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ON THE JAPANESE B—WEST NILE VIRUS COMPLEX OR AN ARBOVIRUS PROBLEM OF SIX CONTINENTS*

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Ten years ago the expanding role of arthropod-borne viruses in tropical medicine was reviewed at the Harvard School of Public Health Conference on Industry and Tropical Health in Boston.[1] This report dealt with a decade of evolution of this new and major field in tropical medicine, largely led and supported by The Rockefeller Foundation. The discovery of new arbovirus diseases, definition of epidemiology of those previously known, isolation of a plethora of new agents, the unscrambling of systems for taxonomy and classification and the exemplary collaboration of scientists around the world has been documented by an informal arbovirus information exchange, the *Catalogue of Arthropod-Borne Viruses of the World*[2] and a variety of formal journals and monographs. No publication of these contributions to scientific knowledge has been more devoted to this new field in tropical medicine than our own *American Journal of Tropical Medicine and Hygiene*.

You have all witnessed the ramifications of this development in arbovirology. Some, I am sure, would tire of another discussion of new central-nervous-system and hemorrhagic fevers or another account of the discovery of more mosquito-borne viruses. Even I have reached the point where I savor most the description of a new worm or elucidation of a new approach to the control of some of our oldest but still unconquered tropical diseases. But one purpose of a presidential address is to encapsulate the significance of a career in tropical medicine, and since nigh onto 20 years of my professional life have been associated primarily with arboviruses, it is in this area that I am most qualified to speak. But these remarks will focus on a group of arboviruses that were already known before the explosion of arbovirology was fused 20 years ago.

The agents referred to are those of the Japanese B–West Nile complex of arbovirus Group B shown in Table 1. Other, more recently isolated arboviruses are on the threshold of antigenic inclusion in the complex, but they will not be considered here because their human disease potential and epidemiology have not been elucidated. Work on West Nile (WN), Japanese B (JBE), Murray Valley (MVE), and St. Louis encephalitis (SLE) viruses has been a central theme in this career. The story commences with first exposure to awareness, and to the antigen, of Japanese B encephalitis just a quarter century ago in this city of San Francisco. It was at the end of the Pacific War. I was being trained as an independent duty medical officer in the U. S. Naval Hospital on Treasure Island. At one of our weekly Wednesday afternoon staff conferences, former Society President Bill Hammon presented the problem of Japanese B encephalitis, the presence in Okinawa, the threat to occupation personnel in Japan and Korea, and the multifarious unknowns which pointed to the possibility that wild birds were an epidemic reservoir of this mosquito-borne virus. He was looking for an ornithologically trained junior officer to participate in the studies that were being implemented in Japan. I volunteered, but my independent duty medical qualification precluded assignment to research.

Shortly thereafter, however, my Group B antigenic virginity was lost to the Navy when I was vaccinated with the Sabin killed-mouse-brain Japanese B encephalitis and 17D yellow fever virus vaccines prior to sailing out of this Golden Gate aboard the USS Monongahela (AO-42). The Navy had literally launched me on a career in tropical medicine. For the next year and a half this auxiliary navy oil tanker sailed the seas of Japan, the Indian Ocean, the Persian Gulf, the Red Sea, Suez, the Mediterranean, the Atlantic, and the Caribbean, exposing my biomedical education and training to diseases of the tropics, a number with a viral etiology. One volume avail-

* Presidential Address given before the 19th Annual Meeting of the American Society of Tropical Medicine and Hygiene, Hilton Hotel, San Francisco, California, 3 November 1970.

TABLE 1
The Japanese B–West Nile complex of arbovirus Group B

Virus	Abbreviation	First isolated	Geographical range	Vectors	Nonhuman reservoirs
St. Louis encephalitis	SLE	1933	North, Central, and South America	*Culex pipiens* *C. p. quinquefasciatus* *C. tarsalis* *C. nigripalpus*	Birds
Ilhéus	JL	1944	Tropical America	*Culex*, *Aedes*, *Psorophora*, *Sabethes*, and *Haemagogus* species	Birds
West Nile	WN	1937	Southern Europe, Africa, Mediterranean, Western and South Asia	*Culex univittatus* *C. antennatus* *C. molestus* *C. "vishnui"*	Birds
Japanese B encephalitis	JBE	1935	Eastern India, East and Southeast Asia	*Culex tritaeniorhynchus* *C. gellidus* *C. "vishnui"*	Birds
Murray Valley encephalitis	MVE	1951	Australia, New Guinea, West Irian	*Culex annulirostris*	Antibodies in birds

able from the Navy Medical Supply Depot was Zinsser's Harvard symposium on viral diseases[3] which was companionable reading in my cabin through these tropical and subtropical travels.

The next known insult to my antigenic experience came in 1952, when, as preparation for participation in the new Rockefeller Foundation arbovirus program, the staff members working under Dr. Max Theiler in the New York Laboratories (RFVL) received almost weekly a sequential series of mouse-brain and chick-embryo vaccines prepared under Dr. Joseph Smadel of Walter Reed for Japanese B, Russian spring–summer (RSSE), Western equine (WEE), and Eastern equine (EEE) encephalitis. Wil Downs and I were working with Ken Smithburn on mouse neutralization tests for arbovirus antibodies in the serum collections made by Taylor in Egypt[4] and Kerr in India.[5] On top of previous 17D and JBE vaccinations, the ultimate effect was a serum with more than two logs neutralization of West Nile virus. For almost 4 years this antibody gave a false sense of security and subsequently provided a useful source of positive control serum for West Nile neutralization tests in Egypt and India. The failure of these antibodies to protect against overt West Nile virus disease is a point to be made later in this presentation.

By the end of 1952 the Foundation had sent me to NAMRU-3 in Egypt to work with R. M. Taylor during the last 5 months before he was supposed to undergo mandatory retirement. We gracefully overlooked that retirement, as so many others since, and I had the good fortune to have those 5 months extend into almost a year and a half with this master preceptor of arbovirus epidemiology. Reeves and Hammon's University of California group and the Communicable Disease Center groups in Greeley and Montgomery were turning up clues to the role of birds in the dissemination of WEE, EEE, and SLE viruses, but little was known to us about this, other than a few *Proc. Soc.* reports.[6,7] The ecology of West Nile virus had become a subject of major study undertaken by Taylor and Hurlbut at NAMRU-3,[8] after Melnick had isolated three strains from Egyptian children's sera,[9] 13 years after the original isolation of West Nile virus by Smithburn[10] in Uganda. In February 1953, the 2nd month of my apprenticeship with Taylor, I broached the suggestion that wild birds might be involved.

Responding with a now familiar clearing of throat, he did not exactly discard my suggestion, but detailed the disappointingly negative results for yellow fever in birds in Brazil as one reason that this might not be a productive line to follow; the other being that mouse production and accom-

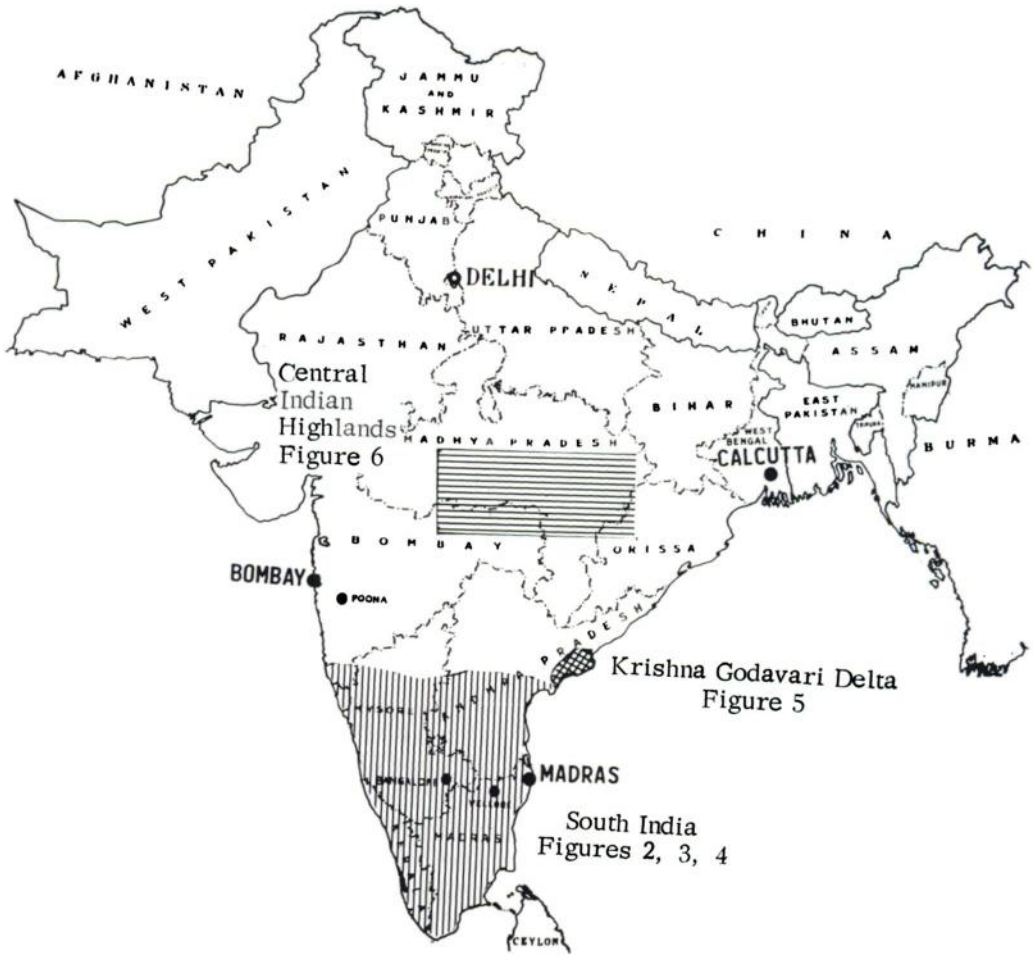

FIGURE 1. Map of India showing areas where investigations on JBE–WN viruses were made, 1955–58

modation was already fully taxed by the febrile children's blood study, isolations from mosquitoes, and neutralization tests on domestic animal sera.[11]

Nevertheless, a counter request for use of discard mice (those groups not showing signs of illness after inoculation and being chloroformed on the 21st post-inoculation day) and permission to spend Saturdays collecting birds in the Nile Delta enabled this study to commence. In second-hand mice, West Nile virus neutralization tests of these sera—largely from a common bird, the Hooded Crow—gave the first clues to the pattern of wild birds as circulating virus reservoirs of West Nile virus in the Nile Delta.[12] These studies were further facilitated by Dick and Mary's departure on retirement home leave in May. By the time of his return as a consultant in August, we had actually isolated West Nile virus from blood of a wild Hooded Crow and a feral pigeon.[13]

This led to investigation of *Argas arboreus* and *Argas hermanni* ticks as reservoirs of West Nile virus.[14] This is another fascinating story and mentioned here only to state that from these ticks we did get a West Nile-like virus which was lost in the Suez Crisis of 1956 before definitive study of its antigenic nature could be undertaken.

In recent years Chumakov's group, working in the Volga Delta have reported repeated isolation

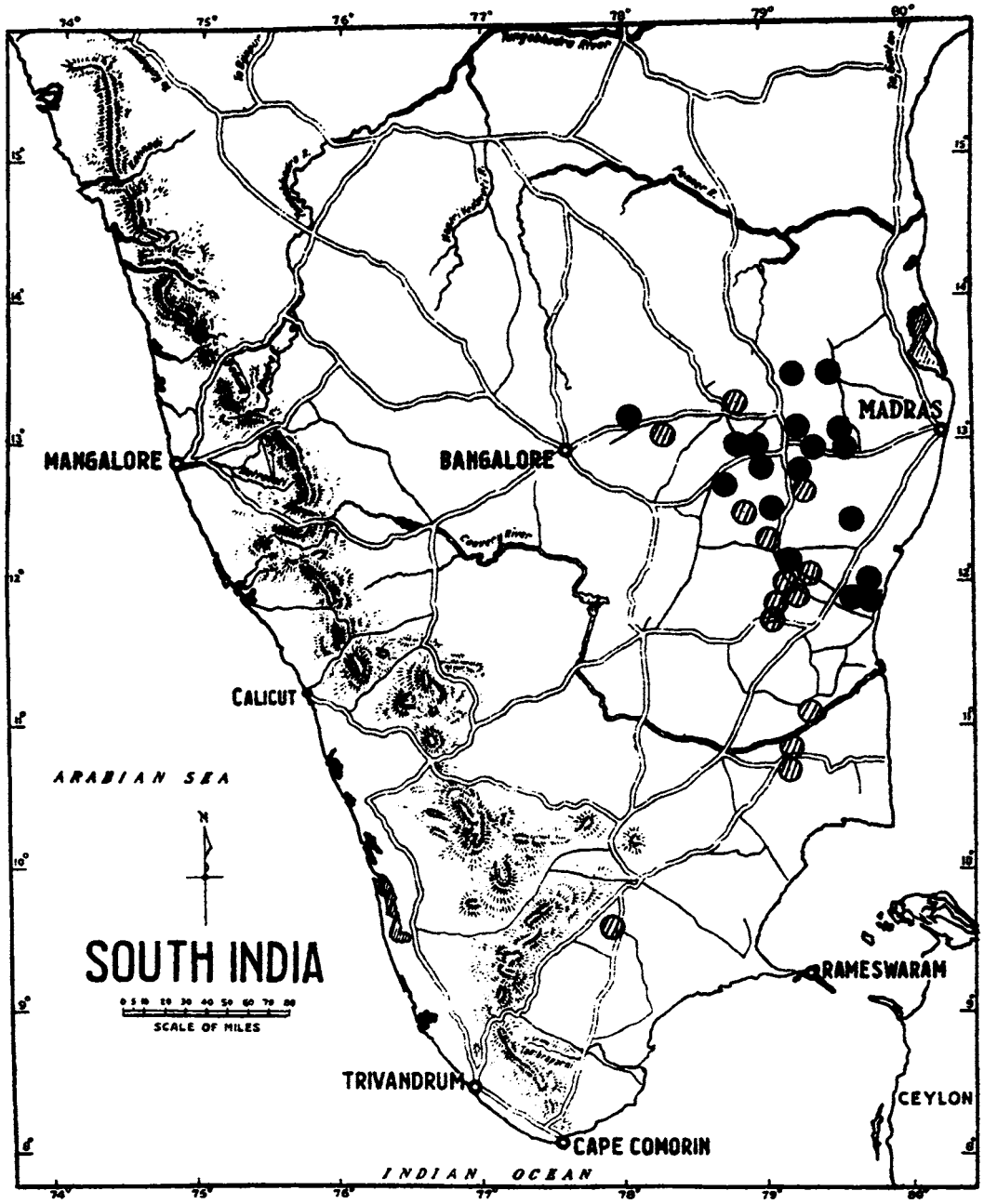

FIGURE 2. Map of South India showing location of confirmed (solid circles) and presumptive (shaded circles) cases of Japanese B encephalitis during the epidemic of 1955.

of West Nile virus from ticks.[15] Ours was the first clue to a subsequently experimentally demonstrated replication of a mosquito-born West Nile virus in ticks.[16] But assignment to India in April 1954 precluded follow-up of this tantalizing observation that is so germane to the problem of overwintering of West Nile virus in the temperate zone.

The Implications of Serological Surveys

Cross comparison studies of what were then called arthropod-borne neurotropic viruses by Bugher, Kerr, Johnson, Smithburn, and Taylor began in 1948 when Dr. Taylor was Director of the RFVL in New York.[1] Subsequent serological surveys by mouse neutralization tests set the stage for much that was to be illuminated by indigenous virology in Egypt, India, Trinidad, Brazil, and South Africa in subsequent years. Casals' refinement[17] of the Sabin-Buescher-Chanock hemagglutination-inhibition tests for arbovirus antibodies subsequently accelerated the accumulation of information from serological surveys. These NT surveys were the usual first step for a newly established laboratory effort abroad, initial tests being done in New York and subsequent tests being carried out indigenously once the actual viruses had been isolated in the area.

One of the most remarkably informative surveys was that accomplished by Kerr and Gatne[18] in India. I was the junior-most in the traditional daily coffee klatch at the RFVL in the fall of 1952, when Smithburn brought in the first five mouse NT cards that showed neutralization of RSSE virus by sera collected in Saurashtra, little anticipating the drama of Kyasanur Forest Disease that would unfold in India less than 5 years later.[19] But of great continuous interest was the finding of widespread neutralization of West Nile virus all over India, while NT positives for JBE were confined to a very few sera collected between the Satpura Mountains of Central India and the Coromandel Coast on the Bay of Bengal (Fig. 1). This suggested that Japanese B encephalitis was a disease of eastern India, an implication borne out by our discovery of JBE in Madras State in 1955 (Fig. 2).[20]

Human epidemics of JBE are rare in tropical regions probably because there is endemic transmission in infected areas throughout the year immunizing the exposed at an early age. This one in South India, and another recently reported from Cheng Mai in Thailand, are notable exceptions worthy of careful study.

To isolate the etiologic strains and to seek the endemic source of JBE virus in peninsular India, extensive serological and entomological studies were undertaken in 1955–1957, based at a field laboratory established in an abandoned outpatient shed at the rear of the Christian Medical College in Vellore where the first cases of JBE had been recognized in 1955.[21] Most of these studies have never been published because they were abruptly displaced by the appearance of Kyasanur Forest Disease in March 1957,[22, 23] when we had to divert most of our field and laboratory resources to this new problem. However impressive the elucidation of KFD was in giving visibility to the importance of arbovirus disease in India and to the arbovirus capability of the Virus Research Centre in Poona, it was a scientific catastrophe to the long-term study of what I believe to be far more important disease problems in India and elsewhere: West Nile fever and Japanese B encephalitis.

A Zoogeographical Interface for Japanese B and West Nile Viruses

Additional serological studies and subsequent isolations confirmed that the range of West Nile virus extended over most of the subcontinent of India while similar evidence localized JBE in eastern and southeastern India. The sera collected in a more comprehensive and detailed survey of South India in 1956 (Fig. 3), were tested by Dr. Khorshed Pavri for complement-fixing antibodies to detect recent infection. This clearly outlined two areas of extensive virus infection, associated with rivers draining into the Bay of Bengal (Fig. 4). Three hundred miles to the north, the Krishna and Godavari Rivers form a delta that appeared to provide a milieu that would support the endemic maintenance of JBE virus transmission. Human antibody studies (Fig. 5) and isolation of JBE virus there (Table 2) confirmed this hypothesis. In accomplishing this work, the pressures on the mouse colonies in Poona and Vellore forced us to consider the use of tissue culture. Even though some rather senior vanguards of tissue culture virology cautioned that it couldn't be done, persistent application by Dr. Pravin Bhatt adapted both WN and JBE viruses to chick-embryo and monkey-kidney cells with cytopathic effects.[24] This substantially augmented our neutralization-test capacity. Subsequently, as you know, tissue culture became a standard procedure for study of arboviruses in American laboratories.

By the end of 1955, weekly mosquito collections were instituted in villages where cases had occurred.[25] C. N. Dandawate, a technician recently recruited for training at the VRC Poona was assigned the task of inoculating the species-sorted mosquito pools into suckling mice.[26] Isolates were

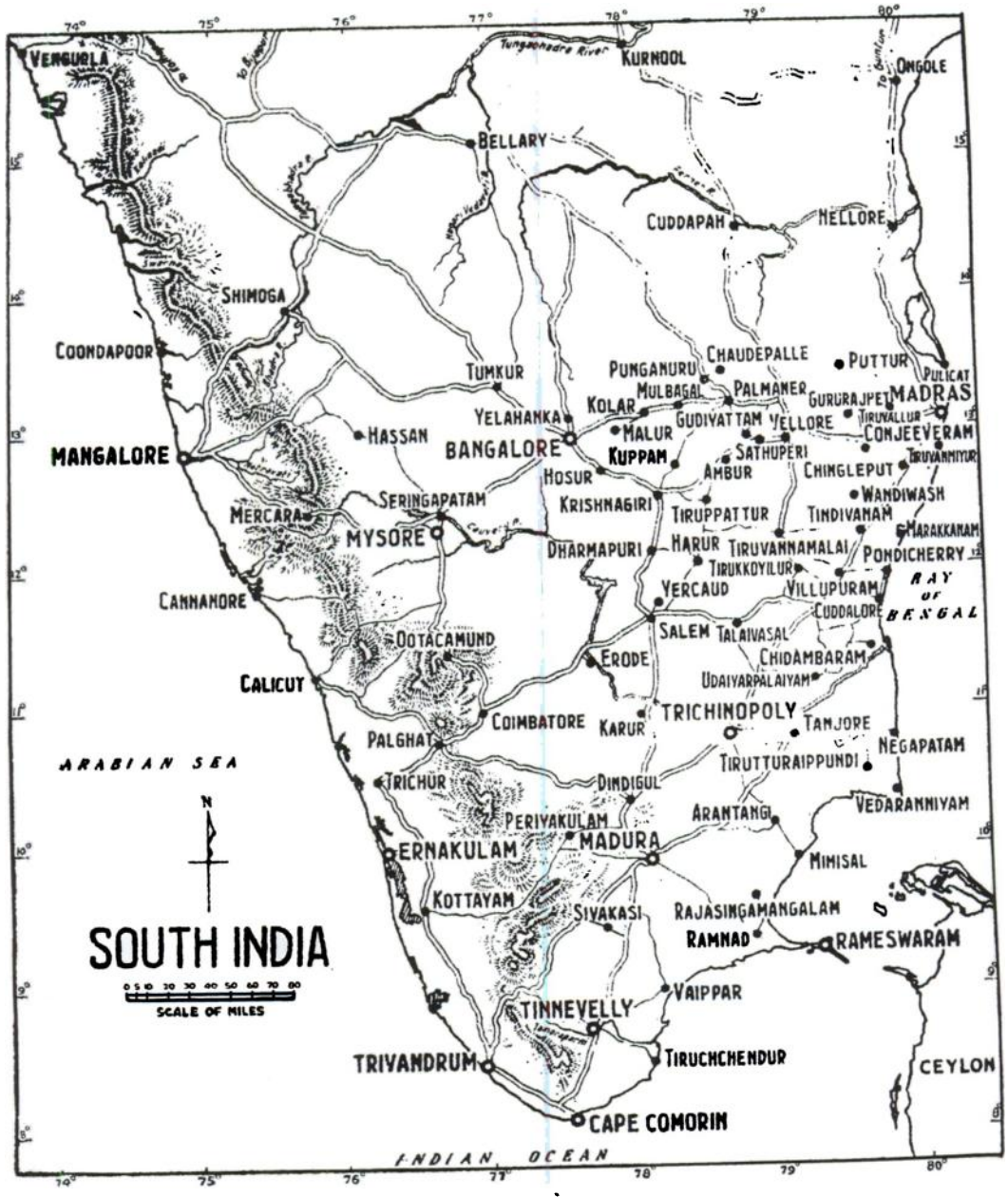

FIGURE 3. Map of South India showing localities from which serum was collected in the survey of 1956

forwarded 700 miles to Poona for identification. Actually, 34 of these isolates were characterized and grouped in the period 1959–60 when I worked at the RFVL in New York. Of interest here are those identified as West Nile and JBE (Table 2) for they provided evidence of the validity and accuracy of the original serological survey.[5]

The first virus we isolated near Vellore (G 2266) turned out to be West Nile, the first isolation of this virus in India.[26] Subsequent isolations of West Nile and JBE viruses from *Culex vishnui* complex mosquito pools, collected from the same locality—Sathuperi—posed a question as to the mutual exclusiveness of antigen-

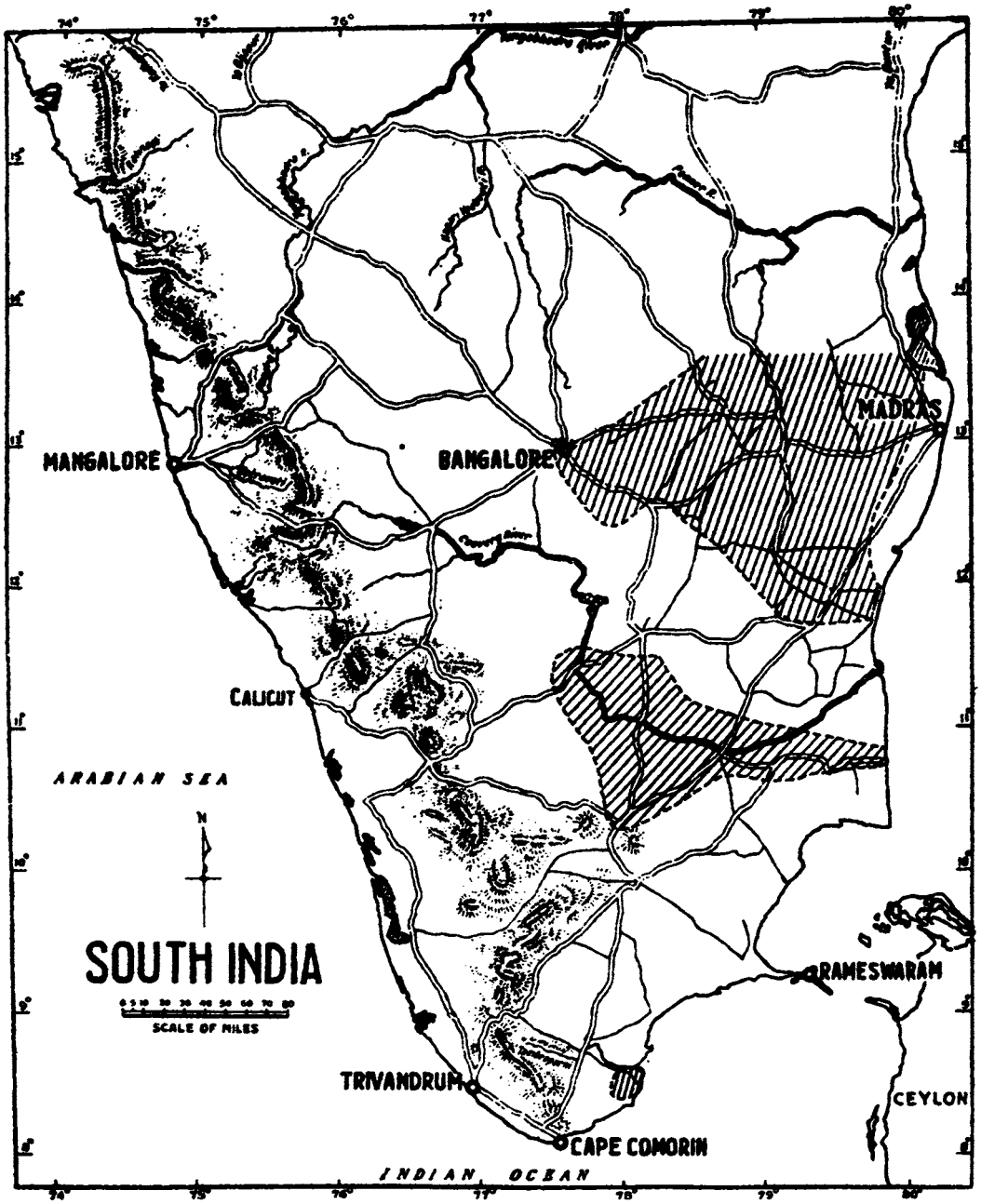

FIGURE 4. Map of South India. In the shaded areas, complement-fixing antibody to viruses of the JBE–WN complex were found in serum from residents in 1956.

ically related strains of virus of the same complex. This was to be recognized later with the dengue complex, but was unexpected with WN and JBE viruses in South India. In carrying out cross HI tests in the RFVL to characterize these viruses, it was surprising to find that hyperimmune mouse sera for WN and JBE had indistinguishable HI affinities for two of the JBE strains (G 8049 and G 9473). However, by neutralization tests these agents have been found to be recognizably differ-

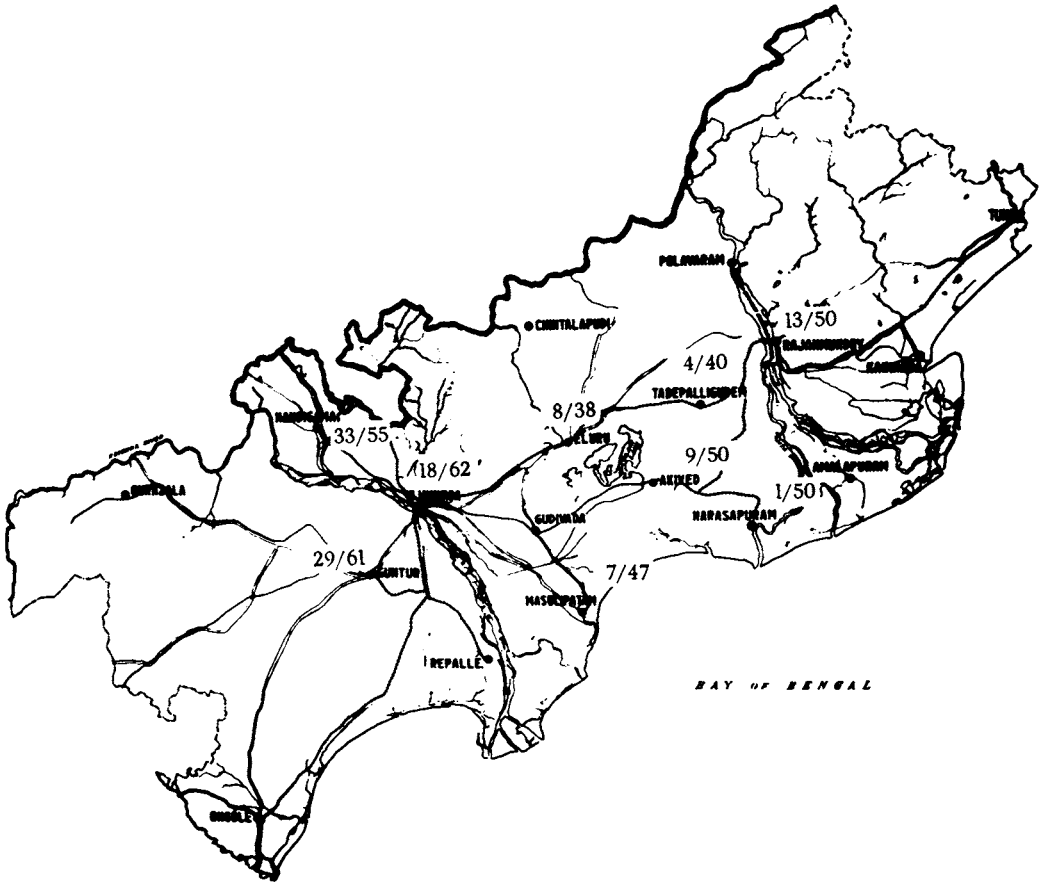

FIGURE 5. Map of Krishna-Gadavari delta region showing ratios of residents positive for neutralizing antibody to Japanese B encephalitis virus. Numerators, persons positive; denominators, persons tested.

ent from, but similar to, the Nakayama strains of JBE.*†

In an old map, shown in Figure 6, are the Central Highlands of India, which, almost at the geographical center of the country, serve as a subcontinental divide. This divide separates by less than 25 kilometers the Tapti-Narbada River drainage which flows west into the Arabian Sea, and the Godavari River which drains south and east into the Bay of Bengal. To the west of the divide there is evidence only of West Nile virus; this continues northwest to the Volga Delta and west to the Rhone Delta of Europe, the Israel-Arab Mediterranean Coast to the Atlantic Ocean, and all the way down through East Africa to South Africa.

East of the divide, West Nile and Japanese B viruses intermingle in the southern part of the Indian Peninsula, but Japanese B is the type that is manifest through eastern Asia to Siberia, Manchuria, Korea, Japan, Guam, and southerly through the Malay Peninsula into western Indonesia.

Another serological study in 1955 of coastal and inland residents of West Bengal showed that NT antibody to JBE was present in rice cultivating indigenes north of Calcutta, linking south and eastern India to the recognized JBE endemic areas of Burma and Southeast Asia (our unpublished observations). Out of these observations emerged

* W. Price, Johns Hopkins School of Public Health, Baltimore, Maryland 21205. Personal communication.

† Charles L. Wisseman, Jr., Department of Microbiology, University of Maryland School of Medicine, Baltimore, Maryland 21201, 1970. Personal communication.

TABLE 2
Strains of West Nile virus isolated from Indian mosquitoes

Strain	Origin	No. in pool	Locality/District	Date collected	Identity test
G 2266	*Culex vishnui**	100	Sathuperi North Arcot	31 Dec 1955	NT, CF, HI
G 2267	*Culex vishnui*	74	Sathuperi North Arcot	31 Dec 1955	NT
G 7247	*Culex vishnui*	12	Akividu West Godavari	24 Sept 1956	NT, HI
G 15578	*Culex vishnui*	150	Sathuperi North Arcot	22 July 1957	NT, CF, HI
G 16146	*Culex vishnui*	140	Sathuperi North Arcot	4 Aug 1957	NT, CF, HI
G 16919	*Anopheles subpictus*	40	Kammavanpeth North Arcot	3 Sept 1957	NT, CF, HI
Strains of JBE–WN complex virus with hemagglutination-inhibiting affinities for both JBE and WN viruses					
G 8049	*Culex vishnui*	148	Akividu West Godavari	3 Sept 1956	NT, HI
G 9473	*Culex vishnui*	47	Kammavanpeth North Arcot	30 Nov 1956	NT, HI
Strains of Japanese B encephalitis virus isolated from Indian mosquitoes					
G 8924	*Culex vishnui*	160	Sathuperi North Arcot	15 Nov 1956	NT, HI
G 9044	*Culex vishnui*	198	Sathuperi North Arcot	22 Nov 1956	NT, HI

* Subsequent taxonomical studies by Dr. Rachel Reuben have shown that *Culex vishnui* as identified here is really a complex of *Culex tritaeniorhynchus*, *Culex pseudovishnui*, and *Culex vishnui*.

the hypothesis of a zoogeographical interface between WN and JBE viruses.

Polyvalent-Sequential Immunization by Group B Arboviruses

In early 1956 attention was focused on identification of these early mosquito isolates, which we then assumed were the strains of JBE for which we were searching. I sustained a febrile illness with headache, malaise, muscle and joint pains, and cervical and epitrochlear lymphadenopathy. It was during the hot, dry season in Poona when mosquitoes were absent and one could only cool off by a plunge in the Poona Club swimming pool. The typical macular rash appeared on sudden exposure of the wet skin to the cooling effect of the dry air. Despite the multiple Group B arbovirus immunizations—17D, JBE, and RSSE, and high-titer WN-NT antibodies—it was a classic clinical course of West Nile fever.

To confound the picture there was an immediate high titer rise in HI and NT antibody to JBE virus. Only after 3 months did the antibody titer for West Nile virus reach and exceed those of the antibody response to JBE which might well have been accepted serologically as the diagnosis had we not isolated the P 4230 strain—typically West Nile virus—from the acute-phase blood. The implications of this, and similar human Group B arbovirus infections in others, in the presence of pre-existing neutralizing antibody, point to the need for some other avirulent live strain of Group B for prophylaxis against exposure to a multiplicity of Group B arboviruses such as occur in the jungle, rural, and urban areas of tropical Southeast Asia, Africa, and Latin America. Recognition of zoogeographical arbovirus interfaces such as that which became visible in India may be the source of a naturally circulating mutant which could serve as a broader immunizing agent than those now available to us either in live attenuated or killed vaccines.[27]

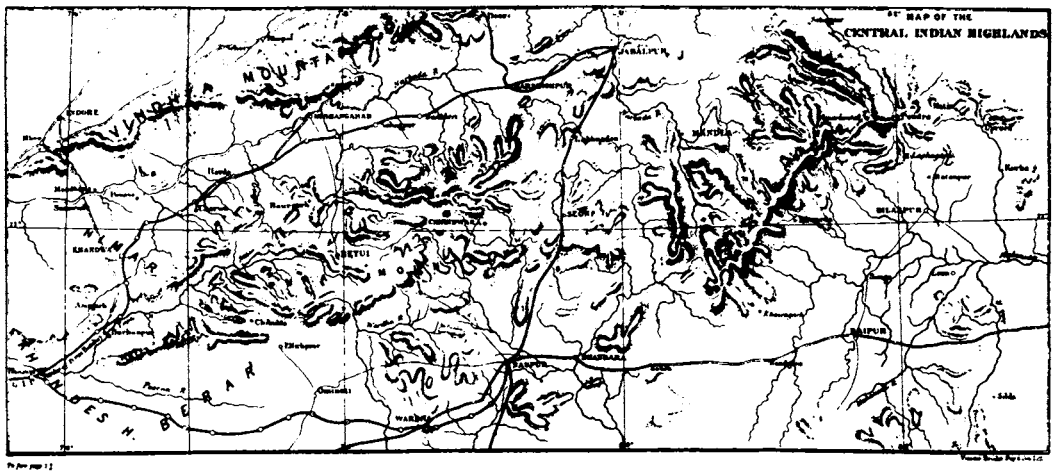

FIGURE 6. Map of Central Indian highlands

It is hoped that the search in India will be resumed by our arbovirus colleagues who are in such an excellent laboratory-supported position to do so.

In a presentation such as this, biography is an important unifying element. Lytton Strachey once defined the requirement of biography to be "serious, complete and of a certain magnitude." In attempting to be complete and to project a certain magnitude, the information presented here has ranged from unpublished data to anecdote. Before leaving the subject of West Nile virus I must by anecdote include the presence of this virus in Southern Europe.

One May afternoon in Paris, in 1965, the day before the U. S. Mission on Hemorrhagic Fevers[28] departed for Moscow, I was visiting the Pasteur Institute. One of the French workers intermittently smiled and nodded to me as we moved from room to room discussing their arbovirus program. Finally he said, "You don't remember me! When I visited your laboratory at CDC in Atlanta, you pointed to the map and said that if West Nile virus existed anywhere in Europe, it should be in the delta of the Rhone River where the Camargue appears to have all of the mosquito vector-wild bird elements for maintenance and transmission. Our finding of West Nile virus, with consequent human infections is what we are relating to you now." The speaker was Claude Hannoun.[29] The published French reports of this unique finding of tropical West Nile virus in temperate Europe helps to complete the geographical perimeter of this arbovirus, apparently the most wide-ranging of all viruses of the JBE-WN complex. Extrapolating from the febrile children's study in Egypt this infection must cause millions of cases of febrile illness each year in the vast area of West Nile virus activity.

St. Louis Encephalitis in the New World

That West Nile virus has the greatest range may someday be challenged when the extent of SLE virus activity in South America is better defined. From virus isolations and serological evidence it obviously has wide distribution. In fact two of the only four known isolations of SLE virus from human blood came from infections sustained in the Darién of eastern Panamá[30] and establish that this is a potentially serious disease problem to be encountered should it be decided that the new sea level Atlantic-Pacific Interoceanic Canal be excavated in that area.* Much of this speculation derives from experience of the past decade when SLE has proved to be the most serious arbovirus disease problem of the United States, somewhat of a replay of the 1930's when the etiology and epidemiology of urban SLE was first discovered in the City of St. Louis.[31]

In 1960, the venue for my arbovirus research shifted from the RFVL in New York to the U. S. Public Health Service Communicable Disease Center in Atlanta, in new laboratories occupied

* T. H. Work, 1967. Unpublished report to the Ecology Section of the Atlantic-Pacific Interoceanic Canal Studies Commission.

by Chamberlain, Kissling, Stamm, and Sudia who had moved from Montgomery, Alabama, the CDC facilities where they had contributed so much to the field and laboratory elucidation of EEE, WEE, and SLE. Within the context of organizing a virological laboratory resource at CDC to serve progress in the state capabilities for diagnostic and epidemiological virology, we put together the Arbovirus Unit.

Few states can afford to maintain an adequate entomological, epizootiological, epidemiological, and virological capability sufficient to deal with epidemic arbovirus encephalitis when it suddenly occurs. There is no more evident justification for a flexible, mobile national resource from a federal Center for Disease Control than a competent and experienced arbovirus laboratory and field team. The purpose of our unit was to provide epidemic and technical aid on request of the state; to define and elucidate persistently new knowledge on these problems; and to develop continuously more effective laboratory means for surveillance and characterization of arboviruses and diagnosis of arbovirus infections. The hub of our new Arbovirus Unit was an expanded laboratory operation under Dr. Philip Coleman with initiation of long-term field studies in the suspect VEE area of southern Florida. This operation was used as a training facility for doctor draft recruits to the Public Health Service, the first being Dr. Donald Quick, an Epidemic Intelligence Service officer.

In 1960 we had found serological evidence that neotropical Venezuelan equine encephalitis virus had infected a substantial number of Seminole Indians in Southern Florida.[32] This exciting clue, which subsequently led to the isolation of VEE[33] and other neotropical viruses in this subtropical extension of the continental United States, obscured the significant incidence of antibodies to St. Louis encephalitis in the same rural population of southern Florida.

The first recognizable epidemic experience with arbovirus encephalitis in Florida had occurred in the Tampa Bay area in 1959. What serology was done pointed toward EEE virus as the cause of a few cases,* but retrospective serological and epidemiological studies showed that SLE virus was also involved, perhaps to a larger extent.[34] Sixty-eight cases of suspect, acute, febrile central-nervous-system disease were analyzed; five died.

In October–November of 1961, Florida reported cases of encephalitis in elderly retired persons residing in Bradenton and Sarasota, south of Tampa Bay. Investigations by Dr. Quick and Dr. James Bond, the state epidemiologist, backed by the state and CDC arbovirus laboratories, established that a small epidemic (25 cases with 7 deaths) had occurred; etiology, SLE virus.[35] On 9 May 1962, in St. Petersburg, the Florida State Board of Health organized an all day Symposium on Arthropod-borne Virus Investigations in the Tampa Bay area.[35]

In retrospect a number of noteworthy statements were made by participants of that meeting. Among them were two that pointed toward the essence of Florida's problem. Chamberlain suggested that the most likely vector of SLE to man in Florida was *Culex nigripalpus*, a mosquito not yet implicated in the epidemic transmission of arbovirus encephalitis. Called upon to summarize and conclude the days' deliberations, I compared what was known about the epidemiology of the JBE-WN complex in the Nile Delta of Egypt, the Krishna-Godavari Delta of India, the Kanto Plain of Japan and the Great Central Valley of California, and stated that the Tampa Bay area appeared similar in so many ways, that the stage might well be set for rather extensive transmission of SLE sometime in the future.

In July, the 1962 Tampa Bay epidemic of St. Louis encephalitis struck.

Investigations of St. Louis Encephalitis Epidemics

Those of you who have dealt with epidemics of arbovirus diseases know that even though the first order of business is to establish the identity of the etiology and the specificity of the vector, these efforts can be overwhelmed by pursuit of confused cross purposes, involving those officially responsible for the public health and those associated by some possibly useful expertise. The apparition of "sleeping sickness" leads to panic in the inadequately informed residential population. The local health officers rise to protect the integrity of their community. The state must place in perspective the higher economic and social interests, defining resources that can be shifted to help solve the problem. Academicians are on hand to offer special knowledge and expe-

* Dr. E. Russell Alexander, Department of Preventive Medicine, School of Medicine, University of Washington, Seattle, Washington 98105, 1961. Personal communication.

rience, and, sometimes, special technical support with the long term view of education and defining significant future research. On invitation only by the state, the federal experts may enter under the "pall" of varying suspicions which often stem from the age-old distrust of the federal establishment, which was constitutionally defined almost 2 centuries ago. The problem is therefore not one of single-minded scientific endeavor but rather delicate diplomacy and negotiation towards a collaboration in the common interest.

Only facts, reliably reported, can allay the panic. Only such facts will provide a logical basis for developing and applying appropriate control and preventive measures. In an arbovirus disease epidemic, the laboratory is the spearhead of the epidemiological investigations. The 1959 and 1961 Tampa Bay epidemics had occurred in late fall. The 1962 epidemic began in July and therefore threatened to become a much more devastating experience. What was needed was substantial laboratory capability, not at arms length in Jacksonville or Atlanta, or both, but at the site for expeditious handling of a variety of specimens with prompt reporting of information to the local authorities and populace who needed to know the dimensions of the problem.

Amid a daily proliferation of misinformation by the press, and national coverage by television, which necessitated a calming visit by the Governor, the Board of Health selected a site for the laboratory in rooms over the garages of the state tuberculosis hospital just a mile from the Tampa International Airport. CDC moved a laboratory into these quarters with capability for serological diagnosis and virus isolation. Florida contributed substantial field and laboratory entomological and zoological resources. This collaborative effort became the Tampa Bay Regional Encephalitis Laboratory under Dr. James Bond, the state epidemiologist. In 3 months of transition it became a state epidemiological and grant-supported research operation that did not previously exist, and which has, to the present time, continuously provided new and essential information on encephalitis and other viral diseases in Florida. Within 24 hours of its establishment, data were emerging which miraculously allayed the public panic and served as a unifying focal point for the public health agencies of the four counties (Pinellas, Hillsborough, Bradenton, and Sarasota) involved.

Regional Dissemination of SLE Virus

Quick's 10-day spot maps of case onset in Pinellas County for the month of August showed a northwesterly drift from St. Petersburg to Clearwater. Looking at the previous inland evidence of SLE transmission it seemed peculiar that there were no cases reported elsewhere in the Tampa Bay area. The mosquito collections made early in September by Chamberlain and Sudia yielded strains of SLE virus from *Culex nigripalpus*, establishing this mosquito as a new and important, if not only, vector of SLE virus in the Tampa Bay area.[37]

With a mouse colony now established in Tampa we, with Dr. Richard Dow of the Florida Entomological Research Center, distributed mosquito traps in the four counties all around the Bay. The SLE virus isolates from *C. nigripalpus* in these collections[38] established that transmission was widely distributed throughout the Tampa Bay area, indicating that cases should be occurring in the other three counties. Cases surfaced in Hillsborough County as soon as we set up a 24-hour autopsy watch. From the first post-mortem examination of an urban resident of Tampa the TBH-28 strain of SLE virus was isolated.[39]

Intensification of acute- and convalescent-phase serum collection and persistent follow-up of survivors gave sequential sera that were tested for CF, HI, and NT antibodies to SLE virus. These provided patterns that clearly established that NT antibody was first to appear, usually before onset of overt disease, shortly followed by HI antibody, which was usually present by the 3rd day of illness. CF antibody appeared a week to 10 days later. The early results of these tests defined the utility of HI tests of acute-phase sera for an early presumptive diagnosis of SLE. Results of the more time consuming NT tests done at CDC were turned over to the Florida investigators and became the basis for more long-term elaboration of the rise and fall of SLE antibodies, subsequently published by Bond and other collaborators.[40] The significant advance here was the possibility of an early presumptive diagnosis of SLE, which proved so useful in the 1964 epidemics in providing physicians with early information on their patients and in accurately defining what segment of the reported cases were actually due to SLE. These detailed serological interpretations also more clearly defined the pre-

sumptive and confirmed cases that totaled 222 with 43 deaths as two dimensions of the 1962 Tampa Bay SLE epidemic.[40]

SLE Returns to the Urban Environment

The 1964 SLE epidemic in Houston, Texas, was first recognized by an astute and experienced professional City Health Officer, the late Dr. C. A. Pigford.[41] In early August while diligently reviewing the July death certificates, Dr. Pigford noted an unusual number of febrile, nervous-system-disease diagnoses as the cause of death. He sent his laboratory director, Reuben Wende, to the refrigerator to see if there were any serum specimens from July encephalitis cases. Sera of seven patients, four of whom were suspect viral encephalitis, were sent to the state public health laboratory in Austin. Texas had the advantage of a career laboratory director, Dr. J. V. Irons, who in his early experience had worked on the etiology of EEE. Shortly, the laboratory returned results which indicated that at least three of the patients had suffered infection with SLE virus.

Notified of the situation associated with an increasing number of admissions to the Ben Taub Hospital, Dr. J. E. Peavey, Director of the Texas State Health Office, called Dr. David Sencer at CDC. Our response was immediate, with dispatch of entomological and zoological personnel with equipment by road the 800 miles to Houston, and Dr. Sencer and I by air to assist in any way we could in the formulation of collaborative efforts involving the City, Harris County, the State, Baylor University, and the medical profession. Mobilization of the vector-control resources was a major element with which I am not directly familiar. But, here again in Houston, the provision of laboratory and field expertise was the cement that bound these diverse concerns together.

Our entomologists arrived late the following day and we visited the residential sites of diagnosed cases to hang out the CDC miniature light traps, which had been unfailing in catching mosquitoes in previous investigations. Few mosquitoes were in the bag the following morning. It gradually became apparent that we were dealing with the age-old peri-domestic *Culex pipiens quinquefasciatus* that frequented the human-contaminated sumps of urban concentrations, refractory to seduction by bright lights in the dark. This campaign required the slogging persistence of hand-catching in culverts, privies, and chicken houses that were the hallmark of SLE virus-infected mosquito catches in the vicinity of case exposures.[42]

Rex Lord instituted catches of backyard birds from which four isolates of SLE virus were obtained in the latter part of August. This illuminated the peri-domestic *C. p. quinquefasciatus*-backyard bird source of SLE virus.[43]

Dr. Brian Henderson, another of our doctor-draft CORD appointees, received his baptism of fire in Houston when he was assigned responsibility for the receipt and distribution of serum specimens from throughout the city for test. The Red Cross provided twice-a-day courier service for serum specimens from hospitals and physicians with suspect cases. These were referred, depending on daily demand, to the Baylor–City Laboratory effort under Professor Melnick, the State Laboratory in Austin, and the CDC Laboratory in Atlanta. A series of 14 possible serological interpretations were printed on gummed paper to be attached to a form derived from the 1962 Tampa Bay experience. When the 72-hour laboratory results were received, Xerox copies of these detailed master forms were made in the City Health Office and distributed to submitting physicians, patient's hospitals, the Epidemic Intelligence Service, and the City Laboratory as the pulse perception of the epidemic.

It was on a bedspread in the ancient Sam Houston Hotel that we analyzed the first 10-days' data and cautioned that only 44% of reported cases were SLE. Six weeks later, after subsidence of the epidemic, a complete analysis of laboratory results confirmed our estimate as 41%.[43]

The success of this city, county, state, and federal collaboration was marked by a publication of signal importance to the medical profession which, after all, is the ultimate dealer with the citizens who suffer disease. We all got together on writing the informative omnibus report for the *Journal of the American Medical Association*.[43] It was considered so significant that the attention of practitioners and specialists was attracted to it by a special editorial.[44]

The third dimension of any arbovirus epidemic is the incidence of inapparent infection. Ratios ranging from 1 in 16 to 1 in 500 had been reported in previous epidemics.[45,46] Of 243 laboratory-documented cases in Houston, 37 had died. Also of interest was whether the lower socioeconomic

areas with environmental features favoring high exposure had a higher infection rate. A post-epidemic serological survey was designed, which sampled different population groups by socio-economic residential area, and such controlled populations as on-duty firemen and nursing-home residents. Performance of this survey was undertaken by Dr. Brian Henderson and a number of PHS-VD investigators.[47]

The difficulty of interpreting nonspecific Group B antibodies, which are manifest in HI tests, was anticipated and solved by mouse-neutralization tests for specific antibodies resulting from prior exposure to dengue (Texas epidemic of 1922), yellow fever (17D vaccinations in military service), and SLE in 1964. When these were analyzed it became quite clear that the infection rate was the same for all age groups even though most overt disease and mortality were in the older persons who also had a high incidence of durable NT and CF antibodies to dengue. There was a difference between the residents of high-incidence, low-socioeconomic areas (33.2%) compared to 4.5% in residential areas affording screening and air conditioning. The overall average of 6.8% indicated that upward to 68,000 of Houston's one million population were infected, giving an overt disease case rate of 1 in 250.

Still another doctor draft EIS officer, Dr. James Luby, gained his knowledge of SLE by participating in the epidemiological studies and by looking over the shoulder of the autopsy pathologist.[48] It was Dr. Luby who signalled the onset of the 1966 Dallas epidemic when, as an infectious-disease resident, he recognized the first cases admitted to Parkland General Hospital.[49]

These episodes in Houston and Dallas, along with annual reports of cases in Corpus Christi, focus on a sizeable vector-borne disease problem in Texas, which presents three facets of the problem in the United States. Houston was hit for the first time reflecting the accumulations of organically polluted standing water, the ideal situation for *Culex pipiens quinquefasciatus* which retrospectively was implicated as the vector in St. Louis 31 years before.[31] This may well be the consequence of rapid urbanization on the flats of eastern Texas. Dallas appears to have been affected intermittently when nature reinforced the environmental requirements for the bird-mosquito reservoir. The annual recurrence of cases in human sentinels of Corpus Christi points to the possibility that the mechanisms for continuous maintenance of SLE virus lie close to this city in the rural regions of southern Texas.

What has come out sequentially from the series of SLE epidemics in the 1960's constitute an accumulation of knowledge that could be obtained in no other way. Some cynical laboratorians will term our kind as "epidemic chasers," and deprecate the importance of the new and basic knowledge that has come from careful laboratory and field examination of epidemic episodes. These epidemics are nature's experiments which to the appropriately trained scientist provide results that would be impossible for even the most wealthy scientific research establishment to produce. It was demonstrated for the first time in 1962 in Florida that in a disease like SLE and JBE, where virus can almost never be isolated from a living patient, an early diagnosis can be presumptively established by the HI test of an acute-phase serum. That a suburban distribution of cases resulted from a new mosquito vector, *Culex nigripalpus*, was also shown there.

In 1964 the value of local, state, and federally organized collaboration provided services to eight states (Texas,[43] Tennessee,[50] Kentucky,[51] Illinois,[52] Indiana,[53] Ohio,[54] Pennsylvania,[55] New Jersey[55]) that were involved in the largest and the most extensive activity of SLE in recent times, and recognized the disease east of the Appalachian Mountains for the first time. Significant scientific information was obtained from the outbreak by the diverse assignments of personnel components of an arbovirus research group supported at the national level.

The Houston epidemic demonstrated the utility of the early presumptive 72-hours diagnosis of SLE as useful not only to the physician while he was still concerned with the welfare of his patient, but also by defining the probable incidence of actual cases, which is so useful in predicting the course and fate of the epidemic.

In 1966 the benefits of physician training of EIS officers was manifest in early recognition of cases which signalled onset of the epidemic in Dallas, Texas. By this time there was a well-coordinated chain reaction of response which allowed early identification of the etiologic virus (SLE) and vector *C. p. quinquefasciatus*, which brought into play massive aerial-delivered vector control that probably effectively prevented a number of cases which otherwise would have

occurred. The accumulated experience indicates that with SLE, in contrast to JBE, constant surveillance and vector control, rather than vaccination, is the appropriate means for preventing or aborting an epidemic.

St. Louis Encephalitis in the Arid Zone

With that signal achievement of *scientifically defined collaboration* of local, state, and federal public health workers in 1966, one might logically ask if anything has been done since, relative to the JBE-WN complex.

Late in 1966, the prodigal returned home to southern California after 30 years of educational, medical, and scientific wanderings. With the most biologically interesting deserts on earth—Mojave, Colorado, and Sonoran—virtually at the doorstep of UCLA, we started as a field component of our research-training program in Infectious and Tropical Diseases, a study of arboviruses in an Arid Zone.* In the first 2 years our initial two questions were answered. 1) In contrast to the data of Reeves' studies in Kern County, is *Culex tarsalis* active throughout the year in the Imperial Valley? The monthly mosquito collections show significant *C. tarsalis* yields in every month of the year with the minimal catches in July–August when they are only declining from a seasonal peak in the Great Central Valleys. 2) WEE, SLE, Turlock, and California encephalitis viruses are transmitted in the ecological study area located in the Wister Refuge on the southeastern shore of the Salton Sea.

Looking at the SLE isolates as possibly being at the perimeter of radial distribution, we initiated parallel studies at the Finney Lake Refuge about 15 miles distant and inland, a large roost and breeding colony of Redwing and Yellowheaded Blackbirds. The mosquitoes collected there and examined so far in 1970 have yielded nine strains of SLE virus. This is the beginning of another approach to find a mechanism for inter-epidemic maintenance of JBE–WN complex viruses—epizootic transmission in warmer areas of southern North America or cyclic reintroduction by migrating birds.

The newest endeavor focused on this complex is a component of a developing collaborative infectious and tropical disease teaching and research training program with Airlangga University School of Medicine in Indonesia. In August and September with Dr. Biroum Noerjosin, Professor of Microbiology, Dr. Soewigno, and Dr. Suharjono of Indonesia, we commenced a serological survey of East Java, Bali, Lombok, and Sumbawa, islands which straddle Wallace's Line.[56] This was to determine, among other issues, where we might look for JBE or MVE viruses, or both, in defining another possible zoogeographical arbovirus interface that might contribute much to the solution of the general problem of diseases caused by viruses of the JBE–WN arbovirus complex on six continents. Preliminary serological results indicate that we may be on target.

Philosophical Comments

In concluding this odyssey of arbovirology and epidemiology on six continents, some philosophical comments appear to be appropriate. There are those who will think it fortuitous that I am called to account for a quarter century of biomedical performance at the heartland of my education and medical training where the odyssey began just 25 years ago. My Moslem friends and colleagues might say, *Mekhtub*! "In the great book, it was written."

California was then the leader of our nation in public education. The recently popularized subject of ecology was, in the middle thirties, an important topic of biology as taught at University High School of West Los Angeles, under the growing influence of UCLA where it is now a fundamental preparation for those studying infectious and tropical diseases. The biological sciences at Stanford University were second to none in any university of the nation. Stanford School of Medicine was then at Clay and Webster Streets, not far from the venue of this meeting. The Museum of Vertebrate Zoology of the University of California in Berkeley was a fountainhead of field studies in zoology, and particularly ornithology. My teachers were exceptional in that there was less didactic than preceptor guidance with special interest in the students' individual pursuits. Now that I wear the cloak of a preceptor I can feel grateful for the special stimulation and patience of C. V. Taylor, George Beadle, Willis Rich, Edith Mirielees, Elton Trueblood, Loren Wiggins, Alden Miller, Edwin Schultz, Jim McNaught, George Barnet, A. L. Bloomfield, Frederick Reichert, Yank Chandler, and many others.

* T. H. Work, R. Vanis, and H. G. Wallace, Arboviruses in southern California. Manuscript in preparation.

In the post-doctoral years were Sir Philip Manson Bahr, John Kessel, Gilbert Otto, Lloyd Rozeboom, David Davis and my appreciated preceptors of The Rockefeller Foundation, especially Hugh Smith, Dick Taylor, Wil Downs, and Jordi Casals. Although varied in background and expertise they all had two guiding criteria in common: broad preparation and maximal individual performance.

There is a paradoxical satisfaction in the retrospective review of the sequence of events highlighting almost 20 years of work with the Japanese B–West Nile virus complex. Order of authorship begins in the middle, moves to the first position, and then drifts backward to last place as the sphere of collaboration expands and the young disciples take their place, with a few achieving the eminence of independent scientific achievement. It not only reflects the common pattern of scientific aging, but the imposition of responsibility and administrative obligations which so often separate the older from doing what they know best.

By now it must be clear that the style and direction of this address is to our younger members who are seeking their own way toward individually satisfying careers, frustrated perhaps by the engulfing wave of technology, depersonalized relationships, and the demand that they publish in the sacred scientific record everything they do. It wasn't really much different 25 years ago.

To enjoy and appreciate ones obligated military service as an opportunity to exploit unique challenges and experiences for long term career development was considered then not only "square," but almost treasonous to the higher conformity of organized medical training. To enter a career in tropical medicine when the United States was pulling back its huge military involvement in the tropics was considered foolhardy. To pursue interests in infectious disease when chemotherapy, antibiotics, and disease eradication predicated the final conquest of communicable disease, marked one as out of phase with contemporary medicine. And to devote the most precious and formative years of a professional life to what two Nobel Laureates of the Rockefeller Institute in 1952 termed "yellow fever's hobby horse," required determined devotion to persons and concepts that were yet to emerge as arbovirology, an entirely new field in tropical medicine.

Actually, the present prospects for a meaningful career in tropical medicine were never brighter. The health problems and productivity of the human race will focus increasingly on the billions expanding and residing in tropical regions. I would like to be present 25 years from now to hear from one of you the account of how the career went in the last part of the 20th century.

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