine whether the Modoc virus in *Peromyscus* mice can be transmitted by the *Dermocenter andersoni* ticks that spread CTF virus. Powassan virus is spread by *Ixodes* ticks and that is another potential arthropod host. I am

* H. N. Johnson. Isolation of Powassan virus from a spotted skunk in California. *J. Wildlife Diseases* 1987, 23:152-153.

Johnson: currently interested in finding someone to do research on the gopher tick Ixodes holdenreidii as a vector of Powassan virus. I isolated this virus from a spotted skunk. There is plenty of research to be done. The reason these two viruses get little attention is that they do not seem to be a major disease problem. It's more an intellectual problem.

Hughes: You can't get funding?

Johnson: Funding is one problem. In my early training in research, there was plenty of money to study yellow fever. The next was rabies, which was a major problem. Then poliomyelitis got tremendous funding. Later came funding for German measles (rubella), because of congenital disease problems. There was funding for the big epidemics of St. Louis and western encephalitis, and now of course it's the AIDS virus. So there's emergency money for epidemic situations. Yet there should be an interest in basic studies of nature, and how in nature viruses persist with no evidence of illness but can be a source of big epidemics.

European tick-borne encephalitis was a tremendous problem when they moved people to Siberia to cut down the forest and build buildings. The Russians had a high attack rate and morbidity and mortality from this European tick-borne encephalitis. This virus is clearly related to Powassan virus, which is found in Canada and the United States.

A big epidemic of Kyasanur Forest disease occurred in India about two years after I left. That turned out to be a close relative of the European tick-borne encephalitis. But in the Indian situation, what caused it to become a problem was that they moved large numbers of people to work on a dam, with a lot of head-carrying and hand work. About ten thousand people were cutting down forest and brush, and that led to an overpopulation of small mammals and ticks. It was in that area that this disease started appearing, and the people working in the forest would get sick and then die. That became a very scary situation and the big project at the Virus Research Centre in Poona from 1957 on, for ten years. And it still is a problem there.

Disturbing a natural environment by irrigating large tracts of land stimulates the cycle that leads to outbreaks of encephalitis in man. Epidemics occurred previously in central USA when there were periods of high rainfall. When farms are abandoned because of crop failures, the overgrowth of brush and trees result in overpopulation of small mammals. This occurred in the southern states when the boll weevil decimated the cotton plants. This was the underlying cause of outbreaks of fox rabies and Rocky Mountain spotted fever. Outbreaks of tick-borne encephalitis and rabies appeared in eastern Europe after World War II for the same reason.

Hughes: In 1954 you isolated Rio Bravo virus. Will you tell me about that work?

Johnson: Well, as I have mentioned, Rio Bravo virus came from a study area called Rio Bravo School, Kern County, California. We knew it was a good place to get western and St. Louis encephalitis viruses from Culex tarsalis mosquitoes. That was one of the areas in which we studied all the desert animals. But in addition, I was very interested in the bats that were around that school at night.

So one night in 1954, I put up twelve by twenty-seven foot Japanese bird nets for collecting bats. [looking at lab notes] On October 1, 1954, the next morning at dawn, I collected twelve bats. We tested the salivary glands, spleen, and the brain of these healthy bats, [using] separate sterile instruments for each organ. Out of the salivary glands of three Mexican freetail bats, Tadarida mexicana, M64, M65, and M70, we isolated a new virus. This virus turned out to be a flavivirus (group B) related to St. Louis encephalitis and yellow fever viruses.

Subsequent studies of that virus revealed that there have been at least some cases of human infection, but only one in California that we're sure of, where the patient developed neutralizing antibodies to this virus and had had encephalitis. The patient had moved when we tried to follow up the case, but we did have the two blood specimens, one taken at the onset and one during convalescence. Dr. R. E. Kissling at CDC was studying Rio Bravo virus, and one of his technicians was bitten by a hamster that had been inoculated with the virus. She had a skin rash, headache, muscle pain, and spasticity, very much like the disease caused by the dengue virus. She developed antibodies to Rio Bravo virus. There was [also] a lab infection at the state health department lab in Austin, Texas. We have no evidence as yet that Rio Bravo virus is transmitted by mosquitoes or ticks.

Hughes: Do you suspect that?

Johnson: I would suspect that some species of ticks or mosquitoes would get infected by it, but in bats it is transmitted directly via the saliva. It is a major study to do tick and mosquito transmission studies. I was unable to infect Culex tarsalis mosquitoes. I hope someone will do cross immunity studies of Rio Bravo virus and the dengue viruses.

Hughes: Was Dr. Constantine involved with the Rio Bravo virus studies?

Johnson: Yes.* In 1964 he reported persistent infection of the salivary glands of Tadarida mexicana bats with Rio Bravo virus. In the early studies of bat rabies at Carlsbad Caverns in New Mexico in November, 1955, there were two isolations of a virus from salivary glands, identified as rabies on the basis of the microscopic test for Negri bodies.

Subsequent studies showed that these isolations were Rio Bravo virus and that if the salivary glands are positive for rabies, the rabies virus is also in the brain. The initial studies of Tadarida bats at Carlsbad Caverns in 1955 and 1956, in addition to the identification of rabies in some of the bats, led to a decision to establish the USPHS, CDC Rabies Investigation Station at the University Park Branch, Las Cruces, New Mexico, and this was completed in 1958. Subsequently, Dr. Constantine and his assistant Dora F. Woodall were able to do some long-term studies showing that the same bat that once proved positive for Rio Bravo virus in the saliva would continue to excrete the virus for several months, showing it was a true carrier of this virus.

This virus has had a very interesting history in research. It's an example of how a virus can persist in an animal without causing disease and without any evident arthropod host. Rio Bravo virus was also isolated from an Eptesicus bat in California during the survey in 1954 at U.C. Davis veterinary school.**

Kern Canyon and Klamath Viruses

Hughes: Well, the next virus that you isolated was the Kern Canyon virus, in 1956. Now, is that also a virus for which the arthropod vector is unknown?

Johnson: Yes. The Kern Canyon virus has little possibility of being arthropod-borne in bats. The reason I found this virus was my interest in the large populations of Myotis yumanensis bats in some of the electric power stations in Kern Canyon.

* D. G. Constantine. Latent infection of Rio Bravo virus in salivary glands of bats. Public Health Reports 1969, 79:1033-1039.

** J. B. Enright, W. W. Sadler, J. E. Moulton, D. G. Constantine. Isolation of rabies virus from an insectivorous bat (Tadarida mexicana) in California. Proc. Soc. Exp. Biol. Med. 1955, 89:94-96.

Johnson: Having obtained Rio Bravo virus from *Tadarida* bats, I thought, well, I'll see what I might find in the *Myotis* bats. So I collected fifteen of these bats, June 9, 1956, in the Borel power station. A virus was isolated from the spleen-heart pool from M206, one of these bats. These bats were all pregnant, near term, each carrying a single embryo.

When studied by electron microscopy, to my great surprise, this virus didn't look like the other arthropod-borne viruses, which are usually small and round. This looked like rabies, which is rod-shaped. It is a rhabdovirus and it does multiply in mosquitoes. The type strain for the rhabdovirus genus is vesicular stomatitis virus (VSV) of cattle. Rabies virus is classified as a member of this genus, as is Hart Park virus, Kern Canyon virus, and Klamath virus. On July 19, 1961, I collected twenty-three *Myotis yumanensis* bats at the Borel power station. I isolated Kern Canyon virus from one of these bats (M1039). This shows that this colony of bats was still infected with Kern Canyon virus five years after the first isolation. We don't know where the virus comes from in nature, but it shows that there are rhabdoviruses in wildlife.

Another example of persistence of a virus in Kern Canyon, also in bats, was the isolation of rabies virus from *Tadarida* bats on two occasions a mile up the canyon where there was a colony in a cleft in the granite wall on the south side. I had seen the bats emerge from their roost one evening when doing a bat survey. There was a pile of guano on the ground which marked the presence of the bats.

On July 30, 1958, a group of Rockefeller Foundation staff members were in Bakersfield to visit this study area, and we drove up the canyon early in the morning so I could show them the Borel power station where the Kern Canyon bat virus was obtained. On the way I stopped to show them the bat roost in the canyon wall. We had parked about fifty feet away and when we came to the guano heap, there was a paralyzed *Tadarida* bat (M552) lying there. Harold Trapido said, "You planted that one," and we all laughed. Harold took pictures while I collected the bat and put it in a container and placed it in my Stanley steel thermos. It proved to be positive for rabies. Robert Kokernot, Tommy Aitken, Calista and Ottis Causey were present also.

I stopped at the same place many times, and on July 19, 1961, I found another paralyzed *Tadarida* bat (M1018) at the same place. It was positive for rabies virus. Subsequent visits showed no fresh guano and one can assume that the bats had all died.

Now to tell about another rhabdovirus called Klamath virus. In 1962 there was an overpopulation of *Microtus montanus* mice in the alfalfa fields of northern California and adjacent Klamath

Johnson: County, Oregon. A graduate student from the University of Oregon, Edward Hansen, was studying population dynamics of *Microtus* mice at Geary Ranch, Wocus Valley, near Klamath Falls, Oregon. I was interested in the method used for trapping these animals in the fenced enclosure in the pasture on this ranch. It was wintertime and there was about two feet of snow on the ground. I decided to collect some mice for virus studies. One technique was to take out a twelve-inch block of snow at intervals, looking for a mouse tunnel. If one was seen we put a walk-in trap facing the opening. The traps were baited with old-fashioned Quaker Oats.

On the morning of January 29, 1962, I caught ten *Microtus montanus* mice. These were taken alive to the laboratory in Berkeley and on February 5 one mouse was dead (M1056). From the brain and lung specimens of this mouse we isolated Klamath virus.* Recent surveys show that wildlife in Alaska and Oregon have antibodies to it. Humans are probably infected with it. Serologically you can show it, but it's still not a disease problem.

The Klamath and Kern Canyon viruses should be studied for tissue tropism following subcutaneous and intramuscular inoculation. On the basis of VSV causing disease in cattle, these viruses should be inoculated into Caesarian or colostrum-deprived calves and tested for viremia and the presence of the virus in nose and throat swabs and urine.

Other Disease Agents in *Microtus* Mice

Johnson: During the studies of the *Microtus* population at Geary Ranch in 1962 and 1963, we isolated thirteen strains of rickettsiae by intracerebral inoculation of *Microtus* blood into one- to two-day-old mice. Serum obtained from mice immunized with M1193 blood isolate was positive for antibodies to the eastern Montana strain of Rocky Mountain spotted fever rickettsia. Tularemia organisms were isolated from the blood of M1361 *Microtus* bled February 7, 1963. A new virus was isolated from M1146 *Microtus* blood of June 13, 1962. The same virus was reisolated from the blood specimen, heart containing blood, liver, and embryos found in the uterus of this female mouse.

* F. A. Murphy, H. N. Johnson, A. K. Harrison, R. E. Shope. Ultrastructural characterization of a new member of the rhabdovirus group. Klamath virus. *Archiv fur die gesamte Virusforschung* 1972, 37:323-331.

Johnson: On July 25, 1962, the pasture at the Geary Ranch was flooded and I found five young *Microtus* mice in a nest floating on the water (M1191-M1195). A pool of the intestines of these immature mice was processed and tested intracerebrally in infant mice, and this yielded another virus strain identical to the M1146 type strain.

Dr. James E. Froeschle, who had done some research at the arbovirus lab on western encephalitis virus tissue tropism,* was chief of the enterovirus unit, CDC, Atlanta. I asked him if he would study this virus, because its pathogenicity and tropism for rhesus and human cell cultures were similar to the Coxsackie B viruses. Froeschle and his technical assistant Paul M. Florino found that the *Microtus* virus was similar to the human enteroviruses as determined by ether sensitivity, stability at pH 3, size, and magnesium chloride stability when heated to fifty-six degrees celsius. The virus was not neutralized by human gamma globulin nor by antisera for human enterovirus, Coxsackie A 1-22, 24, and Coxsackie B 1-6, polio I, II, III, or echo 1-29. It is not an adenovirus. The virus showed a tropism for the liver and intestinal tract, and infected human and monkey cell lines.

The reason I have summarized these studies is that Andy Main, working at the Yale Arbovirus Research Unit, isolated four strains of a similar virus, M2233, M2309, M2313, and M2537, from the liver of red-backed voles, *Clethrionomys gapperi*, collected at Hockomock Swamp in Massachusetts in November, 1969.** He also obtained one strain, M2268, from a blood specimen of these mice. He reported serological studies that identified his mouse enterovirus and one isolated by Whitney from *Clethrionomys gapperi* and *Microtus pennsylvanicus* trapped in New York State in 1964 as identical to the M1146 *Microtus* virus isolated at the California State Viral and Rickettsial Disease Laboratory from *Microtus* mice collected in Klamath County, Oregon in 1962.*** The ecosystems where these viruses were found are typical of water sources used for human consumption, and they should be investigated further to determine whether they are pathogens for people, especially nonhepatitis A or B type liver pathology.

* J. E. Froeschle. Propagation of western equine encephalitis virus in mice following intramuscular and intranasal inoculation. Proc. Soc. Exp. Biol. Med. 1964, 115:881-884.

** A. J. Main, R. E. Shope, R. W. Wallis. Characterization of Whitney's *Clethrionomys gapperi* virus isolates from Massachusetts. J. Wildl. Dis. 1976, 12:154-164.

*** E. Whitney, A. P. Roz, G. A. Rayner. Two viruses isolated from rodents (*Clethrionomys gapperi* and *Microtus pennsylvanicus*) trapped in St. Lawrence, New York. J. Wildl. Dis. 1970, 6:48-55.

Hughes: It's the similarity to the Coxsackie viruses that makes you suspect that the M1146 Microtus virus might be pathogenic in man?

Johnson: Yes.

One thing more about the Modoc virus. The only publication that goes into the problem of arthropod-borne versus nonarthropod-borne viruses was the paper I gave at the Pacific Science Congress in 1966.* It led to a study of viruses associated with large populations of birds.

Other Viruses Associated with Ticks

Johnson: The reason I got interested in that subject was that in 1965 the Hooper Foundation in San Francisco was doing some studies on the Farallon Islands. I heard about the ticks that they had found there. They were interested in psittacosis. Frank Radovsky and David Stiller were doing taxonomic studies of the *Ornithodoros* ticks and collected some ticks for me.

So I tested these ticks, which were *Ornithodoros denmarkii*,** and from them we isolated a virus in 1965 which we call the Farallon virus. It's related to Hughes virus isolated from ticks collected at the Dry Tortugas bird colonies off Florida. That virus is named for one of the men that isolated it. This is the species type virus, and related variants are found in bird ticks around the world, such as the Punta Salinas virus I isolated from ticks collected in Peru.

We do not know whether man is infected with the Farallon virus. But some of the people who harvested guano at the sea bird colony at Punta Salinas developed illness related to tick bite exposure. I developed a boil-like infection on the ulnar side of my right hand where one of the ticks began feeding before I removed it, when I collected ticks there in 1967.

* Ecological implications of antigenically related mammalian viruses for which arthropod vectors are unknown and avian associated soft tick viruses. Jap. J. Med. Sci. Biol. 1967, 20:160-166.

** F. J. Radovsky, D. Stiller, H. N. Johnson, C. M. Clifford. Descriptive notes on *Ornithodoros* ticks from gull nests on the Farallon Islands and isolation of a variant of Hughes virus. J. Parasit. 1967, 53:890-892.

Johnson: On May 18, 1966, I collected ticks from gull nests at Mono Lake, from which we isolated Mono Lake virus. This virus belongs to the Chenuda-Kemerovo group of viruses. The Chenuda virus is from Egypt; the Kemerovo virus is from Russia. The type strain is AR861 from a pool of ten adult Argas cooleyi collected May 18, 1966 from nests of the California gull, Larus californicus. Mono Lake lies east of the Sierra Mountains at an elevation of 6,409 feet.

Hughes: These viruses are all associated with bird nests?

Johnson: The Mono Lake virus is, but the Kemerovo virus was isolated in Russia from Ixodes persulcatus forest ticks. The gulls at the Farallon Islands were Larus occidentalis. More than a hundred thousand of these birds nest there.

Hughes: Was it these studies that brought you into contact with the Russians?

Johnson: Yes, specifically the ornithologists. I had met the virologists L. A. Silber and V. D. Soloviev in 1946 when they visited the Rockefeller Institute, and M. P. Chumakov and A. A. Smorodintsev in 1958 at Lisbon, Portugal. The reason I was invited to give a paper in Russia was the Mono Lake virus was related to the Kemerovo virus isolated from ticks collected on the ground in Siberia.* That virus was not known to be associated with marine bird ticks. It caused a disease in Siberia spread by ticks found in the woods. So I was asked to speak at an international congress on the role of migratory birds in the transmission of arboviruses, and I gave that paper in Science City, Russia, in 1969. This research establishment is located near Novosibirsk, Siberia.

Subsequently, the virologists in Russia became intensely interested in this field and probably have done more work than other countries on the ticks associated with water or marine birds and isolated additional new strains of virus. The most interesting one they isolated was a virus called Tyuleniy virus, which is a flavivirus like St. Louis [encephalitis] virus, and is transmitted by Ixodes uriae ticks. According to [Dmitri K.] Lvov, the virus multiplies in mosquitoes, but in marine birds, it is transmitted by Ixodes uriae ticks. A field study of marine birds at Three Arch Rocks, Oregon, by the virologists and entomologists of the Rocky Mountain Laboratory at Hamilton, Montana, resulted in their isolation of Tyuleniy virus from Ixodes uriae collected there in 1969.

* H. N. Johnson, J. Casals. Transcontinental connections of migratory birds and their role in the distribution of arboviruses. Symposium on the study of the role of migrating birds in the distribution of arboviruses, Novosibirsk, USSR, 1972, 200-204, A. I. Cherepanov, ed. AS. USSR Siberian Branch.

Johnson: Lvov's team made their tick collections on Tyuleniy Island, Sea of Okhotsk, on August 17-18, 1969. They isolated a second new virus from ticks collected there, which they named Sakhalin virus. The Hamilton team's field study was later in 1969, and neither team knew about the other's project. C. E. Yunker summarized the story of the tick-borne viruses associated with seabirds and he refers to Dick Emmons and me having isolated a Sakhalin virus and a Uukuniemi virus from Ixodes uriae ticks collected at a murre colony on Flat Iron Rock, Trinidad Head, California, June 22, 1972.* Flat Iron Rock is a nesting site for both murre (Uria aalge) and cormorants (Phalacrocorax penicillatus). There was also a colony of sea lions on the island. Our strains of these viruses were studied by Jordi Casals at Yale University and he reported that they were identical to those described by C. E. Yunker.

Hughes: Does your finding tie in with the pattern of bird migration?

Johnson: All the sea birds tend to have long-range migration, even the cormorants.

Hughes: So you could explain finding these spots of infection around the world by bird transmission?

Johnson: Yes, primarily by the nymphal ticks carried on them. That interested me because the Chenuda-Kemerovo type Huacho virus we isolated in 1967 from ticks collected in Peru was related to Chenuda virus of Egypt and Kemerovo virus of the USSR.

Hughes: You were involved with ecological surveys in the sixties. Were they in connection with the tick studies?

Johnson: Yes. During 1966 I was visiting arbovirus projects in the Pacific Ocean region and while in Australia I went to Cairns, near the Great Barrier Reef, to look for marine bird colonies. I learned from the owner of a motorboat that there was a colony of terns on a small cay on the reef. He said he could drop us off early in the morning as he was taking some people fishing and would pick us up in the afternoon. I had my usual kit, so on July 21, 1966, we boarded the boat. It was eighteen miles to the cay. It was a low sand island with no trees. My wife Fran and teenage son Michael were with me. There was a large colony of sooty terns, Sterna fuscata and a lesser number of crested terns, Sterna bergii. There were no ticks under the flotsam. There was no nesting material. I finally discovered that the ticks were in the sand near the eggs where the succulent vegetation shaded the sand. I collected ticks with some sand, trying to sample as many nest

* C. E. Yunker. Medical Biology 1975, 53:302-311.

Johnson: areas as possible. I divided these tick collections into several plastic bags, tied these with string and put them into a thermos cooled with ice.

When we returned to Cairns that evening I shipped the ticks to Ralph Doherty of the Queensland Institute of Medical Research at Brisbane and went on to Mitchell River Mission on the gulf of Carpentaria where Ralph had a field station. The ticks were processed later at the Brisbane lab and two different viruses were isolated. One was related to the Quaranfil virus from Egypt; the other was a new one which was named Upolu virus.*

During 1968 and 1969 Frank J. Radvosky and David Stiller of the Hooper Foundation gave me some Ornithodoros capensis ticks that W. A. Foster had collected at Lake Shalla, Shoa Province, Ethiopia, in July, 1968 and January, 1969. From AR908 ticks collected from a sacred ibis nest on July 27, 1968 and AR913 collected from a white-necked cormorant, January 26-27, 1969, we isolated Soldado Rock virus. Jordi Casals at Yale University identified these strains.

My wife and I visited Lake Shalla in 1973 on a field trip arranged by Wesley Ota of NAMRU3 at Addis Ababa. Wes Ota was the senior technician at the Berkeley arbovirus lab from 1954 to 1963, when he left to study at Johns Hopkins University at Baltimore, where he received a D.Sc. degree. Our isolation of Soldado Rock virus from the ticks collected at Lake Shalla is referred to by Hoogstraal.**

Hughes: Do you want to say anything about the surveys that you did in Sweden and Finland?

Johnson: Yes. The reason for my being involved in surveys outside of my own basic interests is in helping virus laboratories in other countries to plan wildlife virus studies. Those of us who have specialized knowledge are encouraged to act as consultants.

After the 1958 meeting of the International Congresses of Tropical Medicine and Malaria at Lisbon, Portugal, I went to Sweden, because they had received a report from Joseph Smadel that some sera sent in for diagnosis were positive for European tick-

* R. L. Doherty, R. H. Whitehead, E. Judith Wetters, H. N. Johnson. Isolation of viruses from Ornithodoros capensis neumann from a tern colony on the Great Barrier Reef, North Queensland. Australian J. of Science 1969, 31:363.

** H. Hoogstraal, M. N. Kaisek, E. R. Easton. J. Med. Ent. 1976, 12:703-704.

Johnson: borne encephalitis. Dr. Arne Svedmyr, who attended the Lisbon conference, arranged a field trip to a farm at Nortalje, Sweden, where some cattle sera had been found to have antibodies to the tick-borne encephalitis virus.

Arne Svedmyr, Gerolf von Zeipel, B. Zetterberg, and I collected Ixodes ricinus ticks on the low brush and under the fir trees. It was this collection that provided the first tick strain of European tick-borne encephalitis virus from Sweden.* It is an occasion such as this that is so memorable. I have visited Sweden and Finland several times.

In 1969 I was interested in cases of a renal disease, nephropathia epidemica [NE], related to exposure to field mice, and visited sites in northern Sweden along the Baltic Sea where there had been cases of this disease. There was a history of overpopulation of field mice and exposure to mice in barns and hay stacks. In 1971 I visited Dr. Nils Oker Blom, director of the virus laboratory of the University of Helsinki. He arranged field trips to foci of NE and to Tvarminne on the Hanko Peninsula of Finland, a research field laboratory. I was accompanied by zoologists and we netted birds for serology and for tick studies, and collected bats and other small mammals. I made cell cultures of the kidneys of these animals, but these were not tested for viruses. The Rockefeller Foundation had given grants to Dr. Oker Blom for research on European tick-borne encephalitis, and they had excellent equipment for field studies.

My first field visit there was in 1958 when I did some trapping near Kotka. We caught many Clethrionomys and Apodemus mice, the latter are much like our Peromyscus mice. It is the red backed vole, Clethrionomys glareolus, that is the source of the NE virus in Scandinavia. The "Hallnas" strain of nephropathia epidemica was isolated in 1981 from this animal.** This virus is related to Hantaan virus, the cause of Korean hemorrhagic fever.

Hughes: In each of these cases, did you come into contact with local scientists?

* G. von Zeipel. Isolation of viruses of the Russian spring-summer encephalitis-louping 11 group from Swedish ticks and from a human case of meningoencephalitis. *Archiv Virusforsch.* 1959, 9:760.

** R. Yanagihara, A. Svedmyr, H. L. Amyx, et al. *Scand. J. Infect. Dis.* 1984, 16:225-228.

Johnson: Yes. Before you can plan a cooperative field study, you write to the scientists you know and try to arrange a visit when they will be doing field work. This has to be done well in advance of a trip and for the most part is correlated with international scientific congresses. In 1971 I was invited to present a paper at the Second International Symposium on Circumpolar Health, Oulu, Finland, June 25-26. My subject was, "Viral nephritis derived from wildlife." I also took part in a post-congress tour to Murmansk, USSR, visiting their research facilities there and in Leningrad.

I mentioned previously my field studies in Australia in 1966. These were arranged to coincide with the Pacific Science Congress in Tokyo, August 23-25. This was one of the major international congresses dealing with arthropod-borne viruses. There was a special symposium on this subject. Prior to this meeting, I visited New Zealand, Australia, Singapore, Malaya, Borneo, the Philippine Islands, Taiwan, and Hong Kong. Dr. H. Elliott McClure, the ornithologist who had been associated with Bill Reeves at the Bakersfield encephalitis laboratory, had subsequently been directing a U.S. Army Medical Corps study of the role of birds in the epidemiology of Japanese encephalitis. I was in Japan in 1957 because the Pacific Science Congress that year was in Bangkok.

On that occasion, I visited the 406 Army Lab and their field stations. In 1966 they had special funds so that they could supply a jeep, bird nets, and training for local scientists to collect birds, bleed them, and band them at selected places from Malaya to the Philippine Islands. During World War II, Japanese encephalitis and malaria were the major arthropod-borne diseases. Of course, there was mite-borne typhus and dengue also. So knowing McClure and through other ornithologists, I arranged to visit his field stations.

In Malaya, we went to the Cameron Highlands where we netted bats and birds. There is a strain of tick-borne encephalitis in Malaya caused by Langat virus, which is a group B virus. It is related to Powassan, but Langat virus is not known to cause human disease in Malaya. At Kuala Lumpur I visited the bird field study in the forest nearby and the famous Batu bat cave.

The next field station was Jesselton, Borneo, then the island of Negros in the Philippines. Professor D. S. Rabor had the army project there. He had worked with S. Dillon Ripley on the birds of Negros, and it was most interesting to visit his field station. Rabor was on the faculty of Silliman University there. Dr. George Beran, DVM, was teaching microbiology at the university and had organized a rabies control program for the island of Negros, using the LEP Flury avianized virus vaccine.

Johnson: While in Japan I visited Hachinohe at the north end of the island [of Honshu] and collected Ixodes signatus ticks at a bird island there. These were negative for virus at the NIH lab in Tokyo and also at my lab in Berkeley.

The Border Public Health Meetings

Hughes: Had there been international cooperation in Mexico prior to 1960?

Johnson: Yes. The reason for going to Mexico in 1960 was that I had been asked to help with the identification of encephalitis. I arranged to get serum specimens, and these would be tested in the virus laboratory in Berkeley for antibodies to polio and for arboviruses.

We knew there was evidence of eastern, western, and St. Louis encephalitis in Mexico. We had a good relationship with Mexico because of annual border public health meetings. I used to go to this meeting as often as possible. The mutual concerns were in this order: venereal disease, tuberculosis, and, in my field, encephalitis and rabies. [The latter] were a real problem on the border, particularly rabies in dogs.

I had maintained contact with public health staff since I was in Mexico in 1944. We had discussed joint field training, but it was not until 1960 when the annual meeting was held at Hermosillo in Sonora State that we were able to hold a field demonstration. We had an excellent team from California: Dr. Reeves from the School of Public Health, a top-notch medical entomologist; Mr. Keith Murray (zoology) of the health department vector control, who was a real good medical zoologist; two veterinarians from the staff, Dr. Orland Soave and Dr. George Humphrey; and myself as physician and virologist. From Mexico, Dr. Carlos Ortiz Mariotte, physician and chief of epidemiology, Dr. Julio de Mucha Macias, virology, Dr. Luis Vargas, entomology, and Dr. Raul Silva, DVM, were the main participants.

I brought dry ice, stainless steel thermoses, the field equipment for netting birds and bats, traps for getting small mammals, and even lard-can traps, which had screen funnels at each end and [could be] baited with chickens for collecting mosquitoes. That was Dr. Reeves' major interest. So in a period of a little over a week we did an intensive survey of arthropods--mosquitoes, fleas, ticks. We bled large numbers of horses, chickens, and people. We collected mosquitoes in areas that had all the earmarks of being a good place for amplifying the virus.

Johnson: One was a place where they raised chickens for eggs, a hatchery, and we trapped the birds which were there. We collected mosquitoes there for testing. But it was early in the spring, not the season of virus activity. So we didn't get any virus out of the mosquitoes, which were tested properly, but we did learn a lot from the serological survey. We knew pretty much by age groups which had had infection with western and St. Louis encephalitis and dengue. Some had antibodies to dengue, which had been an epidemic there some years previously.

As a special demonstration, I put up bird nets in the compound of the health department. It was made up of old, Spanish-type buildings, and there was a colony of freetail bats in the roof. In the morning, I had five freetail bats. From the salivary glands of those bats, we isolated Rio Bravo virus, M687.

It was an opportunity to get close association with this wonderful team of scientists from Mexico, and we just had an absolutely marvelous time together, intensively trapping and observing. Dr. Carlos Ortiz Mariotte, who was on my medical team, was so thrilled to learn how to use flags to collect ticks. One of their top entomologists was Dr. Miguel Gonzalez Mora, who was doing the Aedes egypti control work. It was a very good way for establishing friendship between the two countries. I'm sorry that it was never done again, but we had good cooperation for many years, as encephalitis was a major problem.

Many of their young men later were trained in virology at the Rockefeller labs. Julio de Mucha was trained at the Rockefeller lab at Yale. He now is one of their top virologists. Others received similar training, so it was a means for getting special training in virology and cooperative work in immunology. So that was the 1960 ecological survey in Sonora State.

1966, I mentioned, was a major study in the Pacific, and '67 was a major work in South America. I was asked by PAHO to go to Argentina and see if I could help them in the study of vampire bat rabies, which was epidemic in cattle in northwest Argentina. I collected a large number of bats, which were all negative for rabies in the salivary glands, which proved they were not carriers, because they were collected where the disease had been active. I found skeletal remains and carcasses of vampire bats, indicating a high mortality in these animals. I believe they die of rabies and do not survive to be carriers.

There has been relatively little work done since, except one small study in 1977 in the Misiones area of Argentina, in which the PAHO team happened to be studying vampire bats in an area where cattle rabies was just breaking out. They isolated rabies virus from vampire bats and showed that some of the cattle had developed antibodies to the virus.

Turlock Virus

Hughes: Well, should we talk about Turlock virus?

Johnson: During the 1954 Culex tarsalis surveillance study by the Viral and Rickettsial Disease Laboratory, forty-two strains of an unidentified virus were isolated.* Additional strains of the same type were isolated in the 1955 surveillance study and also in the Rockefeller Foundation arbovirus section. One of the 1954 isolates (781-19 San Joaquin 1954) from the California State Culex tarsalis Surveillance was sent to the Rockefeller Foundation virus lab in New York, and Jordi Casals reported that it was a new virus.

During 1955 we isolated several strains of the same type of virus from my Bakersfield study area (AR42 deplete (74) Hart Park July 2-3, and AR43 blooded (39) of the same date). My AR43 strain was found to be identical to the type strain 781-19, 1954 and also identical to a previously isolated virus (MP306 July 9, 1947, Turlock) from the 1947 USPHS surveillance study. Therefore, Dr. Max Theiler of the New York lab suggested calling it Turlock virus from the site of the first isolation.

In the Rockefeller Foundation arbovirus project, we isolated Turlock virus from a blood pool of sentinel chicks (C17, Rivermore Ranch) and a pool of horn flies, Siphora irritans, (AR56 (25)) collected at the same place, July 13, 1955. We isolated another strain of the Turlock virus from a house finch, Carpodacus mexicanus (B219, spleen-heart pool), collected at Stockdale Ranch, August 22, 1955. There were other isolates of Turlock virus from my mosquito collections, but our main contribution was the finding of this virus in a wildlife vertebrate host.

In 1956, two small mammals were sent in to the rabies diagnosis section. They were negative for Negri bodies but when tested in mice some of these sickened. But when the brain tissue was subpassaged in adult mice, they remained well. However, when the same material was passed in infant mice, they died. These specimens were gopher 540 LA6-0597 of April 6, 1956, from Los Angeles, and Citellus beecheyi 6-596T of April 13, 1956, Alameda County. I visited the place where the Alameda County specimen had come from and interviewed the boy that found the sick squirrel at Niles, California. I believe that these two animals were infected with Turlock virus and should be considered natural vertebrate

* E. H. Lennette. Proc. CA Mosquito Control Assn., 22nd Annual Conf., Dec. 7, 1955.

Johnson: hosts of this virus. Since that time, Turlock virus has been found commonly in surveillance studies of mosquitoes but does not seem to produce disease in man. It is a Bunya group virus.

I inoculated two calves with Turlock virus, but neither developed a viremia. We bled several calves, and these two were negative for antibodies by the serum virus neutralization test. However, others were positive, and the ones we tested may have had some residual antibody from the colostrum milk of the mother cow. I have suggested that someone who has access to colostrum-deprived or preferably Caesarian-delivered calves test one of these in the same manner. This would provide better information on susceptibility.

To get back to the Turlock virus: When we isolated this virus, it was true that adult mice seemed not to be susceptible to it. But if the adult mice were inoculated intracerebrally with more dilute suspensions of infected brain, some would sicken and die. In a way, this explains the production of disease with the field specimen which would contain less virus.

That led me to a genetic study of the Turlock virus, which was published in abstract form at the Federated Societies meeting at Atlantic City.* There were two ways to adapt the virus to adult mice, one by passing Turlock virus at the 10⁻³ dilution, the other by cloning and testing ten different clones for pathogenicity. In three passages, we were able to select a population of virus particles that would consistently kill adult mice as well as the baby mice, showing that there is variability in a population of virus particles.

This has the same explanation genetically as that there are no two people alike. There are no two spotted skunks that are alike. When you get test bacteria, we find there are always variants--big colonies, small colonies--and that these [differences] are built in so that even if you test a colony from one bacterium, you can still demonstrate differences. I found out with both western [encephalitis] and Turlock virus that by plaquing and testing from a single virus particle, you can demonstrate all sorts of differences. You can demonstrate differences in the incubation period, the pathogenicity, and ability to kill by different routes of inoculation. So having demonstrated that we could select a western strain which was almost nonpathogenic for primates; with Turlock, we could select a variant that is not pathogenic for adult mice and use this to immunize mice by intracerebral inoculation. This is valuable for cross immunity tests.

* H. N. Johnson, J. L. Hardy, R. W. Ross. Selection of pathogenic and nonpathogenic variants of Turlock virus. Federation Proceedings 1968, 27:425.

Johnson: I believe that was why the [virus in the] early vaccines, like yellow fever vaccine, where we passed the virus in chick embryo cultures, was not really a mutation but selecting out a phenotypic population which didn't happen to be pathogenic. The low virulence strains of rabies and poliomyelitis were obtained in a similar manner. This was selection rather than mutation.

This phenomenon is probably best illustrated by the three vaccine strains of poliomyelitis virus. You had to be very careful what temperature you used to grow the tissue cultures. A higher temperature would tend to select pathogenic strains. In yellow fever it could have been the temperature of the incubators--occasional low titer rather than a mutation. Most of the work on viruses has shown that there is a heterogenous population of virus particles. In Colorado tick fever and dengue too, there is a relapse and recurrent fever. The virus particles that grow out first will not immunize against the minority of the virus population, so they will increase as immunity slows the growth of the virus particles that grew out first.

Another example of that is relapsing fever, which is a Borellia infection. People who have been bitten by infected ticks get a fever like malaria and get over it, and all of a sudden they get it again, and then they might have a third relapse. It shows that you are screening out a population of organisms before you get immune to the entire population.

Hart Park Virus

Hughes: We haven't discussed the Hart Park virus.

Johnson: The Hart Park virus was isolated from AR70, a pool of Culex tarsalis mosquitoes collected at Hart Park, Kern County, August 5, 1955.*

Hart Park was a recreational park just east of Bakersfield. It was a little ecological jungle. It had beautiful trees, lots of birds, lots of bats. It turned out to be a good place to get western and St. Louis virus from mosquitoes, probably because migratory birds roosted there, and they would bring in the virus and infect the mosquitoes that fed on them. This means that if a bird happens to be infected with encephalitis virus and spends one

* H. N. Johnson. Diseases derived from wildlife. California's Health 1965, 23:35-39. T. O. Berge. International Catalogue of Arboviruses, second ed. Hart Park, AR70, p. 302.

Johnson: night at Hart Park, it can infect one hundred mosquitoes, which in turn lay eggs and feed again, because there's an intrinsic period of virus incubation of about ten days. By ten days after the mosquito took that meal, it could infect some more hosts, at least one other host.

Besides getting western and St. Louis virus from Hart Park from mosquitoes, I isolated the Hart Park virus which I could not adapt to mice except by passing it in dilute solution. It was not pathogenic for adult mice, but baby mice got sick from the first passage. I made a ten percent suspension of the brain tissue and injected other baby mice. They didn't get sick. If I passed it at 10^{-3} dilution, they'd get sick. By a series of passages, we adapted the strain so it would consistently kill baby mice. That was the strain that we supplied to other labs and to our central lab. It was tested at the Yale laboratories against other viruses, and it was not related to previously isolated viruses.* It was listed as a nongrouped virus. It is a rhabdovirus, like the Kern Canyon and Klamath viruses we isolated from small mammals.

The reason we didn't worry much about its pathogenicity was that it didn't grow in the human HeLa cell cultures. It seemed to be a disease of birds only. Baby chicks got infected but showed no illness. In virus surveys where we put out sentinel chickens, they didn't develop antibodies to it either. So I was not too excited about following it up, except that it is interesting because it is a rhabdovirus transmitted by Culex tarsalis mosquitoes.

Dr. Loring Whitman of the Rockefeller in New York City inoculated mosquitoes with the virus and showed that it grew readily in the mosquito. He could pass it from mosquito to mosquito. So it is an arthropod-borne virus. I've isolated it from yellow-headed blackbirds, tricolor blackbirds, and sparrow nestlings. These were chosen for testing because they appeared to be sick, as compared to their nestmates. There is no evidence that it infects poultry, whereas if the influenza A virus, which is related to the fowl plague virus of Europe, gets into turkey flocks, they quit laying eggs. That is an economic problem. So Hart Park now is a virus curiosity.

* J. Casals. Procedures for identification of arthropod-borne viruses. Bull. World Health Org. 1961, 24:723-734.

Johnson: There is another strain called Flanders virus,* which was isolated in New York state in 1961. It's now called the Hart Park group of viruses, after the first isolate, which was obtained here in California. There are many isolates of that virus. Every year in the surveys in Texas and elsewhere in the United States there are many isolates of Hart Park virus from mosquitoes. I believe that it is pathogenic for wild birds. It should be tested in ducklings.

The Legacy of Biological Research in the United States**

Johnson: Each of us that had the opportunity of working on the staff of the International Health Division of the Rockefeller Foundation realizes the unique opportunity this organization offered for the study of the natural history of infectious diseases. From the beginning, it was necessary to develop field laboratories to check on the effectiveness of the methods available for the control of diseases. This was the beginning of local public health laboratories and clinics, the early projects on hookworm, malaria, and yellow fever. Later it was possible to study the natural history of other disease agents, including those with reservoirs in wildlife.

* E. Whitney. Amer. J. Trop. Med. Hyg. 1964, 13:123-131.

** An addendum written by Dr. Johnson in April, 1989, on "the special experience I had working for thirty-four years for the IHD of the Rockefeller Foundation." (personal correspondence, April 14, 1989.)



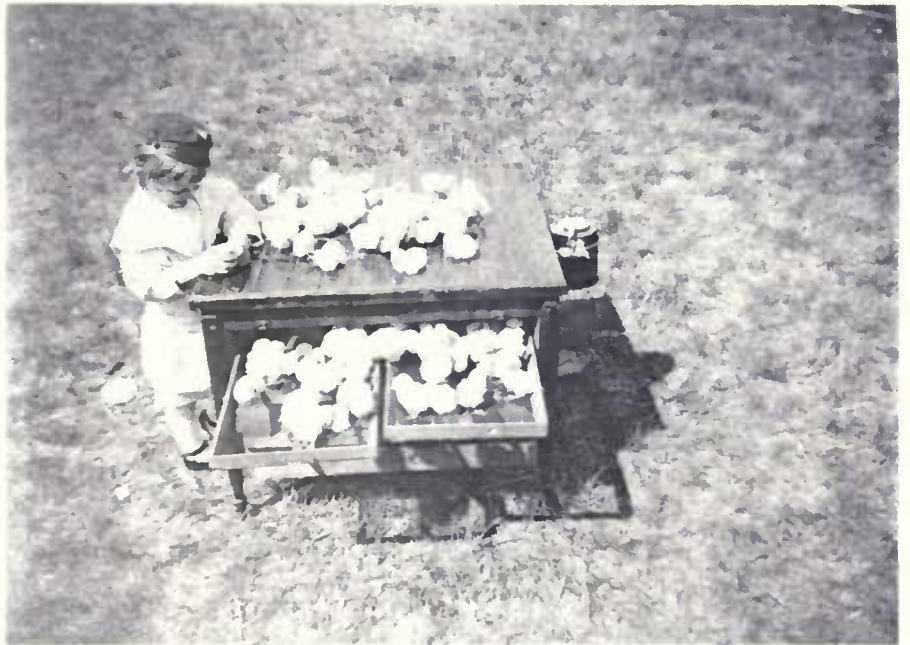
Above: The Gust J. Johnson homestead, Loomis, Nebraska, 1898.

Below: The Johnson family, 1917. Front: Gust, Manny Amalia, Harald. Back: Reuben, Philip, Linnea, Joseph, Paul.





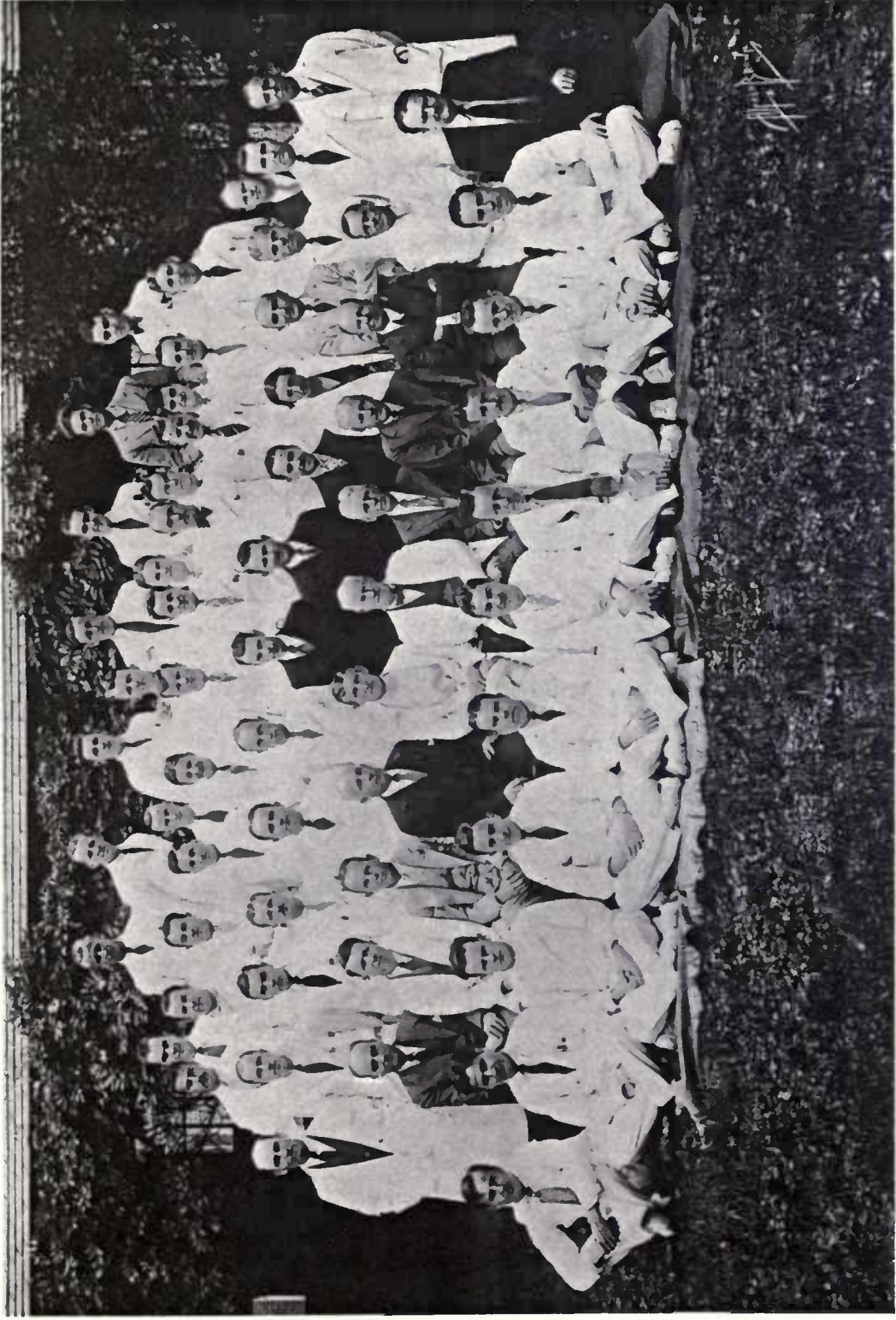
Harald, one year old,
1908.



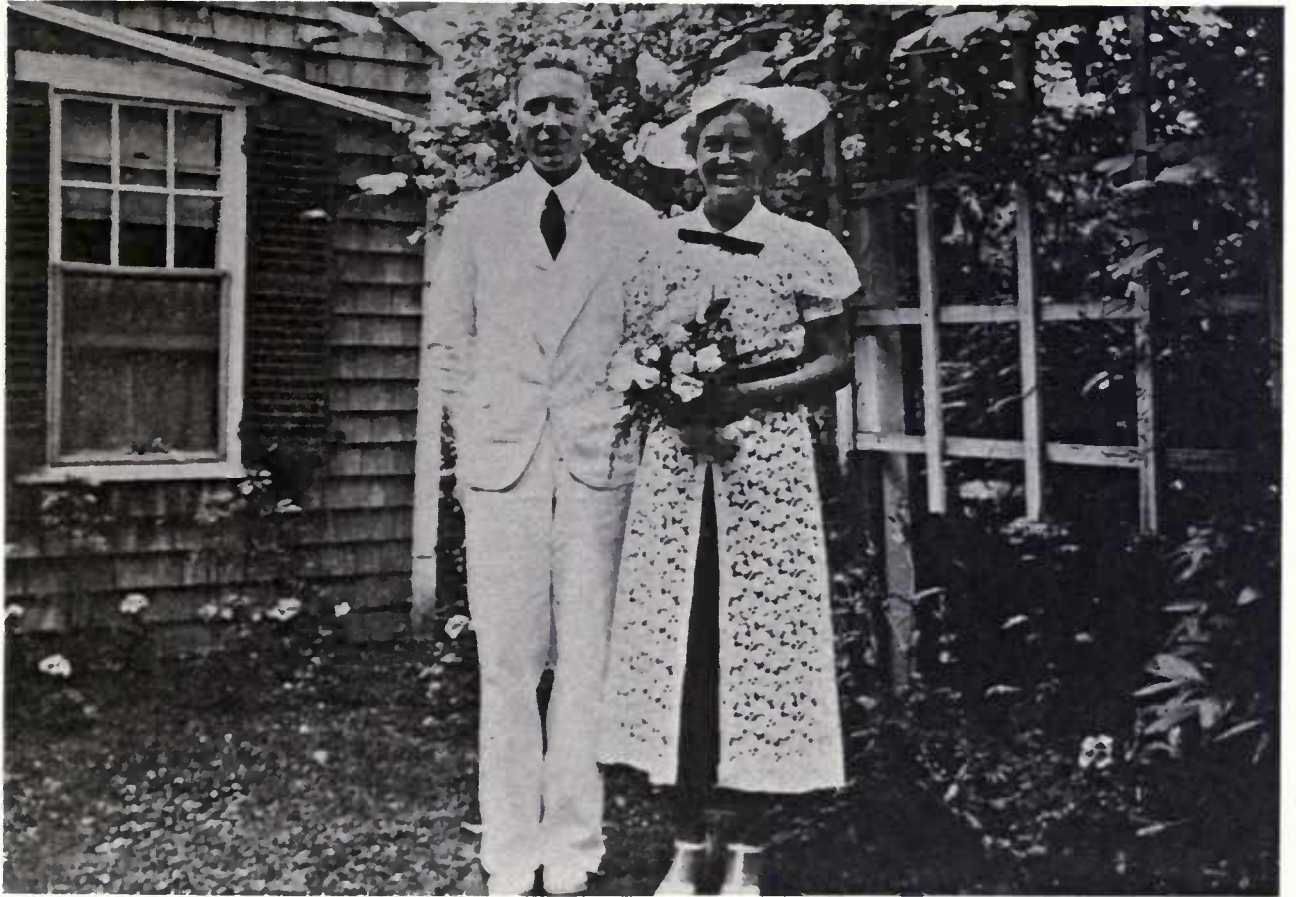
Harald and chicks hatched in kerosene-heated incubator,
1911.



Harald N. Johnson, 1927, at farm home of Alfred and (sister) Linnea Mattson, Phelps County, Nebraska.



Medical, Surgical, and Pathology Staff, Peter Bent Brigham Hospital, Boston, Massachusetts, 1934.
Harald N. Johnson, last on right, fourth row. Second row, seated: fourth from left, S. B. Wolbach,
Prof. Pathology; fifth from left, E. Cutler, Prof. Surgery; sixth from left, H. Christian, Prof.
Medicine.



Harald and Frances Johnson on their wedding day, June 27, 1936, at Mrs. Johnson's family home, Scituate, Massachusetts.



1938 staff, rabies study, Alabama State Health Department and the Rockefeller Foundation International Health Division. Left to right: Harald N. Johnson, scientific director; Charles N. Leach, administrator; Rachel Gorrie, technician; Mary Sue Moore, secretary and lab assistant; Reginald Reagan, technician.



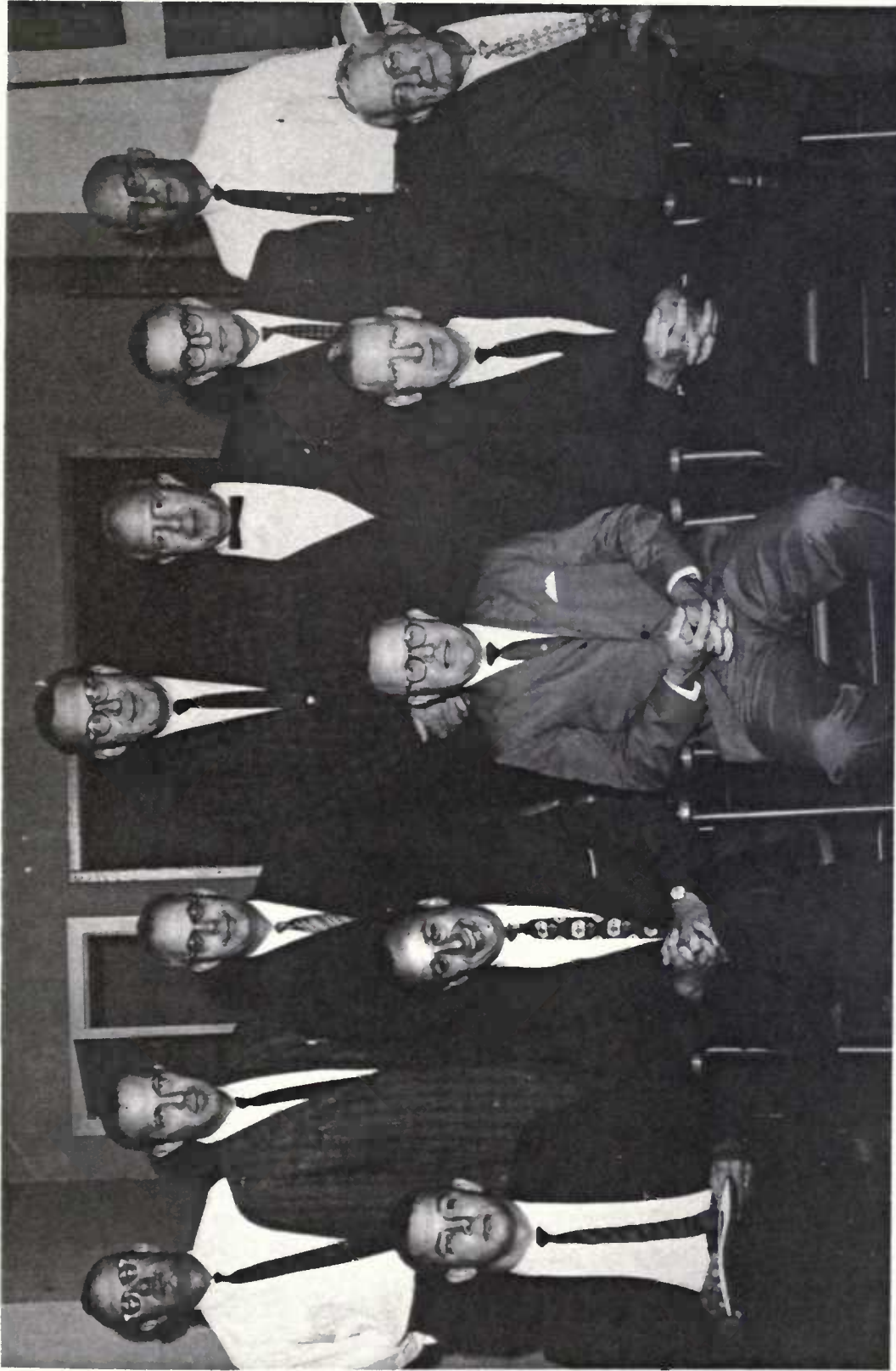
Harald N. Johnson taking a blood specimen from a cow.



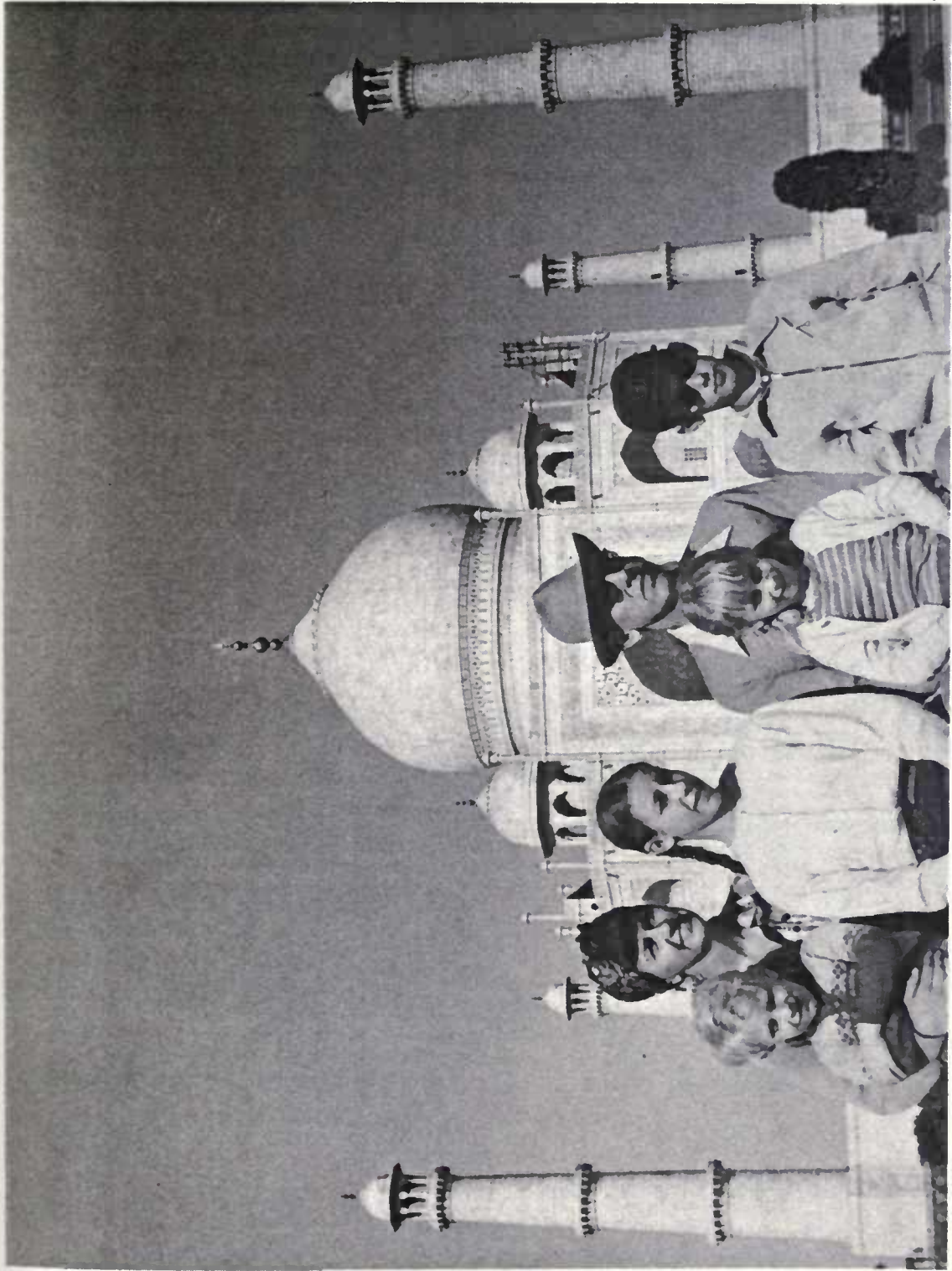
The Derriengue Study, Mexico, 1944.
Harald N. Johnson with a vampire bat.



Recuperating at Warm Springs Foundation, Georgia, December 1944.



CDC Rabies Advisory Committee, 1949. Front: W. G. Winkler, E. S. Tierkel, R. K. Sikes, H. N. Johnson, T. F. Sellers. Back: R. L. Parker, R. E. Kissling, D. Dean, J. H. Steele, J. P. Fox, K. Habel, R. Courter.



The Johnson family at the Taj Majal, 1952. Front: Michael, Marion, Susan, John.



11/9/52 piano
leaving our home

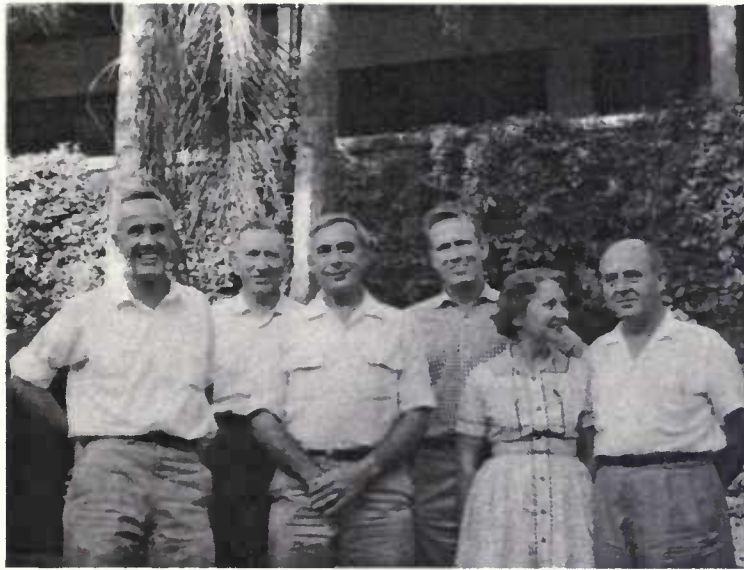
Our piano leaving the bungalow for use at a concert by Richard Farrell,
November 9, 1952.



Panther shoot, Dehu
District, India, March 28,
1953. Harald N. Johnson,
back row, second from
right. Panther had
attacked animals and
people, a hunting tribe
had located the panther
using dogs but had
only bow and arrow and
spears.



Farewell party for the Johnson family, Virus Research Center, Poona, India, February 1954.



Thomas Aitken, Harald N. Johnson, Harold Trapido,
Robert Kokernot, Calista and Ottis Causey,
Bakersfield, California, July 1958.



The Acton Street Laboratory, California Department of Public Health, 1955.



Frances and Harald Johnson, 1958, at home of Dr. and Mrs. E. H. Lennette.

Photograph by David Lennette



WHO Expert Panel on Rabies in former dining room of Louis Pasteur, Pasteur Institute, Paris, May 11, 1961. Front: E. Tierkel, M. Kaplan, P. Lepine, H. N. Johnson. Back: K. Yoshino, R. Schindler, H. Koprowski, K. Habel, D. Dean, ? (not on panel), P. Atanasiu.



Harald N. Johnson showing nest site where he collected ticks, Upolu Cay, Great Barrier Reef, Australia, July 1966. Note recently hatched sooty terns.



Harald N. Johnson collecting vampire bats near Salta, Argentina, 1967. Spaces left at the top of the concrete on both sides led into cave-like chambers. After the nets were set, the bats were driven out by insecticide fumes.

Subcommittee on Rabies, National Research Council [NRC]

[Interview 9: February 12, 1988]

Hughes: Dr. Johnson, I know you were on the National Research Council Subcommittee on Rabies. Could you tell me how that appointment came about?

Johnson: The National Research Council was concerned about the problem of rabies. In June, 1945, Dr. Robert F. Griggs, chairman, Division of Biology and Agriculture, wrote to me asking whether I would accept the chairmanship of a subcommittee on rabies to draw up a plan for the control of rabies in the United States. The other members selected for this committee were Dr. H.W. Schoening, the Bureau of Animal Industry, USDA; Dr. A.L. Brueckner, director, Livestock Sanitary Service, State of Maryland; Dr. R.A. Kelser, dean, School of Veterinary Medicine, University of Pennsylvania; Dr. Karl Habel, the Division of Infectious Disease, NIH; and Dr. T.F. Sellers, director of laboratories, Georgia Department of Health.

Dr. Habel had written to me previously about plans to develop a rabies control program in the USPHS [United States Public Health Service] to be administered through the state health departments. So I wrote to Dr. James H. Steele, USPHS, through Dr. Charles Armstrong, chief, Division of Infectious Diseases, NIH, requesting that Dr. Steele attend the morning session of the meeting and tell the committee about the proposed program of the Veterinary Public Health Division of the USPHS. The meeting of the subcommittee on rabies of the NRC was held in November, 1945, at the NRC building in Washington, D.C. The report of the committee was published in the Journal of the American Veterinary Medicine Association,* and also as a NRC reprint and circular series printing no. 126 in May, 1946.

In January, 1946, Dr. Seward E. Miller of the USPHS visited the rabies study of the Rockefeller Foundation at Montgomery, Alabama in response to my letter suggesting that they take over this facility to continue the studies of rabies vaccines in dogs, and use this as a viral diagnostic laboratory. We had prepared antigens and antisera for EE, WE, and VE encephalitis viruses, there was an animal unit producing white laboratory mice, and there were ample cage units for dogs. The USPHS immediately began arrangements with the Alabama State Health Department to take over this laboratory, and it became the regional Communicable Disease Center (CDC).

*1946, 108:293.

Johnson: Dr. E.S. Tierkel was assigned to direct the rabies vaccine studies. Dr. Beatrice F. Howitt, previously employed at the Hooper Foundation in San Francisco, was in charge of the virus laboratory, assisted by Rachel Gorrie, the senior technician at the Rabies Study. Dr. Roy W. Chamberlain was assigned to the Montgomery CDC laboratory as chief of the entomology section. Dr. Robert E. Kissling was in charge of the veterinary vaccine studies when Dr. Tierkel was absent. Later Dr. R.E. Sikes took over the dog vaccine studies. This was a long-term study, and a special animal facility was later built at Lawrenceville, Georgia, when the CDC virus project was moved to Atlanta in 1960. The preliminary studies of the Flury LEP [low egg passage] avianized rabies vaccine virus in dogs were done at the Montgomery CDC lab.*

In July, 1946, I took part in a conference at the Bureau of Animal Industry, USDA, called by Dr. B.T. Simms, to discuss production and testing of rabies vaccines. By that time we had published the results of the one-year duration of immunity following a single subcutaneous injection of the Semple type phenol-treated rabies vaccine. We also reported the results of tests of the Kelser type chloroform-treated rabies vaccine. This was a potent rabies vaccine, even after storage in a refrigerator for sixteen months.**

In April, 1947, I attended the session of the committee on rabies of the AVMA [American Veterinary Medicine Association] held at the University of Pennsylvania in Philadelphia. The AVMA adopted the NRC committee report and this secured the cooperation of the veterinary profession which was essential for the success of the rabies control program. It was Dr. James H. Steele, chief of the Veterinary Public Health Division, CDC, who organized the rabies control program on a national basis and assigned public health veterinarians to assist in these programs.

I had been appointed a special consultant to the Veterinary Public Health Division, CDC, in December, 1948. In June, 1949, there was a conference at the Georgia State Health Department, where the CDC rabies laboratory and the Lederle Laboratories presented their experimental and field studies of the Flury LEP avianized rabies virus vaccine. Following this meeting, I visited the CDC laboratories at Montgomery, Alabama.

* E.S. Tierkel, H. Koprowski, J. Black, R.H. Gorrie. Am. J. Vet. Res. 1949, 10:361.

**H.N. Johnson. Experimental and field studies of canine rabies vaccination. Proc. Forty-Ninth Ann. Meeting, U.S. Livestock Sanitary Assn., December, 1945, 99-107.

Johnson: The first large-scale field test of the Lederle Flury strain rabies vaccine had been carried out on Staten Island, New York City, from July to September, 1949. Lederle Laboratories furnished the vaccine free of charge and over 6,000 dogs were vaccinated by im [intramuscular] injection of the vaccine in the hind leg. This study was done under the supervision of Dr. Ralph Muckenfuss, Dr. Morris Greenberg, and Dr. Hubert Baum of the New York City Health Department. There were no vaccine-related deaths and no more cases of rabies on Staten Island. Further studies were done elsewhere in New York state under the direction of Dr. Alexander Zeisigg, state veterinarian. At that time there was an outbreak of fox rabies in New York state.

At about the same time, another field trial was in progress in Georgia, directed by Dr. L.E. Starr, state veterinarian. This study included vaccination of more than 100 cattle in a focus of fox rabies. This provided the evidence that the vaccine was safe. The three-year duration of immunity study following immunization with the Flury vaccine was published in 1953.* The evidence that this vaccine would provide complete protection against challenge with dog salivary gland rabies virus for a period of three years provided the information needed for prompt use of this vaccine virus for the control of endemic dog rabies.

The CDC Rabies Advisory Committee continued to meet regularly, but I could not attend in 1952 and 1953 because I was in India. When I was assigned to the California State Health Department in June, 1954, my primary responsibility was to direct the field studies of the natural history of the arthropod-borne viruses, but I was asked to serve on the Rabies Advisory Committee of the state of California. I attended the meetings of the CDC Rabies Advisory Committee each year between 1964 and 1969. Endemic canine rabies was eliminated in 1967. Small outbreaks have occurred along the Mexican border but these have been controlled quickly.

There was a meeting in 1947 at the New York Academy of Sciences at which I presented a paper on rabies control. It was published in the Annals.** That was where I met Martin Kaplan, DVM, who became head of the veterinary section of the WHO. Martin had obtained a master's degree in public health. So these were [steps] leading up to my first connection with the WHO in 1950.

*E.S. Tierkel, R.E. Kissling, M. Edison, K. Habel. Proc. 90th Ann. Meeting, Amer. Vet. Med. Assn., 1953, 443-446.

**Rabies. Ann. N.Y. Acad. Sci. 1947, 48:361-383.

Expert Panel on Rabies, World Health Organization,
1950-1986

Johnson: The idea was to get world control [of rabies]. The United States was not just interested to control rabies in the United States, but we [also wanted to know] what to do with dogs being brought into the country from overseas. Almost every year some dog coming in from another country would come down with rabies. England was very strict. One of the most important needs was to have some means of [screening dogs coming] into the country for rabies.

The new development that led to the WHO involvement in rabies control worldwide was the availability of the Flury live virus rabies vaccine. It was evident that it was a very excellent antigen that would protect with one injection for at least one year.*

Hughes: Why was it important to use the salivary gland virus?

Johnson: Well, I mentioned previously that when we did the mouse potency tests, the Habel-type tests, if we gave more than 10,000 MLD of the virus intracerebrally into an animal, it produced a booster effect, with a less than fifty percent mortality; whereas when we gave the intermediate doses of 1000 and 100 MLD, the mortality rose to greater than fifty percent, then dropped off to zero at less than 1 MLD. So then we wanted to challenge the dogs with something approaching natural-bite exposure. When the dog bites, the virus is from the saliva and not from the brain.

When I began working on rabies, all the leading virologists said that rabies did not occur in any of the organs except the brain. It entered through the nerves and then it would get to the brain. One of the first things we did when we started studying rabies was to test all the different organs in dogs naturally infected with rabies, and when they died, we'd test the various organs. To my surprise, the salivary gland often contained more virus than the brain. The brain would have a titer of 10^{-3} and the salivary gland 10^{-4} , or something like that.

When I told Dr. Goodpasture, who had studied rabies, he said, "Oh, it just leaks out of the neurons." [laughs] I said, "Well, that looks far afield when the brain itself has less virus." Then we found the virus in the pancreas and the kidney and occasionally in the lung. So rabies virus had tissue tropism for tissues other than the nervous system.

*See reference, Tierkel et al., p. 281.

Johnson: When I came to California and studied the skunk and bat strains [of rabies virus] we found that these strains of virus had a high tropism for the lungs. And that's evidence of natural selection. Rabies virus, to succeed in a dog, had to select for making the dog mad so it would become vicious and bite. Number two, it had to be in the salivary gland rather than the lung, and so that was why I say rabies is an aberrant disease in dogs. Yet most virologists say rabies' natural reservoir is the fox and the coyote and the dog. Well, it looks that way, but these are aberrant hosts.

To get back to the WHO: Dr. Martin Kaplan had been appointed chief of the Veterinary Public Health Division of Communicable Diseases of WHO at Geneva, Switzerland. In 1950, Dr. C.R. Klimt of the WHO invited me to serve on the committee on rabies. The first meeting was held at Geneva in 1950.* All the committee members had been working on rabies. Karl Habel, Hilary Koprowski, and I were the American members. We wanted to test the Flury live virus vaccine in other countries, especially in places where there were many cases of dog rabies.

One of the obvious places at the time was Israel, where there was an outbreak of jackal and dog rabies. There was an excellent veterinary profession and we could be certain that every dog that died would be examined. They also had a vaccine they'd developed themselves that they wanted to test. But the major test would be of the Flury avianized live virus vaccine. The other field studies were done in Malaya and Rhodesia (now Zimbabwe). There was a big dog rabies problem in Malaya. Dr. C.W. Wells, the public health veterinarian, was in charge of that study. Dr. J.S. Adamson was in charge of the study in Rhodesia, where they were having rabies in jackals and dogs.

The next meeting of the WHO Expert Panel on Rabies was held in Rome in September, 1953, in conjunction with the International Congress on Microbiology. I presented a paper entitled, "Experimental studies on the duration of immunity in dogs vaccinated against rabies."** In the same publication were the papers by C.W. Wells of Malaya, J.S. Adamson of Rhodesia, and M.M. Kaplan, Y. Goor, and E.S. Tierkel, summarizing the field studies in Israel. These studies showed that this was a very good method of rabies control, and there was no evidence of vaccine rabies in the dogs. The Expert Panel on Rabies functioned at its peak up through 1969.

*H.N. Johnson coauthor as member of Expert Committee on Rabies. Report of the First Session. World Health Organization, Technical Report Series, 1950, 28, 1-26.

**Bull. WHO 1954, 10:725-729.

Johnson: We prepared a book on techniques in rabies.* The panel issued three editions in 1954, 1966, and 1973. They were made available to all countries, so they would get more uniform results in diagnoses and checking vaccines. But the real value of a disease control program is if it works, it works pretty fast.

Hughes: How were people chosen for the panels?

Johnson: Well, first of all, there were certain political [considerations] in covering the various countries. Dr. Pierre Lepine, director of the Pasteur Institute of Paris, was a key person. Another key person in the studies was Dr. M. Baltazard, director of the Pasteur Institute in Tehran, Iran. It was in Iran that the early studies on immune serum and vaccine treatment in man were done after wolf bite exposure, which had been almost 100 percent fatal.** Dr. N. Veeraraghavan, director of the Pasteur Institute at Coonoor, India, was an important figure in the studies of the human vaccine treatment.***

The big problem was how do you get good vaccine for immunization in man. Again, there was the same story: A lot of vaccines were probably of no value at all, and certain ones were dangerous because they had too much live virus. So you brought in a problem and then dealt with it on an international basis.

The best example I can give you is the yellow fever panels. Once the Rockefeller Foundation got the 17D yellow fever vaccine by working through the World Health Organization Expert Panel on Yellow Fever, there was a uniform immunization program. [It ensures] that people are immunized in the yellow fever endemic zones so they won't start an epidemic of yellow fever if they move elsewhere in the country or overseas. Another good example was polio vaccine where, through WHO, we got the Sabin live virus polio vaccine approved promptly, and this vaccine was used in every country in the world where there was polio. It was unbelievable how by the 1970s polio was controlled. But the virus is still present around the world. The expert panels were a good way of developing control of infectious disease around the world.

If you're dealing with worldwide diseases, you need international panels on bubonic plague, smallpox, influenza, and cholera. WHO has programs on each of these diseases. The new one is the retrovirus disease (AIDS) caused by the human immunodeficiency virus type 1 (HIV-1). The WHO panel on smallpox was instrumental in coordinating the elimination of smallpox.

*Laboratory Techniques in Rabies, WHO Monograph Series, 1954.

**M. Baltazard, M. Bahmanyar. Bull. WHO 1955, 13:747.

***N. Veeraraghavan. Bull. WHO 1954, 10:789.

Johnson: I was on the WHO panel on rabies through 1986. The major meetings I attended after 1953 were held in 1961, 1965, and 1969. Arrangements had been made to hold the 1961 meeting in Moscow, but a few weeks prior to the meeting, Dr. Kaplan was informed that they had withdrawn the invitation because the place they were to hold it was not ready. Dr. Lepine invited Dr. Kaplan to hold the meeting in Paris. Dr. Kaplan suggested I might go to the Soviet Union afterwards as a consultant. Dr. Selimov of the USSR was supposed to attend the meeting in Paris but was unable to come. It was wonderful to have the meeting at the Pasteur Institute in Paris. I was particularly interested in doing library research there.

The development of the program of cooperation with the Soviet Union came out of the meetings of the International Congress of Tropical Medicine and Malaria held in 1958 in Lisbon, Portugal. That was the first time a delegation of doctors came from the Soviet Union. Drs. M.P. Chumakov and A.A. Smorodintsev were there. Dr. Robert Morison of the Rockefeller Foundation arranged a dinner where they were invited and I was chairman. I asked them to tell their history of the early research on Russian spring summer encephalitis. Chumakov was a member of the field expedition to Siberia in 1937.

Dr. Chumakov was one of the three doctors that developed encephalitis at Khrebet Sikote Alin, Siberia, in 1937. Pomeransov died; Chumakov recovered but was left with residual paralysis of his right arm and severe deafness; Kurinsov recovered completely. Chumakov told about the early studies of RSSE [Russian spring summer encephalitis] and the isolation of the virus from brain specimens by Drs. L.A. Silber and V.D. Soloviev.

Dr. Smorodintsev was arrested in 1937. I do not know the charges. He got at odds with E.M. Pavlovski and P.A. Petrisceva of the Gamaleya Institute. The virologists wouldn't work with them after that, so virology became separate from parasitology.

I asked Smorodintsev, who knew English, to speak about the epidemic of Japanese encephalitis which occurred in Vladivostok in the fifties. We Americans had little information about this epidemic. He told us that they had identified Aedes togoi as the vector. This mosquito was breeding in coastal rock pools where cormorants were roosting. All they needed to do to control the disease was to eliminate the breeding of Aedes togoi. They have had only a few cases of Japanese encephalitis since that time. This was a most interesting way to exchange information and this led to the Arthropod-borne Virus Information Exchange.

When I returned home after this meeting I began attending an evening class in Russian and continued this for three years. This made it possible to travel independently in the USSR.

Johnson: After the Paris meeting in 1961, I went on to Moscow and visited the newly built institute for poliomyelitis and encephalitis. Dr. Chumakov arranged for me to meet Dr. M. Selimov and other members of the staff in the rabies section. So that was a key meeting to bring the Soviet Union into the international WHO work.

The meetings of the WHO rabies committee were held at Geneva in 1965 and 1969. Dr. Selimov invited me to visit several regional rabies laboratories in the USSR in 1969 before I took part in the international symposium on the role of migratory birds in the transmission of arthropod-borne viruses. This meeting was held at the Siberian Science Center near Novosibirsk, July 19-28. I visited Moscow, Kiev, Tbilisi, Baku, Ashkabad, Tashkent, Samarkand, and Alma-Ata before the meeting at Science City. On the return trip, I stopped in Moscow and Leningrad. My next stop was in Helsinki where I visited the arthropod-borne virus laboratory at the university.

Some of the leading Russian virologists have been trained in this country. The depository of arthropod-borne viruses is now at the Yale University epidemiology department lab (YARU). The building was built with Rockefeller funds in 1964, and that has been a training center for virologists from all over the world. Dr. Richard E. Shope is now director. Some of the Russian virologists have been trained at the California State Department of Public Health. Dr. Sergei T. Drozdov, now director of the poliomyelitis and encephalitis institute in Moscow, was at the Berkeley lab in 1962 and 1968. I took him on a field trip to Bakersfield in 1962. He was at our home in Berkeley in 1968 and I remember him helping my daughter Susan with her Russian lesson.

Dr. Sophia Gaidamovich of the Ivanovski Institute of Virology in Moscow had an extended period of training at the California Health Department virus lab under the direction of Dr. Edwin Lennette, in 1968, and Dr. Lydia Fadyeva in 1976. Dr. Michael Chumakov visited the Berkeley lab in 1961 and Dr. Anatol Smorodintsev in 1964. It was in 1964 that Dr. Smorodintsev played the piano at my home in Berkeley and I learned he had received a good education in classical piano before the revolution in Russia in 1917. Dr. D. Lvov and Dr. S. Klimenko of the Institute of Virology in Moscow were in Berkeley in 1970 and I took them on a field trip to Modoc County where we were studying Colorado tick fever.

The epizootic of bat rabies that began in the United States in 1954 raised the question of a carrier state of rabies virus in vampire bats. This had been reported by J.L. Pawan in Trinidad in 1936.* I found no evidence for this in my study of vampire bat rabies in Mexico in 1944. The carrier state of rabies in vampire bats was accepted by the veterinary profession.

*Ann. Trop. Med. 1936, 30:401.

Johnson: In 1967 there was an outbreak of vampire bat rabies in northwest Argentina, and at the invitation of the WHO I took part in a rabies conference in Buenos Aires. After the conference I collected more than eighty vampire bats in areas where the disease was prevalent, and found no evidence of salivary gland infectivity in the bats that were alive. We found many that had died. I didn't bother with them. I just wanted to see if there were any carriers, and there weren't any.

Later, we went through the same story in the United States. I had isolated Rio Bravo virus, which is a group B [virus] from Mexican freetail bats in California, and I had notified Dr. Denny Constantine at the CDC lab in New Mexico to look out for this virus.

But in the meantime, some bats were sent to the Newton, Georgia, CDC lab. They tested the salivary glands of these bats and reported isolation of rabies virus from the salivary glands of bats whose brains were negative for rabies virus. The mice died like rabies, and they had inclusions and were called positive for rabies. That was what led to a period of time when it was assumed that there was a symptomless carrier state of rabies in insectivorous bats. But subsequent studies, particularly at the state health department in California, clearly demonstrated that there is no evidence of the carrier state in insectivorous bats.

The WHO virus panels have not been as active in recent years as previously, because there were no emergency situations. I would regularly report problems in rabies [when I was serving on the rabies panel]. For instance, in Thailand they had reported identification of rodent rabies. It became obvious to me that there were errors in their tests, and what they were doing was not boiling their instruments. They were getting the virus from rabid dogs on the instruments onto their rat specimens. Subsequent field tests failed to confirm the presence of rabies virus in the *Rattus* genus there.

The reports of rodent rabies in Czechoslovakia were not confirmed. I believe that they had lymphocytic choriomeningitis virus in their mouse colony, so they got mixtures of rabies and LCM. They don't talk about rodent rabies in Europe anymore; there probably wasn't any. Other scientists in Czechoslovakia studying tick-borne encephalitis tested large numbers of field rodents but did not report finding rabies virus. Another thing was the character of the rabies viruses used in research studies. Some of them had undoubtedly been hybridized with LCM virus.

The WHO expert panels are fine, but the selection of the staff members in the public health section is the key to success. Dr. Martin Kaplan was an excellent person as head of the veterinary public health division at WHO. He's still in Geneva and now associated with the Pugwash Conference program.

Hughes: Was the problem with the earlier killed rabies vaccines that they just were not as effective as the live?

Johnson: We found that some vaccines were actually contaminated with pathogenic bacteria, but the main problem was that some were not antigenic. It was a money-making biologic. [The biologics companies] didn't know their vaccines were no good. They'd inoculate calves, horses, or sheep intracerebrally with the Pasteur rabies virus and harvest the brains as a source of virus for making a killed virus vaccine.

Hughes: What was the USDA's rule?

Johnson: Well, the USDA's rule then was that these vaccines were supposed to pass the Habel test. The difference in the ic titer of the challenge virus in the mice that had been vaccinated and the controls indicated the antigenic value of the vaccine.

Several biologics firms ceased production after they were required to test each lot number of vaccine and they failed to pass the test. The major biologics firms were able to produce a satisfactory vaccine. The factors that were found to be important in producing a good vaccine were the strain of Pasteur rabbit fixed virus, the titer of the virus in the vaccine pulp, the pH, and the inactivating agent.

The use of phenol as an inactivating agent in the Semple type vaccine was a misnomer in that the phenol did not destroy the infectivity of the rabies virus when kept at ten degrees centigrade, but in the presence of 0.25% phenol the antigen was preserved on incubation at thirty-seven degrees centigrade. Beta propiolactone is the inactivating agent in the currently used killed virus vaccines for dogs.

More on Arboviruses

Hughes: Well, Dr. Johnson, I have a few more questions about arboviruses. You wrote about two epidemiological types of arthropod-borne virus diseases,* and one you called the silent or jungle type, which is maintained in nature in animals, birds, and arthropods. The other is the urban type, where man or domestic animals or both are affected. Was the original model for this yellow fever?

*Annual report for 1952 field program. [Typescript on deposit at the Bancroft Library.]

Johnson: Well, the yellow fever problem [of where the virus is maintained in nature] is still with us. As for the rest of the arboviruses, where do they survive in nature? Originally it was believed that yellow fever was maintained in man and mosquitoes (Aedes aegypti). Only in the late thirties and early forties, did it become evident that there was another host cycle in monkeys. They called it the sylvatic, the forest, infection. But even up to the present time it is believed that the long-term host is the monkey. But I think it's very similar to rabies, in that they expect the natural host to exhibit disease when inoculated with the virus. The monkeys of South America, such as the howler (Alouatta spp.) exhibit a high mortality when naturally infected. During the early studies of yellow fever in Africa, they tested the local monkeys, and they didn't seem to be susceptible, that is, when inoculated intramuscularly or intracutaneously they did not sicken and die.

Then they tested some rhesus monkeys from India, and that's what led in 1927 to getting one animal they could use as a test animal. In South America, the howler monkey was particularly susceptible. You would find a dead monkey, and it would be positive for yellow fever virus. Nobody seems to want to consider the possibility of another host to maintain the virus. My feeling is that there has to be another one. In South America I've visited all the foci of yellow fever and, in fact, in Africa as well, and it seems that monkeys are incidental or aberrant hosts of yellow fever.

Hughes: Why is there resistance to the idea that there is another yellow fever host?

Johnson: Well, for example, they say it's not the bat, because originally they had inoculated some bats in South America with yellow fever, and they didn't get sick. That's one thing. And then the technique they had for testing for virus in the blood was using adult mice, and some strains of mice are not susceptible to any strain of yellow fever injected into the brain as far as disease is concerned. The baby mouse is the most susceptible test animal for yellow fever virus.

My feeling on the basis of epidemiology is that fruit bats in both Africa and South America may be at least an amplifying host, like birds are for the encephalitis viruses in the United States. Everywhere there are outbreaks of yellow fever in South America, you usually find Carollia perspicillata, one of the fruit bats. It is common in all the endemic foci of yellow fever in South America. You wouldn't expect them to get sick, but like adult birds with western encephalitis virus, they may be infected. There is a nice lab in Belem, Brazil, and they catch Carollia bats in their animal traps on the ground, because they use bananas for bait. They seldom test them for evidence of viremia, using baby mice as test animals, and do not test organs, such as the salivary glands and kidneys, for virus.

Johnson: There have been similar instances as far as the American encephalitis viruses are concerned. I do not believe that birds are the natural host in the sense of reservoir hosts. They are a tremendous epidemic amplifying host, and the host that moves the virus.

This led to the meeting I went to at Science City in the Soviet Union in 1969, which was an international meeting on the role of birds in the movement of arthropod-borne viruses. We know birds come from far north to the tropics and vice versa every year. One reason I studied the wagtails in India, looking for arboviruses, was that they came in such large numbers during the first week in October. I got Sindbis virus out of them, which is a group A like western encephalitis virus. Look what's happened in the last ten years: They now find the same virus in Sweden, Norway, and the Soviet Union, where the white wagtail breeds. The virus is actually being carried from one marsh to another and is amplified on the way and then finally gets to India. So that gives you an idea that this is one way it can move.

We also learned in the Rockefeller studies in Trinidad and Belem, that western and eastern encephalitis have a different seasonal involvement in places like Jamaica. Eastern was there at one time, and it hits a certain part of Texas or Mexico, and you'll find it in Belem, Brazil, in December, and then you'll find another cycle later in Argentina.

Hughes: You can tie that into bird migration?

Johnson: Well, there is really no other explanation of how the viruses move, but those birds do move that way. Swallows from California go all the way to Argentina. We isolated western encephalitis virus from nestling barn swallows in California. They migrate to Argentina in the fall.

Hughes: But you say that birds are not the natural host?

Johnson: Well, it's like rabies. Some epidemiologists now believe, like I have believed for years, that canines are not the natural host, largely thanks to the studies on skunk rabies. With monoclonal antibodies, we can identify specific wildlife strains of rabies in skunks. We'll never, I guess, really understand the details of the wildlife cycle. I look for the animals found occasionally infected with the disease, like the spotted skunk and the weasel, from the far north in Alaska to Argentina, and where they never suffer an epidemic. The spotted skunk was called the phoby cat where I grew up on a ranch in Nebraska, the name recalling the fact they were known to cause hydrophobia, that is rabies, in man. There was no rabies in Nebraska when I was a child, but in 1964 a rabid spotted skunk was identified a few miles from where I was born. There was no dog rabies, but a striped skunk and some cows developed rabies there in 1964.

Johnson: In 1988, a woman living in Lake County, California, was bitten by a spotted skunk that was positive for rabies. It is the most recent spotted skunk rabies virus isolated in California. A woman in Kern County was bitten by a spotted skunk in 1954 while she was sleeping in a sleeping bag while hunting deer on Greenhorn Mountain, Tulare County, California. This spotted skunk was killed, and it was positive for rabies. She refused the vaccine treatment because the immediate microscopical examination was negative for rabies. The mice inoculated with the skunk specimen developed rabies three weeks later and the vaccine treatment was started, but she developed rabies five days later and died. These are examples of isolated cases of skunk rabies.

Hughes: Why would you seek the occasional host?

Johnson: Well, because that points to a focus where the disease is present. We know that there is an old history of foci of yellow fever. There are also old histories of outbreaks of encephalitis virus in horses.

There are certain areas in which western encephalitis virus recurs, and they're usually in the line of flight of migratory birds, like blackbirds in the central United States. But the true understanding of natural hosts is going to involve studies, which I've been suggesting for a long time, where you test all the small mammals, as well as birds, in areas which seem to be endemic foci. And then you try to get the virus, not from the blood, which is only going to be positive two, three, or four days, but by cell culturing certain organs, like the kidneys, salivary glands, and lungs.

I isolated the Powassan tick-borne encephalitis virus in California from the kidney cultures of a spotted skunk. That skunk was healthy, so the virus was latent. When we tested the kidney tissue specimen, it was negative for virus, but the cell cultures produced Powassan virus. That's a general method.

I've done a lot of looking at wildlife populations in California and studying the viruses that are present. All wildlife has pretty much the standard type of viruses. They have paramyxoviruses, adenoviruses, herpes viruses, rhabdoviruses, such as Kern Canyon and Klamath viruses, orbiviruses, such as Colorado tick fever virus, and flaviviruses, such as Modoc and Rio Bravo virus.

Modoc virus survives in two ways in *Peromyscus* mice. It's an encephalitis-producing virus, and we've shown that you can collect that particular mouse in nature and grow the virus right out of the kidney. I've never gotten it out of blood, but I can show it's present in Modoc County, California, almost every year I test the mice where I found the virus in 1958. Rio Bravo virus persists in *Tadarida* bats as a salivary gland infection.

Johnson: Yellow fever should have a pattern like that. An experimentally infected hamster excreted the Modoc virus in the urine for five months, and at a year and two months after infection, the cell culture of the kidneys produced the virus. That would be the logical way a virus would survive in its natural host. It's like Theobald Smith says, to be a successful parasite, it shouldn't do much harm to the host. Whenever [an infectious agent] gets into an aberrant host, the most disastrous things happen, like bubonic plague in the Norway rat (which I don't think is the reservoir host) or yellow fever in the howler monkey, or rabies in dogs. That's more or less my concept.

Hughes: Do people listen to you?

Johnson: There is little interest in studying the natural virus infections in wildlife. The arthropod-borne western encephalitis and eastern encephalitis viruses are controlled by insecticides directed at the mosquito species found infected, Culex tarsalis for western encephalitis and Culiseta melanura for eastern encephalitis viruses. You control rabies by either vaccinating the dogs or strict dog control. In this country, because of the amount of rabies in wildlife, you have to vaccinate dogs. Once you get rid of the epidemic canine type of [rabies] virus, which is dog-to-dog, a very virulent strain, the disease problem is controlled. In California, where there is a tremendous amount of virus in bats, we still haven't had an outbreak in coyotes like they had in 1915-16; we haven't had any outbreak in foxes, and no new outbreaks in dogs, although we get an occasional dog infected from a skunk.

So it's the nature of the virus. Once it's able to establish a successful aberrant cycle, which happened in the dog, it may be difficult to stop. Epizootics in dogs did not occur until the 1700s. There were great wildlife epizootics in foxes in Europe and the United States since World War II but these run a rapid course. But once we had large populations of dogs in urban areas, dog rabies would persist as an enzootic disease.

There has to be some change in the ecology. When you develop these great agricultural systems to irrigate cotton and rice fields, then you create a fresh-water swamp which results in production of large populations of Culex tarsalis mosquitoes in California, which serve as the vector for the western encephalitis virus. When the breeding of this mosquito is reduced by managing the surface water and if necessary by insecticides, this is sufficient to prevent human infection.

Well, what goes on in the great natural swamps? Mostly it's migratory birds, but there are plenty of bats in places like that. That's where they rest. It's a place where they're safe. Also in tule marshes. Such places have the variety of mosquitoes that can pick up arthropod-borne viruses from either the local or migratory

- Johnson: species that serve as reservoir or natural hosts. I believe that the encephalitis-producing viruses are able to survive in some hosts without the necessity of insect transmission.
- Hughes: After the Rockefeller Foundation stopped supporting the arbovirus program, what happened to the funding of that type of research?
- Johnson: The great advantage of having a nongovernmental type of program like the Rockefeller Foundation's was that it helped certain doctors working at a university or in a state health department because they were interested in studying viruses. They would do simple studies which didn't require much money. Our basic studies of rabies in Alabama or the yellow fever in Africa or South America didn't cost much money. In fact, mostly what you needed was money to buy equipment for laboratories.

The Importance of Field Work

- Johnson: Most medical doctors are not interested in field work but like to stay in the laboratory. The tragedy right now is there are really no field teams out studying disease problems that derive from wildlife. I've been so interested in the Wildlife Disease Association because it encourages the study of wildlife.

Ducks play a very significant role in the maintenance of influenza A virus. I've been involved with the Cooper Ornithological Society for thirty years, because that's where I learned about the ecology of birds. Wherever I go in the world, I study birds and small mammals.

- Hughes: Do you think the lack of field work is more a question of lack of interest rather than lack of money?
- Johnson: Yes. [The need is] to encourage young people to study nature.

When I was a child in an ordinary country school, everybody learned the names of flowers, small mammals, birds, trees, and plants. Now there seems to be hardly any teaching of natural science. Medical students used to have to study botany and zoology. It was a complete study of natural science.

The big money now, of course, is in AIDS, but retroviruses are also responsible for diseases in wildlife. The monkey has one type of AIDS virus. Then there is the Rous sarcoma [virus] of chickens, which is no problem in chickens, but it's a retrovirus like AIDS. And there is infectious equine anemia, which is very similar to AIDS in its epidemiology. There was an epidemic of equine anemia virus at a racetrack from using the same needle and syringe for giving penicillin to several horses that were ill of a

Johnson: respiratory infection. One was evidently a carrier because some horses died later of infectious equine anemia. The epidemiology is like the spread of AIDS virus in heroin addicts.

One reason I was able to study rabies in vampire bats in Mexico was because I grew up on the prairie in Nebraska at a time when horses were used to pull farm machinery and for transportation. I was used to riding horses and that was a necessity for doing field studies of vampire bats on the large cattle ranches in Mexico. You have to collect bats after dark. It's almost impossible [now] to hire people that will go out with you at night to net bats, especially vampire bats.

The best help I had in California was graduate students in zoology and ornithology. They would do it for the fun of it, and I didn't have that much money to pay. Gordon Orians helped me in the study of blackbirds in California. He's now a professor of zoology at the University of Washington, one of the best ornithologists in the USA. All I could do for him at the time was to pay his gas bills, with Rockefeller Foundation money. He showed me the places where he was studying colonies of red-winged blackbirds.

Don Roberts, who worked for me at the Rockefeller [Arbovirus Study] Project got his master's degree in zoology at Cal, and finished his work for a doctorate but did not complete his doctoral thesis. He was invaluable, because he loved field work. Several of the staff in vector control at the state health department, like Keith Murray and me, used to do field studies of small mammals, setting traps, and then you had to be out at the crack of dawn and collect the animals. At night, you'd often work till midnight collecting the ticks and fleas and taking blood specimens. You really had to enjoy what you were doing.

Same with the work with plague. This project is restricted now. California's had one of the best medical zoology programs dealing with plague and encephalitis in the country, but the health authorities now seem to feel that the problem has been solved. We can control plague; we have a good drug for it and can cure it. There's very little money for the vector specialist in zoology. And then there's the need for studies of resident and migratory birds. The bird organizations, like the Cooper Ornithological Association, are usually [only] interested in the natural ecology of birds. They do not study birds as a cause of disease or predation.

You can't prove that birds really die of encephalitis virus, but I'm convinced from my studies of small nestling birds, like sparrows and swallows, that birds die of western encephalitis. In fact, where I get most of my isolates is from sick or freshly dead nestlings. So encephalitis viruses do cause die-offs of nestling birds.

Johnson: This was true in some of the studies I made around the world of marine bird colonies where there had been die-offs of pelicans, boobies, murre, gulls, and terns. Nesting sites of such birds sometimes contain enormous populations of ticks. I don't know how many different viruses have been isolated from bird ticks, probably twenty different ones. I found eight. We don't really know what they could do to man. We know some of them infect man.

Hughes: When an agent isn't known to be infectious to man, does that make the funding more difficult?

Johnson: Yes. The real money always is in disease which is a real crucial problem [to humans]. In the early days, it was polio. You could get plenty of money for polio research. Then we had the problem of rubella. The reason I came to California in 1952 was because of a real big problem of encephalitis in children in Kern County. There was money available. But it has to be a problem [to humans]. AIDS is a very serious problem and consequently there's a lot of money for it. Some of the wildlife diseases [caused by] retroviruses should be studied to determine how a virus like AIDS is maintained [in nature] and where it came from. As I said, we know that there are AIDS-type viruses in wildlife. AIDS is a new disease.

Well, so is encephalitis. Dr. K.F. Meyer isolated western encephalitis virus from a horse in 1930. Mosquito and fly transmission was suspected but at the time it was called equine encephalitis and regarded as a disease of horses.

Hughes: You mean encephalitis is a new disease in the sense that it was first recognized then?

Johnson: Yes. The disease was known but not the source in wildlife and the method of transmission. Then when the 1941 epidemic occurred, Dr. Hammon and Dr. Reeves succeeded in isolating western and St. Louis viruses from mosquitoes in the Yakima Valley of Washington. That was the year we had the tremendous outbreak of western-type encephalitis in humans in the central United States, some three thousand cases and hundreds of people severely affected, some in a coma for months and a few for years. That's when money became available for encephalitis research.

Dr. [S.Y.] Gaidamovich started to study some viruses isolated during wildlife studies in the Soviet Union, and she found that some were vaccinia virus. One of these field isolates from the Soviet Union was sent to the YARU lab in New Haven. During preparation of immune serum from the peritoneal fluid from mice inoculated with the Russian isolate followed by mouse sarcoma virus, it was found that the specimen contained vaccinia virus. All of this material had to be destroyed. [Vaccinia virus contamination] has been one of the big problems in [virus] research labs all over the world. Vaccinia [is essentially the

Johnson: same virus as the one causing] ectomelia in mice. LCM, which is found in house mice, has been a hidden infection in some mouse colonies, and like ectomelia [vaccinia]-type virus, it may spread when introduced into virus laboratories.

Hughes: When did monoclonal antibodies become available?

Johnson: In 1975.*

I've just finished the chapter on rabies for the new edition of Procedures for Viral, Rickettsial and Chlamydial Infections, of which Dr. Emmons is the editor.** The identification of the different strains of rabies virus from bats, skunks, raccoons, foxes, and dogs has been done in the last two or three years. The technique was developed, but then you had to get monoclonal antibodies for all the different viruses, which is quite a job. I think that the real key is getting monoclonal antibodies and identifying different strains of the wildlife viruses. I don't think they yet have any good studies with monoclonals of the western encephalitis virus strains. It's a brand-new technique, and it's going to be the biggest aid in identifying natural sources of viruses.

Tissue Tropism of Arthropod-borne Viruses

Hughes: Well, is this the time to say something about your non-neural adapted strain of western encephalitis virus?

Johnson: Yes. I sent you the transcripts of the arthropod-borne virus session of the 1958 meeting in Lisbon.*** Dr. Sabin and I presented topics that we felt needed to be studied. What was the natural epidemiology of different viruses? At that time I brought up some studies we had done of western encephalitis viruses isolated from natural sources by test inoculation of one- to two-day-old mice. What tissues in the body are infected?

Mother mice eat the baby mice that die, and they are infected in turn. They have the virus in the blood two days later. The mother mice usually remain healthy, but if killed at

*G. Kohler, C. Milstein. Nature 1975, 256:495-497.

**N.J. Schmidt, R.W. Emmons. American Public Health Association, 1989.

***Proceedings of WHO Informal Meeting on Arthropod-borne Viruses, Lisbon, September 8-9, 1958. [mimeographed typescript]

Johnson: ten days after ingesting the dead infants, the virus is found in the mammary gland, kidney, and pancreas. If the mother mice die in ten to fifteen days, the virus is found in the kidney, pancreas, or brain. In some instances the brain is negative for virus, whereas the kidney or pancreas is positive. So this shows that the virus has the ability to multiply in organs other than the blood and brain. This prompted me to establish a strain of western encephalitis virus not passaged in neural tissue.

Hughes: How have you used this non-neural strain of western encephalitis virus?

Johnson: Well, we isolated western encephalitis from blood, spleen, and pancreas of birds. It was the B628 house sparrow which I collected as a nestling that was the source of my non-neuroadapted strain of western encephalitis virus.* The pancreas was grossly enlarged and contained more than 4 log 10 of western encephalitis virus. I reisolated the virus from this specimen by inoculation of a primary culture of hamster kidney cells. Later it was cloned in chick embryo cells. So it's never been brain passaged.

Now, when we started with viruses directly from brain passage from mosquitoes, we never could get a strain of western encephalitis virus that didn't kill adult mice. But from this B628 strain, by selecting individual virus particles which we called clones, we were able to get a strain that did not kill adult mice, ponies, or rhesus monkeys, inoculated intracerebrally. It is a very low virulence strain of western encephalitis virus, which could be used in an epidemic situation to protect humans if there was a real serious problem. At least it is a good live virus vaccine for horses.** To date this vaccine virus has not been licensed for immunization of humans or horses in the USA.

Last year [1987], there was a good-sized epidemic of horse encephalitis in Colorado. There were human cases, too. It was almost the first real serious outbreak of human and horse western encephalitis since 1965.

Hughes: Will the vaccine be used?

*H.N. Johnson. Selection of a variant of western encephalitis virus of low pathogenicity for study as a live virus vaccine. Am. J. Trop. Med. Hyg. 1963, 12:604-10.

**J.P. Hughes, H.N. Johnson. A field trial of a live virus western encephalitis vaccine. J. Am. Vet. Med. Assn. 1967, 150:167-177.

Johnson: That is a question for the medical and veterinary medical professions. Horses had such a high mortality from western encephalitis during 1937 and 1938 that farmers had to shift to tractors because they didn't have enough horses for their farming.

Hughes: Do you maintain any other non-neuroadapted virus strains?

Johnson: I have non-neuroadapted strains of SLE [St. Louis encephalitis virus], CTF [Colorado tick fever], Modoc, Rio Bravo, Powassan, and rabies virus, that is, never passed by the intracerebral route in laboratory animals. Such viruses are passed in cell cultures of mammal kidney or in chick embryos.

I have a strain of St. Louis virus which has low pathogenicity for laboratory animals. This non-neuroadapted strain was maintained by yolk sac passage in chicken embryos. In other words, we did not pass the embryo tissue, and the virus multiplied in the cellular lining of the yolk sac. It lost its pathogenicity for adult mice, even when inoculated by the ic [intracerebral] route. That strain of SLE virus was plaqued, and a stock virus was prepared from chicken embryos inoculated by the yolk sac route. I recommend this strain for people who do diagnostic work with SLE virus, because it is less pathogenic than other strains.

Teaching

Hughes: You taught for over thirty years at the University of California School of Public Health. Tell me how that came about.

Johnson: In 1954, Dr. Charles Smith, dean of the School of Public Health, invited me to join the faculty as a lecturer. Dr. Lennette already held an appointment as lecturer and was in charge of a laboratory-training course at the School of Public Health. He and I used to lecture in that regularly, and the handout I gave you today gives you something about the course and how it was set up.* Dr. Nathalie Schmidt, who died in 1987, played an important role in this teaching program. She was honored by a memorial lecture at the School of Public Health. I regularly lectured in the epidemiology and zoonoses courses and served on the doctoral committee. Dr. Emmons got his doctor's degree working with me on Colorado tick fever.

*Virus Lecture and Laboratory Schedule, Public Health 150B, Spring Semester 1956, University of California School of Public Health. [typescript]

Johnson: When Dr. James L. Hardy joined the faculty of the School of Public Health, we were involved together in studies of WE, SLE, Turlock, Modoc, and Rio Bravo viruses. The field programs of the School of Public Health and the State Health Department were coordinated.

Hughes: The State Department of Public Health had its own courses, is that true?

Johnson: Yes, but these were for special training in virology. The courses for university credit were always run out of the School of Public Health.

Hughes: Who took these courses?

Johnson: The students were usually graduate students, such as veterinarians and MDs taking degree courses to obtain a master's or doctoral degree in public health. In my seminar on rabies and arboviruses, I tried to put across the ecological aspects of controlling disease problems and the methods used in field studies. In fact, a lot of those students had Rockefeller Foundation fellowships after they had worked at field projects of the foundation, including the Poona Virus Research Centre where I was stationed before coming to California. When I was at Harvard for five years, I was regularly involved in teaching Harvard medical students. I was a resident in pathology at Children's Hospital. Besides teaching and doing the path conferences, we also were teaching graduate students and medical students there.

No one should be too proud of what he's accomplished because somebody always has to teach you. The way that I learned virology was from other people. Hopefully you can add certain things [in the way of] safety and certain techniques in your own particular field.

My interest was in developing better methods for collecting animals, not just shooting them. I'm for live netting and trapping so you can work with live animals and bleed and study them. With Colorado tick fever, we'd take blood specimens in the field and bring some back to the lab. If we isolated a virus, such as Colorado tick fever virus, and the animal was healthy, we could see how long the virus remained in the blood. That's when I found that the virus was actually in the red blood cells, because we tested serum versus red or white cells.

So the real teaching of virology is technique. It is not safe to have people working in a lab who have not completed courses in microbiology. They are dangerous because they do not know the basic methods. Dr. Emmons, Dr. Lennette, and I have been interested in teaching absolute technique: Don't get it on your hands; don't get it in your mouth; don't mouth pipette. We developed a very tight system to prevent technicians from getting infected in the lab. In many laboratories, technicians do get

Johnson: infected with viruses they work with, because they're careless. The virus gets on the tables, on their clothes, and on the outside of containers. The big problem is in preventing spilling or aerosols. Viruses seem to fly through the air when you have automatic pipettes and homogenizers. Tiny droplets may escape when grinding specimens in mortars.

Hughes: Don't most virology departments emphasize technique?

Johnson: I suppose they do, but the best technique is going to be in places where they've been forced to improve it.

The early rabies work, like at the Pasteur Institute in Paris, progressively became more secure in handling specimens and record keeping. In the old days, the US Public Health Service usually had only one person working on a disease, plus a technician. It's not a bad idea. Dr. Richard Emmons' father was in charge of the mycology lab at the NIH. There would be similar small units for tularemia, rickettsia, rabies, and general virology and bacteriology. There was the public health plague lab, and later public health labs worked on various diseases, like rickettsial diseases at Hamilton, [Montana]. There's where you got your best technique, because these were dangerous organisms. They'd say, "Well, how can we best protect our people?"

The Ecological Approach to Virus Studies

[Interview 11: February 26, 1988*]

Hughes: Dr. Johnson, one of the important themes of our talks, I feel, is your ecological approach to virus studies. Would you describe that approach?

Johnson: Well, it is really natural history. My observation is that the associates I've had that worked with yellow fever or the encephalitis viruses, whether they were entomologists, zoologists, veterinarians, or doctors, had been interested in not just one field but all fields. Several of my doctor friends had PhDs in physics as well as MDs. Many of them were interested in birds. I hardly know one of my associates in the foundation that didn't keep a list of the birds he'd seen. Dr. Emmons, Will Downs, and others do. Almost all of them are interested in nature. Natural history is a field that you really should have a good knowledge of if you are going to study any of our infectious diseases.

* For better continuity, the transcript of Interview 11 appears before that of Interview 10.

Johnson: We don't know where Salmonella came from, or typhoid bacilli. But think of the bubonic plague organism; it's got to be somewhere [in nature]. And then from there it gets to man periodically, and then it causes devastating disease. Bubonic plague in the 500s and again in the 1300s killed at least fifty percent of the people in the world, as far as we know.

Let's name some diseases that might be just human parasites--poliomyelitis. Most people still feel that that is a natural disease of the human race. Currently, the epidemic strain or the field strain in this country is almost eliminated. But it is still present in many parts of the world. Well, was it always in man? The reason I don't believe that is, if you study ecology and go back in history, up until relatively modern times, you could not maintain a large population of people in any place because of the lack of sufficient food. Even in biblical times, there were cities like Jerusalem which would be periodically sacked and completely destroyed. They could last for months and sometimes years without bringing in any food. [The human species] had to develop a form of agriculture where you didn't just gather food, but you could grow and store it.

I'm convinced that the infectious diseases that occurred when people lived--like they still do in certain areas of the world--in groups of twenty-five or thirty people in one area, couldn't be maintained. Like polio: You get the infection; you will have it in the intestinal tract for say a month or two or even up to four months. But when it gets into a community like New York City, you don't realize how many people get infected. There are, say, thirty cases of polio. But then they put down swabs in the sewers where the water's clear, and they can isolate polio virus. The degree of dilution would make you believe that they had to have 100,000 cases at that time, showing that these infections could cause a disease, but the majority of the people would not be obviously ill. The polio virus had to come from somewhere else.

The Cocksackie viruses produce the disease called devil's grippe or Bornholm disease. Well, where did they originate? Bornholm is an island, so what started it off there? In Norway, it's been epidemic at various times. We know there are six Cocksackie B's and that there are twenty-three of the Cocksackie A's. Do they come from nature?

Then we have over one hundred viruses that produce respiratory tract infections. Where did influenza A, influenza B, and influenza C come from? I'm very suspicious that they have a reservoir in bats, because the lung cultures of bats are one of the best host cells in which to grow the A and B types. I have not tried to cultivate influenza C in bat cells.

Johnson: The yellow fever virus was supposed to be limited to humans until the rhesus monkey was found to be susceptible. At present the monkey species is believed to be the natural reservoir. But, as I've said, I don't believe that, because I've been in the yellow fever foci in Africa and South America, and there just are not enough monkeys. They only have the virus in the blood for four days. So where is it the rest of the time?

The Rockefeller Foundation scientists in South America and Africa collected a great variety of animals and inoculated them with yellow fever virus, but they used the neuroadapted mouse passage strain. The Asibi strain has been passed by peripheral inoculation. So when you use a strain that you pass by the ic route in mice, it's neuroadapted and you've eliminated the majority of the virus particles that like to live in organs other than the brain.

Hughes: The Asibi was not neuroadapted?

Johnson: No. His blood was inoculated subcutaneously into monkeys.

Hughes: Why didn't they use the Asibi virus in serological tests?

Johnson: Well, in serology, you want something that will kill mice well. The trouble with the early work in yellow fever was that certain strains of laboratory mice wouldn't even get sick if you put the virus in the brain. This was true also for wild rodents.

They'd inoculate various animals with viruses that they had in the lab, usually mouse-fixed strains. Then they'd bleed them to see if they had a viremia. This is an important test for susceptibility, but the neuroadapted virus is less likely to produce a viremia. When I began working at the yellow fever lab [at the Rockefeller Foundation] we used the adult mouse as a test animal. It's not very susceptible to yellow fever. If you test the blood of a monkey at daily intervals after inoculation with the Asibi non-neuroadapted strain, there is virus in the blood, and the adult mice inoculated by the ic route get sick but will recover. If there is little virus in the blood, they remain well but may be immune. We thought that the vaccine virus, 17D, didn't circulate when we were vaccinated, because we'd take the blood, test it in mice, and you could hardly make an adult mouse sick. If we had used baby mice, it would have been different. If you use baby mice, which are more susceptible, you will find that the virus does circulate in the blood. So what we need to do now is to do all those wildlife studies over again in small mammals in South America and include the different species of bats. Of course, if they are the natural hosts, some will be immune if collected in foci of yellow fever.

Hughes: Is there anybody interested in doing this?

Johnson: No. I have been unable to get anybody interested in working with bats in yellow fever or dengue foci. I would say that the thing that needs to be done now is to repeat studies at field laboratories. But it's difficult to get money for working in field studies of infectious diseases. We have very little money in California for doing field plague studies.

So back to the natural history story. To study infectious agents, you have to study the whole environment where people are getting infected with certain diseases. My feeling is that it is to everybody's advantage in a country to have, besides geodetic surveys, a natural history survey. In the United States, the Interior Department geodetic service in 1860s to 1880s described the natural history of the western states. They are a wonderful source of the description of the geology, climate, rainfall, forest, open land, flora and fauna. We need to continue studies in which there are records of when there are overpopulations of animals and birds.

At the Manomet Bird Observatory in Plymouth, Massachusetts, the number of birds they net and study varies a lot. In some years, certain bird populations have dropped markedly. If such studies are continued, it is possible to correlate such findings with climate change, drought, and excessive rainfall.

Hughes: Did the Rockefeller Foundation and the California State Department of Public Health recognize the importance of having multi-disciplinary fields participate in field surveys?

Johnson: Yes. The reason that the foundation contacted Dr. Lennette in the 1950s after the [encephalitis] epidemic in 1952 was to get assistance in a more general study. They were collecting mosquitoes, and there was a good program on small mammals in California related to bubonic plague. It was an opportunity to enlarge these studies so that there would be more study of wildlife. I think that's the key. Until the Rockefeller Foundation program ended in 1972, it was possible to continue the wildlife studies.

Hughes: What was the reason for the budget running out?

Johnson: Well, the Rockefeller Foundation discontinued the research program on human virus diseases. The agricultural sciences were continued, including the rice research institute in the Philippine Islands, the grain research in Mexico and Colombia, and the tropical agriculture field stations in Africa and India.

Hughes: From reading some of your papers on the ecological approach, it seems to boil down to three basic questions in the study of infectious diseases of plants and animals. The first question is

Hughes: where are disease agents maintained in nature? Second, what leads to dispersal of disease agents to new hosts? And third, how can the diseases be controlled?

Johnson: That's pretty good. It's like I used to say in my ecology lectures: where, when, how, and what. Where is the virus in the long run? [When does it appear?] How does it get out of its usual cycle, and what can you do to control it? Those are the steps. That's the natural history approach. The amazing thing is that when all the work has been done, we really know very little of where the parasites persist, even diseases like amoebic infection in man. You can go to Strawberry Canyon [on the UC Berkeley campus] and find amoebae in mud that looks exactly like Amoeba histolytica in man. One of my daughters did this for her microbiology course in college. [laughs]

Worms you see in wildlife of all kinds, and so where did hookworm come from, for instance? It must have had a cycle before it reached man, because small populations of people moving about could not really maintain hookworm. Malaria would not become established in man unless you get a pretty [large] population.

Hughes: Do you want to say more about the origin of yellow fever virus?

Johnson: In the early 1940s everybody was pretty well convinced that yellow fever was a natural disease of man and the Aedes aegypti mosquito.

Hughes: Originating in Africa?

Johnson: Yes, and possibly also in South America. There was no idea that it came from anything other than man at the time. Geneticists and evolutionists [argue over] whether the original source of the virus is in the mosquito, and whether it's maintained in mosquitoes or other insects. The Russians seem to think that all the encephalitis viruses are maintained in an insect host. Now we know that it's possible for a virus to go through the egg. But there are many problems with that, and one of the first things they did with yellow fever was to test the male mosquitoes versus the female mosquitoes where the yellow fever was. And we didn't get yellow fever virus from the male, but we did out of the female mosquito. And the female has to take blood for laying eggs.

The consensus of opinion after about twenty or twenty-five years of work at field studies in Africa and South America [is that] the monkey [is the natural host]. One reason was that the rhesus monkey of India, where there is no yellow fever virus, when you'd inoculate it with yellow fever, will get sick and die, but essentially all the African and South American monkeys, if you would inoculate them peripherally with the virus, do not get sick and therefore the virus does not destroy the host.

Hughes: There has also been a debate about whether the yellow fever virus was imported from Africa, maybe on slave ships.

Johnson: Yes. In India, we were particularly interested in the ships, "dhows," that go to India from East Africa. They have water in them all the time, and mosquitoes have been found that would be capable of harboring the yellow fever virus. So that was one reason we made a particular study in the first year to see if [the local Indian population] on the west coast had antibodies to yellow fever, and there were none.

Everything now points to the fact that yellow fever can move, even in sailing ship days, from some port in South America to Baltimore. They had epidemics there [and also in] New Orleans and Philadelphia. So all you need then is a person that's been bitten by an infected mosquito--there's an incubation period of five to ten days before you get sick--and after the onset of the viremia a person will probably have the virus in the blood for five days. That gives an opportunity for him to travel from South America to North America, even in a sailing ship. There's reason to believe that the virus has been in South America a long time.

Theobald Smith said that parasites must have alternate means to survive. And there are so many, we can't quite understand what they may be. And then in their natural system, the way they are passed, the way they are maintained, and when they infect, whether it's early in life of the animal or not, the organism may not be so virulent. But we do know, of course, that these monkeys in South America will get infected and circulate virus but usually won't get sick. But each one can only produce virus for five days, and then the immunity lasts for life. The total populations do not appear sufficient to maintain yellow fever virus if the virus must infect insects to survive.

Howler monkeys actually get sick and die of yellow fever--one of the few [species] in South America that do--and I said, "Well, it can't be them, because there are only five or ten left in endemic foci, and they're immune." There is no yellow fever for ten or fifteen years, and all of a sudden it's there. In Trinidad it could come from the mainland of South America.

My basic contention is that the natural history of diseases in man is very poorly known. There are acid-fast bacteria all over in nature. There are Salmonella. You can go to a beautiful stream in Yellowstone and it has B. coli. Studies in Canada show that all the common strains of influenza can be isolated from ducks, wherever you have a place they can stop and feed. You get it either out of the throat or the cloaca of the ducks, and sometimes out of the water.

Each virus disease you study seems to go off in tangents. Smallpox now has been eliminated, but there are all kinds of very

Johnson: similar viruses. In fact, there is one that's been isolated from a hedgehog in Nigeria that's very close to human smallpox. Then there are pox viruses in birds, small animals, and even domestic animals. The monkeys in Africa are occasionally infected with the pox, which will actually spread to people in the villages. So far this strain has not produced a sustained epidemic. But it's possible.

Evolution of Viruses

Hughes: Tell me what you mean, please, by the evolution of viruses.

Johnson: Well, we are very fortunate now to have monoclonal antibodies. We really don't know much about the stem viruses, but we do know that the viruses that we see today can change as they go from host to host. The host itself puts in certain components of the proteins, so that we can now in this country detect viruses which have come into bats only recently. There is no evidence that there was bat rabies here until 1953, and it spread all over the country.

Well, the viruses adapt to the different species of bats. The strain of [rabies] virus in the big brown bat, Eptesicus fuscus, can be identified as being different from the one that's in Myotis bats, or different from the one that's in the red bat, Lasiurus borealis. The Mexican freetail bat, Tadarida brasiliensis, is the source of an epidemic variety in the bat caves. Now, each of these viruses has undoubtedly changed in the way its transmitted, because the freetail bat virus in the caves has a very short incubation, like dog rabies. It is very dangerous, because it goes by aerosol. People can get infected just from being in the cave. There is also the possibility that such viruses have hybridized with certain bat viruses.

Now, some of the bat [virus] strains are probably of low pathogenicity and really would require a bite to transmit them. However, we know that foxes can get infected by eating bats, and you can tell that because the strain that comes out of the fox is similar to [that of] a bat strain. Almost all of the cases of fox rabies that occurred recently in the East were typed as bat rabies strains. Foxes either were bitten by a bat or had eaten it, because that's the strain. There were epidemic fox strains [of rabies]. There's still a red fox rabies epidemic going on in Canada, getting into New York and Maine. That strain you can pick up by monoclonal antibody [tests].

The evolution of viruses, like all forms of change, are phenotypic, and they are selective on the basis of the host. I call it phenotypic selection by host. Then the most exciting of all that's coming out now is the hybridization of viruses. It

Johnson: seems yellow fever or rabies [virus] can hybridize with even the chicken sarcoma retrovirus. So when we worry about things like the AIDS virus, we know that virus is going to be easily changed by hybridization with other viruses, because it is a retrovirus. The parvoviruses [infect] dogs and humans. There is a hybrid of an adenovirus and a human parvovirus.

So viruses change in two ways: If the skunk or bat rabies virus gets into a dog, most often it won't spread. That particular dog doesn't get mad; it just gets paralyzed and dies. So, it has to be a selection of a phenotype that makes the dog vicious. And then he will bite a number of dogs and start a chain of infection. If that happens through two or three passages, and virus phenotype is selected by going to the salivary gland and making the animal bite, it can produce a highly virulent disease, because it is selected by causing an infectious insanity, and the virus has been selected for tropism for the salivary gland. To get to man, rabies has to go through the dog, because we have so little chance of being infected by any other animal.

We have no definite knowledge of where Colorado tick fever is maintained, but I feel that it's not going to be in the animals which sicken and die if they're infected. To me, the pocket mouse, *Perognathus*, is the logical host. It is present in the CTF-foci and has been found infected. The larval ticks will feed on it, and then the virus will be present in subsequent stages. Finally, of course, man and the deer are the aberrant hosts. They aren't needed [to maintain the cycle].

All species of birds and mammals have a complement of protozoa, spirochetes, borellia, bacteria, and viruses. They all have their parasites. Then evolution [determines] how these will be phenotypically selected to produce disease in man.

Hughes: Why do you say that there is not much hope of finding the long-term permanent hosts of these viruses?

Johnson: You're dealing with organisms which have a very low pathogenicity [for the long-term host]. We're better equipped now where we use cell [and tissue] cultures. Almost all my viruses are passed in a way so that they have not been neuroadapted. For instance, I've gotten Modoc virus out of the cell culture of the kidney of the wild *Peromyscus* wood mouse. So that virus has never been passed in another species; it just came directly out of the kidney. Dr. Hardy worked with that [non-neuroadapted] strain at the School of Public Health. Then you could take a strain like that and inoculate mice sc [subcutaneously], like we did with the Asibi strain in yellow fever, and then bleed them, and then you have blood tissue-adapted virus. So the method of isolating an organism may make you believe you have an extremely dangerous virus, where in nature it probably doesn't cause nervous system infection.

Hughes: Is that why your viruses are not neuroadapted?

Johnson: Well, I want to keep one non-neuroadapted strain to find out if it produces a viremia. I've advised people to do that around the world. In Czechoslovakia, Dr. V. Bardos has isolated a California-type virus which they call Tahyna virus. He wanted to do viremia studies. He did what I suggested. He took his original isolates from mosquitoes and then injected a hamster peripherally and then bled it. That way he could show that the virus produced good viremia. There was less virus in the blood of hamsters inoculated with the neuroadapted Tahyna virus.

The best example I can give of tissue tropism is illustrated by rabies virus. One of the first things I did when I found out that rabies virus was in the salivary gland of rabid dogs in very high titer, higher than the brain, was to test the Pasteur virus that had been passed in the brain of rabbits a thousand times. I couldn't get the virus out of the salivary glands. That's the reason I started passing the Flury strain intracerebrally. This was continued until the incubation period was fixed at six days. At that passage the virus did not invade the salivary glands. I passed it intracerebrally in one-day-old baby chicks for four years, and that became the Flury-avianized strain of rabies [virus]. I passed it 138 times, and then I sent the 136th passage to Lederle and various other laboratories. Lederle passed the virus in chick embryos for thirty-five times. This was the low egg passage (LEP) vaccine virus used to immunize dogs.

Later, the strain that Dr. Koprowski kept going in the yolk sac of chick embryos became the high egg passage (HEP) Flury strain that lost its virulence for adult mice inoculated intracerebrally, but kept its pathogenicity for baby mice. But that in itself was not a big change. It was not a mutation, because all you had to do was to pass it once intracerebrally, and it would revert to pathogenicity for adult mice. It's important, when you work with a virus, to try to keep it as near as possible to the natural cycle of infection.

I wanted to study the natural dog rabies virus, so I finally made a standard virus from infected salivary glands of dogs. We would challenge the dogs intramuscularly with the virus derived from infected salivary glands. If we inoculated the virus by the intracerebral route, it killed the vaccinated dogs. Pasteur claimed he had immunized dogs against intracerebral trephining, which was the way he used to pass the virus. There is no evidence in dogs that you can have immunity to intracerebral inoculation unless they've had an infection in the brain previously.

We have had dogs recover after showing symptoms of rabies, but dogs that had been given forty-five injections of live Pasteur rabies vaccine were immune to peripheral inoculation. But if you

Johnson: put the virus in the brain, they all died, except an occasional one which had some symptoms that made you believe that the dog had had rabies and recovered.

The Viral and Rickettsial Disease Laboratory*, California State Department of Public Health

Hughes: Would you comment on the role of the virus laboratory of the California State Department of Public Health in diagnostic virology?

Johnson: Well, the beauty of the history of virus research is that all of us that have had the opportunity to be involved with research in certain institutions have been able to learn things which you really can't work out for yourself. It's an accumulation of knowledge which has come through years of research in the universities of Europe, England, and the United States, and applied to diagnostic work.

You have to go where the research has been done, where the best libraries are, where people are actually working with their hands. I've written book chapters [about viral] technique for years, and you try to inculcate safety precautions in the diagnostic work. But there is nothing like going to a place and working there. Even if you are going to do a simple thing, like repairing your car or something, you need to see it done.

There have been several places in the United States that have accumulated a good deal of basic knowledge [in virology]. The California State Health Department is one of the best in the country. Texas, New York, and Georgia are others where there are diagnostic virology laboratories. The commercial biological firms, such as Lederle Lab, have provided virus vaccines and have developed laboratory methods for processing viruses.

When I arrived in Berkeley in '54, they were just finishing [the Virus Laboratory at UC Berkeley]. Wendell Stanley took me up to the top to show me the view, and look what came out of that laboratory: the studies of polio virus, the electron microscopy by Robley Williams and others. That was a wonderful department to have near the state health department.

Hughes: Stanley apparently was a strong spokesman in Sacramento for the needs of the California Department of Public Health.

*This laboratory is often referred to simply as "the virus laboratory."

Johnson: Yes. You have to pick people, good people, and give them the reins to work. He had a good staff. He had the same interests as the virologists in public health because he had been working with the veterinary and plant viruses.

The Rockefeller Foundation assigned staff to the California Health Department who had been trained at the Rockefeller Institute, beginning with Dr. Monroe Eaton in 1939, and later Dr. Lennette and myself. This brings in new knowledge and techniques. The same thing happened in Los Angeles. You had the universities there, so that Los Angeles and Berkeley developed outstanding virus laboratories.

The reason that the virus laboratory at the California State Department of Public Health is so good was that from the very earliest days it was necessary to diagnose rabies and provide rabies vaccine. Wilbur Sawyer, who had been director of the diagnostic laboratories of the state of California, later told me, "I've got to tell you about the big epizootic of coyote rabies in Modoc and Lassen counties in 1915." There was [also] knowledge of rabies in the miners that were bitten by spotted skunks and got rabies in the gold rush days in California.

One of the earliest men that studied rabies in California was Dr. John Marsh. He described rabies in dogs in Los Angeles, and he also described rabies in one of the members of his expedition across the country through Wyoming, a person by the name of Harris, who was bitten by a wolf and developed rabies. He ran away from their camp when he developed hydrophobia. So there is a long tradition of making the old Pasteur vaccine in California.

At the recent meeting we had at the School of Public Health honoring Nathalie Schmidt was one of the ladies that prepared the Pasteur rabbit spinal cord tissue vaccine in the Life Science Building in the 1920s.

Hughes: Who was that?

Johnson: Amy Darter. [interruption]

Over at the university are the best zoologists and ornithologists in the world. Here you get specimens identified; they have all the life forms collected and stored and indexed at the Museum of Vertebrate Zoology [at the University of California, Berkeley].

At the school of public health, Dr. Bill Reeves was professor of epidemiology, with a field laboratory at Bakersfield, California. He was a graduate of the department of entomology at

Johnson: Cal. Dr. [Deane P.] Furman in the entomology and parasitology department was a world authority on mites. So, in Berkeley you could learn about the general ecology of California.

At the state health department, we would conduct field surveys in the spring. We were often four, five, or six people. We'd be working together in a certain area, trapping and collecting ticks, mites, and fleas. Where I really got into the Colorado tick fever work was seeing the work on plague. I would say that the University of California at Berkeley and the California State Department of Public Health have had a very wonderful association for exchange of information, and there was always somebody to help with identification.

For example, I collected the second bat found positive for rabies in California. I was not certain of the identification, so I took it to Dr. Lloyd Benson at the museum [Museum of Vertebrate Zoology at UC], and he identified it as Myotis californicus.

The state health department having had for many years a Rockefeller project on the respiratory viruses directed by Dr. Monroe Eaton, the virus lab flourished after Dr. Lennette became director in 1947. In 1956, a new building was completed and Dr. Lennette was able to develop a well-equipped virus laboratory. In the meantime, I came with Rockefeller support to study what he was not in a position to do at the time, which was studying nature in general for the arboviruses. The beauty of it was that I could work with the veterinary section and the vector control section, and we would all win as far as getting more information.

Similar vector control studies were done at the departments of health in Texas, New York, Georgia and Alabama. The Rockefeller Foundation Rabies Study at Montgomery, Alabama for a time was the only laboratory in the South that could identify arboviruses. Now there are modern laboratories just to study wildlife viruses. The University of Wisconsin has a big basic laboratory for wildlife virus and bacterial diseases.

Hughes: With no connection with human pathogenicity?

Johnson: Their major concern is disease and die-offs of game birds and helping to maintain good populations of ducks and geese, also deer. Wildlife disease is a specific study now in many universities, often tied in with the veterinary school. Beautiful virus work has been going on in veterinary medicine.

One project I like very much was the Athabasca Field Study in Alberta by the University of Wisconsin, a study of snowshoe hares, which are a wildlife host of the snowshoe strain of California virus. They also isolated eastern encephalitis from snowshoe hares, which is a very significant finding. The short-tail shrew distribution includes Athabasca.

Johnson: I think that sort of explains that you need to have certain laboratories to aid the development of a science. If you have one high tech lab in a city, it stimulates knowledge of other sciences, from mathematics to simple laboratory equipment. Technology is the key. That's why California has been a very fruitful and wonderful place to work.

Hughes: What about the Hooper Foundation?

Johnson: That was similar. Actually, I was thinking of that as being tied into the school of public health. Dr. Meyer was actually in the Life Sciences Building at UC Berkeley before the Hooper Foundation was built. He was a professor on the campus here. The Hooper Foundation was endowed by the Hooper family. Dr. Meyer developed studies of bubonic plague, equine encephalitis, polio, brucellosis, and chlamydia.

Hughes: Were there cooperative studies between the California State Department of Public Health and the Hooper?

Johnson: Yes. Dr. William McD. Hammon, who began his virological studies at Hooper Foundation with K.F. Meyer, became dean of the UC School of Public Health. Bill Reeves was associated with Bill Hammon at the Hooper and moved over to the school of public health with him, later becoming dean.

About the time that I came to California, Dr. Ralph Audy, who was trained in Great Britain, became director of the Hooper Foundation and developed an international program with a field station at the University of Malaya at Kuala Lumpur. I visited their field project in 1966 and the field station working on dengue virus. Ralph Audy died of heart disease. I enjoyed his friendship. We shared a special interest in ecology.

Davis has been another [institution] where we have had cooperative research studies. We tested our live virus western [encephalitis] vaccine there.

Hughes: And there hasn't been a problem with rivalry?

Johnson: Not at all. We exchange information by phone. Anything you find that's of mutual interest you can share. I have given lectures there in Dr. C.W. Schwabe's epidemiology course.

Monoclonal Antibodies

Hughes: Would you say something about the significance of monoclonal antibodies to virology?

Johnson: It was in 1975 that the theory was developed by G. Kohler and C. Milstein. Prior to that we really had very little idea what cells in the body made antibodies. But now we are able to get specific populations of cells, and it was found that the cells that make the antibody are lymphocytes, T-cells from the thymus and B-cells from the bone marrow. I studied lymphocytes in cell culture while at medical school.* Albert H. Coons of Boston originated the antibody staining using the fluorescent group.**

The principle [of the monoclonal antibody technique] is that you have to get a culture of cells derived from a single cell. During my studies of rabies, I learned that we could extract a substance, that is, rabies virus neutralizing antibody from the bone marrow and lymph nodes of immunized dogs that had been perfused with isotonic saline solution. Monoclonal antibodies are produced by cell hybrids of spleen lymphocytes from an immunized animal with myeloma cells which retain their ability to continue to reproduce. Each colony would be the progeny from one cell, one lymphocyte.

Then it was learned that these hybridomas only make one antibody. You have to do some rapid tests to pick out the ones that produce antibody to, say, rabies. This can be done by the radioimmune assay. Then you would test those cells for their ability to produce antibodies, and you'd find these were different. You might have a thousand cell clones, but only, say, twenty-five or thirty which produce antibodies to the rabies virus.

You might have cells that were producing antibodies to the surface of the virus; others would be producing antibodies to the internal structure of the virus. So that immediately made a way of determining the differences in strains of rabies virus. The basic work was done by Dr. Hilary Koprowski and Dr. Tadeusz Wiktor of the Wistar Foundation. Dr. Wiktor died recently, which is a very sad loss. I was very fond of him. I told you that Dr. Koprowski and I used to play two piano music together in New York City.

Hughes: Did you ever consider using yourself as a guinea pig in your research?

*See previous discussion.

**Proc. Soc. Exp. Biol. Med. 1941, 47:200-202.

Use of Humans in Research

Johnson: Oh, the only way we did that in the Rockefeller Foundation was if we needed to take a vaccine to be able to work with a dangerous virus. I don't think anybody in the foundation was reckless in testing vaccines in humans. But, as you know, there were six staff people that died of yellow fever while doing research on yellow fever.

When I came to the Rockefeller Foundation, Dr. Thomas Rivers was making smallpox vaccine using chick embryos as a source of virus. I had a natural infection with smallpox in 1923. So I was given a booster inoculation of the chick embryo virus and developed a severe bacterial infection at the vaccination site. That batch was contaminated with bacteria.

In 1938 I was given the newly developed 17D strain of yellow fever vaccine. I got an obvious disease from the vaccine. I had fever and body aches for a day or two. Now we know you get a viremia with this virus. But at that time, I had to be immunized in order to work in the yellow fever lab. Same when I was going to work with rabies. I had no doubt that I'd have to take the vaccine. And I also would see to it that anybody that worked in the lab was immunized against the virus we studied. Every five years we had to repeat the yellow fever vaccine, as we often visited foci of yellow fever. I didn't like the idea of taking that vaccine repeatedly because I would have an allergic reaction to the chick embryo vaccine.

Hughes: It was Rockefeller Foundation policy that you had to be immunized?

Johnson: Well, it was a WHO regulation for international travel to countries where yellow fever was endemic.

When I went to India, I had to have typhoid, paratyphoid, plague vaccine, cholera vaccine, and Rocky Mountain spotted fever vaccine. Dr. Lennette found that if you repeated the Rocky Mountain spotted fever vaccine too many times, you'd break out in a necrotic lesion at the place that you'd been vaccinated previously.

So there are hazards from vaccination, but you don't blame anybody or yourself; you do the best you know how to save lives. I always said that when it came to rabies vaccine or any other vaccine, you'd say, what would happen if you don't do it? So that's the reason that anybody working in my lab had to be immunized with rabies vaccine. We had a high passage Flury vaccine when I was working with rabies in California. Dr. Emmons and the technicians were vaccinated with a live rabies virus, the HEP Flury Lederle vaccine. It's not used now, but if we ever were

- Johnson: to have an epidemic of the disease where you wanted to have immunity starting right away, I would still recommend it. But we have now some very good killed rabies virus vaccines.
- Hughes: Did the Rockefeller Foundation have a policy on the use of human volunteers in research?
- Johnson: No. It was like with standard bacterial vaccines; you used those that had been approved by licensing the product. Public health procedures are mostly directed at avoiding exposure by controlling food handling, washing hands, and boiling drinking water.

IV MEETINGS, MEMBERSHIPS, AWARDS, PUBLICATIONS, AND
CONCLUSION

[Interview 10: February 19, 1988]

The International Congress of Tropical Medicine and Malaria

Hughes: Dr. Johnson, do you have anything to say about the International Congress of Tropical Medicine and Malaria that you attended at various times?

Johnson: I attended the International Congress of Tropical Medicine and Malaria regularly from 1948 through '73 and usually presented papers. This is where you kept your contacts with all the people who were doing the same [research] around the world, and were able then to find out what they were doing, before it was published, in the control of malaria, yellow fever, and whatever other diseases we were interested in. In fact, very little of that type of work is published. Papers on current problems were presented, and then you'd have group meetings.

The Rockefeller Foundation was much involved in this, because they were the first to plan specific disease-control procedures, which started, as I said, with hookworm and malaria and then later yellow fever.

My contribution to the discussions on arthropod-borne virus infections appeared in the Proceedings of the '58 congress in Lisbon.* A lot of [the congress attendees] had had Rockefeller support for equipment and short-term training in the United States.

*H.N. Johnson. Discussion on arthropod-borne virus infections. Proceedings Sixth International Congresses on Tropical Medicine and Malaria 1959, 5:146-148.

Hughes: Why is malaria singled out in the title?

Johnson: It has been--it still is--a major disease problem. Malaria has come back in India. It was controlled pretty well, but then by relying on insecticides and neglecting the management of surface-water drains, dengue spread by the mosquito, Aedes albopictus, became more prevalent.

The Pacific Science Congress and the United States-Japan
Cooperative Medical Science Program

Hughes: Please comment on the Ninth Pacific Science Congress in Bangkok in 1957.

Johnson: This organization became important after World War II, also the U.S.-Japan Cooperative Medical Science Program. Dr. Lennette was on the panel on virus diseases of the latter organization. A special meeting was held in 1970 on the problem of rabies in the Pacific region. My chapter, "General epizootiology of rabies," was published in the Proceedings.* The war with Japan resulted in a long-time presence of the US armed forces in that country. American bases were built at Guam and in the Philippine Islands.

Japan had a terrible problem with Japanese B encephalitis [JE]. The major medical facility of the United States armed forces in Japan was at the 406 General Hospital near Tokyo. The studies of mosquito transmission of Japanese B encephalitis were done there. Dr. H. Elliot McClure was in charge of the bird studies, bleeding nestling herons at the imperial heronry. They used horses and pigs as bait in traps for mosquitoes. It was a tremendous study of this disease. Dr. E. [Ed] L. Buescher was in charge of virology. James L. Hardy, now a professor at the UC School of Public Health, was a technician at the army lab.

Dr. Masami Kitaoka was head of the National Institutes of Health in Tokyo. He later visited Berkeley, stayed at my house, and we became good friends. I spent over a month in his laboratory and at the 406 army lab in 1957, en route to and from the meetings of the Pacific Science Congress in Bangkok. One of the NIH staff, Dr. Kamasaburo Yoshino, had been in the United States for training. He arranged to spend time translating the Japanese papers on encephalitis, describing the isolation of Japanese encephalitis virus in 1935 and subsequent research. In 1956, there were 4,284 cases of JE, and 1,574 deaths. Those that recovered were apt to have residual paralysis and brain damage.

*Rabies. Proceedings, Working Conference on Rabies, Y. Nagano, F.M. Davenport, eds., University of Tokyo Press, 1971.

- Johnson: The foundation at that time was giving money to the [Japanese] National Institute of Health for laboratory equipment and granting fellowships for training in the United States.
- Hughes: Wasn't there a strong microbiological tradition in Japan?
- Johnson: Oh, yes, excellent. One of their distinguished scientists, Dr. Takeo Tamiya, had completed a major study on scrub typhus. He had been trained in Germany, a scientist much like Dr. K.F. Meyer, the old school. Japan had some well-trained entomologists. There was a good relationship with the United States, and that led to the founding of the US-Japan Medical Science Program.
- Hughes: The name of the Pacific Science Congress implies that it had a very wide focus.
- Johnson: Yes. The 1961 meeting was held in Honolulu. There were sessions on volcanology, oceanography, infectious diseases, and population problems. It's a big meeting and an interesting one.
- Hughes: With simultaneous sessions?
- Johnson: Yes. I went to the volcanology session because that's been my side interest ever since I saw the Paracutin volcano develop in Mexico. I have a choice story: At the Honolulu meeting, they were showing an active volcano in Indonesia. The speaker told about an American scientist he had escorted, who looked over the crater edge and said, "It looks like hell," and the Indonesian said, "Well, you Americans have been everywhere." [laughter]

The American Wildlife Disease Association

- Hughes: You are a member of the American Wildlife Disease Association.
- Johnson: That's [a group] that I've been very much interested in. I felt from the beginning of the arbovirus studies that you had to study nature as a whole before you could find the source of the diseases derived from wildlife. Blood-feeding insects, such as mosquitoes, Simuliids, Culicoides, and ticks, are going to feed on a great variety of animal hosts.

You have to know medical entomology, medical ornithology, and medical zoology. These are taught in most schools, [but] there is little information about disease agents. Dr. Carlton Herman and I were much involved in writing and talking about this and in bringing in people from microbiology and associated sciences, particularly the veterinarians and wildlife control people who are aware of the great die-offs of birds and small mammals of mysterious toxins and infections.

Johnson: Clostridium botulinum toxin will cause tremendous die-offs of water birds. There is so much toxin in the blood of the paralyzed birds that a little blood serum injected intraperitoneally in mice will kill them in less than an hour. Avian cholera caused by the Pasteurella organism was responsible for the death of over 7,000 snow geese and ducks at the Tule Lake and Lower Klamath National Wildlife Reserves in California in November, 1986. There are a variety of other bird diseases. Many of them insect-transmitted--bird malaria, Hemoproteus [infection], Leucocytozoa [infection], trypanosomiasis, and filariasis. They are killers, too.

When I came back from India, I was convinced the vector of the Hemoproteus parasite was Culicoides, which we found in crows' nests in India. The crow nestlings would have this parasite in the blood by age eighteen days. The parasite would not infect the mosquitoes that fed on their blood.

The scientific study was by A.M. Fallis and his co-workers of the Ontario Research Foundation at Algonquin National Park in Canada. They set out one-day-old ducklings in little cages in areas along streams in the Algonquin Reserve. The ducklings would get sick, and they found Hemoproteus parasites in the blood. Then they covered the cages with different size screen. They discovered that the Hemoproteus vector was the little no-see-um, Culicoides. The vector of Leucocytozoa was the black fly, a Simulid, the buffalo gnat. You can use sentinel birds or animals to find out which insects transmit viruses.

Hughes: Ducklings were used because they hadn't developed antibodies?

Johnson: Right. The parent bird has immunity; there's some in the yolk of the egg. It will last for a couple of weeks after the young are born. So there's a bridge in which they can survive in the presence of a parasite. But they quickly lose that [protection] and are then susceptible. But a nestling bird or a little animal without feathers or fur, like baby mice or baby Microtus, is very exposed to insect bites.

Hughes: I know that you have used baby animals quite a bit in your work, including the suckling mouse. Were you one of the first to suggest that they were good hosts?

Johnson: That's not my discovery. Max Theiler in 1930 noted that newly born mice inoculated intraperitoneally with yellow fever virus died of encephalitis.* John Bugher in 1941 showed that up to age ten days the mice sicken and die when inoculated with yellow fever virus by subcutaneous injection.** Ed Lennette applied this high

*M. Theiler. Am. Trop. Med. Parasit. 1930, 24:249.

**J.C. Bugher. Am. J. Trop. Med. 1941, 21:299.

Johnson: susceptibility of three-day-old mice to the neutralization test, especially for WE, EE, and VE viruses.*

The white mouse was not used as a test animal in virology until Theiler reported his use of this animal in 1930. In 1935 the white laboratory mouse was found suitable for use as a test animal for the diagnosis of rabies.** Certain strains of white mice injected in the brain with St. Louis virus or yellow fever virus don't get sick. So there is a genetic susceptibility. But there are selected strains that have been developed which we use which are susceptible to the disease. There is susceptibility to infection without illness, and that's true of birds, too. Some of the most dangerous hosts in nature are the ones that don't get sick but have virus in the blood and so infect the insect population.

Hughes: In 1974 you received the Distinguished Service Award of the American Wildlife Disease Association. Was there some specific work on which that award was based?

Johnson: I don't know. In fact, I was very surprised [to get it]. I was very pleased, because one of the things they said that had been appreciated was my sharing my knowledge with people that do field work or study wildlife, and having been one who tried to get cooperation between the different groups of medical scientists and wildlife-control people, ornithologists, and zoologists.

That still is not done as much as it should be. You need a team. That was realized when we had money from the Rockefeller Foundation, where you could actually hire staff members to work on a problem like yellow fever or, later, arthropod-borne viruses. They were veterinarians, zoologists, and entomologists, and then you would develop a team. That's what we tried to do in the Rockefeller Foundation field programs. The beauty of this, we had people that were knowledgeable in these fields working on vector control and veterinary public health problems.

Dr. Graham Kemp, DVM, of the California Department of Public Health veterinary section, Bureau of Communicable Diseases, and I conducted field tests of an attenuated western encephalitis virus vaccine.*** Dr. George Humphrey of the same division and I in 1960 did an immunity survey of horses in northern California. The horse sera were tested for antibodies to western encephalitis, St.

*E.H. Lennette, H. Koprowski. *J. Immunol.* 1944, 49:175.

**L.T. Webster, J.R. Dawson. *Proc. Soc. Exp. Biol. Med.* 1935, 32:570.

***G. E. Kemp, H. N. Johnson. Immunization of horses with a live virus western encephalitis vaccine. *Scient. Proc.*, 101st Annual Meeting, Am. Vet. Med. Assoc. 1964, 202-205.

Johnson: Louis encephalitis, and Colorado tick fever. That was one way you could see whether these viruses had been present in a region where we had little information about arthropod-borne viruses.

The big thing in the Wildlife Disease Association was to encourage various scientific groups to be involved with diseases derived from wildlife. And what isn't from wildlife? As I've said in some of my ecology writing, when man lived in family units of twenty-five or thirty, very few infectious diseases could persist. Influenza and other viruses causing respiratory tract diseases on Svalbard (Spitzbergen) Islands, north of Norway, would cease soon after the last boat left prior to the long winter season. Influenza runs a very rapid course, and most of the viruses do. Such infections would recur in the spring when the boats returned.

It is only in very recent times that the ability to produce food and store it led to the establishment of large urban populations. The Moravian missionaries that set up missions for the American Indians in what is now called Indiana recorded that if there was a bad winter, there would be deaths of children and old people and often only a few would survive.

Imagine what happened during the little ice ages, 400 BC, 400 AD, and 1300 to 1700 AD, when the so-called barbarians from northern Europe encroached on southern Europe. The great epidemics of bubonic plague occurred during these cold periods. To survive people congregated in countries that had the best food supply. The food supply attracted rats which with their fleas would amplify and spread the plague organism once it was introduced. That is what happened in the 500s and the 1300s. Thirteen hundred was the beginning of a little ice age that started in the 1200s and lasted until 1700. That's when the Viking colony in Greenland disappeared. You can understand how difficult it was for a small population to survive when it was necessary to hunt all the time; if there was an epidemic, there was nobody to bring in the food or even get water.

One of the famous smallpox epidemics in North America occurred in 1619, before the Pilgrims came to Cape Cod.* Smallpox swept through that area, coming up from Florida. There were very few people that were left alive in any village. In fact, there were only a few people that were left in the Wampanoag tribe living on Cape Cod and in what is now Boston. So these villages were uninhabited or limited to a few families. The reason shown later is that even if the Indians were not sick enough to die, they would be unable to bring in water or hunt and fish.

*F.B. Rogers. Pox acres on old Cape Cod. New England J. Med. 1968, 278:21.

Hughes: How was the smallpox transmitted?

Johnson: That was by the release of the smallpox virus from skin lesions, more so probably than from the throat. The dried virus from these skin lesions got into the dust and was viable for a long time. There are many stories how smallpox was started by just handling blankets or clothes that had been used by the people that died. Audubon wrote about one instance which occurred when he was traveling in the central United States. They had a burial of a smallpox victim and warned the local Mandan Indians not to touch the body, but it was dug up, and there was an outbreak of smallpox among the Indians in June, 1837. It was estimated that 30,000 died and only thirty were known to have survived.*

Hughes: Well, getting back to your receiving the Distinguished Service Award, I quote from the presentation speech: "Perhaps his most significant achievement is his artful communication of his concepts to colleagues, students, and other young people."**

Johnson: I don't know who wrote that.

The fun of anything, whether it's a hobby or the arts and sciences, is talking about the work. Anytime you hide information until you've published it, I think that's when you lose all your contacts. At these international meetings, we talk about our work, and how we work, and what can be done. That particularly came out of the Rockefeller Foundation groups, because they were involved in specific projects, knowing they had a limited time to see what they could do with information we already had. It's not so much the discovery of new information as the application of it.

When you come to the wildlife story, we probably will never know the exact way that the virus, rickettsia, or chlamydia survives [in nature]. There are all sorts of chlamydial diseases in birds and small mammals. But under what conditions do they become diseases in man, like trachoma, chlamydial pneumonia, and chlamydial venereal disease? The chances are that very few of these disease agents could have persisted in small [human] populations. But when you build up city populations around the world and have rapid movement of people from one area to another, almost any infectious agent is going to get into people. Even new diseases like Lyme disease have been present but not recognized as a specific infection. The spirochete of Lyme disease looks just like those found in syphilis, yaws, and the disease called "pinta" in Mexico. Perhaps syphilis is an aberrant offspring of one of these.

*M.R. Audubon. Audubon and His Journals, vol. 2, N.Y.: Dover Publications, 1960, p. 48.

**J. Wildlife Dis. 1974, 10:490-491.

Johnson: The Rocky Mountain spotted fever story goes like this: They cut the forest first in New England, and then in Michigan, Wisconsin, and Minnesota, and finally they were cutting down the big pine trees in the Bitterroot Valley of Montana. Nobody got sick then, as far as I remember. Then, as the brush grew up and people went into farming, they noted high tick populations. In certain areas, about thirty percent of the people that moved in there died of Rocky Mountain spotted fever. Now we know it's not a disease just of that area, but the basic organism is present in the southern states, the southeast, New York state, and New England. There are old stories of the spotted fever in Massachusetts in the early 1800s.

As we mentioned before, we really still don't know where yellow fever persists in nature. That's [also] true of St. Louis encephalitis and western encephalitis. It is like Alfred North Whitehead said: There are few questions in science, whether it is about weather, disease, or atomic physics, that you're going to get a final answer to. You can get general types of information, and you can learn to control animal diseases, but you can't control the weather.*

Diseases come and go, and what makes big populations of animals? Well, in the old days, great forest fires would upset the natural balance. Later man did this when he cut down the forests. There was a forest fire in Russia in 1915 that burned for three years, and they hardly saw the sun during that time. The trans-Siberia railroad probably started this fire from the sparks of the engines. That probably had a lot to do with tick-borne encephalitis in Russia, because when an area is reforested, that's when you get the big population of ticks. Abandonment of agricultural land during World War I and World War II produced the same result.

In the United States and Canada, there were forest fires in the earliest times, but many of them did not succeed in burning down large areas, because some of them were very wet forests. But people used to say, "Well, it's man-made fires," but anyone that's lived in the rural areas of the United States knows that lightning starts fires in all sorts of conditions. When I was working in Modoc County, California, we were at a forest station where they

*"Nature appears as a complex system whose factors are dimly discerned by us. But, as I ask you, is not this the very truth? Should we not distrust the jaunty assurance with which every age prides itself that it is at last has hit upon the ultimate concept in which all that happens can be formulated. The aim of science is to seek the simplest explanations of a hypothesis, but if you think you have, distrust it." (A.N. Whitehead. The Concept of Nature.)

Johnson: spotted something like ninety fires from lightning in one day. Some of those will just smoulder in one tree, and only if they persist will the lookout send out a team. Now they even parachute in rangers if it looks like the fires are going to take off. I guess this last summer [1988] was one of the worst for forest fires. So that is a natural event.

When you burn out large areas, like occurred in Canada in the 1800s where literally thousands of square miles were burned, then you get a big ecological change, a hundred-year cycle of reforestation. It goes through grass and brush and softwood trees, such as chokecherries. Finally you get the forest re-established pretty much the way it was to begin with (climax forest). But it takes a long time. And during that time you have a succession of animal populations.

The primeval forest is pretty quiet. It is not populated by small mammals and birds, like the edges of forest where you get insects, grass, nuts, and fruit for birds to eat. What ecology is all about is to study the natural factors that produce large populations of wildlife. Wildlife maintains all the basic viruses and bacteria. The famous example is bubonic plague. Where was that all the time? It looks like the high desert is the natural home of bubonic plague.

Hughes: In what animals?

Johnson: In the USA it's the kangaroo rat, the *Dipodomys* genus, the gerbil family. In India it is *Tatera*. Same with South America. The *Meriones* in the Middle East are very much like kangaroo rats. They have one genus in South America called *Grayomys* that look like a gerbil and I suspect it is the wildlife host there. These animals have a similar ecology. They live in high desert environments. When injected with the plague organism, some of these species get sick; others get infected but remain healthy. That's what you would expect.

I visited the WHO plague field lab in Akinlu in northwest Iran in 1968. It's in the Kurdistan area, where plague broke out in November, 1947. There were no rats of the *Rattus rattus* genus. The rat is supposed to be the endemic and permanent host of the plague organism, because it dies in great numbers during epizootic plague. Theobald Smith said, if you find a parasite infection of any host, it better not kill it because [if it does] then the disease agent won't be able to survive.

Other Memberships

Johnson: In New York, there was an organization called the Bug Club, a local group that met regularly to discuss current research in bacteriology and virology. It had the leading scientists of the Rockefeller Institute, the New York City Health Department, and the New York City Research Laboratory. It was a casual group, a sort of information exchange.

The New York Academy of Medicine is a real active one. They have regular meetings, and I enjoyed that because I also belonged to the medical society there. The beauty of the New York Academy of Medicine is their regular meetings, which they still hold. I still have a membership. The New York Academy of Medicine Bulletin always has very good articles.

Hughes: Well, the last organization with which I understand you were associated is the Alameda-Contra Costa Medical Society. Did you do anything particular with that group?

Johnson: No. It is a working organization of medical doctors, with no salaried officers, that sponsors correct certification of physicians and supervision of medical practice. I have been an active member in the local medical societies wherever I worked. You keep up on your medical training, and that way you know what's going on. Now I take part in the continuing medical education programs at local hospitals. We have to attend twenty-five hours of such meetings each year to keep our license to practice.

The medical society is able to present problems in disease control to the state legislature. I took part in that in California when we were trying to inform the legislature about rabies control and what needed to be done.

Hughes: Would you testify?

Johnson: Oh, yes. Health departments will have people with more or less specific tasks. Dr. Ben Dean, who was chief of the veterinary section of the Bureau of Communicable Diseases in California, was very effective in dealing with the legislature. Dr. George Humphrey organized the control work and was absolutely excellent at that. Later he took over as chief of the veterinary section.

If you present to all the various interested groups of people what the problem is, then you can do something about it. But they have to know why. For instance, you need to say why you need to quarantine dogs, vaccinate them, or get rid of stray dogs. The Society for Prevention of Cruelty to Animals has to be involved, so you can explain what the problem is. You have to

Johnson: inform all the public. Newspapers, of course, are one of the best ways of getting information. If you ever want to find out what's going on in health in the country, read the newspapers.

Hughes: Were you ever aware of any prejudice when you were a member of a group, such as the Alameda-Contra Costa Medical Society, stemming from the fact that you were a public health physician rather than a private practitioner?

Johnson: No, not if you join the medical society. I think that Dr. Lennette agrees with me that if you are a member of the CMA [California Medical Association] and hold a medical license for that state, you will benefit from it [by learning] what's going on in clinical medicine. I think that most people engaged in public health work, such as epidemiology, will join, but some do not want to pay the dues. But membership gives you an entry into the practice of medicine.

In India, I met with the medical society there, and I was very active in the Council of Medical Research and the Armed Forces Medical College, which was a relatively new department. I was on good terms with them.

You can call upon the medical society for cooperation in control work, as was done in California with the Sabin live virus vaccine campaign from September, 1962 to March, 1963. The physicians and nurses gave their services free of charge. Those vaccinated could donate some money by putting cash in a container at the clinic. After paying for the vaccine, about \$70,000 was left which was donated to local hospitals. There was an outbreak of smallpox when I was in New York City in 1947. Anybody that had a license to practice helped vaccinate. You had to carry out the vaccination work rapidly.

I don't think that anybody that has a medical license [will experience prejudice]. I think the problem would be that MDs that are strictly lab oriented or teachers but not licensed would not have much social contact with the practicing doctors.

Hughes: Well, I asked that question because I know that Kaiser Permanente doctors were not always allowed membership in local medical societies.*

Johnson: I do not know that to be true.

*See the interviews with Drs. Morris F. Collen, Ernest W. Seward, and others in the oral history series, The History of the Kaiser Permanente Medical Care Program, Regional Oral History Office, The Bancroft Library, University of California, Berkeley, 1986-1988.

Johnson: I withdrew my membership in the New York State Medical Society because they were going to assess all members for a fund to fight socialized medicine. Dr. John Fox and I were members, and we did have licenses to practice medicine in New York, and that was not an issue. I said, if they wanted money to study how to provide better medicine, then we'd be willing to give, but not for the other purpose. So both Dr. Fox and I for a time withdrew from the medical society.

Hughes: Did you tell them why?

Johnson: Oh, yes. Perhaps that question came up in California. I didn't hear about it when I came in 1954. I lectured on rabies at Kaiser Permanente for their medical staff. I gave lectures annually at UC, San Francisco, and several times at Stanford, the University of Southern California, and UCLA.

Hughes: You weren't aware of any prejudice against Kaiser?

Johnson: No. The employees on my budget could choose either Blue Cross or Kaiser Permanente [for health coverage].

I believe that we should have private, university, city, and county hospitals. Free medical care should be available for the indigent at the last three. Health plan hospitals, such as Kaiser Permanente, Associated Hospital Service of New York, Baltimore Health Insurance Plan, and Harvard Health Plan, based on insurance contracts with or without management by the Blue Cross Plan, are the major sources of acute medical care.

Hughes: Did the New York plan last?

Johnson: The Rockefeller Foundation gave \$250,000 to help organize the Associated Hospital Service of New York, a nonprofit community service using Blue Cross, sponsored by 250 hospitals in the New York area. I was covered under this plan until my retirement, when I became eligible for social security health insurance. Additional private insurance coverage was provided by the Rockefeller Foundation. The Rockefeller Foundation also assisted in the organization of the Baltimore plan.

One feature has been lost in the development of medical care, that is, the county health clinics and hospitals. They are a good place for providing medical care for everyone. I worked at Kern General Hospital in Bakersfield, California, during 1954-1957. They had the contagious disease service and there is where the suspected encephalitis cases were admitted. It was an excellent hospital.

Many local hospitals which we aided by the Hill-Burton Act have been closed, because the gravitation now is to larger hospitals where they have more facilities. But in all events, the

Johnson: basis [of medical care] should be the county and state university hospitals. They should be involved in any kind of free medical coverage.

The Importance of Publication

Hughes: Well, I would like your views first of all on how important you think it is to publish, and secondly, how easy was it for you to publish?

Johnson: Well, several people that have become quite famous in research of all kinds published very few papers. You can go back to people like Einstein. In this so-called "publish or perish" practice, a lot of papers become not really research [reporting] but almost newspaper reporting on tests. It's almost like publishing polls. The fact is that [publishing] is the way you get support.

The nice thing about the Rockefeller Foundation, and the Howard Hughes Foundation is now doing a similar thing, is you select people that are just finishing their training in medicine, and then you give them a good salary and expenses for their work. So there is no competition for publication, and there never really was for me. They used to fuss at me for not writing up current [research], which we always shared. I think what is important is to share information openly. I am very much against keeping medical studies secret.

Hughes: Who was fussing?

Johnson: Well, a lot of people in any type of research try to keep secret how they do things, like making vaccines or developing a new theory. The Rockefeller Foundation never patented anything; I did not even hold a copyright. That's what led to the difficulty in maintaining the quality of certain virus seed strains.

Hughes: Because they felt that everything should be--

Johnson: Available. For example, the avianized Flury vaccine virus was made available to any firm in the United States and other countries that wanted to make it. The trouble was that Lederle withdrew in '67, because it was costing more to make the original chick embryo LEP (low egg passage) Flury vaccine than to make tissue culture vaccines. After all, the dog rabies problem had ended by 1967. But then many of the biological companies were making vaccines which had potentially pathogenic viral strains. In others, the virus was dead. Donald Dean in the New York State Health Department tested vaccines from some nineteen companies,

Johnson: and only a few of them had enough viable virus to immunize against rabies. So this is the danger. There's no way of assessing the relative pathogenicity of some of the live virus vaccines.

Today in the United States, I and many others would favor not having any live virus rabies vaccine for dogs, because of the strains that have been used and changed because of secrecy and [the drug firms] wanting to have a patent on their particular variety of strain. Now you can always get the old Pasteur strain in Paris. That's a rabbit-passed virus originating in a rabid bovine in France. The danger in certain countries like the United States is losing the old Pasteur strain because there is a lack of continuity in passage history. That's been countered to a large extent by developing national storage places for viruses and bacteria--national type strains, we call them.

Hughes: Where are those storage areas?

Johnson: Most of the type culture collections are in Washington, D.C. and Maryland.

Hughes: Well, getting back to the question of publication, was there any pressure from the Rockefeller Foundation to publish?

Johnson: Not really. The big thing that we had to do was submit reports. I would report the results of current studies. I'd send in reports as certain experiments were going along, and that information would appear in the annual reports of the Rockefeller Foundation. One way to publish such results was to present a paper at a meeting, which was to spread the word about what was new and interesting. That would be published in the proceedings, and I liked that.

Hughes: When you became associated with the California State Department of Public Health, did the department feel it was necessary to publish?

Johnson: Well, again, my technique anywhere I go is to supply reports. Dr. Lennette was director, and I regularly reported to him. Certain ones would go into their reports. One of my early ones was in California Health.* It's up to the director of a program, whether it's epidemiology or the laboratories, to put what is significant for the public into their regular reports. Now you can find out by computer transmission what diagnoses of viruses are made all over the state. Results of tests for viruses and antibodies in serum specimens are reported as soon as obtained to the doctor submitting the specimen.

*1965, 23:35-39.

Johnson: So it depends on whom you're working for. I think that one of the problems with the university is that if you're working for a professorship, and you're going for tenure, you may want to achieve positions of power. Such people tend to move more often and press for publication. I think that's been overemphasized. It's not necessary to be the one that publishes the most, but it's how well you teach.

Research Grants

Johnson: The Rockefeller Foundation's grants were so different, because they usually were [awarded to] people that were doing good work in public health. Most of us never got our names in the newspaper, but had adequate support. Now, the grants given by the NIH tend to be compartmentalized, and you get people that tend to perpetuate a certain type of study, and new people in the field may not get support. I think the big thing is to pick really dedicated people to work in a learning situation, and then they can develop new studies.

Hughes: Are you arguing for longer-term grants?

Johnson: Well, I would like to see grants go through health departments and universities where it is possible to encourage research by those interested in studying specific problems.

I do believe that information exchanges [newsletters] are the way to go in science research. They are present in almost all fields, including astronomy and mathematics. When you get a committee of professors deciding who can publish, there is an inner circle, and people that have no monetary or political power will have difficulty in getting papers published. There is one type of editing that's good and that has to be done. Dr. Lennette was a very good editor, and we need that. Those that get on editorial boards are sometimes resistant to new ideas. It's not good.

Hughes: Did the fact that you were never an NIH investigator pose any problem in getting grants?

Johnson: It was not a problem for me, because I was employed by the Rockefeller Foundation, International Health Division, and it always worked through university, governmental, or state health departments. The National Institutes of Health and the National Science Foundation became the major source of research grants in the United States when the Rockefeller Foundation stopped supporting projects in the United States. The Rockefeller Foundation gave a large grant to the National Research Council, and this was to become the source of grants in the United States.

Johnson: We had field directors in the foundation who had a large view, whatever field was involved, whether it was in education or not, and they would select candidates for grants. The foundation continued to provide grants in the international field. They would pick the people to work in the United States. All staff members were involved in recommending candidates.

The NIH became a conglomerate of institutions. It is for solving specific problems like the national institutes for stroke, heart disease, dental research, genetics, and cancer. Originally, the National Institutes of Health were more or less like the foundation. You had one person with very wide knowledge of a particular field, and he'd be head of, say, the mycology department, like Dr. Emmons' father was, another studying viruses, like Dr. Armstrong, one of the first to isolate LCM virus, and then Dr. Karl Habel in charge of the rabies lab. They had relatively small staffs. Since the NIH developed into these large-scale institutes, they are often just testing drugs and organizing information. Whoever's head of the institute will determine what it's going to do, with not much opportunity for young medical doctors to develop original ideas.

I'd like to see the National Institutes of Health money go to the universities directly, where the teachers would choose new graduate students, so they would have plenty of money for postgraduate research. Now the postdocs from the universities come to the lab here to work in fields--like we used to in arboviruses--where you get people from zoology, ornithology, and microbiology to work together at a health department or in departments of microbiology and epidemiology.

Each health department has a vector control department, and I'd like to see people with NIH grants working in vector control, the veterinary section, and microbiology, so that you would tie these fields in more, rather than all seeming to be in molecular biology. [Nowadays] you don't study trees, but you study the chemistry of the leaves, or something like that, rather than looking at the large natural mystery of biology generally.

The big problem in medicine now is disease related to cultural inheritance. So much of our disease is sort of home-grown, and it's not being dealt with properly. There is no reason why anybody in the United States can't have a good diet. We have frozen food; we have fresh vegetables of all kinds; you can move food around the world rapidly. So there is no reason why people can't have a good diet. But that doesn't mean they will have it. The type of eating today is in many ways not conducive to a good diet. But, of course, the worst thing is the smoking and drinking.

Foreword, CRC Handbook Series in Zoonoses, 1981

Hughes: I want to turn to the foreword that you wrote for the CRC Handbook Series on Zoonoses.* How did you come to write that?

Johnson: They asked me to write it. [laughs] That was true also for my chapter, "Natural ecology" in Methods in Virology.** [A foreword] is an opportunity to give a general view of problems. You get only experience from seeing all the mistakes. In the foreword of the CRC Handbook Series in Zoonoses, I begin with the quote, "It is not the things I don't know that bother me, it is the things I know that aren't so." (Anonymous)

We've talked about the problems you can get into in research where you think you've found a new virus; perhaps there is a hidden virus in your animals or in your cell culture systems. It's amazing how you can have a cell line, and you're working with one virus, and you think you're working with just this new one, but you have [another] one already in the cell line.

For instance, parainfluenza 3 was present in a widely used swine kidney cell line. It was used as a standard cell culture line for a long time before they knew they had para-3 in it. Even several of the mosquito cell lines have been found to be infected with laboratory strains of viruses. I think it's largely from using mechanical pipetting devices. You get aerosols, and certain viruses, if droplets escape and dry quickly, will remain infectious for a long time at room temperature.

Hughes: Were you one of the first to warn of the danger of these unknown infections in laboratory animals?

Johnson: I made a note here about when I began to study rabies in Alabama. It's a good example. Dr. Dalldorf and I both got involved with a virus problem. I wanted to get a good distemper vaccine for dogs, so I wrote Lederle Labs to send me some dog distemper virus from

*Foreword in volume one and two, CRC Handbook Series in Zoonoses, G.W. Beran, section editor, J.H. Steele, editor-in-chief, Boca Raton, Florida: CRC Press, 1981.

**K. Maramorosch, H. Koprowski, eds. New York: Academic Press, 1967. Dr. Johnson adds: "I wrote chapter I, 'Natural Ecology': 1. The Ecosystem, 2. Natural Fluctuations in the Ecosystem, 3. Parasitism and Disease, 4. Geographic Distribution of Disease and Natural Ecology, 5. Field Study Methods, 6. Comparative Studies of Viruses."

Johnson: the spleen of a dog. It contained distemper virus, because it produced distemper in dogs. But that particular pool also had lymphocytic choriomeningitis virus in it. I tested all such specimens in mice, and when the mice came down with a certain pathology, I realized that this was probably lymphocytic choriomeningitis.

Well, who knows how it got into the dogs used for passing the distemper virus. But it wasn't just myself. Dr. Gilbert Dalldorf, who later became famous for working with the Coxsackie viruses and polio, for some time studied dog distemper virus in monkeys, thinking that it was related to measles. Actually, dog distemper is very closely related to measles virus and also to rinderpest of cattle. The problem was, he produced a disease in monkeys, and it turned out that the encephalitis was from lymphocytic choriomeningitis virus and not dog distemper virus.

Remember when pet hamsters in the United States and Germany were found to be infected with lymphocytic choriomeningitis virus in 1973-1974 and many persons were infected with LCM virus from handling hamsters? The hamster colonies in the United States and Germany were undoubtedly infected by LCM virus from house mice. They are the natural host. In the 1940s, Dr. Beatrice F. Howitt isolated lymphocytic choriomeningitis virus from the spinal fluid of a young man living in a fraternity house in Berkeley.

The new Department of Public Health building on Berkeley Way was in the process of building in 1955 but was not completed until 1956. However, in October, 1955, we moved our mouse colony from Acton Street Lab to the new building. We took special precautions to see that all the ducts were screened in order to prevent entry of wild mice. In December, 1955, I made three isolates of LCM virus from mice injected with bird bloods. We found out later, in sweeping, the grills of hardware cloth had been displaced and wild mice had entered. Other isolations of LCM virus were made from March to April in 1956. It was necessary to destroy the mouse colony and start another. In 1956 we sent the LCM virus to Dr. Whitman in New York, and he injected three different genera of mosquitoes with the virus. The virus did not multiply in mosquitoes so it could not be spread by mosquitoes.

I believe that every big research facility in the world is subject to invasion by house mice. They do seem to get into trouble with lymphocytic choriomeningitis. So you learn by experience how you can safely work using animal hosts, and then try to be sure that whatever you're using as a host for virus is not naturally infected with a latent virus.

One of my children had measles when we were living in New York City. I tested a throat swab specimen by inoculating chicken embryos, and we obtained a virus that hemagglutinated red blood cells. I made inquiries about Newcastle disease, and Dr. F.R.

Johnson: Beaudette said, "Oh, they've had a Newcastle disease outbreak in some of those hatcheries." So the chick embryos were infected with Newcastle disease virus coming through the egg. So the virus was not measles virus and did not originate in my child.

[There are] various viruses now which we cannot isolate in the usual test systems, but you can see them with electron microscopy. Even some of our cell lines, like the hamster kidney cell lines, have viruses which look like cancer viruses.

So it is very difficult because in any system you work, any animal you use, has its own complement of viruses. House mice have herpes viruses, adenoviruses, and mouse polio viruses.

Hughes: How does an investigator work around those viruses?

Johnson: This is the problem. It turns out that Pasteur chose a very good method when he passed rabies intracerebrally in rabbits, because then you bypass the intestinal and respiratory tracts, and you tend to concentrate whatever you're passing in the brain. But you can get into other things. The first reported isolation of chicken pox virus turned out to be herpes virus of rabbits. Well, chicken pox is a herpes virus. It produces the same type of inclusions.

So whatever you're studying, be alert for hidden viruses that may replace or hybridize with the viruses you are studying. In our studies of yellow fever, on one occasion this virus was replaced by mouse polio virus. The incubation period was about the same, but it was not neutralized by the yellow fever immune serum.

In St. Louis in 1933 during the epidemic, one strain of virus was different from others. That turned out to be lymphocytic choriomeningitis. Whether a monkey was naturally infected with the lymphocytic chorio virus, or it was actually from one of the patients, we will never know.

When preparing biological vaccines, there has been incidental infection, which is not necessarily any fault of the investigator. But you must be alert to the system you're using for making the vaccine. An example is the polio vaccine, which had SV40, a cancer-related virus, in the vaccine. It was harder to kill, actually, than the polio virus. Of course, the big disaster with the Salk vaccine was when it was supposed to be killed and it had residual live polio virus. In the original field tests in Pittsburgh, the immunity rate was evidently high. But when they started using the vaccine in California, Idaho, Nevada, and Montana, a high percentage of the kids were nonimmune and developed vaccine virus polio a week later.

Johnson: Any cell system that you're using [could contain extraneous virus]. Just think if you were using one that contained AIDS virus or hepatitis B virus. We had no way of testing for these in the 1950s.

Hughes: Getting back to the foreword for the handbook, I want to quote you: "Scientists, physicians, and veterinarians must be open to new ideas about animal-human disease interrelationships, but must be skeptical of all theories regarding the way viruses survive and spread in the natural environment."* Would you enlarge on the reasons why you think skepticism is called for?

Johnson: Because we'll probably never really know where many of these viruses come from. The famous example is foot and mouth disease (FMD) of cattle.

This disease occurred in the United States in 1924-1925 and again in 1929, also in Canada in 1952, and in Mexico from 1946-1954. I became involved with the FMD problem in 1948 while serving as a consultant to the USDA during planning for the Plum Island Animal Disease Center at Greenport, New York. The outbreak of FMD in Mexico in 1946 was a serious threat to the US livestock industry. Given the necessity of using quarantine and slaughtering cattle on affected premises, it was necessary to destroy more than a million head of cattle. When I was in India during 1951-1954, I saw the effect of FMD virus and rinderpest virus on the cattle. We had to use water buffalo milk rather than cow's milk.

The recent classification of FMD virus as a rhinovirus of the picornavirus family reminds me of the isolation of rhinovirus no.86 from a throat swab specimen taken from me in December, 1961. I had an acute "cold" and suspected that I was infected with one of the influenza viruses, but the tests in chick embryos failed to reveal any hemagglutinating viruses. I kept the specimen in the dry ice box hoping to have a chance to test it later in other cell systems.

When Dr. Jack Schieble began working in the respiratory disease section in 1964, I asked him to test the 1961 specimen in human lung cells. It proved positive for rhinoviruses, and when he sent it to the WHO reference center at the Children's Hospital at Columbus, Ohio, it proved to be a new strain. Jack asked me where I had been traveling just before I got sick in 1961, so I checked my notes and told him that I had been in Berkeley at that time but had a delegation of Russian doctors visiting the laboratory. We laughed and said it must have been a Russian virus.

*Unpaginated, fourth page.

Johnson: There are horse and cattle rhinoviruses, the latter closer antigenically to the human strains.

Before leaving the rhinovirus story I should mention that the rhinoviruses account for about forty percent of the identified agents found to cause common colds in humans. They are found most frequently in children and seem to be transmitted primarily by contact infection, where the virus is on the fingers contaminated from nose and mouth secretions.

The rhinoviruses are one of the major groups of viruses in the picornavirus family, which includes the enteroviruses called Coxsackie A and B. There is a disease of children called hand, foot, and mouth disease. It is caused by Coxsackie A virus, and the symptom complex is similar to that of FMD in cattle. The veterinary pathologists have described a heart lesion in that disease of cattle. It is called tiger heart because of the blotches of gray or yellow on the heart surface. The heart lesion appears to be the major cause of death in FMD of cattle. This reminds me of the carditis pathology observed in Coxsackie B virus infection in children. I believe that Coxsackie B viruses are the primary cause of myocardial infarction in humans and pericarditis in addition to pleuritis (devil's grippe).

What about domestic animals and Coxsackie viruses? Lundgren and his associates isolated Coxsackie B1, B2, B3, and B5 viruses from dogs and studied the disease produced in dogs by experimental infection with Coxsackie B1 virus.* What about other domestic animals and human enteroviruses? Verlinde and his co-workers described the isolation of Coxsackie B5 virus from swine.** There is a vesicular virus disease of swine which is similar to FMD clinically. It has proved to be closely related to Coxsackie B5 virus.

I mentioned rinderpest virus as one of the diseases of cattle in India. That virus is a paramyxovirus related to human measles (rubeola) and canine distemper. Other members of this group of viruses are the parainfluenza viruses 1, 2, 3, and 4. Parainfluenza virus 3 is the cause of shipping fever in cattle as well as being one of the croup-related viruses found in humans. All of these paraflu viruses have a wide host range and may be found in various laboratory animals.

Then there is the mumps virus and respiratory syncytial virus, the latter known to infect cattle as well as causing croup in children. Pneumonia virus of mice is in this group, and serological studies indicate that this virus does infect humans.

*Proc. Soc. Exp. Biol. Med. 1968, 128:463.

**Nederland T. Geneesk, 1958, 102:1445.

Johnson: I should mention also that Newcastle disease of chickens is caused by a paramyxovirus. I isolated this virus from a cuckoo bird in India, Eudynamis scolopaceus.

Wild primates can be infected with the three types of polio virus of the picornavirus family. The tedious experimental studies required to expose other wild animals to polio viruses and then take samples of feces over a period of several weeks to determine whether there is an incubation period and evidence of production of new virus is not likely to attract students looking for a research project for a M.A. or Ph.D. degree.

From this long expose of related human and animal viruses, we are left with the question of which species is the long-term natural host. Most likely where they do the least harm. With the means for rapid spread of viruses in today's worldwide society and the penchant which some people have for cuddling up to strange wild animals, one can readily understand that we will sooner or later get infected with every bacterium or virus of wildlife to which we are susceptible.

Vesicular stomatitis virus (VSV) appears once in a while in cattle, and I'm wondering if it could come from *Microtus* mice, where I got Klamath virus which is very similar in its appearance. There has to be a source in nature for VSV. The veterinary medical profession is able to work with domestic animal viruses better than MDs can, because they're dealing with pets and other domestic animals and birds.

We wonder about the origin of the human parvovirus (HPV) and whether it may be related to the parvoviruses of domestic and wild mammals. We are familiar with the human adeno-associated parvovirus, a defective parvovirus that requires a helper adeno-associated virus. It commonly infects humans and is found in clinical samples of throat washings, eye swabs, and feces but does not seem to cause disease in humans. An outbreak of erythema infectiosum (fifth) disease in England was shown to be serologically associated with a human parvovirus infection. It was already known that HPV infection was responsible for the aplastic crisis in children with sickle cell anemia.

I have previously mentioned my concern about the retrovirus that causes Rous sarcoma in chickens and whether that virus could be related to human sarcoma. In my studies of a Rous sarcoma strain that I isolated from chickens we were using in the study of avian malaria, I found that this virus would produce an encephalitis when inoculated intracerebrally in one-day-old chicks. We know that the human retrovirus that is responsible for AIDS causes encephalitis, especially when transmitted from mothers to their babies. Then there is herpes virus of chickens. That's the Marek's herpes virus which causes avian neurolymphomatosis.

Johnson: We have several herpes viruses; herpes I called herpes simplex, herpes II which is venereal herpes, cytomegalo herpes virus, chicken pox herpes (varicella), infectious mono[nucleosis] which is caused by Epstein-Barr virus, and Burkitt's lymphoma virus. Well, did they all start with man? Did AIDS start with man? I strongly suspect that we are aberrant hosts for these viruses. I have yet to work with any mammal species which does not have its own strain of herpes virus. The wild rodents have their own herpes viruses, adenoviruses, enteroviruses, and paramyxoviruses.

Where does influenza A come from? One of my interests is avian influenza A found in terns in South Africa, fowl pest chicken influenza A, and turkey influenza A. We know you can isolate Hong Kong virus A from ducks in Canada. We wondered why Asian flu started in south China. Well, that's where they raise ducks in special ponds and they have been found infected with the influenza A Asian strain. Whether the duck is an amplifying or reservoir host of influenza A is not understood. There is an influenza A virus of horses, and swine influenza A. Cell lines from insectivorous bats are susceptible to influenza A and B viruses.* We should test lung specimens of insectivores and frugivorous bats for influenza viruses.

Conclusion**

[Interview 12: March 18, 1988]

The Ecological Approach to Man in Nature

Hughes: Dr. Johnson, in 1968 you presented a paper entitled, "Disease related to cultural inheritance," at the First American Institute of Biological Science [AIBS] Interdisciplinary Meeting on Environmental Biology, held at the University of Wisconsin. How did people at the meeting receive your paper?

Johnson: My paper was well received and I had many requests for copies. I expected that it would be published in Bioscience. I expected some editorial change and prepared a revised edition, but it was not published, though other papers were published. I am interested in writing a book on disease related to cultural inheritance.

*H.N. Johnson, J.H. Schieble. Proc. Soc. Exp. Biol. Med. 1970, 135:228-231.

**Dr. Johnson rewrote the transcript of interview 12. The result includes a summary of his major scientific concerns and his thoughts on some new topics.

Johnson: It was a most interesting meeting. There were several hundred in the audience when I spoke. The central theme was, "Eco-systems, evolution and revolution." The first session was introduced by the chairman, Herbert H. Ross. His subject was, "Man and nature." The afternoon sessions on marine biology and on the physical environment were held separately. The sessions on the next day were called, "Remote sensing in ecology" and "Algal nutrition and biochemistry."

Here are portions of my paper: The ecological approach to the subject of man in nature is to study humans in relation to the whole environment, the ecosystem. Man has been given the capacity to think and wonder about nature. The natural beauty of the universe is evident to all who study it. One of the most thrilling experiences of life on this planet is to be in a place of great natural beauty on a clear night and lie in a bedroll in the open and watch the stars wheeling overhead and to think about the universe and about the earth on which we live. It is difficult to enjoy sunrise in the city; the sun usually is well up in the sky before we can see it. But when we look at the sun as it appears over the horizon, we are reminded that we are living in the atomic age and that we are watching a controlled hydrogen fusion process. Why should we not postulate that there is a hydrogen fusion in the deeper layers of the mantle of the earth and this is what produces volcanism? I spent an entire night in 1944 watching the eruptions of the volcano Paracutin in the state of Michoacan, Mexico. This gave me a life-long interest in volcanos. They are the source of water and the various elements necessary for life on earth. Volcanic sand is the ultimate soil source. We can expect that the earth has increased in size as hydrogen is transformed into the various elements, and volcanism and fissuring have caused the continents to move apart.

The animal kingdom is dependent on the vegetation type and in turn the vegetation type is determined by the temperature, precipitation, and solar radiation, and the constancy or variability of these factors. Certain regions have a rather uniform climate because of the constancy of the temperature and precipitation, for example, the great equatorial tropical evergreen forests. On the other hand, the mountain ranges and inland plateaus are subject to a great variability in temperature and precipitation. This, in turn, results in variation in the wildlife species.

We are familiar with the way successive storms carry moist air masses against the mountains and how the air mass is deflected upward into colder air, resulting in precipitation. On the other side of the mountain there is a rain shadow as the air mass proceeds inland at a more constant temperature. Thus we have moist grassland, marshland, and forest on the coastal side of a mountain range and dry grassland and desert brushland in the inland valley. Farther inland, a higher mountain range associated

Johnson: with a large plateau will have rain and snow where the moist air mass again is deflected upward. There will be flooding of the valleys during the rainy season and when the snow melts. On the other side of the mountain ridge there will be irregular woodland of pine and juniper alternating with sagebrush and grass desert on the plateau. The succession of storms at one season is followed by a dry season. Similar conditions are found on all the major continents.

Moving from north to south in the northern hemisphere, we find vegetation zones of treeless tundra; northern coniferous forest or taiga with stands of birch, alder and aspen; temperate deciduous forest, grassland, and brushland. As we approach the equator, the grassland and forest become more lush and there is greater variability of species, although the vegetation looks more uniform at a distance. We find similar vegetation zones at different elevations. If we ascend the mountain, we will pass through the various vegetation zones observed in the northern hemisphere, and with each 1,000 feet of ascent it is like moving 170 miles to the north, other conditions being equal. The angle of declination of the ground to the sun has a profound effect on the vegetation. This can be observed when we compare the southern exposure to that of the northern exposure of a mountain valley. The same differences can be found in hills and rock rims. In this way the topography brings about the development of a great variety of biomes.

Where do we find the vegetation types which favor a large and relatively stable population of plants and animals which would be suitable for human habitation? The semiarid plateaus and mountain valleys of the temperate zone with forest, park woodland, grassland, and desert brushland do have a large population of wildlife. When man lived as a food gatherer and hunter, he liked this environment because of the abundance of game and a great variety of plant food. In dense deciduous or evergreen forest, there is little ground vegetation and there are no cereal grasses or other plant foods which attract herbivorous animals. The moist, dark floor of the tropical evergreen forest is very quiet and it is difficult to find any animal life.

This is a general description of the ecosystem. The subject for discussion is disease related to cultural heredity. Perhaps it is best to begin by trying to define disease. The definition in the English dictionary is: Illness, sickness, interruption, or perversion of function of any of the organs, a morbid change in any of the tissues or an abnormal state of the body as a whole, continuing for a longer or shorter period. We also must consider a definition of health. This is defined as absence of disease, a condition of body and mind in which all the functions are normally performed. It seems that disease or health is a relative state.

Johnson: It is like trying to define freedom or democracy. You may feel ill and yet be healthy when compared to a person who says he or she feels fine.

The major problem for modern man is to be at peace with self. In today's urbanized culture, people tend to be constantly with other people, talking or listening to others, listening to the radio or watching television. There seems to be a fear of being alone. When man is alone, he has to think about why he is in this world, whether there is a god or whether this complicated universe was assembled in a series of accidental events. If we conclude that there is no god, we have to find a purpose for life. If we believe that there is a god who planned the universe, we have to determine our relationship to him. There is a tendency in urban culture to deify man because so much of what we see was built by humans, and it is difficult to see the natural beauty of the world.

We must also define cultural heredity. This is all experience acquired after birth, that is, put-in information as compared to instinct or built-in information. Without cultural information there is no speech, singing or writing. Our brain, that marvelous computer, stores all information we experience. We cannot zero out this computer so we have to keep the bad information as well as the good. This contributes to our frustration because we are exposed to a great deal of both good and bad information compared to the era before the development of printing and the various mass information media.

When we think about a subject such as disease and culture, we naturally ask questions such as why, what, where, and how. Why do we have disease? What diseases do we have? What causes them? When did they arise? Where do they come from? How do they spread? What diseases are the result of man's culture? How long have we had a culture? I suppose we should begin with the last question. From what I can learn, our culture goes back about 10,000 years. We have had written information for about 5,000 years, but there is little information from the period prior to the fifteenth century. How was man living 10,000 years ago? What was the total population, several hundred thousand or several million? I would choose the former figure. Natural foods were fruit, berries, nuts, wild vegetables, and grass seed, such as wild barley, emmer, einkorn, oats, and rice. There were honey, insects and insect larvae, shell fish, crabs, and turtles. To what extent fish and game animals contributed to the diet we do not know but it was possible to subsist on a vegetarian diet and also on fish, fowl, and mammals.

Johnson: The account of Ishi gives us an idea of how man may have lived 10,000 years ago.* We have examples of food gathering and hunting families today in Australia, Malaysia, India, and Africa. It seems that prior to domestication of plants and animals, man lived in small family units. We can conclude that there was little contact between such food-gathering population units. It is unlikely that man would have caused much disturbance in the natural vegetation and animal populations.

Compare this with what we see today. There is little arable land which does not show the effect of agriculture. The great deciduous forests of the riverine plains of Asia Minor, Asia, Europe, and North and South America have been cut and the land plowed and seeded with domesticated varieties of plants. Man has increased from a relatively rare species to the most abundant species of large mammal on earth. Likewise, the domesticated animals have almost completely replaced their wild ancestors, and large herbivorous mammals, other than domesticated ones, seem to be on the way to extermination. The American bison, which less than two hundred years ago numbered in the millions, is now extinct, except in national parks. The deer and the antelope would soon be gone if they were not protected by hunting regulations and sanctuaries.

When we consider the paradomestic wildlife associated with man, we can list the house mouse, house rat, house sparrow, pigeons, and the great variety of birds, rodents, tree-living mammals and bats which find the structures built by man suitable for occupancy during part or all of the year. Storage of water in clay jars resulted in the domestication of certain species of mosquitoes. Certain flies adapted to the piles of garbage and dung heaps associated with villages. Cockroaches, bed bugs, fleas, ticks, lice, and mites became associated with man, and each brought with it the capability of transmitting certain diseases to man.

The plants and animals domesticated by man appear to suffer a great deal more from disease than related wild species. The selected varieties of plants and animals have suffered disastrous epidemics of disease, for example, stem rust of wheat, blight of potatoes, rinderpest and foot-and-mouth disease of cattle, cholera of swine, encephalitis of horses, Newcastle disease and fowl pest of chickens, and rabies in dogs. Archeological investigations have revealed temples dedicated to the god of wheat and other cereal plants. Without a normal crop there was starvation. The

*T. Kroeber. Ishi, Last of his Tribe. Berkeley, California: Parnassus Press, 1964.

Johnson: field of arthropod-borne virus diseases of plants is as complex as that of arthropod-borne virus diseases of animals. The sources of the pathogenic viruses in nature are difficult to find because the pathogens in the natural hosts are not apt to produce evident disease. Whenever a species becomes very abundant, it is apt to suffer epidemics of disease because it encroaches on other species and so becomes exposed to parasitic organisms with which it has had no prior experience.

From contamination of natural water sources by wildlife, intimate association with domesticated animals, and the great variety of wildlife brought into captivity as objects of curiosity or pets, man will sooner or later become infected with all the parasites in nature to which humans are susceptible. Under certain conditions, some of these will spread from one person to another. With the development of cities, the environmental conditions and population numbers were suitable for the spread of contagious diseases. From what we know of the epidemiology of our most serious epidemic diseases, they could not have been maintained in humans when they lived in family or small tribal units.

What are the great scourges of man? The great killers have been bubonic plague, smallpox, cholera, typhus, and malaria. There seems to have been no infectious disease which has killed so many people as bubonic plague. This disease has been called the great teacher because so much has been learned about the control of infectious diseases from the study of plague. There have been three pandemics of bubonic plague in modern times: the Justinian plague, the Black Death, and the widespread epidemic of the nineteenth century. The Justinian plague of the sixth century killed half of the inhabitants of the empire. At the peak of the epidemic, 10,000 persons died a day in Constantinople. The second great pandemic, the Black Death, began in Asia during the first half of the fourteenth century. This was the most frightful epidemic of recorded history. From fifty to seventy-five percent of the population of Europe and Asia died of the plague.

What do we know about plague today? Where does it come from? We know that there are foci of endemic plague in certain areas of the semiarid high plateau regions of North and South America, Russia, Africa, and Asia. From time to time there are die-offs, of marmots, ground squirrels, and other small mammals, and at such times it is easy to isolate the organism, Yersinia pestis, from fleas and from tissues of the sick or dead animals. Between such outbreaks there is no sign of the disease, and we can only assume that some species of animals in these regions harbour the plague bacillus as an inapparent infection. Once introduced into villages and cities where there is a large population of rats, plague can be maintained as a migrating enzootic infection of rats, and it may be several years before the rat epizootic

Johnson: subsides. The infected rats ordinarily die of the infection, and the fleas associated with the rat must find a new host. This is the way the infection spreads to humans.

The usual series of events in an outbreak of urban plague is to have a rat-fall or die-off of rats. Subsequently the infection spreads to humans as flea-borne or bubonic plague. Secondary infections spread as pneumonic plague, and this type of infection produced a near one hundred percent mortality prior to the introduction of antibiotic therapy. The early control measures were quarantine and reduction of the rat population. The development of insecticides active against fleas has been a major factor in the control of plague.

Cholera is another disease associated with large urban populations where cultural practice results in heavy fecal contamination of drinking water. It seems unlikely that cholera could have been a disease problem for humans prior to the development of cities. Typhus is a louse-borne rickettsial disease. This disease has been a major cause of death during international conflicts. Rocky Mountain spotted fever is a rickettsial disease contracted from exposure to the bite of wood ticks in areas where there is reforestation and high populations of small mammals. Malaria has been a major cause of death in the densely populated regions of Asia and Europe. The malaria parasite is transmitted by domestic or parodomestic mosquito species.

Yellow fever epidemics have caused great loss of life in Africa, Europe, and North and South America. The epidemics of yellow fever can be controlled by killing the domestic mosquitoes. Jungle yellow fever is a disease of woodcutters who are exposed when felling trees to the bite of canopy-living mosquitoes. The reservoir host in the forest is unknown. Epidemics of dengue and encephalitis are related to domesticated mosquito species, that is, mosquitoes breeding in the city or in irrigated or flooded fields used for agriculture. Filariasis is transmitted by domesticated mosquitoes. Phlebotomos flies or sandflies, which breed around human habitation, transmit sandfly fever and kala azar. The dog is an amplifying host for the kala azar parasite.

We know of more than one hundred viruses derived from wildlife which infect men. Many of these are transmitted by arthropods and others by contact or ingestion. Since 1960 there have been outbreaks of hemorrhagic fever in South America, which are caused by members of the Tacaribe virus group. In this instance the virus is eliminated in the urine of house mice or parodomestic wild mice and man is infected from contaminated food or water. The epidemiology of lymphocytic choriomeningitis is similar, and in this instance the house mouse is the vertebrate host.

Johnson: There are more than 160 respiratory and enteroviruses which parasitize man. Where did we get this museum of viruses? There are three types of influenza virus, four of parainfluenza virus, three reoviruses, thirty-seven adenoviruses, 110 rhinoviruses, plus measles, German measles, mumps, and respiratory syncytial virus. Among the enteroviruses we have three types of polio virus, thirty Coxsackie viruses and thirty-three echoviruses. There are viruses such as rabies, chicken pox, infectious mononucleosis, and infectious hepatitis virus which are not classified with the respiratory or enteroviruses. Of these, influenza, measles, polio, and infectious hepatitis have been the most serious disease problems.

Evidence is accumulating that many of these viruses have been derived from wildlife. However, once introduced into a species as numerous and mobile as man, they can continue indefinitely and in the process change so that one cannot find their counterpart in nature. Measles virus is closely related to dog distemper and rinderpest of cattle. Influenza A virus has been isolated from swine, horses, chickens, ducks, and terns. The epidemic of Spanish influenza of 1918-1919 was responsible for the death of more than twenty million people, and the epidemic of Asian influenza beginning in 1957 produced a mortality of more than five million.

Polio has been one of our most dreaded diseases. There were certain years, such as 1916, 1927, 1931, and 1935, when the disease was epidemic in the United States, but after 1943 the disease continued for several years at about the same rate. I suspect this was related to the high mobility of the population during the war years. The development of a live virus oral vaccine for polio has made it possible to control this disease. We are now witnessing a nationwide immunization program against measles. Rabies, although uncommon in humans, is a serious public health problem in some countries. The control of canine rabies in the United States by vaccination of dogs with a live virus rabies vaccine eliminated the highly virulent dog virus, but the rabies virus remains active in wildlife. The disease problems associated with some of the enteroviruses appear to be new, for example the epidemic pleurodynia and carditis caused by Coxsackie viruses. These viruses are contracted from contaminated food, water, or hands, so the control method is to wash the hands before eating. Psittacosis is a pneumonic infection of humans derived from pet psittacine birds, such as parrots and parakeets.

There are other diseases related to our cultural inheritance which do not seem so important but are responsible for much ill health. These are the worm infestations such as hookworm, Ascaris, Toxocara visceral larva migrans, hydatid disease, schistosomiasis, guinea worm, and a variety of tapeworms. Amebiasis is also a serious public health problem. Typhoid fever used to be a major cause of death in the United States but is now

Johnson: uncommon as the result of vaccination and sanitary measures to ensure pure drinking water. The Salmonella organisms are now a major cause of enteric fevers.

There are certain other bacterial diseases which are related to domestic animals, for example, scrofula or bovine tuberculosis. This type of tuberculosis of the lymph nodes, contracted from drinking the milk of infected cattle, used to be a common disease, but in the United States it has been eliminated by eradication of the disease from cattle. Brucellosis or milk fever, derived from drinking milk of infected goats and cattle, has been controlled by pasteurization of milk in the United States. Tetanus and anthrax are related to human infection from association with infected domestic animals. Human tuberculosis, which was a major cause of death until recently, was a disease associated with overcrowding and malnutrition in cities. Epidemics of scarlet fever, streptococcus and pneumococcus pneumonia, erysipelas, and diphtheria are related to urban populations. The problem of venereal disease is related to the urban environment.

What is the nutritional status of modern man compared to that of 10,000 years ago? We learned that a diet composed primarily of polished rice results in a fatal illness called beriberi. A diet of bread made from white flour is no better. Freshly ground whole cereal grain is a nutritious food. It is the wheat germ and the rice polishings that contain the B vitamins which are necessary for proper nutrition. Then there is vitamin C which is present in fresh vegetables and fruit, and the absence of this vitamin results in scurvy and death. Of the minerals, it is calcium and magnesium which are so important. These elements are present in plants, but in meat-eating cultures they have been obtained from fish and animal bone by use of vinegar in the preservation and cooking process.

What about stress? This can be the result of too much heat, too much cold, too much work, either mental or physical, too much or too little food, and from frustration in our association with other people. One of the most common causes of stress is anger and resentment, blaming other people or the government for personal problems and dwelling on this. It leads to adrenal exhaustion, arthritis, asthma, and degenerative diseases. Anxiety is associated with frustration and anger, and our tendency is to hypoventilate when suffering from this type of stress. As with carbon monoxide poisoning, the lack of oxygen leads to severe headache. This is encountered after fainting attacks. Another common cause of headache is coffee withdrawal. In treating this symptom, people consume a variety of drugs, especially aspirin.

Drug taking is not new but is related to the urban culture. Opium and hashish have been used for centuries. Smoking tobacco is another cultural practice which is responsible for disease. The excess of deaths per year in the United States from lung

Johnson: cancer, cardiovascular disease, and disease related to the lungs in smokers versus nonsmokers is in the order of half a million persons. There are at least two million useless alcoholics in this country and many more whose health and efficiency is impaired by consumption of alcohol. With about ten million car accidents, with at least two million hospitalized and 40-50,000 killed each year, with more than one half of these the result of driving cars when intoxicated, we have a real bad disease problem. Some consider beer a safe drink. It so happens that hops extract used in the manufacture of beer is a psychedelic drug similar to cannibinole of hashish and marijuana. Cocoa leaves have been used as a drug in South America for many years. When it became possible to extract pure cocaine, this became a widely used addictive drug. Freud used it for his own depression and recommended it for others. Aldous Huxley advocated psychedelic drugs to achieve self realization, especially the drug LSD.

It is the egotistical nature of man that he wants to think well of his achievements. Folklore and recorded history cite certain illustrious individuals and how the human race has gone forward in its culture. There is the idea of a general trend towards improvement and how we have harnessed the forces of nature to create a better world. There will be other discussions at this meeting about man's effect on his environment, [but] the nature of the human species remains the same. We are mortal and the three score and ten [years] mentioned in the Bible is what we can expect today. As regards health, I suspect that we have not improved on the situation as compared to that of 10,000 years ago.

The Rockefeller Foundation doctors who were engaged in field operations in other countries learned that there were certain diseases that were related to the culture of the people. I mentioned previously that in 1954 I presented a paper, "Arthropod-borne viruses in the tropics," at the second conference, Industrial Council for Tropical Health, at the Harvard School of Public Health.* In this paper I described some of the problems of public sanitation and contaminated water sources. I noted two special physical characteristics of the people of India which differed from those seen in the United States. One was the absence of acne and the other was the near-perfect teeth of both children and adults. By the way, they do not put fluorine in the drinking water. Milk is rarely a part of the diet. Those who can afford it boil fresh milk and ferment it, and this is called "curds." We call it yogurt. A spoonful of this may be served with the rice and curry. So how do they get their calcium? Of course they get some from the cereal grain and vegetables used in making curry, especially the plant called ragi. The major source

*New York: Robert W. Kelly Publishing Corp., 1954.

Johnson: is from the lime paste they consume at the end of the meal. Each person takes a "pan" leaf, and on this he puts about the equivalent of a large pat of butter of the lime paste, and add some condiment such as cinnamon or cardamon, roll it up, and eats it slowly. This will give each one more than 1500 milligrams of calcium carbonate and also some magnesium. The adults usually use some soporific, such as betel nut, opium, or hashish, with this paste. It is the betel nut that makes the intense red dye when mixed with calcium carbonate. When they spit out the dye from the betel nut it leaves the red dye on floors, walls, and clothes, and of course also on their teeth and lips. Anyway, they do have fine teeth and strong bones. They get plenty of calcium.

Before leaving the subject of teeth, I wish to mention that the rural people in Mexico also have fine teeth and do not appear to have any disease related to vitamin deficiency. Milk of cows is not an important dietary item. Their source of calcium also depends on the use of raw lime (calcium carbonate). They grind the corn (maize) each day and soak the cornmeal over night in the natural lime water. The next day they gently squeeze out the excess water and make flat cakes, called tortillas, which are cooked the same way we cook pancakes. With the additional calcium derived from the lime water, each tortilla contains more than 130 milligrams of calcium.

What about the absence of acne in India? I believe that acne is the result of stimulation of the fat-secreting glands of the skin by the lactogenic hormones in cow's milk. Infection of the skin is secondary and related to scratching the swollen fat gland. It is amazing how much cream is ingested by young people, mostly in the form of ice cream, and some subsist on milk shakes made with ice cream. It is not necessary to stop drinking milk and of course growing children ordinarily need a quart of milk a day. If they get acne, use low fat milk and less butter. If they have a lactase deficiency which results in failure of the metabolism of the lactose in the milk and get abdominal discomfort after drinking milk, it is possible to add lactase to the milk. Some dairies provide milk to which lactase has been added. One of the B vitamins, pyridoxine, has an inhibitory action on the lactogenic hormone, so acne to some extent could be related to deficiency of the B vitamins. Of course, those who have acne can be given the necessary calcium in the form of calcium carbonate, two parts to one of magnesium oxide, both of which are needed and ordinarily derived from milk.

There is also a serious public health problem in India from intestinal parasitism of Ascaris and hookworm. Hookworm infestation is very common and an important cause of death in young children. Reinfestation is almost constant in anyone going barefoot because of the fecal contamination of street and roads from drain water coming out of houses. There is a religious taboo in India against drinking boiled water. The natural river, lake,

Johnson: or stream is regarded pure. The reason that the surface water is so heavily contaminated is that people are taught that their excreta should return to the sea.

For example, in March, 1952 there was a cholera epidemic in Poona. Processions of people from remote villages arrived in Poona carrying prayer (puja) flags. Hindu priests had calculated a time for the puja, held at a temple located at the conjunction of the Mootha and Moola rivers. The city had assigned a place for the pilgrims and provided chlorinated water for drinking. At the assigned time the people moved into the river near the temple. They took up some water with their hands and poured it on their heads as a ceremonial cleansing, then took some water in their hands and drank it. The effluent of the city sewers from a population of about a million people drained into the river. Within a few days, hundreds of people developed cholera. Some of these were taken to the infectious disease hospital. I had been there previously, so I went there to see what I could do. I offered to drive to Bombay to get some physiological saline solution for intravenous injection, but they said this had been ordered. It did not arrive for several days and in the meantime treatment consisted only of spooning in saline solution and giving sulfadiazine by mouth. The problem in caring for cholera patients is that they continue to vomit and pass watery stools. In a few hours they become dehydrated, lapse into coma, and die. Several hundred died and the bodies were cremated along the river. We lived near the river and this was one of the most chilling experiences I witnessed in India.

Hughes: They didn't put the two events together?

Johnson: One of the older physicians in Poona, who was associated with the department of public health, said it was not from the ceremony because the river purifies itself. It was just an epidemic of cholera brought on by the coming together of a large number of people from small villages. One can consider this point of view because there was no epidemic of cholera in Poona before the puja. It seems more likely that there were carriers of cholera organisms who had no symptoms among the people in Poona and the river was highly contaminated. Likewise, there may have been carriers among the pilgrims who contaminated the shoreline before the ceremony.

Vibrio cholerae is the name of the organism that causes Asiatic cholera. It was responsible for many deaths among the British who served in India. The cemeteries left by the British contain many graves of children with the inscription, "died of enteric." Intestinal infection with amoebae and Giardia is also very common, and systemic invasion of Entamoeba histolytica may produce cysts in the liver and brain. An unusual parasite encountered in India is the guinea worm, Dracunculus medinensis. The intermediate host of this parasite is a snail, and people get infected with the cercariae from well water. The adult worm

Johnson: migrates to the surface, usually on the legs, and forms a boil-like ulcer and discharges larvae that get into the well water and repeat the cycle.

Bacillary dysentery is very common in India, and the most dangerous form is caused by Salmonella typhi, which is responsible for typhoid fever. I developed symptoms of typhoid fever while at Sakleshpur, Corg State, India in July, 1952. Fortunately, I had chloromycetin with me and this proved effective in preventing serious sequelae.

In August, 1952 when I was visiting Christian Medical College, at Vellore, Tamil Nadu State, South India, I saw several acutely ill children at the college hospital who were positive for S. typhi and were under treatment with sulfadiazine. Dr. Ruth M. Myers, who was the bacteriologist there, said that the college had an ambulance which would pick up sick children at designated places in the nearby villages. These were usually almost moribund and the parents had assumed that they would die. The admitting body temperature would be about 105 F. Sulfanilamide or sulfadiazine was used for treating these cases, and with the use of hydration with intravenous fluids and liquid diet, most of these children would recover.

The key public health measure to prevent bacillary dysentery is to boil water before drinking it. I collected drinking water from wells or surface water and boiled it for five minutes using a two-wick kerosene stove (called the Beatrice Stove). This was the first objective after making camp at an official rest house (forest or inspectors' bungalow). I had three tin-lined metal containers of different sizes so they would fit together. All were filled and the water boiled. In the morning I would pour off the clear supernate into a water can fitted with a spigot and into bottles or thermos jugs. There would be a gelatinous sediment after boiling, which we would discard. If we needed boiled water for cereal or powdered milk, we would use it immediately after boiling. The various organisms in the water probably had some nutritional value.

The principal sources of food toxins are Staphylococcus aureus, Clostridium perfringence, and Clostridium botulinum. The staph toxin is not heat labile, but it is not a problem in boiled water. Food poisoning from staph toxin is produced in meat and fish after surface contamination after cooking, usually warmed-over cold meats and salads. The perfringence and botulinum toxins are destroyed by boiling. As you might expect, milk powder mixed with surface water containing these common organisms and not boiled will be an ideal culture medium for these organisms. It is not the fault of Nestle's milk formula that children sicken in Asia and Africa; it is because they do not boil the water before mixing it with the powdered formula. Surface water is potable if boiled.

Johnson: When we speak about health, such as I mentioned in disease related to cultural inheritance, what did I learn about cultural heredity in India? I like what Mr. Walsh, secretary to the Poona Club, said to me when I first arrived in India: "Do you have patience?" He said if I did not have patience when I arrived I would get it, and if I had it I would lose it. It seems that health has much to do with how we handle frustration in dealing with other people. It surely is important not to insult and criticize other people.

Here I want to mention a question posed to a person in the United States. He arrived in a town and inquired whether the people were nice and kind there. The person he spoke to replied by asking how he found the people where he lived previously. He replied that they were mean, ugly, and vicious. The person he spoke to then said, "Well, that's probably the way you will find them here."

I am very grateful to so many people I met in India--Hindus, Muslim, Sikhs, Jews, Parsis, Christians, Harigans (outcasts), and aborigines. I found some from each of these groups who were helpful, kind, and friendly. I am especially indebted to individuals belonging to hunting tribes who taught me all they knew about wildlife and methods of collecting game for food and how they dealt with marauder panthers and tigers. I felt safe in the villages where we were doing field work. I always contacted the local state health stations, mostly anti-malarial officers and others engaged in public health projects. The society in India is structured by caste and family units. When they travel in India, they stay with family contacts and not in hotels, and marriages are arranged within their caste and family line. Child marriage contracts are still common and are arranged early in life.

Controversies

Hughes: Well, you hoped that the paper on disease related to cultural inheritance would be published in Bioscience. Who in AIBS did not like it and why?

Johnson: There must have been several criticisms. One was my statement on the origins of the earth, geology, what we know about human culture, the origin of oil, gas, coal and tar, which are called fossil fuels. In 1965, I heard George Beadle, the geneticist, speak at Cal, and his dating of human culture, writing, and language seemed the same as mine.

Dating of volcanic eruptions and deposits of volcanic sand reminds me of when I was walking about San Joaquin, Mexico in 1944 where the village was almost completely covered with fresh volcanic sand. The sand was about twenty-five to thirty feet

Johnson: deep, and when I was returning from a volcanic ridge where the jets of steam and gas were coming from the lava ridge, I looked back at my footsteps and realized that a rainstorm would leave a permanent footprint, soon to be covered by more volcanic sand. I have flown over the Gulf of Aqaba, between Israel and Jordan, sailed through the Red Sea, and these are rifts in the earth. I have traveled the length of Africa and flown over the Rift Valley. I find these rifts similar to the Grand Canyon and even the central valley of California. When we look at the geodetic surveys made of the ocean bottom, we find similar rifts and plains, all indicating spreading of the earth's crust.

Sir Edward Bullard, who was one of the scientists who conducted the study of the Atlantic Ocean during the 1960s, spoke at Cal in 1975, and he told about the more or less constant volcanic rifting along the Atlantic Ocean Ridge, and the ocean was only about one hundred million years old. He also told about the changes in the earth's polarity as revealed in the lava flows, as they tested the magnetic field at various distances from the Atlantic Ridge. If we work with electric motors, we know that changing the polarity makes the motor stop and turn the other way. This would make it look as if the sun had stopped. Would gravity be affected? Probably not very much.

Hughes: Did your broad ecological approach to research run up against the targeted view of research, reflected in NIH's grant-giving philosophy?

Johnson: When I reported cloning western encephalitis virus and obtaining clones of different size and of greater and lesser pathogenicity for mice, this raised a philosophical problem as regards mutation.

Hughes: Why was that controversial?

Johnson: Well, that raised questions about the discoveries of smallpox vaccine virus and yellow fever vaccine virus. These were regarded as examples of genetic mutation. The mutant strains were regarded as fixed and therefore safe as to pathogenicity. Our cloning studies of western encephalitis virus and Turlock virus demonstrated that pathogenic and nonpathogenic variants could be selected from the natural population of virus particles, and one could back-select from selected variants.

Let me illustrate by what happened to the Flury avianized low egg passage rabies vaccine virus. I emphasized the importance of maintaining a seed virus at the local passage level between forty and fifty, and if biological laboratories other than Lederle were to produce the vaccine, it should be maintained within five additional passages and using the same chick embryo host system. So what happened? Some laboratories evidently had difficulty in getting a high titer of the virus when passing it using the chick embryo pulp from the earlier passage, and passed the virus in the

Johnson: brain of baby mice, which does result in a higher titer of virus. They used that for inoculating the chicken embryos by the yolk sac route. Other laboratories have adapted the LEP Flury vaccine virus to canine kidney cells. This is a mammalian cell and will back-select for tissue tropism and mammal pathogenicity.

The monoclonal antibodies prepared from hybridomas of cells producing antibodies to rabies have made it possible to show differences of the Flury LEP avianized vaccine virus prepared in chicken embryos by different manufacturers. These had been tested because some dogs developed rabies from the vaccine virus. It is not yet evident what produced these differences; they could be from hybridization with mouse viruses or avian viruses. There is, of course, a rapid antigenic change of a virus from cultivation in another host. One major step is developing a greater neurotropism by one passage through the brain of a mouse. If we had a true mutant it should not change in pathogenicity. Some of my colleagues and administrators of vaccine committees evidently felt safe in approving new vaccines. Because of this, I believe the current live virus vaccines used for immunizing dogs against rabies should be withdrawn. As shown by the isolation of the ERA-SAD vaccine virus from cats given this vaccine and developing the equivalent of an encephalitis that would not be recognized as clinical rabies, we can expect the same would be true for dogs and even humans.

Hughes: Did the fact that some of your findings did not fit with current practice make it difficult to get papers published?

Johnson: Oh, yes, even the paper I gave at the International Wildlife Disease Conference at Uppsala, Sweden in 1985. I presented a view of the natural history of the various flaviviruses we had isolated in California, that is St. Louis encephalitis virus, Powassan virus, Rio Bravo virus, and Modoc virus. I wanted to present evidence about the natural history of these viruses and how they might survive without an insect vector. The papers given at the meeting were supposed to be published in the Journal of Wildlife Disease. I have been active in this organization since it was founded and I gave a paper at the first international conference of the Wildlife Disease Association in 1962 and this was published on microfilm.*

I suppose that only a few of the members of the association have read that 1963 paper. I have sent out many copies. The reviewers stated that it was not suitable and that I might rewrite the 1985 paper as a research note, which was published.** I am

*The ecological approach to the study of wildlife disease. J. Wildlife Disease Assoc. 1963, 11:423-431.

**Isolation of the Powassan virus from a spotted skunk in California. J. Wildlife Diseases 1987, 23:152-153.

Johnson: glad the abstract was published in the proceedings of the conference at Uppsala. This did contain the basic information I wished to present.

Hughes: What was the problem with it?

Johnson: No doubt, the philosophical comments on the origin of arthropod-borne viruses. I believe that most if not all of these viruses must survive in nature without depending on an arthropod host. Now that doesn't mean that an arthropod host is not involved in the transmission of the virus to various mammals and even humans during an epizootic. However, the majority of persons now doing research on arthropod-borne viruses believe that these viruses are maintained in the arthropod host by transovarial infection. What I presented in Sweden was that my studies of Modoc virus and Rio Bravo virus showed that these viruses survive year to year in a single host species.

Around the world today there is evidence from field studies that the alphaviruses of the family Togaviridae are associated with freshwater swamps during epizootics. If we set our mosquito traps within a few feet of the edge of such swamps, this is the best place to find the group A viruses. For thirty years I have been studying the natural history of eastern encephalitis virus [EEV] in Massachusetts. This virus is associated with the great freshwater swamps, and in recent years both this virus and the western encephalitis virus [WEV] have been isolated from Culiseta melanura mosquitoes collected near the edge of several different swamps. The WEV appears first, usually in mid-July, and the EEV in August. There seems to be no interest among epidemiologists to study the mammals associated with these swamps. From the results obtained by the virus laboratory of the Massachusetts State Health Department, the isolates of EEV are in an ecological biome inhabited by the broad-tailed shrew, Blarina brevicauda, which likes heavy leaf mold, dead tree stumps, stone walls, and banks of stone and gravel. The WEV is related to the swamp itself, with overturned dead trees, floating vegetation at edges of the streams entering the swamps, which is the biome preferred by the water shrew, Sorex palustris. I know of no zoological studies related to these two animals among the scientists investigating the group A viruses. The distribution of these two genera of small mammals coincides with the presence of WEV and EEV. These animals are primarily insectivores and, though population numbers may vary from year to year, they are often very abundant.

I have become very familiar with the Blarina shrew at our summer home in Scituate, Massachusetts. I hear them some years during the day, a very piercing squeak possibly associated with the capture of a katydid or cicada. They are difficult to catch in the small Sherman traps, but one day I saw one of the shrews run along a stone wall and immediately set a Sherman trap exactly where I saw the shrew and within twenty minutes I caught it. I

Johnson: tried to keep it alive and it did eat a seaworm I obtained from the local fish bait shop. I intended to keep it and prepare cell cultures of the kidneys and salivary glands, but it escaped from the wide-mouth jar, which I used for a cage. It had to leap at least eight inches to get out. I caught two more after this but they entered the traps after dark and were not alive in the morning.

Another unusual capture of this shrew was in a storage pantry near a stone wall. There had been a narrow opening along a shelf where I have caught *Peromyscus* mice from year to year, and on one occasion I caught a *Blarina* shrew with a snap trap at this place. It would have had to climb up about five feet to this spot and reminded me that it does live in stumps and walls where its nest is protected.

By the way, during the summer when the group A viruses are found, there are few birds in the swamp. There is a large population of shrews in freshwater swamps in northern United States and in Canada up to the northern edge of the cultivated regions.

Support from the California State Department of
Public Health

Hughes: How did the California State Department of Public Health regard your ideas?

Johnson: As I mentioned previously, Dr. Lennette supported my studies of small mammals and birds in relation to wildlife viruses. He has had the same problem in obtaining grants for research studies. These have to fit with public health problems, such as polio, rubella, and the mosquito-borne viruses. Fortunately, I had the financial support from the Rockefeller Foundation so I could pursue such studies until 1972 when this source of funds ended. In California I have had excellent support from the vector control department and the veterinary public health section.

I would like to see graduate students interested in zoology recruited for public health research in virology, especially in cell culture studies of organs, such as the kidneys, lungs, and salivary glands of small mammals, such as shrews, to look for viruses, such as Rio Bravo virus which is found in the salivary glands, and Modoc virus which is found in the kidneys. The epidemiological problem is, why do the encephalitis-producing viruses keep reappearing in certain places at intervals of several years. Are they introduced from wildlife populations hundreds of miles away, or are they found locally, as observed in Massachusetts?

Hughes: Is there interest in that topic?

Johnson: That is the problem. It is my observation that governmental funds for research in public health are not available for basic studies of wildlife unless there is some alarming situation, such as epidemics of disease in agricultural crops or in humans, like when I went to Mexico in 1944 because cattle were dying by the thousands from derriengue. Cattle are an important food item and the Mexican government was anxious to get some help in dealing with this disease. There have been agricultural emergencies in California related to disease of grapes, citrus, and pears. Another is the potato yellows virus. The Rockefeller Foundation Agriculture Program has established research studies of corn and rice to select for resistance to virus diseases, but not on finding sources of these viruses in the natural vegetation. A major effort has been made to collect varieties of wheat, corn, and rice, and to select variants for high yield and resistance to disease. The rapid increase in the population of the world, now over five billion, was made possible by the control of the major infectious diseases and the ability of modern farmers to produce a high yield of the cereal crops and to store them properly.

Hughes: When you had trouble getting papers published, did you have other ways of getting your viewpoint across?

Johnson: Well, my feeling--this is very honest--is that, in all fields of endeavor, the best means of putting across ideas from research is not in publishing long papers but by word of mouth, person to person. I'm so grateful for having been able to work for the Rockefeller Foundation and to associate with people who have great curiosity and openness of mind and who are willing to work for a modest salary and see what they can do to control diseases and how to improve the yield of food crops. It is necessary to control diseases of domestic animals as well as to develop better varieties of grain. I have enjoyed teaching in courses of public health and presenting the ecological approach to the study of plant and animal parasites and how they affect people. Teaching ecology, ornithology, zoology, and natural history is done in general best on field trips, and laboratory techniques [are best taught] by showing exactly what you have learned from experience and guidance of others. It is not, "Here are the instructions." My cultural inheritance from my medical training is, show the new staff member exactly how to do the work and then let him do the procedure himself, with you there to help if necessary.

I would say that the biggest problem in research laboratories is keeping records. There should be a workbook where each person records the day's work. It is important to have continuity so that the records are kept for posterity.

Johnson: I liked to visit the Pasteur Institute in Paris. They have the workbooks of the laboratory responsible for keeping the Pasteur rabbit fixed rabies virus. The passage was numbered serially from the original cow from Melun, France. They showed me the current passage number. In the United States, the government laboratories have strains of the Pasteur virus, but they do not know how many passages have been done in rabbits, which was the only laboratory animal used to maintain the basic strain. Passage history should include all passages, the hosts used, and the route of inoculation. Facilities have now been developed for storage of type strains of bacteria and viruses. There is still a problem in government records, both state and federal, because of the profusion of records and with numerous changes of directors.

I do a lot of reading. I take several technical journals and I read them. I have been a member of the AAAS [American Association for the Advancement of Science] for fifty years and have files of tear sheets on several different subjects. I enjoy visiting the university libraries, especially the Bancroft Library. I like to browse in the stacks, looking up books on the history of the United States. Only a small percent of students reads anything but his regular assignments, and these may lean to the bias of the teacher.

Hughes: Did you ever use yourself as a guinea pig in research?

Johnson: The practice of medicine is really experimenting on each one you treat. When I was house officer in medicine at the Brigham Hospital in Boston in 1938, we had no life-saving medicines. The main treatment was bed rest and TLC (tender loving care) plus a good diet. It seemed that most of the patients who came to the cardiac clinic were suffering from arrhythmias, which were caused by toxicity of the drugs they're taking, especially theophylline, quinidine, and digitalis. The same holds true today. If a person comes in with a generalized skin rash, the first thing to do is ask what drug they're taking. The common drug phenolax, prescribed in the 1930s for constipation, was apt to cause a skin rash in long-term users. Later it was the sulfa drugs and antibiotics.

What about myself and family? I have mentioned all the different biologic vaccines we had to take. I took vials of vaccine with me to India in case we needed boosters. Our daughter Susan was cut with barbed wire in India and, because tetanus was a common cause of death in that country, I gave Susan a booster dose of DPT vaccine, which included tetanus. She had a severe reaction to the vaccine, with fever (103+) and aching. I inquired about reaction to DPT vaccine and found out that such reactions increased with repeated doses of vaccine. Later we used only a small dose intradermally for boosters. Repeated vaccination with

- Johnson: any biological is apt to result in allergic reactions. I did give boosters of cholera vaccine to my family and other staff members at the time of local epidemics of the disease in India.
- Hughes: Did the Rockefeller Foundation have a policy about the use of human volunteers in research?
- Johnson: Only that we voluntarily took jobs that had an inherent risk of infection and possible death. For instance, in 1938 when I began working at the yellow fever laboratory, past experience of deaths among the staff from yellow fever mandated that anyone that was to work in the lab had to be immunized against yellow fever. By 1938 the Rockefeller Foundation yellow fever lab had an experimental vaccine, the avianized 17D Asibi yellow fever virus. Beginning with staff members, a small number had been vaccinated with this virus. I was vaccinated with this virus and experienced a low-grade fever and symptoms involving muscles and joints, but this was expected.
- Hughes: But inoculation to protect staff is a little different from using human volunteers who may not benefit from the research. Was there a policy?
- Johnson: Well, the ethical test is, would you use [on another] a drug or vaccine that you would not be willing to try on yourself. There was no written policy statement.

Medical Disasters

- Hughes: What are some medical disasters in your own lifetime?
- Johnson: One example is the Salk polio vaccine, where the vaccine virus had not been completely inactivated by the formalin that was supposed to have killed the virus. This was mentioned previously.

The history of medicine has many examples of treatments which were responsible for adverse reactions and deaths. The bleedings, purges with drugs such as mercury and arsenic, and lancing boils by barber surgeons are examples. George Bernard Shaw, in his preface to The Doctor's Dilemma, says that doctors have an intense dread of doing anything that everybody else does not do or omitting to do anything that everybody else does. This is a problem at the present time because of lawsuits claiming injury or negligence. Drugs and vaccines have the possibility of adverse effects and death. Surgery requires anesthesia; there may be secondary infection, loss of blood requiring transfusion, the necessity for respiratory assistance with the artificial lung, and the ever-present intravenous line for medication. Any of these may contribute to death or serious sequelae.

Johnson: I want to begin with a classic example of acute poisoning by a new drug, the elixir of sulfanilamide (Massengil), that occurred in 1937. Sulfanilamide (Prontylin) had been introduced in the United States, and Prontosil in Europe, and these drugs had been found to be safe and effective in treating streptococcal infection. In September and October, 1937, there appeared newspaper reports of deaths in south central United States from the new elixir of sulfanilamide. The AMA [American Medical Association] received hundreds of inquiries from doctors about the elixir, and tests were ordered immediately to learn what was wrong with the drug. The active components were found to be diethylene glycol and sulfanilamide. There had been no reports of serious reactions among people treated with sulfanilamide in New England, and there was no information about the chronic effects of diethylene glycol. This [latter] substance was a palatable sweet syrupy liquid which had been used in small amounts as an additive in bootleg liquor.

The AMA gave a grant to the University of Chicago chemists E.M.K. Geiling and P.R. Cannon, who conducted toxicity experiments on rats, rabbits, and dogs.* The undiluted diethylene glycol produced a toxic syndrome of lethargy, coma, and death, and pathology studies showed renal tubular degeneration and central lobe degeneration of the liver. It is pertinent to mention here that Chauncey D. Leake in a discussion of drug policy presented at the meeting of the Section on Pharmacology and Therapeutics of the AMA in 1929 stated: "Many drug firms make the mistake of believing that their chemists can furnish trustworthy pharmacologic opinion. Indeed, some eminent chemists, impatient with careful pharmacologic technic, have ventured to estimate for themselves the clinical possibilities of their own synthetics....There is no short cut from chemical laboratory to clinic, except one that passes too close to the morgue."** I met Dr. Leake when I was in medical school.

There is a current problem with diethylene glycol. In the New York Times of March 19, 1986, there is an article entitled, "Wine test conclusions," telling about spiking of wines with a chemical used in antifreeze called diethylene glycol. This had been done as a flavor enhancer, using an average of one gram per liter. The article also noted that the Food and Drug Administration had found that a dose of twenty-five grams could be lethal. Prestone II Antifreeze contains 95% ethylene glycol. This chemical is used in windshield cleaner, radiator flush, squeeze deicer, brake fluid, and detergent household cleaner. Diethylene glycol is twice as toxic as ethylene glycol. It is used in skin lotions and in coating pills.

*E.M.K. Geiling, P.R. Cannon. JAMA 1938, 111:919.

** Proc. Annual Meeting AMA 1929, Section on Pharmacology.

Johnson: My current interest in these compounds is to learn whether low-dose exposure could be the cause of giant cell arteritis and giant cell myositis, both of which are associated with neuropathy. My suspicion is based on our use of 50% diethylene glycol or polyethylene glycol to obtain fusion of splenocytes from immunized mice to mouse myeloma cells and so produce hybridomas that will secrete specific monoclonal antibodies. This is an effect of cell membranes similar to that producing giant cells.

The compound itself is not toxic. It is the metabolism of ethylene glycol to glycolic acid and lactic acid that produces acidosis and toxicity.

We have learned a form of therapy from treating people who have tried to commit suicide by drinking antifreeze. Primary treatment consists of putting in an intravenous line and administering sodium bicarbonate to treat the acidosis and then giving a slow drip of 10% ethanol in a 5% solution of dextrose in water to block the metabolism of ethylene glycol and allow its excretion in the urine, or if anuria has supervened, to allow elimination of glycolic acid by hemodialysis therapy using bicarbonate dialysate in a batched delivery system.

I want to continue with the problem of toxicity of chemicals having long-term effects. Most people are familiar with lead poisoning from house paint, but the first description of a disease related to lead in the United States dates to the colonial period when Cotton Mather presented evidence that the "new" disease was limited to beer drinkers and incriminated the lead lining of the containers used for brewing the beer. Evidently, the carbonic acid released from the fermentation process acted on the lead to produce lead acetate. While I was in training at the Brigham Hospital in Boston, the medical staff presented a play about the poisoning from beer described by Cotton Mather. The players were all dressed in colonial-type clothing, and Tom Evans and I presented incidental music from the period, Tom playing the violin and I the piano.

Wilson's disease occurred in Scotland soon after the introduction of Scotch whiskey. Copper containers were used for distillation of the alcohol. At the present time we read of reports of copper poisoning where someone has prepared lemonade and left this in a copper container at room temperature or in the refrigerator for several hours before serving the drink. This can produce acute copper intoxication, resulting in nausea and vomiting. I remember visiting the Vanderbilt estate at Newport, Rhode Island, and on entering the kitchen I saw a long row of gleaming copper containers. When they told us that Mr. Vanderbilt died soon after moving in, I envisioned the long-term effects of cooking with acidic vegetables, such as tomatoes and lemons as well as with vinegar. Of course the word vin-agar means sour wine.

Johnson: My current concern with metal poisoning is aluminum intoxication. Aluminum cooking containers became popular after World War II. An example of the rapid release of aluminum can be observed if we place a tomato on a sheet of aluminum and cook it in the oven. You will note that the aluminum foil under the tomato has dissolved. We can expect that cooking in an aluminum container using tomatoes, lemon juice, or vinegar will release ionic aluminum. The studies of brains of people who have died of Alzheimer's disease show aluminum deposits in the amyloid substance, in the plaques, and also in the neurons. There is also some copper and iron.

The studies of the geographical distribution of multiple sclerosis, amyotrophic lateral sclerosis, and Parkinson's disease show a relationship to areas where the drinking water is acidic and the bauxite (aluminum ore clay) is acted upon by the carbonic acid from vegetative decomposition to release ionic aluminum. Where there is alkaline soil, these diseases are not present. Furthermore, it is known that calcium carbonate and magnesium oxide are protective in that the aluminum is not deposited when these substances are plentiful. Of course, tomatoes and lemons are excellent foods and contain vitamin C (also acid). The point is to cook in iron, stainless steel, enamelware, or glassware. Likewise, if we get plenty of calcium and magnesium from our food, especially milk and milk products, we can handle the aluminum intake.

The word disaster means any event causing great harm or damage, and an alternate word is calamity. There are plenty of examples from what we eat, drink, and smoke, but there are also some special examples of drugs used in medicine. Remember pHiso-Hex that for a time was the all-purpose skin cleaner and disinfectant? The chemical name is hexachlorophene. By the way, it contained polyethylene glycol and some other chemicals containing a phenol ring besides the chlorophene. I associate the phenol and benzene rings with trouble. Well, pHiso-Hex proved to be dangerous. It had a toxic effect on the bone marrow and kidney, and why should we spray babies with this compound? The baby is born with a protective covering of wax and oils. Just wipe dry with a sterile towel. If bathing is indicated, use boiled water and add a little salt to dissolve the mucus. At all events do not use soap and so take off the natural oils.

Remember calamine lotion with phenol? Chronic application of this lotion may cause kidney damage. There is a common analgesic which has been prescribed by doctors for more than fifty years, the APC (aspirin-phenacetin-caffeine with codeine) tablet. I wonder how many thousand people became addicted to codeine from taking this drug. Several companies still sell APC tablets. They do include a warning of possible renal toxicity. From reports in

Johnson: the literature and especially from people I know who have observed end-stage renal disease from phenacetin, this may be a common complication of chronic medication for arthritis.

Tylenol (acetaminophen) with codeine is a commonly prescribed drug. Here again we have the problem of the patient becoming addicted to the codeine and continuing to take acetaminophen just to get the codeine. This could lead to end-stage renal disease and the necessity of beginning dialysis treatment.* A large dose of acetaminophen, which is the active ingredient in Nyquil, can cause complete shutdown of the kidneys and liver in persons that drink the medication to get some alcohol. Aspirin is one of the most common analgesics. About thirty million pounds are sold each year in the United States. Long-term medication with this drug can cause allergic reactions with symptoms of acute asthma (bronchospasm) after taking a single 300 milligram dose. A dose of ten to thirty grams taken at one time can cause death. The most common complication from long-term medication with aspirin is a tendency to bleeding from the depression in the production of blood platelets. The white blood cell count may be reduced. The effect on the blood-forming cells is one feature of the general immunosuppression.

There seems to be an epidemic of degenerative arthritis in the United States. It is well known that most people over age fifty are deficient in calcium and magnesium. When the doctors see the exostosis on the vertebrae of the spine and the distal interphalangeal joints called Heberden's nodes and other signs of degenerative joint disease, and the patient complains of arthritis, they are apt to prescribe analgesic drugs, as mentioned previously, and recommend reducing the intake of milk and cheese. If the patient is given a diuretic, this will increase the loss of calcium. We need from 1000 to 1500 milligrams of calcium carbonate a day. Milk and milk products are the major source of calcium in the diet in the United States. We can expect the development of secondary hyperparathyroidism if the calcium intake is inadequate. So the prevention of degenerative joint disease in the elderly is to see that they get sufficient calcium and vitamin D and C so they can repair the bone. At all events, they should not have long term treatment with diuretics.

Before I leave the subject of disasters in medicine, we must include the use of radioactive elements in diagnosis. The most serious was thorostrast (thorium dioxide) (1930-1950) used to visualize the gall bladder and whether there were gallstones. The tragedy was that most of the people that had this diagnostic test developed angiosarcomas of the liver after a lag period of ten to

*Goodman and Gilman. The Pharmacological Basis of Therapeutics. 7th ed. MacMillan, 1985, p. 692.

Johnson: twenty years. Currently thallium and technetium radioactive compounds are used to visualize blood vessels and tumors. These have a short half-life, but we do not know what will happen in ten to twenty years. It is like x-ray radiation. X-ray radiation of skin for acne and tonsils that seemed too large produced tumors of the parotid glands and the thyroid and an increased incidence of leukemia. Treatment of bony areas, such as the knees or shoulders, produced a similar incidence of leukemia after ten to twenty years. I remember when shoe stores had x-ray machines so the customers could see the bones in their feet. This was stopped by the government when we found out about the effects of x-ray mentioned previously.

One of the major problems today is that people expect magical cures for disease problems that are the result of their lifestyle. They want pills to induce sleep, to wake up, to feel good, and for relieving symptoms such as headache, muscle pain, nervousness, and indigestion (the alkalizers). During the Reformation Period of the sixteenth century, a doctor named Theophrastus Bombas von Hohenheim began to practice medicine in Basel, Switzerland. His father was a physician and his mother the superintendent of the local hospital. He studied medicine with his father and also studied botany and chemistry to learn whether any of the drugs used in medicine had any value as a cure, and which were harmful. He called himself Paracelsus. It probably meant that he followed a system other than that prescribed by the famous doctor Celsus. Research on medicine was primarily concerned to find the "elixir of life." This lasted a long time and the ingredients were hashish, opium, and later cocaine and hops. Paracelsus is known for the drug laudanum, a tincture of opium. He lectured at the university and his criticism of toxic drugs, such as mercury and arsenic, caused the local medical profession to drive him out of Basel.

The advertising in newspapers, magazines, television, and radio telling of magical cures with pills and surgery, has caused some people to be wary of doctors. Others flock to doctors, demanding pills or surgery to relieve their illness. Billions of dollars are earned by drug companies for some of the new drugs each year, especially those recommended for the treatment of asymptomatic high blood pressure or asymptomatic high cholesterol. There is no evidence that these drugs cause people to feel better or live longer. Recent studies indicate that vascular disease maybe related to secondary hyperparathyroidism, which leads to deposition of calcium in the walls of blood vessels and in muscle tissue. The cure for hypertension is to use a moderate amount of salt and eat food providing an adequate intake of calcium and magnesium.

We have some of the best surgeons in the world, but there is a lot of unnecessary or inappropriate surgery. For example, during the last fifteen years there have been up to a million

Johnson: tonsillectomies a year, with 300 to 400 deaths. During the same time period, we have learned that our immunity depends on lymphocytes and that those found in the tonsils have a unique value of providing antibodies for defense against respiratory tract infections. The same is true for the adenoid tissue in the posterior pharynx behind the nose.

Finally a word about the tranquilizers, such as phenothiazines (chlorpromazine) and meprobamates (Equanil and Miltown). They have been useful in short-term treatment, but long-term treatment with this type of drug can lead to irreversible persistent tardive dyskinesia characterized by rhythmical involuntary movements of various muscle groups. We should remember that lithium now used to treat central nervous disease was once used in place of salt. It was found that lithium, which is near to sodium in the periodic table, can produce central nervous system symptoms such as seizures, and a dose of ten to thirty grams can cause death. In other words, as with the tranquilizers, it may be safe to use them for a few weeks but not on a sustained basis.

Naturalist or Virologist?

Hughes: You have described yourself as a naturalist first and a virologist second.

Johnson: Yes, and this has been true for several of my colleagues employed by the Rockefeller Foundation. I'll name them alphabetically: Thomas H.G. Aitken, Marston Bates, Jorge Boshell, John C. Bugher, Calista and Ottis Causey, Wilbur G. Downs, Robert H. Kokernot, Henry W. Kumm, Vernon H. Lee, Ronald B. Mackenzie, Max Theiler, Harold Trapido, Loring Whitman, Telford Work, and C. Brooke Worth. All of these have conducted intensive field studies of the natural environment as well as the insect, bird, and animal life.

I previously mentioned how Brooke Worth introduced me to the tropical evergreen forest of western India at Sakleshpur, west of Bangalore. Brooke had been a professor of zoology at Swarthmore but was an MD. I suppose he had a major interest in birds, but he would identify all the mammals and insects as well. Loring Whitman had an extensive knowledge of the wildlife of Africa, having been a member of the Strong expedition to the Belgian Congo. Max Theiler was also a member of this expedition. Marston Bates conducted an extensive study of the upper reaches of the Amazon at Villavicencio, located in the foothills on the east side of the Andes in Colombia where there had been outbreaks of yellow fever. This is where Jorge Boshell had proved that the Hemagogus mosquito was the vector of yellow fever in the jungle cycle. John Bugher also conducted studies at Villavicencio. He also lived in

Johnson: Uganda as a member of the yellow fever staff there. Marston Bates later became professor of zoology at the University of Michigan. Tommy Aitken conducted field studies at both Trinidad and Belem, Brazil. Wilbur Downs conducted studies in Mexico and Trinidad. The Causeys had assignments in Belem, Brazil, and Ibadan. Bob Kokernot organized field studies in Colombia and South Africa. Telford Work conducted field studies in Egypt and India. He had a special interest in birds. Henry Kumm was in Brazil during the early field studies of yellow fever, and Ron Mackenzie worked in Panama, Colombia, and Brazil. What fun we had talking about natural history.

Hughes: Has it been an advantage in your career to have an MD degree, as opposed to a PhD?

Johnson: Well, in the early days most of the Rockefeller Foundation staff were MDs because they were engaged in public health work. The first specialty other than medicine was sanitary engineering for developing water supplies, malaria control, and the construction of sewage disposal facilities. Parasitology was also a specialty. An MD degree was essential for doing hospital work and doing autopsies. Several of us were specially trained in pathology.

Hughes: How closely have you tried to tie in your research with patient care?

Johnson: In all the projects I was assigned to I was involved with patient care on the clinical side and pathological studies of fatal cases. Of course, I consulted on all kinds of infectious disease, and this was a good way to learn about tropical medicine in general.

Hughes: What I was trying to get at with that question was the importance you place on basic science knowledge as contrasted with applied medical knowledge?

Johnson: The basic sciences teach you how to study problems related to physics, chemistry, botany, zoology, bacteriology, and virology. This is where you learn how to conduct experiments, write notes, and present results. In public health you study new problems using these tools.

There are so many problems related to cultural practices. Hookworm control is dependent on proper disposal of excreta and wearing shoes. Trichina worms require proper cooking of pork [for their destruction]. The same for beef, pork, and fish tapeworms. Visceral larva migrans involves both dogs and cats, and toxoplasmosis is specifically derived from the feces of cats. It's a good idea to keep dogs and cats out of kitchens and wash one's hands after handling pets. One simple public health practice that has not been used to control many of the infectious respiratory infections is staying home at the beginning of cold symptoms and fever. After the third day of fever one is not apt

Johnson: to be infectious except for rhinoviruses and adenoviruses. It should not be necessary to have an official excuse to stay home. In Japan, people that do have early colds wear face masks and that is a good idea. When coughing in crowded places, one should hold a kleenex or handkerchief over the mouth and nose. I do not recommend antihistamines for people that have an acute cold. It is best to sneeze into a kleenex or cloth and let the tears and nasal secretions remove the cell debris. Gargling with salt water is best for treating a sore throat. Headache is a warning to reduce activity.

Dr. Johnson's Scientific Contributions

Hughes: Dr. Johnson, what do you consider to be your most important contribution?

Johnson: For what?

Hughes: I'll leave that to you.

Johnson: Well, the biggest contribution I believe a person can make is not what you publish, but what you contribute to others where you happen to be at the time, all through your life. Spectacular events may attract attention, but if you want to contribute, you work with other people on certain problems or teach how to study such problems.

When it comes to the specific projects of the Rockefeller Foundation, I feel satisfied with what I did at the Rabies Study in Alabama. This work had a lot to do with the elimination of dog rabies from the United States, first by learning about the nature of the disease, the tissue tropism of the virus, how to test a vaccine for potency, and then how to develop a field control program. The development of the avianized Flury strain virus for immunization of dogs was a special piece of virology work.

The expedition to Mexico to study vampire bat rabies was a success as regarding the isolation of rabies virus from vampire bats, which was a new discovery for Mexico. In the process I was bitten by a vampire bat, but it was an unhappy surprise when I developed an acute encephalomyelitis five months later. I was terribly handicapped in 1944 and early 1945. I realized that this had to be a learning experience. Life depends on what you do with the physical strength you have and how hard you work to rebuild your muscles. I shall always be grateful to my physiotherapist, Helen Vaughn, at Warm Springs Foundation in Georgia, who patiently taught me how to train what little muscle activity that remained. Very little was left, but it is amazing how I regained control of facial and eye muscles, and the ability to use my arms and legs.

Johnson: The first big step was to stand upright with a full-length leg brace on my left leg and crutches with a sling support for my elbows to prevent buckling. Later came learning to walk and, as the arms improved, to swing the two legs up to get up four-inch-high steps.

I guess it's the same thing with personal relationships, the big battles are right where you live in your home. If you can deal with these without rancor or anger you have made the big step. One of my friends, a doctor that was recovering from a myocardial infarct, was visited by an older colleague who said, "Why do you work so hard? Do you want everybody to know your name, and you won't know their name?" [laughter] In other words, do you want to be famous and have your name in the newspapers? Back to the subject.

I had a good experience from 1946 to 1951 working at the Rockefeller Foundation lab at the Rockefeller Institute in New York City. I was still recovering from my illness and this required constant training. The studies I carried out on monkey malaria confirmed the existence of an exoerythrocytic stage of Plasmodium cynomolgi malaria in the liver of monkeys infested with sporozoites. This was not accepted by some of the leading malariologists in the United States when I presented my illustrations at the 1954 meeting of the American Society of Tropical Health. I also studied avian malaria, and here again exoerythrocytic forms were found in liver parenchymal cells but also in endothelial cells. It was the study of avian malaria that made it difficult for some malariologists to believe that the liver was the primary site for the exoerythrocytic form of the primate Plasmodium infection. I also did pathology studies of various arthropod-borne viruses.

The assignment to India (1951-1954) was a good experience for me and my family. I appreciate the support and confidence Hugh H. Smith extended to me. I gained in strength from getting back into natural science field studies, the first of which was an ecological survey of India. The main effort was to equip the new Virus Research Centre (now the National Institute of Virology of India) at Poona (now Pune) and then to train the staff and start the field studies. I was very satisfied with the lab and staff and, as I told each one, "Now is your turn to train others." It is still a very fine virus lab. Of course, the time spent in California, 1954 to the present time, at the State Department of Health Viral and Rickettsial Disease Laboratory, running the wildlife disease program on viruses, has been a most rewarding experience.

To return to the subject of publications. I was most concerned with keeping other interested scientists informed about any new information we obtained in our studies and the cooperative studies with Dr. W.C. Reeves of the department of epidemiology of

Johnson: the University of California School of Public Health. There was constant association with the department of entomology at UC and the Museum of Vertebrate Zoology. Even now it's difficult to tell you what I do or what I don't do. But if what I saw is to be passed on, I want to try to be specific. You'll know when I correct my oral history typescript. [laughter]

Hughes: Well, thank you, Dr. Johnson.

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