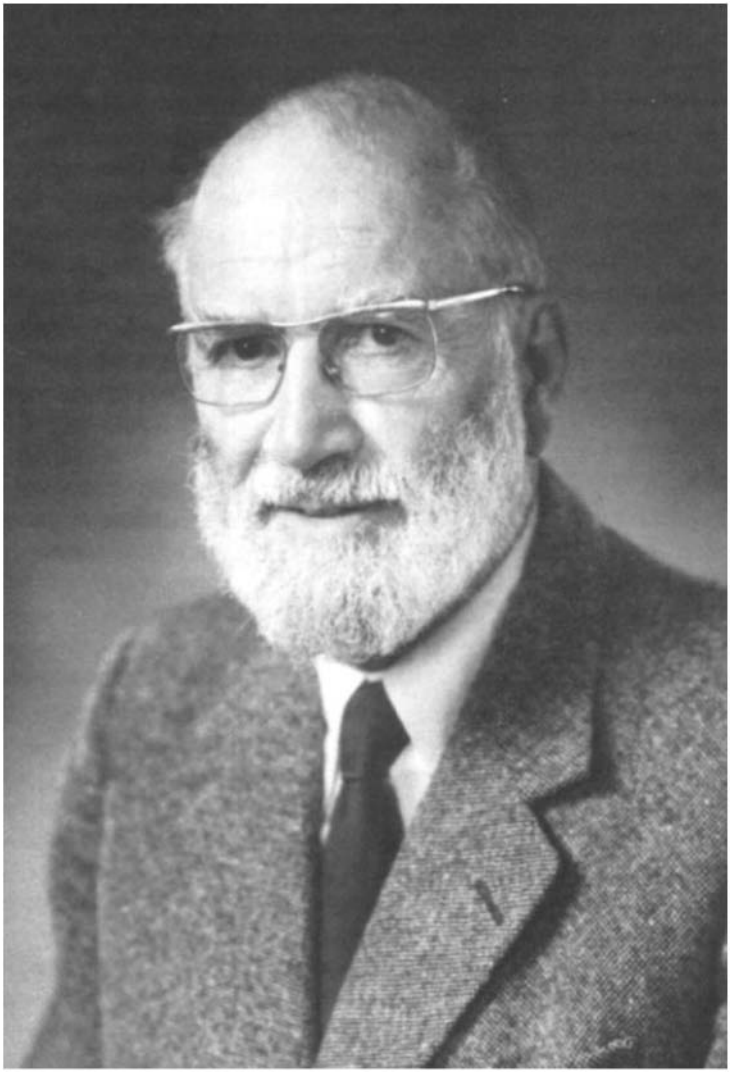

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THE ROCKEFELLER FOUNDATION VIRUS PROGRAM: 1951-1971 WITH UPDATE TO 1981

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The Rockefeller Foundation (RF) was organized in 1913 for "the well-being of mankind throughout the world." The International Health Commission (1913–1916) was established the same year with Wickliffe Rose as the first director. Its objective was "the promotion of public sanitation and the spread of the knowledge of scientific medicine" with the world as its field. In a series of conferences which included William Crawford Gorgas, Henry Rose Carter, and Joseph H. White the eradication of yellow fever was unanimously approved as a practicable and worthy project for the fledgling commission to undertake. The Yellow Fever Commission was established in 1916. The International Health Commission was superseded by the International Health Board (1916–1927), which in turn was replaced by the International Health Division (IHD) (1927–1951). The IHD and the Division of Medical Sciences were merged in 1951 to form the Division of Medicine and Public Health. The history of these early days and decades is well set forth in formal books (1–4) and in informal biographies and accounts (5–9). *The Arthropod-Borne Viruses of Vertebrates: An Account of The Rockefeller Foundation Virus Program, 1951–1970* (10) gives historical data plus detailed information on most of the viruses mentioned in the following pages.

In the prevaccination decades extending into the 1930s there were thirty-two laboratory infections with yellow fever in laboratories maintained by several agencies, including the RF, in America, Europe, South America, and Africa. Five of the thirty-two people infected died, all of them physi-

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cian-researchers. Seventy-six RF staff members were associated at one time or another with the yellow fever program in field or laboratory. Sixty were doctors of medicine, plus seven entomologists, two zoologists, one biochemist, one physicist, and one sanitary engineer. Five of the seventy-six RF staff members who participated in the program died of yellow fever: Howard B. Cross, Adrian Stokes, Hideyo Noguchi, Paul A. Lewis, and Theodore B. Hayne. William A. Young, a British Colonial Medical Service pathologist associated with the Yaba, Nigeria, field laboratory, also died of the disease.

The goal of yellow fever eradication received a rude jolt with the discovery in the early 1930s of the jungle cycle of the virus. The gospel prior to this time held that yellow fever was a disease of population groups, spread by the ubiquitous household mosquito of the tropics, *Aedes aegypti.* The new gospel states that the virus could be maintained in "silent cycles" in the jungles of the New World and the Old World tropics, passed from arboreal monkey to arboreal monkey by the forest-canopy-inhabiting *Haemagogus* mosquitoes in the Americas and by *Aedes* mosquitoes of several species in Africa. This discovery was viewed as threatening to wipe out the gains of the prior twenty years, gains that included the virtual eradication of the disease from the coastal cities of Central and South America and the West Indies.

Laboratory work was progressing in RF-supported laboratories in New York City, in Rio de Janeiro and Bahia in Brazil, in Bogota, Colombia, in Yaba, Nigeria, and in Entebbe, Uganda. The Nigeria and Uganda laboratories were maintained by the Colonial Medical Service of the British government. Work was also going on in French West Africa, with French investigators assigned to Instituts Pasteur of several countries. It was found that the rhesus monkey was susceptible to infection with yellow fever virus and it was further shown that the virus was filterable, this latter being full confirmation of earlier, cruder experiments of Walter Reed and James Carroll in 1902. Johannes H. Bauer and Alexander F. Mahaffy showed the sensitivity of the virus to saline solution, water, or Ringer's solution, and emphasized the importance of using a diluent of 10% normal rhesus serum. Max Theiler found that the adult white mouse could be used as a test animal and after successive modifications, virus titration and virus neutralization techniques were devised, usually relying on intracerebral inoculation of anesthetized mice. Such techniques, with minor modifications, remain the basis for present work, even including the various adaptations required when cell culture systems incorporating fluorescence and radio-immune assay techniques were substituted for live animals. However, an obvious improvement, the substitution of infant mice for the weanling or adult mice employed throughout the 1930s and 1940s, did not suggest itself until Gilbert Dalldorf demonstrated the effectiveness of this extension of the

technique in the isolation of Coxsackie viruses from human feces. Ottis R. and Calista E. Causey, working in the Belem Virus Laboratory, tried the infant mouse intracerebral inoculation technique. This use of infant mice for virus isolation greatly increased the sensitivity of the virus detection system and was directly responsible for the isolations of hundreds of previously unsuspected "new" arthropod-transmitted viruses from arthropods, mammals, birds, cold-blooded vertebrates, and humans in many laboratories in the New and the Old Worlds. Many were viruses that showed no demonstrable pathogenicity for adult mice but that caused disease or death when inoculated into infant mice. The delay in recognizing the importance of the inoculation of infant mice in virus detection is surprising since, on several occasions in the 1930s, the phenomenon of infant mouse susceptibility was observed. Theiler himself remarked twenty years later that he could not understand his own failure to make the jump to application of the phenomenon which he, among others, had observed.

The test systems developed permitted the study of the behavior of yellow fever virus strains, some with both viscerotropic and neurotropic features, in monkey and mouse, studies that progressed to a search for virus strains with modified pathogenicity. Theiler and Hugh H. Smith in 1937 reported that both the viscerotropic and the neurotropic virulence of the Asibi strain of virus cultivated in chick embryo had been greatly reduced, and in the same year this modified strain, designated 17D, was employed in human immunization and was shown to be safe and also effective in inducing antibodies to yellow fever virus. Theiler in his Nobel Prize address (11) gives the story of the 17D strain. A mutation evidently occurred during the course of passage. The mutant strain has been carefully preserved, with as little further passage as possible, as the vaccine strain from which all 17D vaccine currently in use is made. Further attempts to produce the same or a similar mutant through repetition of the steps of the original passage history or by any other technique have met with no success.

Workers at the Institut Pasteur in Dakar, Senegal, working with a strain also developed by Theiler, used this strain (modified by J. Laigret and known as the French neurotropic strain) in vaccinating over 26,000,000 people in French West Africa. This vaccine was effective when administered by the scratch method However, when given to small children there was rare and grave meningo-encephalitic involvement, sometimes fatal. The French neurotropic vaccine has now been completely replaced by the 17D vaccine. The present 17D vaccine has been and remains the most innocuous and the most effective vaccine ever developed for any virus disease.

The new vaccine was able to relieve the consternation that had followed upon the demonstration of the jungle yellow fever epidemiology. It was obvious that any government interested in controlling yellow fever within

its boundaries had the means at hand to do so. At this point, the Directors of the IHD of the RF decided to bow out gracefully from the yellow fever scene. The decision was understandable but time has shown that in the real world, few if any of the underdeveloped countries of the equatorial and subequatorial zones are able to maintain a tightly run *A. aegypti* control campaign and none keeps up a vigilant 17D vaccination service reaching all inhabitants of *A. aegypti*–infested coastal cities and populations at risk in the hinterlands. The RF backed a program for the eradication of *Aedes aegypti* from Brazil in the 1930s, with Fred L. Soper in charge and supported by a staff of RF and Brazilian helpers. The international team achieved eradication.

In the decades of 1940, 1950, and 1960, DDT and other newly developed residual insecticides were powerful aids in keeping *A. aegypti* populations under control or even eradicated, but in time certain geographical strains evolved insecticide-resistant populations. Whole regions formerly rid of the pest, even including parts of Brazil, are now reinfested. Dengue, transmitted by the same vector, has reappeared in the Caribbean, Latin America, and, in 1980, in southern Texas. Where conditions are right for transmission of dengue, conditions may be right for transmission of yellow fever, and a chance introduction of the virus by way of an infected traveller or by an infected mosquito hitchhiking on an aircraft might lead to an epidemic. *A. aegypti* of course poses no threat in an immunized population, but political instability, understaffed and underfinanced health departments, and bureaucratic indifference and inadequacy guarantee that immunization programs are not realized and that yellow fever will continue to be with us. Outbreaks, major and minor, in Brazil, Colombia, Venezuela, Argentina, Paraguay, Bolivia, Ecuador, Peru, Trinidad, Panama, Costa Rica, Nicaragua, Honduras, El Salvador, Mexico, Ethiopia, Nigeria, Ghana, Senegal, Portuguese Guinea, Sierra Leone, The Cameroons, The Gambia, and other countries have occurred since 1950. The Ethiopian outbreak of 1960–1962 claimed thousands of lives and several outbreaks claimed hundreds.

When the RF closed out its yellow fever program in the late 1930s there were many experienced field and laboratory workers separated from their lifetime work. Every effort was made to accommodate these workers. Kenneth C. Smithburn, Richard M. Taylor, and J. Austin Kerr were absorbed into the RF Virus Laboratories (RFVL) housed in the North Building of the Rockefeller Institute (now Rockefeller University) complex at 66th Street and York Avenue, New York City. Bauer was then the head of the Laboratories. The laboratory did not involute. Theiler discovered the mouse encephalomyelitis virus, and his classic studies of the epidemiology of this infection, with fecal shedding of virus, provided valuable guidelines for later human poliomyelitis virus studies. George K. Hirst, William F.

Friedewald, and Frank L. Horsfall, Jr. made significant contributions to knowledge of the influenza viruses, and Taylor described influenza C, a type as uncommonly encountered now as it was then.

The threat of World War II introduced new priorities for the RF Virus Laboratories. Studies were started on typhus fevers, with John C. Snyder and Charles R. Anderson participating. Anderson contracted typhus and recovered. Anticipated demands for heavy production of the 17D vaccine for military personnel plunged the laboratory staff into developing the necessary equipment and techniques for the job. Staff members assigned to this task included, beside Bauer, Delphine H. Clarke and an assigned specialist, Alice Moore. Edward G. Pickels with a well-equipped machine shop and skilled machinists available developed and refined ultracentrifuges, filters, vacuum desiccating apparatus, and electrophoretic apparatus. Much of this equipment served as prototypes for the laboratory-equipment-manufacturing companies that proliferated in later decades. Early production runs of the vaccine had diluted human serum added as a virus protective buffer. A serious outbreak of serum-transmitted hepatitis occurred in US Army personnel immunized with certain of the early vaccine lots. Further lots of vaccine had an aqueous base, no serum, and this complication was thus circumvented.

Malaria became a major problem for Allied forces overseas and the RFVL increased its participation in this area. Lowell T. Coggeshall uncovered a new species of malaria parasite by examining the pheasants from Southeast Asia that were housed in the Bronx Zoo. He was looking for *Plasmodium gallinaceum,* a most useful parasite in malaria laboratory studies, the import of which into the United States was forbidden. He found *P. lophurae* in the blood of a Borneo Fireback Pheasant, *Lophura lophura.* It proved to be a reasonably good substitute for *P. gallinaceum.* Coggeshall, John Z. Maier, Anderson, and Clarke worked on sulfa drugs and drugs of the naphthaquinone series as antimalarials in work that anticipated unwittingly the emergence of drug-resistant malaria strains in the 1960s in Southeast Asia. Anderson made significant studies with the in vitro cultivation of a malaria parasite.

John B. Lynch and Harald N. Johnson worked on rabies in a RF-supported laboratory in Montgomery, Alabama, and there developed egg-passaged strains of rabies which, as live virus vaccines, have had extensive use in canine and livestock immunization. Rabies was then considered as an essentially monolithic (i.e. unchangeable, immutable) virus agent with only one unvarying representative in nature. This view has been modified in recent years, as is discussed later.

Earlier yellow fever studies in South America and Africa had incidentally made available to field-laboratory workers several adult mouse pathogenic

agents which at the time were regarded as virus curiosities, including the viruses of Ilheus, Anopheles A, Anopheles B, and Wyeomyia (from South America) and West Nile, Uganda S, Semliki Forest, Bwamba, Bunyamwera, Zika, and Ntaya (from Africa). Trivittatus virus was isolated from mosquitoes in North Dakota by Carl Eklund working with a USPHS team. The known or suspected arthropod-transmitted viruses important in causing disease in human beings and/or livestock in the period prior to 1950 included yellow fever, dengue type 1, dengue type 2, Japanese encephalitis virus, Negishi virus, Eastern, Western, Venezuelan and St. Louis encephalitis viruses, Rift Valley Fever virus, the virus of Russian spring-summer encephalitis (now known as TBE or tick-borne encephalitis virus), Colorado tick fever virus, Omsk hemorrhagic fever virus, the viruses of Naples and Sicilian sandfly fevers, and California encephalitis virus. There were also the animal pathogenic virus agents of African horsesickness, Vesicular stomatitis (Indiana), Nairobi sheep disease, Louping ill, African swine fever, and Bluetongue of sheep. These 22, with the 12 exotic curiosities mentioned above, gave a total list of 34 agents recognized.

Smithburn, Taylor, and Kerr worked with these viruses in the RFVL in New York, and Peter K. Olitsky and Jordi Casals in the Rockefeller Institute, New York. C. Hallauer in Germany, and Albert B. Sabin and Edward L. Buescher in Cleveland developed the first hemagglutination inhibition (HI) techniques, work carried forward by Casals, L. V. Brown, and Delphine H. Clarke. Casals specialized in complement-fixation (CF) studies and interested himself greatly in the sorting and classifying of the known viruses. The viruses in the cluster of the human and animal encephalitides, Eastern, Western, and Venezuelan, all of them mosquito transmitted, were shown to have at least some degree of serological relationship, demonstrated in CF, HI, and neutralization (NT) tests, and were designated Group A agents (now known as alphaviruses). The viruses of yellow fever, the two dengues, and St. Louis, all mosquito borne, demonstrated strong serological cross relationships and were designated Group B viruses. The viruses of Russian spring-summer encephalitis and Louping ill were shown to be related serologically to the Group B agents and were known to be tick transmitted. The Group B viruses have since been designated "flaviviruses."

This finding of mosquito-transmitted and tick-transmitted viruses within the same serological group was the first of several biological incongruities to be encountered as work with serological classification progressed. The remaining viruses in the lists above showed no relationships, one to another, and each stood temporarily as an individually unique agent with no other apparent counterparts in Nature. It was observed that all of these viruses were RNA viruses. To demolish this generalization, it was later shown that the virus of African swine fever, tick transmitted, is a DNA virus. All of

the arboviruses require, for their continuing existence, a virus growth cycle in an arthropod, and a virus growth cycle in a vertebrate. In simple terms this means an intermediate and a definitive host. From our anthropocentric vantage point, we regard the arthropod host as the intermediate host. There must also be a time-tested mechanism for transmission of the virus from the arthropod to the vertebrate, and in due course from the vertebrate back to the arthropod. Should one wish to consider the realm of virus phylogeny, as viruses have evolved over millenia, along with their intermediate and definitive hosts, there are many questions and no solid answers.

In 1951 the RF-IHD decided that a major program would be started, with an anticipated backing for 15 to 20 years, to explore the field of arthropod-transmitted viruses, not channeled as were earlier programs specifically toward yellow fever, but aimed in a shotgun approach at "what may be out there." The United States Army had already gone heavily into the field in Japan with an extensive program aimed at Japanese encephalitis with supporting US laboratories, Army and civilian. This program, under the civilian direction of Joseph E. Smadel, enlarged its coverage to include a laboratory in Kuala Lumpur, Malaya. The US Navy, operating through the USN Medical Research Units #2 in Taiwan and #3 in Cairo, was active in the field. The US Public Health Service (under DHEW) increased activity in the National Institutes of Health in Bethesda, in the Rocky Mountain Laboratory in Hamilton, Montana, and in the Center for Disease Control in Atlanta, Georgia, as well as in the program of International Cooperation in Medical Research and Training (ICMRT, later shortened to ICMR) with field programs, in collaboration with contracting American university groups, in Colombia, India, Malaysia, Iran, Pakistan, and Costa Rica. Several state health department laboratories, notably in California, Wisconsin, Texas, New Jersey, New York, and Massachusetts, were active.

The RFVL in New York City, then under the direction of Theiler, assumed a central coordinating activity for the RF program. Field laboratories were established in a number of countries over a period of several years. Two staff workers were assigned to the Cairo laboratory of the US Naval Research Unit #3 in 1952. Next to be established were the Virus Research Centre in Poona, India, in collaboration with the Indian Council for Medical Research in 1952; the Trinidad Regional Virus Laboratory in Port of Spain, Trinidad, in collaboration with the Trinidad Government Health Department, and later the Overseas Development Council and the Medical Research Council of Great Britain in 1953; the Belem Virus Laboratory at Belem, Brazil, in collaboration with the Brazilian Special Service Public Health Department (SESP) in 1954; and a laboratory attached to the South African Institute for Medical Research in Johannesburg, South Africa, and under the jurisdiction of the South African Council for Medical Research,

in 1953. Smith and later Robert S. Morison in the RF-DMPH central office in New York gave very careful thought to the location of these field laboratories, carefully considering such factors as geographical location tied in to what might be anticipated as being favorable ecological characteristics of a region, and of necessity also including considerations of ease of communication, political stability of a region, and willingness of a host country to have an outside collaborating laboratory within its national borders.

In the 1960s, personnel and support were withdrawn from the South African laboratory with the knowledge that South African workers including James Gear and Bruce McIntosh were well able to proceed on their own, and two further field stations were established, one in 1961 in collaboration with the Colombian government, the Cali Virus Laboratory, at the Universidad del Valle, and the other, the Virus Laboratory at the University of Ibadan, Ibadan, Nigeria, with the collaboration of the Nigerian government in 1964. Each of these laboratories had one or more RF staff members assigned to it, and, in addition, one staff member was assigned to the California State Health Department Laboratories in San Francisco/Berkeley, in 1954. Workers from the counties involved were recruited to the program and eventually in most instances took over the direction of the local laboratories. The programs in each country were designed financially to be receiving a major portion of support initially from RF funding, and with local funding gradually increasing until, eventually, RF funding and personnel would be completely withdrawn, with the laboratory becoming a completely national affair. The later stages of withdrawal were always difficult. A condensed and incomplete statement at the time of this writing is that the Poona, Trinidad, Belem, and South African laboratories are thriving, although not necessarily adequately financed for support of far-ranging field work. The Ibadan Virus Laboratory is in serious difficulty, essentially nonfunctional because of lack of government financial support, and the Cali Virus Laboratory is no longer heard from as a functioning arbovirus laboratory although some of the RF-associated Colombian virologists have attained high levels of service in national and international organizations.

The several field laboratories engaged in programs of exploration of the virus problems of the local region, studies that involved the detection and investigation of human disease, the surveying of "normal" human populations for evidence of past involvement with viruses, and the search for viruses and for past virus involvement in a wide variety of arthropods, mammals, and birds. Arthropod studies included mosquitoes as a major item, ticks, mites, *Phlebotomus* flies, *Culicoides* midges, Simuliidae (black flies), Tabanidae (horse flies), Cimicidae (bed bugs), Reduviidae (kissing bugs), Pulicidae (fleas), lice, indeed, the major groups of arthropods biting

man and animals, domestic or wild. This far-ranging coverage was usually reduced to mosquitoes and ticks, with *Phlebotomus* and *Culicoides* also receiving much attention.

In addition to this internal program of the RF, an effort was made in many other countries to locate workers who were interested and who needed assistance in furthering their studies. Assistance was given in two ways, usually as an integrated package. Actual financial support was given to laboratories (always through government or university channels) and fellowships and travel grants were given to workers in such laboratories to enable them to gain experience and acquire skills in other centers of investigation, most often the RF Virus Laboratories in New York.

The RFVL in New York City at the beginning of the expanded virus program in 1951 was staffed by Theiler, Casals, Clarke, Taylor, Smithburn, and Loring Whitman. Sonja M. Buckley joined this group in 1957. Other RF staff members were often detailed to it temporarily, for training before proceeding to field assignments. The program for the staff was the refining of existing techniques for virus study, basically the CF, HI, and NT tests, effecting improvements in preparation of the antigens and antisera for the performance of these tests, the development of new techniques, and the application of these techniques to the sorting, identification, and classification of the viruses isolated in the field both by RF field teams and by other groups. With the emergence of cell cultures as working tools for the virologist, Buckley was an early worker in adapting such cultures, both of mammalian and later of insect cells, to arbovirus work.

In 1964 the RF completed arrangements with Yale University, and the laboratory investigators, with much equipment and invaluable and irreplaceable stocks of viruses and immune reagents, were moved to new quarters in the Laboratory of Epidemiology and Public Health of the Yale Medical School in New Haven, Connecticut, where they continue to function as the Yale Arbovirus Research Unit (YARU). The YARU is no longer supported by the Rockefeller Foundation although some holdover endowment funds remain. Support comes from the National Institutes of Health of the US Public Health Service through grants and contracts, from the US Army, from the US Navy, from the Australian Government, from the World Health Organization, and from occasional other sources.

YARU was designated as the World Health Organization International Reference Centre for Arboviruses and works in effective collaboration with WHO Regional Reference Centres in Ft. Collins (Colorado), Belem, Bratislava, Brisbane, Dakar, Entebbe, Johannesburg, Moscow, Poona, and Tokyo, and with many other laboratories around the world.

In 1959 the Rockefeller Foundation sponsored a meeting of US arbovirologists at Gould House, an estate on the Hudson River. From this

meeting emerged the American Committee on Arthropod-borne Viruses (ACAV). A committee on information exchange was formed and developed the present *Catalogue of Arthropod-borne Viruses of the World,* a catalogue that is continuously being revised and updated as new information comes in. This catalogue plus revisions contains detailed information on all of the arboviruses and arenaviruses mentioned in following pages. Taylor played a major role in the catalogue development until 1968, when he was succeeded by Trygve O. Berge, and later by Nick Karabatsos. Telford H. Work, William H. Scherer, and Roy W. Chamberlain played major roles in the Catalogue and Information Exchange developments of the ACAV, along with Casals, Robert E. Shope, and Thomas H. G. Aitken of the RF-YARU staff. The Rockefeller Foundation supported the operation until 1962; since then it has been operated with assistance from the US Public Health Service. The ACAV remains in close touch with arbovirologists around the world.

The Catalogue of Arthropod-borne Viruses of the World (12) contains a vast amount of information on viruses, their geographic distribution, the vertebrate hosts involved, including in some instances man, and the arthropod vectors involved. The first edition, published in 1967, supplied information on 204 viruses, a big increase from the earlier-mentioned 1950 total of 34. The 1975 second edition (13) lists 359 viruses, the 1978 supplement (14) raises the total to 388 viruses, and the 1981 working catalogue (not a citable literature source) lists 440. There are still many viruses awaiting study and classification. To a considerable degree, present virus studies are conducted on viruses isolated during the heyday of field virus investigations. The pace of this virus isolation activity is much slowed at this time, with remaining field laboratories working at a greatly reduced level. Financing of field epidemiological studies does not receive high priority in today's laboratory-oriented virus research world.

At the beginning of the RF expanded program in 1951, only two virus groupings were recognized, Group A (alphaviruses) and Group B (flaviviruses). Alphaviruses included 4 of the then-known 34 viruses, flaviviruses 14, and the remaining 16 were in an "ungrouped" category. A California group of viruses was early separated out and in due course this was absorbed into a Bunyamwera supergroup of viruses, this latter being a large grouping with shared morphological features demonstrable by electron microscopy and with a complex pattern of serological interrelationships. Group C was set up early, but when a continuing spate of new viruses made it obvious that an alphabetical progression for new virus groups would be unsuitable, a naming system for groups was established where the name of the first virus described in a new group would serve as the group name. When an ungrouped virus is shown to be serologically related to another (any other)

virus it is included in the group to which this serorelative belongs. In case both were previously without relatives, a new serogroup comes to be recognized.

The number of groups has increased greatly, as well as the number of viruses assigned to groups. There are now 59 recognized groups. The alphavirus group includes 26 viruses and the flavivirus group 61 viruses. The only other group with such numbers is the Phlebotomus fever group with 29 viruses. Most of the remaining 56 groups have only a small number of viruses per group. However, the Bunyamwera supergroup has a total of 102 viruses, divided among 15 groups in the supergroup. Viruses in several groups, including the Kemerovo group, the Colorado tick fever group, the Bluetongue group, and others, are recognized as being morphologically and structurally identical to orbiviruses. The Tacaribe group, which includes important viruses of lymphocytic choriomeningitis, Lassa, Junin, and Machupo, is actually not an arbovirus grouping. The viruses are known as arenaviruses and are associated with small mammals. Since they are commonly encountered by arbovirus workers, they remain in arbovirus listings. There are 96 viruses remaining in the "ungrouped" category, i.e. none of them is serologically related to any of the grouped viruses or to any other one or more of the 96. Many of these viruses have not yet been thoroughly studied. They are a challenge to those interested in virus phylogeny and in virus classification.

Although serology has remained the base for the current arbovirus classification, electron microscopy is now a valuable adjunct tool. For example, among the 96 ungrouped viruses are several that are rhabdoviruses morphologically. Once observed, this is a stimulus to search harder for serological relationships with other known rhabdoviruses. More recently still, RNA "fingerprinting" is being shown to be a valuable tool for exploring degrees of relationship among viruses already known to be related serologically. Also among certain of the viruses in the Bunyamwera supergroup and the Kemerovo group, recombination of segments of the RNA genome can be effected, and is proving to be a useful tool for exploring relationships.

In all instances tested the serological relationships established have received supporting electron-microscope confirmation of morphological homology. Also, from an epidemiological point of view, the serogroup members appear to have similar epidemiological patterns involving vector and vertebrate hosts. The alphaviruses, as far as studied, are all mosquito transmitted except for Fort Morgan virus, isolated from cimicid bugs collected from the nests of certain birds. The flaviviruses, as earlier noted, diverge with some members mosquito transmitted, others tick transmitted, and some with birds as primary vertebrate hosts, others with mammals. With refined serological tests within the flavivirus group, subgroupings can

be made that do conform to the epidemiological patterns. In the many other groups, there is usually a consistent epidemiological pattern within a group. This suggests a basic phylogenetic classification of the arboviruses and this has indeed been the subject of some speculative writing.

Yellow fever and dengue types 1, 2, 3, and 4 are the only important viruses found in the Old and New Worlds. This can be explained by the pantropical distribution of an exceptionally good vector, *Aedes aegypti.* Wad Medani, a virus isolated from ticks in Egypt, Pakistan, and India, has been recovered from a species of tick in Jamaica, West Indies. It is presumed that the virus was brought to Jamaica on imported livestock. Powassan, a tick-transmitted virus of mammals in Canada and northern United States, has been recovered from ticks in eastern Siberia. The other important arboviruses, important from the standpoint of human health, are limited to one continent, rarely two. Hundreds of the viruses, with no particular involvement in human disease, are similarly restricted, sometimes restricted indeed to very narrowly demarcated geographic regions, i.e. ecotopes.

These virus distributional characteristics highlight some specific features of arboviruses in general. Each virus is a zoological entity unto itself, with its own narrowly defined determinants for its continued successful existence. The virus must have an invertebrate host, an arthropod that is receptive, i.e. will be infected by, propagate and in due course transmit, and a vertebrate host, bird or animal including man, that is also receptive, i.e. will be infected by and propagate the virus in quantity sufficient to infect the arthropod vector that bites it. There are wheels within wheels, since it is also necessary that the infected arthropod is interested in biting the susceptible vertebrate. The virus itself is probably not influenced by climate and physiogeographic details of landscape. The arthropod vector, however, may be profoundly influenced by such, and indeed so also may be the susceptible vertebrate. With this in mind, the seemingly random or senseless distribution of the more than 400 viruses can be shown to be a rigidly determined distribution. Facts to support these statements can come only from detailed field investigations, and speak for the continuing need for such studies.

The unique feature of arboviruses in general and the diseases they cause, i.e. that their occurrence and distribution are determined by geographic and attendant ecological considerations, can be appreciated by acquaintance with the history of each of the several laboratories included in the RF extended virus program.

The Virus Research Laboratory in Poona, established in 1952 under the aegis of the Indian Council for Medical Research, had as its first director J. Austin Kerr accompanied by Johnson of the RF field staff. Indian counterparts were, over the period of several years, T. Ramachandra Rao, Raja

Varma, K. R. P. Singh, Keerti Shah, Pravin Bhatt, Sharda Paul, Khorshed Pavri, Kalyan Banerjee, Rachel Reuben, P. K. Rajagopalan, M. K. Goverdhan, F. M. Rodriguez and Harilal Bhat. Kerr and Johnson left, to be replaced by Work, Harold Trapido, and later, when Work left, by Anderson and Jorge Boshell of RF field staff. The initial program was directed to a serosurvey ranging widely over the Indian subcontinent, from the Himalayan foothills, to the Gangetic plain, the Ganges delta, the central Deccan plateau, and to the coastlines east, west, and south.

Meanwhile virus vector studies, including both mosquitoes and ticks were started in several regions. A substation of the Poona laboratory was established in quarters furnished by the Vellore Christian College and staffed by Donald E. Carey, with Ruth M. Myers of the Vellore staff collaborating. Reuben was assigned to this group as entomologist. Serosurveys established the probable presence of a number of viruses in India, including West Nile, Japanese encephalitis, Sindbis, dengue(s), and phlebotomus (sandfly) fever of Naples and Sicilian types. The word "probable" is used advisedly. Serological findings are not generally considered as definitively diagnostic in connection with serosurveys and in connection with virus studies on a single individual. Definitive diagnosis comes with virus isolation and identification. Early laboratory efforts for several months were directed to an outbreak of meningoencephalitis in small children in the industrial city and region of Jamshedpur. A diagnosis of this outbreak was not attained and some hard facts were learned as far as the Indian scene was concerned. Adequate diagnostic materials, as for example autopsy specimens from fatal cases, were almost unobtainable.

In 1957 an outbreak of a disease, often fatal, with hemorrhagic and shock symptoms, was reported from Mysore State, south of Poona. There were not only human cases, but also cases in the langur and rhesus monkeys of the region. Monkey mortality was high. Early fears that it might be yellow fever were soon dispelled when a virus was isolated and shown to be a serorelative of the Russian spring/summer encephalitis virus (TBE), a known cause of widespread and sometimes epidemic occurrence of encephalitis in the USSR. It was thought that this virus might have been carried over or around the Himalayas into India. However, the earlier serosurveys had not turned up evidence of its past presence. Work, Trapido, and their Indian associates were involved with the Indian epidemic problem and the virus was dispatched to RFVL-NY and shown by Clarke to be distinct from the TBE virus. It was given the name of Kyasanur Forest Disease virus (KFD). Field studies showed the virus to be closely associated with ticks and monkeys, with involvement of other small mammals of the region and with birds also possibly involved. KFD continues active in Mysore State with the same general region of a few hundred square miles involved, but

with the center of activity shifting year by year. The epidemiology of this virus infection has been the subject of continuing research on tick vectors, vertebrate hosts, and the interaction of the human beings of the region with the ecosystem harboring the virus. Did KFD or a progenitor, with the recession of the glaciers in millenia past, invade the Siberian taiga from the south and differentiate enough to be considered a distinct agent (i.e. TBE)? Or did TBE move southward, invade the Indian subcontinent, and differentiate enough to become what is called KFD? Incidentally, while we are musing, another tick-borne flavivirus related to TBE and KFD, namely Langat, was found on the Malayan peninsula in 1956 by C. E. Gordon Smith of the London School of Hygiene and Tropical Medicine. It causes no significant human illness. Another relative is found in Canada and the northern United States, Powassan virus, which is also tick borne, with vertebrate reservoir in small mammals. Powassan virus is associated with encephalitis in human beings. It has also been reported recently from far eastern Siberia. None of this subgroup of tick-transmitted flaviviruses had been found south of the Equator until Australian workers reported a new member of the subgroup isolated from ticks associated with birds.

The Vellore field laboratory soon became involved with several virus problems. Dengue was shown to be endemic in the region and three types were found. One expatriate doctor working in the hospital over the period of several years became infected with each of the dengue serotypes of the region, and had a typical course of illness with each episode. Virus was isolated in each of the infections. This unique history has incidentally provided material for thought in connection with the development of an effective dengue vaccine. Even with the three dengue serotypes in the region, the syndrome of dengue hemorrhagic fever–dengue shock syndrome (DHF-DSS), which has been and continues to be a grave problem in various countries of Southeast Asia and Indonesia, has not been reported from the Vellore region (even though there was an outbreak in Calcutta). There have been many dengue outbreaks in India in recent years including major outbreaks in Calcutta and New Delhi.

Chikungunya virus is an alphavirus, mosquito transmitted, which was first reported from a major outbreak of illness with fever and joint pains in East Africa in 1956. Outbreaks were seen in Southeast Asia a decade later. In Bangkok, the disease occurred concurrently with dengue. Both share the same vector. The Vellore laboratory was involved in a major outbreak in the Madras Presidency of India where it was estimated that there were more than a million cases. Carey was impressed by the clinical similarities, and differences also, between dengue and Chikungunya and analyzed literature reports of "dengue" outbreaks in Southeast Asia over the past 100 years, concluding the Chikungunya had been responsible for many outbreaks.

Japanese encephalitis (JE) was responsible for the siting of the Vellore

laboratory. Smithburn at RFVL-NY had established the presence of antibodies in sera collected in India by Kerr and Johnson. These findings in serum specimens collected from suspected human cases from the Vellore region were confirmed by Work. Carey, Myers, and Reuben, based in Vellore, carried out epidemiological studies on people, mosquitoes, birds, and animals of the region, made many virus isolations from mosquitoes, man, and birds, and confirmed the epidemiological details elaborated by earlier workers in Japan and Malaya. Since the Vellore studies there have been several outbreaks of JE in various parts of eastern India, some of them large.

West Nile (WN) virus is another flavivirus, mosquito transmitted, known from studies ranging from central Africa to the Middle East. Smithburn at RFVL-NY detected antibodies in specimens from the Vellore region in 1954. The Vellore laboratory group encountered the virus, and the Poona laboratory group found it more common in western India. JE and WN are closely related flaviviruses, both serologically and in details of mosquito transmission. JE is associated with fatal human disease, encephalitis, whereas WN is usually innocent. St. Louis virus, a cause of encephalitis in the United States and the Americas from Canada to the Argentine, is a close serorelative, also with similar epidemiological details. The same holds for Murray Valley encephalitis virus in Australia–New Guinea, and for South American viruses Ilheus and the recently described Rocio. India is particularly interesting as far as JE and WN are concerned since it is the common ground for JE from the East and WN from the West. Overlap is observed in the Vellore region.

There are many other achievements of the VRC-Poona in the RF association days, a couple of which merit special mention. African horsesickness virus was found for the first time in India in connection with an epizootic in cavalry horses stabled at Jaipur (it had earlier been recognized in Pakistan). A virus named Chandipura was isolated in 1967 from humans during an epidemic of "dengue" in Nagpur, which turned out to be a mixed outbreak of Chandipura, Chikungunya, and dengue. Chandipura virus has been shown to be a rhabdovirus, related serologically to the viruses of vesicular stomatitis (of livestock) found in the New World, and is proving to be a very useful tool in the study of rhabdoviruses. K. R. P. Singh in Poona became interested in the insect cell culture work being carried out in Australia, and developed a technique for cell culture that has been most useful in laboratories the world over. Singh's original article, incidentally, was turned down by *Nature,* and was published in *Current Science,* an Indian journal. It is a much cited reference.

The VRC-Poona remains a very active center, under the Indian Council for Medical Research, with no continuing RF participation, but indeed with active collaboration with the YARU.

The Trinidad Regional Virus Laboratory (TRVL) was established in 1952, in empty US Army barrack buildings on the waterfront in Port of Spain. Wilbur G. Downs was the director, with Anderson supplying background laboratory talent and experience from prior work in Colombia. The Trinidad government seconded Leslie Spence and later Elisha S. Tikasingh and Kenneth Delpeche to join the group, and the RF, over several years, added Aitken, C. Brooke Worth, and Andries H. Jonkers. The traditional starting gambit of a serosurvey, specimens being sent to the RFVY-NY, gave evidence of the probable presence of a number of the then-known viruses on the island. The discovery of yellow fever antibodies in the sera of several schoolchildren was a surprise. No yellow fever activity had been reported from the island since 1914. This hint was confirmed in 1954 when a yellow fever outbreak developed on Trinidad, with many thousands of cases, most of them subclinical or mild, and with four confirmed deaths. The TRVL had made a very early diagnosis while the outbreak was brewing and this resulted in an early island-wide immunization program and extensive *A. aegypti* control efforts, which probably aborted an even more extensive outbreak. Trinidad and Tobago then constituted a Crown Colony, and the British government, through the Overseas Development Ministry and the Medical Research Council gave very welcome financial and personnel help to the laboratory, help that continued for many years.

An epidemic of dengue was going on in 1954 also, and several strains of dengue type 2 virus were isolated. These represented the first strains of dengue virus from the New World and indeed, no further New World strains were available to workers until the extensive dengue outbreaks of the late 1960s swept the more northerly West Indian islands, Colombia, and Venezuela. Dengue outbreaks continue throughout the circum-Caribbean and Gulf of Mexico regions, and cases were seen in Texas in 1980. Dengue had moved through these regions and the southern United States for centuries, unchecked until mosquito control measures were introduced. With the advent of DDT as an insecticide, and its application to the *A. aegypti* problem, the mosquito was eradicated from many countries, and almost eradicated from most. Hemisphere eradication was a goal of the 1960s. Dengue ceased to be seen, although it must have been hanging on somewhere. Surveys carried out by the TRVL in many of the northern islands failed to detect evidence of recent disease, and no more cases were seen in Trinidad after 1954–1955 and the transient eradication of the vector mosquito from that island. But the mosquito developed resistance, first to DDT and then to other insecticides, and insidiously reestablished itself throughout the West Indies and northern Southern America and Central America. This was followed by the current dengue resurgence.

There had been an outbreak of Venezuelan equine encephalomyelitis in Trinidad in 1943–1944. Virus was isolated and studied at the Walter Reed

Army Medical Center in Washington at that time, and carefully preserved. TRVL workers encountered a VEE relative in mosquitoes and small mammals 15 years later. The virus was not as virulent as the earlier virus and had detectable serological differences. It is known as Mucambo virus. Whether it represents a clonal derivative from the earlier VEE is not known. It causes no livestock disease whereas the earlier virus did.

Both St. Louis encephalitis virus (SLE) and Eastern equine encephalitis (EEE) virus were recovered in Trinidad from mosquito and vertebrate hosts. One isolation of SLE was made from the blood of a child with no fever or symptoms of disease. Nearly 15 years later two fatal human cases of EEE infection were reported. Ilheus virus had been isolated from mosquitoes in Brazil in 1947 by Hugo Laemmert and Thomas P. Hughes. Trinidad surveys showed immunity to be widespread and prevalent in forest-dwelling people, findings parallel to those encountered in large areas of Brazil and northern and northeastern South America. Virus isolations were made from the blood of three Trinidadians, one with no fever or symptoms of illness, one with mild illness and low grade fever, and one with a severe encephalitis. This experience on a small scale is illustrative of the spectrum of disease seen with several of the important arboviruses, SLE, Western equine encephalitis, Japanese encephalitis, and even yellow fever.

TRVL personnel carried out investigations of disease epidemics (EEE and WEE) in the Rupununi Savannah region of Guyana, and serosurveys in Venezuela, Curacao, Tobago, Barbados, and more northerly West Indian islands. A virus group headed by Louis Grant at the University of the West Indies in Jamaica was in close contact with TRVL, as was also a group under Gernot H. Bergold at the Instituto de Investigaciones Cientificas in Venezuela.

Two new diseases of man were encountered. Mayaro virus, an alphavirus related serologically to Chikungunya virus of Africa, was encountered during the study of the 1954 yellow fever outbreak. The illness was an acute febrile illness, with joint pains as a feature, much like the African disease. Serosurveys carried out in Trinidad, Guyana, Surinam, and Venezuela showed antibodies to be prevalent in peoples of the jungle regions. Epidemics of illness have only rarely been seen. Also during the 1954 yellow fever epidemic, a "new" virus, Oropouche, was isolated from a febrile person. No other isolations of this virus have been made in Trinidad but the Belem Virus Laboratory has reported successive massive outbreaks of illness caused by the virus both in Belem itself and elsewhere in Para State.

Many other viruses in the total of 35 agents isolated in Trinidad are of interest to the virus world but most are not involved in human disease. Epidemiological studies carried out in the Bush Bush Forest region of eastern Trinidad have provided much detail on the movement of a number

of these viral agents through the small mammal, bird, and mosquito fauna of undisturbed tropical jungle.

The TRVL moved in 1964 from the Army barracks to the quarters left by the Colonial Microbiological Research Institute in Port of Spain, and after the RF left the scene in 1970 the name was changed to Caribbean Epidemiological Centre (CAREC), which continues as an active coordinating institution for public health matters in the West Indian region.

The Egyptian program of the RF began in 1952 after an invitation from the US Navy for the RF to post a virus team to the newly established US Naval Medical Research Unit #3 (NAMRU-3) in Cairo. Taylor, on a post-retirement assignment, and Work made up the RF team with Herbert S. Hurlbut and Harry Hoogstraal as Navy-assigned associates. Part of the effort went into the usual initial serosurvey of Egypt and the Sudan, a survey encompassing both people and domestic and wild animals and birds. West Nile (WN) virus was widespread in the region. It had first been isolated from the blood of a febrile woman seen in the West Nile province of Uganda in 1940 by Smithburn, an RF staff member then assigned to the Entebbe Virus Laboratory. Joseph L. Melnick and associates, studying poliomyelitis in Egypt in 1951, made three isolations from the blood of apparently healthy children. One of these isolates was designated Egypt 101 strain. Smithburn had established that WN virus was a flavivirus. Taylor and Work and Navy associates began a detailed study of the epidemiology of WN virus in Egypt, and established its endemicity in Egypt, with clinical disease of mild nature seen mostly in small children. *Culex* mosquitoes of several species were found to be vectors. The mosquitoes feed mainly on birds and also on man and other mammals, but birds were found to be the main vertebrate hosts of the virus. The period of major virus activity is in the summer months, coinciding and indeed dependent upon, the period of greatest mosquito density. This study showed the similarity between this virus life cycle and the life cycles of the other close serorelatives among the flaviviruses, namely the viruses of Japanese encephalitis, St. Louis encephalitis, and Murray Valley encephalitis. Later studies by other investigators in other regions have shown that the disease produced by WN may be quite severe, and that encephalitic involvement is sometimes seen in elderly people, in this respect quite resembling what is seen in St. Louis encephalitis. Hurlbut undertook arthropod infection and transmission studies and showed two *Culex* species to be capable of propagating and transmitting the virus. He also showed that a tick, *Ornithodoros savignyi,* could act as a vector. Coincidentally, Whitman and Aitken at the RFVL-New York showed that another African tick, *O. moubata,* was able to transmit the virus by bite, and also excreted the virus in coxal fluid exudate. WN maintains itself in some very dry regions where mosquito populations may be

very low for long periods of time, and it is tempting to consider that ticks might provide an alternative cycle, permitting virus survival over long dry periods.

The RF involvement in Egypt lasted until 1954 when Taylor returned to the United States, where he remained active for many years in the affairs of the ACAV mentioned earlier, and Work was reassigned to India to head up the Virus Research Centre in Poona.

The RF experience in South Africa began in 1953 and involved the assignment of personnel to an already existing group of virus investigators in the South African Institute for Medical Research in Johannesburg, which was under the leadership of James Gear. M. Paul Weinbren, Hugh E. Paterson, Botha de Meillon, and Bruce M. McIntosh were other members of the South African group. There had been a distinguished history of South African involvement with insect-transmitted viruses of livestock. The Onderstepoort Laboratory, under the direction of Sir Arnold Theiler, the father of Max Theiler who joined the RF, had pioneered around the turn of the century in the investigation of numerous diseases of African livestock, which included the isolation of the viruses of African horsesickness and Bluetongue virus of sheep, and in studies of their epidemiology.

Smithburn and Robert H. Kokernot were the RF staff members assigned to Johannesburg. Again, the early program involved the survey of an immense land area and its people, insects, and vertebrates and included surveys of the Transvaal, Mozambique, Lourenco Marques, the Caprivi Strip, Southwest Africa, Natal, Tongaland, Cape Province, Angola, and Bechuanaland Protectorate. Ill health forced Smithburn's premature departure from the program and Worth was added. Virus isolation studies involved man, animals, birds, and arthropods. Many new virus agents were added to the growing list of arboviruses: Wesselsbron, a flavivirus infecting man and sheep, with epizootics of abortions in ewes and deaths in lambs, and transmitted by mosquitoes; Middelburg, an alphavirus causing epizootics in sheep and having mosquitoes as vectors; and other viruses from mosquitoes such as Pongola, Spondweni, Banzi, Ingwavuma, Germiston, Ndumu, Lumbo (Tahyna from Czechoslovakia), and Mossuril. Some previously known African viruses were encountered: Uganda S, isolated from man and mosquitoes; West Nile, isolated from man and birds; and Bunyamwera isolated from mosquitoes and from man.

Worth was deeply interested in the possibility of transovarial transmission of viruses in mosquitoes, to explain the ability of viruses to persist through prolonged dry periods when mosquito populations are much reduced. He carried out an extensive study at a field station in Ndumuland. His approach was to try to isolate virus primarily from male mosquitoes, and the capture technique involved crawling for hours on hands and knees

through thorny brush with a mosquito aspirator, capturing male mosquitoes one by one. He was not lucky, and it was not until nearly two decades later that transovarial passage of virus was demonstrated in laboratory studies, and then, in Wisconsin, in field-captured mosquitoes. The study was never reported in the scientific literature but a good resume is to be found in a popular work (8).

The South African program of the RF was terminated in 1960. The South African Institute for Medical Research and other centers in South Africa have continued an active program of arbovirus investigations. Kokernot was transferred to Cali, Colombia, to start an arbovirus program there, and Worth went to the Trinidad Regional Virus Laboratory.

The location of a laboratory at Belem, at the mouth of the Amazon, in 1954 was a most fortunate choice. Ottis R. Causey of the RF staff, and his wife Calista, had worked on yellow fever and malaria problems in Brazil for many years, and with the start of the expanded RF virus program, Causey pushed for a laboratory there and in due course headed up its development, until his later transfer to head up the virus laboratory at the University of Ibadan, Nigeria. A highly effective and efficient laboratory was established, with a cadre of Brazilians attached, most of whom still remain with the laboratory. The list includes Francisco de P. Pinheiro, the present director of the Instituto Evandro Chagas, which subsumes the laboratory, Gilberta Bensabath, Amelia Andrade Travassos, and Nazarena de Freitas. Amazonia Toda Tann, an entomologist, is no longer with this group. RF staff members assigned to the laboratory included Shope, later the director, Aitken, entomologist, transferred to Belem from Trinidad, Boshell (after his return from Poona), and John P. Woodall, director after Shope, and now with the San Juan Laboratory of USPHS-CDC.

The Belem Virus Laboratory program got started in 1954 with serosurveys of inhabitants of the lower Amazon region, later extended far up the Amazon and its tributaries. There were many interesting findings in these earlier studies. Yellow fever virus antibodies were widespread, as might be anticipated. Eastern equine groups encephalitis virus antibodies were encountered in some population groups upriver from Belem, in much higher percentage than had been seen with EEE antibody studies in the US. EEE virus infections in man in the US are uncommon and often fatal. In contrast, continuing efforts to find any EEE cases in man in Brazil, even where antibody rates have been high, have been fruitless. There have, however, been large outbreaks of the disease in horses in Brazil. In Trinidad, EEE virus was found in mosquitoes, with only one reported case of horse disease, and no antibodies in human beings. Casals, studying US Brazilian, and Trinidadian strains of EEE, was able to show subtle serological differences between the US strains and the others, a finding which coincides with the

epidemiological differences observed, but does not explain these differences.

The Belem laboratory was soon involved in outbreaks of yellow fever, both in the immediate vicinity of Belem, and farther afield. An epidemic of Mayaro virus infection was also studied. This virus was first encountered in Trinidad but the prime vector was not determined there. Belem studies have incriminated *Haemagogus* mosquitoes as vectors, in a jungle cycle involving monkeys and reminiscent of the jungle cycle of yellow fever. Oropouche virus, also first uncovered in Trinidad, has been the cause of recurrent major outbreaks of febrile illness in Belem and other Amazon population centers. Workers at the Belem laboratory have in recent years incriminated *Culicoides* (sandflies) as the principal vector. St. Louis virus was shown to be widely distributed in the Amazon region, as was Ilheus virus. Disease caused by either agent is rare. St. Louis infection has been reported with a hepatitis-like illness.

Studies in the deep rain forest have resulted in exposing dozens of new viruses, in a number of different virus groups. Most of them are mosquito-transmitted agents. Many have been isolated from forest inhabitants or forest-fringe dwellers with febrile illnesses, including the viruses of Guaroa, Mucambo, Guama, Catu, and the "Group C" agents of Caraparu, Apeu, Oriboca, Murutucu, Marituba, and Itaqui. The definitive studies of the diseases caused are still awaited. Recent construction work on the Belem-Brazilia highway and on the Trans-Amazon highway have been followed closely by field teams and have resulted in a further flood of new virus agents, some associated with illness in human beings.

Other agents of great interest are Piry virus, a rhabdovirus, related serologically to the vesicular stomatitis viruses of the Americas and to Chandipura virus of India; and Amapari virus, isolated from rodents, related to Tacaribe virus from Trinidad, and in turn to lymphocytic choriomeningitis virus and to Lassa virus from Africa, and to the viruses of Argentinian hemorrhagic fever (Junin) and Bolivian hemorrhagic fever (Machupo). Amapari, unlike some of its relatives, appears not to be associated with human disease. Pacui virus received intensive study by Aitken who worked out details of the life cycle involving rodents and terrestrial marsupials and a phlebotomine sandfly of the genus *Lutzomyia.* An endemic and epidemic focus of a frequently fatal disease, called Labrea Fever from a remote interior riverine area (Rio Purus) of the Amazon basin, was studied intensively by Boshell. The cause was never found, albeit hepatitis B is suspected.

The animal capture and recapture programs, the exposure of sentinel animals, both primate and rodent, in the forest for virus isolation, the timed capture of mosquitoes, the tracing of trapped and released animals to their burrows by tying strings to them, the use of mist nets for ensnaring birds both on the forest floor and high in the forest canopy, and the streamlined

procedures of rapid identification of viruses in the laboratory are all areas of work in which the Belem Virus Laboratory staff demonstrated high ingenuity, resourcefulness, and skill. Their techniques have been widely copied by other laboratories. The Belem Laboratory continues as a very active center, a decade after departure of the RF staff in 1971. A close affiliation with the Yale laboratory (YARU) continues.

The RF field programs were not entirely limited to the international arena. In 1954, a collaborative effort was begun with the California State Health Department, with the assignment of Harald N. Johnson, formerly with Kerr at the VRC-Poona, to the post. Funds were provided to install and equip a small laboratory in the State Health Department Laboratory in Berkeley. Edwin H. Lennette, a former RF staff member from the yellow fever program of the 1940s in Brazil, headed the State laboratory. The role of small mammals in the epidemiology of WEE and SLE, and other viruses (some new to science) was studied. Johnson also was instrumental in developing a variant of WEE virus which has been successfully and widely used as a live vaccine for WEE in equines. Although RF support of this program was terminated in 1970, Johnson continues to be associated with the California State Health Department Laboratory.

The Cali Virus Laboratory was activated in 1961, associated with the Universidad del Valle, and in quarters loaned by the University. The RF staff members assigned were Kokernot coming from South Africa and Trapido coming from the Virus Research Centre in Poona. Two additional staff members were later added: Ronald B. Mackenzie, who had first drawn attention to the outbreak of Bolivian hemorrhagic fever (later determined to be caused by a virus named Machupo) in 1963 while working for the United States Public Health Service, and Vernon H. Lee, a medical entomologist. The direction of the laboratory was shared with Carlos Sanmartin, a Colombian virologist who had worked with earlier RF yellow fever programs in Colombia. Trapido, fresh from experience with Kyasanur forest disease in India, was certain he could find a flavivirus of the TBE group in Colombia and searched diligently for this. No such virus was found. The generalization that TBE viruses do not occur south of the Equator has, however, been upset by the finding of a TBE-related virus in Australia very recently. Mackenzie, fresh from his Machupo virus experience, was certain he could find a Colombian counterpart virus in ecological situations comparable to those of the San Joaquin region of Bolivia. He ranged far and wide over the Colombian llanos and did not find his virus. Meanwhile, Trapido had gone into the mountains near Cali, looking for his TBE virus, and there, in the Valle de Pichinde, encountered Pichinde virus, a serorelative of Machupo, endemic in small forest rodents of the genus *Oryzomys*. There is now a large group of these arenaviruses, apparently not

vector transmitted, serorelatives of lymphocytic choriomeningitis, in the New World. The first of these to be isolated was Junin virus in Argentina from man and from small rodents, followed by Tacaribe virus in Trinidad, from bats. Machupo in Bolivia, Amapari and Flexal in Brazil, Parana in Paraguay, Portillo in Argentina and Tamiami in Florida are later additions to the list. Only Junin and Machupo are presently associated with disease in the New World, and both diseases are notorious for high morbidity and mortality under epidemiological circumstances favoring the virus. A later addition to the list of arenaviruses, Lassa, is also a dreaded virus and is discussed later.

While the field teams were scouting in the hinterlands, Venezuelan equine encephalitis (VEE) virus invaded the valley of the Cauca River in the immediate vicinity of Cali, and Sanmartin became involved with a major epidemic, associated with much equine mortality, and with several human infections with encephalitis observed. None of these was fatal. Mosquitoes of many species and several genera were found infected with the virus. An outbreak occurred in a high valley not far from Cali, where there were relatively few mosquitoes. Trapido made an intensive but unsuccessful effort to incriminate Simuliidae (blackflies), which were abundant in the region, as vectors. There could be little doubt that they served as mechanical vectors but biological transmission was not demonstrated. Another large VEE epizootic was studied on the Guajira peninsula. Major outbreaks of vesicular stomatatis in cattle were followed in the region of Medellin but no vector could be incriminated. Lee and Sanmartin established the bromeliad-breeding anopheline mosquito *Anopheles* (*Kerteszia*) *neivai* as the important, perhaps the only, vector of Guaroa virus in the heavy rainfall region of the Choco, near Buenaventura. Guaroa virus, first isolated and studied by Hernando Groot in Colombia, has been recovered several times from febrile human beings. Some infections are severe. No fatalities have been reported. Antibody rates in human beings average out at about 14% for the Amazon region in general, and rates as high as 77% were found in the Colombian Choco. The same *Anopheles neivai* is also the principal vector of malaria in the Choco.

RF support to the laboratory came to an end in 1970. In the terminal few months of the laboratory's existence, dengue, long absent from Colombia, was found to have returned in epidemic waves to the Caribbean coastal lowlands. The Instituto Nacional de Salud in Bogota has been engaged in studies of the outbreaks, with Groot playing a prominant role.

The program in Nigeria began in 1964. The prior RF yellow fever laboratory at Yaba, Nigeria, near the coast and near Lagos was considered. This laboratory was the seat for continuing virus studies by British entomologists and virologists, under the auspices of the West African Commission for

Medical Research. After a survey of West African possibilities by Ottis and Calista Causey, the location at the new University of Ibadan was chosen and buildings were constructed for the Virus Laboratory. Causey was the first director, joined by Carey from Vellore, Graham Kemp, a veterinarian trained in arbovirology, and Lee, transferred from Colombia. Dorothy L. Moore was seconded by the University of Ibadan. Causey had been intrigued by the seeming inability of prior workers in West Africa to capture the masses of mosquitoes he was accustomed to work with in Belem, and by the relative lack of success of the sentinel mouse virus recovery routine that he had developed in Belem. His first efforts were to show that West Africa was like Amazonia. It was not. The mosquito-capturing techniques that produced heavy captures in Amazonian Brazil simply produced thin captures in middle Nigeria, and attempts to modify techniques and boost the catch did not improve the situation. Similarly, the sentinel animal virus recovery routines were of such limited success as to be hardly worth the effort. Ticks were abundant on the livestock of the region, however, and M. H. Madbouly, an Egyptian tick specialist trained under Harry Hoogstraal, joined Causey and Kemp in highly successful studies of virus-tick-livestock interactions in West Africa. Tam David-West, a Nigerian virologist assisted in these studies. An early finding was many isolations of Congo virus from ticks. Congo was known from earlier isolations at Entebbe, Uganda, by workers at the East African Virus Research Institute and by Gervais Courtois, working in Brazzaville. These earlier isolations were from human beings with mild illnesses. Casals, at the RFVL-NY demonstrated the relationship between these viruses and the virus of Crimean hemorrhagic fever, a dreaded disease accompanied by high mortality, in the southern regions of the USSR (and later Pakistan and Iraq). Given this, a diligent search has been made for the disease in West Africa. Virus isolations have been numerous in Nigeria, the disease not seen. The difference in disease epidemiology between the USSR and West Africa remains unexplained.

British workers at the Yaba laboratory had found a rhabdovirus in bats, named Lagos Bat Virus, and workers at Ibadan found a rhabdovirus in shrews. Shope at YARU showed that both of these agents are serorelatives of rabies virus, a topic discussed later.

West Nile virus was prevalent in Nigeria. A series of yellow fever outbreaks were investigated. A number of Bunyamwera supergroup viruses were isolated from livestock and in some instances mosquitoes, forming the Simbu group of viruses. With Simbu (from South Africa) and Sathuperi (from India), the addition of the Nigerian viruses Sabo, Sango, Shamonda, and Shuni gives us alliteration at a high level.

There had been a long history of dengue in Africa, with episodes reported ranging from a major outbreak in Durban to scattered reports of minor

outbreaks or single cases in West Africa. None of these reports had been verified by virus isolation, and the presence of so many flaviviruses in Africa had made interpretation of serological findings a considerably less than convincing exercise. Carey, fresh from dengue experience in Vellore, tackled a number of unclassified virus isolations from children seen in the Outpatient Department of the University of Ibadan Hospital. Many of these were shown to be dengue strains, and it was established that dengue type 2 was endemic in the Ibadan urban complex. These studies led to recognition of dengue outbreaks in several Nigerian population centers. A few years later a group of West Indians attending a cultural conference in Lagos, carried what proved to be dengue type 1 back to Jamaica, starting a brisk outbreak there. Much further work is needed on the epidemiology of dengue in West Africa. The virus (dengue type 2) has been found as far north as Senegal. The complications of dengue hemorrhagic fever–dengue shock syndrome (DHF-DSS) have not been recognized in West Africa.

Lassa fever virus was isolated from the blood of an American missionary nurse working in Nigeria, when she was flown back to the United States for study. Personnel of YARU–New Haven isolated the virus and characterized it as an agent, previously unknown, with morphological and serological affinities to the Tacaribe group viruses of the New World and to lymphocytic choriomeningitis virus. Lassa fever is a dangerous illness with high mortality. Ibadan laboratory personnel carried out extensive field studies on the epidemiology of this virus in the region of Jos, Nigeria, including collecting large numbers of rodents, among them species later shown by USPHS-CDC investigators to be vertebrate reservoirs of the virus. Lassa virus has been responsible for several disease outbreaks, particularly associated with hospitals and hospital personnel, but certainly not exclusively so, in Nigeria, Liberia, and Sierra Leone. Serological studies show it to be even more widely distributed in West Africa. A virus isolation has been made in Mozambique. The existence of this disease has made it necessary to review and stiffen international quarantine regulations particularly with respect to travellers and tourists returning home with fever after sojourns in Africa. The dangerous nature of the virus, and the known history of laboratory infections and deaths, makes it imperative that the virus be handled only with most stringent laboratory precautionary measures. This inevitably slows down the pace of continuing investigations.

RF support of the Ibadan Virus Laboratory terminated in 1972. The laboratory has continued under the direction of Akinyele Fabiyi and more recently David-West. Two Nigerian virologists, O. Tomori and A. Fagbami, have been active in studies of dengue and yellow fever epidemics and of a new virus, Orungo, associated with human disease. The program has,

however, been seriously restricted owing to lack of adequate support funding.

The Yale Arbovirus Research Unit commenced operations in 1965 after the closing of the RFVL-New York activity in December 1964. This unit was originally funded entirely by the RF. It was first under direction of Downs, and after 1974 under direction of Shope. RF personnel have included Casals, Whitman, Buckley, Aitken, Robert Speir, Anderson, and Clarke with other RF personnel in and out for shorter periods of time. Jack R. Henderson, Nick Karabatsos, and Robert B. Wallis of the Yale faculty have been close collaborators. Downs, Casals, Anderson, and Clarke have retired and vacancies have been filled by other individuals including Gregory H. Tignor, Dennis L. Knudson, Barry J. Beaty, Andrew J. Main, Barry R. Miller, Andrew B. Carey, Thomas Scott, James Marouniak, William Krimsky, Robert B. Tesh, and Stephen J. Brown. Support for these workers has come from sources outside the RF. Major support for the laboratory from the RF terminated in 1974 and the appointments of the RF staff members were terminated. Provision was made by the RF for salary continuation of four ex-RF staff members remaining. Apart from some RF endowment money still remaining, support for the operations comes entirely from outside sources, and specifically until 1981 not including Yale University. The staff puts in a considerable amount of time on training and instruction of Yale graduate students, and of scientists from many countries who come to Yale for special instruction. These latter may come for weeks, months, or years.

Designation of YARU by the WHO as the International Reference Centre for Arboviruses carries with it responsibilities for keeping the house in order with respect to the large number of arboviruses already recognized, and those still under study or unstudied. With 440 viruses already recognized, the task is a formidable one. Casals, Whitman, Shope, Clarke, and Main have worked hard on this task. It is a far from routine endeavor, for from the study of the unknown viruses have come exciting new developments, including (*a*) the finding and identification of Lassa virus; (*b*) the finding of a virus of the Bluetongue virus disease of sheep in Australia where BTV was not known to occur; (*c*) the uncovering (by Shope) of a whole series of viruses serologically related to rabies virus, and with some of them isolated from mosquitoes, findings that have shaken the accepted concept of the monolithic character of the rabies virus and have furnished material for the continuing search for better rabies vaccines; and (*d*) delineation of a whole series of new virus groupings, which involves demonstrating relationships from among the several hundred ungrouped viruses still at hand, work largely carried out by Casals, who has as a subset of the endeavor begun to establish order in a large assemblage of viruses isolated from ticks

around the world. He has put in special effort on determining the distribution of immunity to Lassa virus in Africa, and on studies on the Congo-Crimean hemorrhagic fever viruses transmitted by ticks. This latter group has been difficult to study because of inadequate testing methods available, and Casals has made special effort to develop more specific and sensitive testing procedures. Recently he made a trip to Baghdad to investigate a disturbing outbreak of C-CHF there.

The backlog of viruses still unstudied plus continuing annual influx of several dozen "new" viruses from around the world is an embarrassment. Work proceeds slowly. Support is received from the US Army, the US Navy, the National Institutes of Health, the World Health Organization, and the Australian CSIRO. However, the pace could be stepped up with more funds and personnel assigned to the task.

Morison, over 20 years ago, had urged the inclusion of more fundamental virus work in the program of the RFVL-NY. P. Y. Cheng was taken on the staff and carried out some excellent work on morphology and composition of Semliki Forest virus, an alphavirus. Such work was in progress also in several study centers in America and Europe. The RF was uniquely organized to back field epidemiological studies, whereas the university and institute groups were not usually thus organized. There was acute pressure for funds and it was felt that the RF should continue to give heavy support to epidemiological studies and drop the more fundamental studies; this was done. With the passage of twenty years, the present YARU program encompasses considerably more in the field of basic virus research than had been the case earlier. There continues to be an effort to link basic virus studies to virus epidemiological problems of importance for the well-being of the human community.

Shope, Beaty, and Tignor have been effectively exploiting the virus resources of YARU, carrying out investigations on the RNA gene-coding assigments among several viruses of the bunyavirus group and studying biological properties observed when mixing RNA segments from two related viruses by growing them in the same cell. David Bishop from the University of Alabama is an active collaborator in this program, providing the reassortant viruses. Recombinant viruses are studied for composition, for growth characteristics in various live animal and cell culture systems, including pathogenicity, and for ability to infect the virus vector. These studies are coordinated with similar studies on the parent viruses. The RNA segments are "fingerprinted" in gel electrophoresis and are plotted following autoradiography. Closely related viruses can be differentiated by these techniques. Several viruses in the rabies serogroup have been studied with these techniques. Fred J. Murphy at USPHS-CDC-Atlanta, in close collaboration with Shope, carried out basic electron-microscopic studies of

many arboviruses, including several in the rabies serogroup, the reo-like virus groups (orbiviruses), and the Bunyamwera supergroup.

Beaty and Aitken are engaged in studies of transovarial transmission of yellow fever virus in *Aedes aegypti,* and in studies of multiple strains of this mosquito from different parts of the world to determine what differences may exist in mosquito infectability and ability to transmit virus among these different strains. These studies are linked with studies on the isoenzyme profiles of *A. aegypti* strains being carried out by Jeffery Powell and Walter Tabachnik in the Department of Biology at Yale University.

Knudson has been working on the insect pathogenic viruses known as nuclear polyhedrosis viruses. These viruses form inclusions easily viewed under the light microscope. The viruses are of interest as possible means of control of insect pests, including insect vectors of disease agents. The viruses contain DNA which can be mapped and manipulated with the aim of understanding the genetic basis for the ability to kill insects.

Knudson has also attacked the complex group of viruses known as orbiviruses by molecular genetic techniques. The orbiviruses have great genetic diversity. Colorado tick fever causes human disease; Bluetongue of sheep and African horsesickness viruses are serious pathogens of livestock. Knudson has shown how one can use the RNA segments for identification and for tracking the transmission chain as well as the evolutionary changes leading to the genetic diversity. This is an admirable marriage of molecular science with practical epidemiology.

Tignor and Abigail Smith are studying the virus receptor sites in cultured mouse neuronal cells, using histochemical techniques at the cellular level, and coordinating these with electron-microscopic visualizations of the same cell region. These studies are being coordinated with rabies virus studies and promise to provide a better understanding of the progression of events when an encephalitogenic virus invades the nervous system. Main has been combining field and laboratory in studying the viruses of seabird colonies, viruses being isolated from the ticks found on the birds or in their nests. Bird colonies have been investigated on North American and northern European shores of the North Atlantic, particularly including offshore islands. Several viruses have been isolated, and characterized. This work fits closely into the work of the International Reference Centre for Arboviruses. Main has also been associated with studies on the putative tick vector of the as-yet undiscovered causative agent of Lyme arthritis, and with studies of the epidemiology of Powassan virus in the northeastern United States. Powassan virus, first isolated in Canada from brain of a fatal case of encephalitis, is the North American representative of the tickborne encephalitis virus which causes much disease in the USSR. Powassan cases, even Powassan im-

munes, are uncommon in the US and Canada. Mammalian hosts of the virus include squirrels, woodchucks, and skunks. The ticks found on these animals only rarely attach to man, and this may explain the rarity of the disease.

It is not possible to predict what the future course of the Yale Arbovirus Research Unit will be, dependent as this is on staff interests, scientific and technical developments, and level of funding, including success or lack thereof in obtaining approval of grant and contract applications. Some program bias is to be expected internally and this will not necessarily be consonant with program bias determined by external pressures. The writer hopes that the reference laboratory studies will continue to be strongly supported, and further hopes that epidemiological studies will continue to be supported at an adequate level. Such studies in the past have supplied the raw material—viruses—that are needed in the development of further basic virus studies. It may be possible to resolve the conflict between studies at the molecular level and studies at the field epidemiological level by finding people interested in both.

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