VIROLOGY MONOGRAPHS

16

R. W. SCHLESINGER

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SPRINGER-VERLAG
WIEN NEW YORK

Printed in Austria by R. Spies & Co., A-1050 Wien

Library of Congress Cataloging in Publication Data. Schlesinger, Robert Walter, 1913—. Dengue viruses. (Virology monographs; 16.) Bibliography: p. 1. Dengue viruses. I. Title. II. Series. QR360. V52. no. 16 [QR398]. 576'.64'08s [616.01'94]. 77–24108

ISBN 3-211-81406-X Springer-Verlag Wien-New York
ISBN 0-387-81406-X Springer-Verlag New York-Wien

Dengue Viruses

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With 34 Figures

Preface

This monograph is dedicated to the memory of ROBERT DOERR (1871—1951), the founder of the Handbuch der Virusforschung which was the predecessor of this series of Virology Monographs. In 1939, he also founded the Archiv für die gesamte Virusforschung, the first journal devoted to virology. His comprehensive vision, expressed in these publications, marked him as a father of modern virology as a distinct science. The tradition established by him was ably continued by his long-time collaborator and editorial successor, CURT HALLAUER, with whom I share the privilege of having been introduced to viruses by DOERR.

More specifically, the subject of this monograph was one of personal interest to DOERR. His studies of sandfly (pappataci) fever stand as a classical demonstration of the role of arthropods in the transmission of human virus diseases[1,2] and led him to write an authoritative review of that disease and of dengue[3].

During the second World War, Japanese and American investigators independently succeeded in propagating dengue virus in mice and in developing attenuated strains suitable for experimental vaccination of human subjects. Professor S. HOTTA, now at Kobe University, was a member of the Japanese team. He and his associates have continued to make valuable contributions to dengue research. I worked with the group led by Dr. ALBERT B. SABIN in the United States. When I was asked by Professor HALLAUER to write this monograph, I invited Professor HOTTA to be a coauthor. In 1968, he supplied valuable references and summaries, many pertaining to work published in Japan to which I did not have access. Unfortunately, various distractions forced me to postpone my own writing, and the

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[1] DOERR, R., FRANZ, K., TAUSSIG, S.: Das Pappatacifieber. Leipzig-Wien: Deuticke 1909.

[2] DOERR, R., RUSS, V. K.: Weitere Untersuchungen über das Pappatacifieber. Arch. für Schiffs- und Tropen-Hygiene **13**, 693—706 (1909).

[3] DOERR, R.: Pappatacifieber und Dengue, in: Handbuch der pathogenen Mikroorganismen (KOLLE, WASSERMANN, eds.), pp. 500—546. Jena: Fischer 1930.

rapid flow of new knowledge made sustained long-distance coauthorship virtually impossible. The resulting delays compelled Professor HOTTA to meet deadlines in the publication of his book "Dengue and Related Hemorrhagic Diseases" (St.Louis: W. H. Green, Inc. 1969). In order to avoid duplication, the emphasis of this monograph had to be re-oriented. Professor HOTTA graciously agreed to change coauthorship to an acknowledgment of our early collaboration in portions of the text. The greater part represents my reflections and interpretations for which Professor HOTTA should not be held responsible. I am grateful for his contributions and understanding.

Most of the text was written and re-written during brief periods of undisturbed quiet at the Rockefeller Foundation's Study and Conference Center, Bellagio, Italy (October 1972), and at the Library of the Marine Biological Laboratory, Woods Hole, Massachusetts.

Piscataway, N.J., March 1977 R. WALTER SCHLESINGER

Table of Contents

I. Introduction

Dengue fever is a mosquito-transmitted disease of man which has afflicted untold millions of people over the past two centuries. It is caused by viruses classified as a subgroup of the group B togaviruses. Along with other members of that group as well as group A, the dengue viruses have been investigated intensively during recent years. Certain unique aspects of their structure, composition, antigenicity, replication, and antigenic relationships have established the togavirus family as quite distinct from other families of enveloped RNA viruses (see recent review of PFEFFERKORN and SHAPIRO, 1974). The basic studies leading to this conclusion have coincided with epidemiological field investigations which have resulted in a continuing increase in the number of viruses now designated as group A or B togaviruses. This, in turn, has led to a growing appreciation of their immense importance as actual or potential pathogens of man and beast.

All group A togaviruses (or alphaviruses) are antigenically and structurally related to each other, yet each one has its own antigenic and biological specificity. The same is true for group B togaviruses (or flaviviruses). Most viruses now unequivocally classified as togaviruses (cf. HORZINEK, 1973a, b) are also arthropod-borne (arbo)viruses, *i.e.*, they are transmitted to vertebrate hosts by arthropod vectors in which they multiply and establish life-long infection (cf. BERGE, 1975). What is the evolutionary significance of this infection cycle? What are the selective pressures that such a complex, apparently mandatory, alternation between invertebrate and vertebrate hosts imposes on viruses? What are the consequences of these pressures in terms of antigenic and pathogenic variation? These are among the questions for which carefully designed laboratory models may ultimately provide some answers.

In addition to the dengue viruses, group B contains some 50 other viruses. Although logic might suggest that a monograph ought to cover all of them rather

than just the dengue subgroup, we know very little about many members of the group. A few—notably Japanese, Murray Valley, and St. Louis encephalitis (JE, MVE, SLE), Kunjin, certain tick-borne encephalitis (TBE) viruses—have been analyzed to about the same extent as dengue viruses. By and large, the information obtained is remarkably similar for all of them. Comparative data will be included in this monograph whenever they supplement in essential detail our knowledge about dengue viruses.

Why then have the dengue viruses been selected as the subject for this monograph? One reason is purely historical. Dengue fever was the second specifically *human* disease (after yellow fever) whose etiology was critically identified as a "filtrable virus" (Ashburn and Craig, 1907). Immense efforts were made subsequently to learn more about the disease and its epidemiology and to make the virus amenable to laboratory investigation. This work was summarized in the classical monographs by Siler *et al.* (1926) and Simmons *et al.* (1931). In the decade immediately preceding World War II, however, the importance of dengue was eclipsed by that of its close relative, yellow fever, and by the success in development of an effective YF vaccine (see Strode, 1951). The Pacific phase of World War II brought dengue fever to renewed prominence, and that era signified a beginning of the detailed knowledge about dengue viruses and the diseases they cause that we have now acquired. Therefore this seems to be a good time to amplify earlier résumés of the work accomplished in the 1940's and 1950's (Sabin, 1952a, d; Hotta, 1965, 1969).

Like yellow fever, dengue has a relatively simple transmission cycle which involves only certain species of mosquitoes and man (and occasionally monkeys). In this respect these two viruses differ from most other serious pathogens in the group, notably the encephalitis viruses, which have a far more complex transmission cycle and for which man is a relatively minor, accidental, or dead-end link. Moreover, while these latter viruses do infect man in nature, the vast majority of human infections remain subclinical.

In contrast, a first infection with one of the dengue viruses almost invariably leads to some kind of illness. Man, being the single major natural host, is bound to reflect in his response to infection any variable property of the viruses that may affect their pathogenicity. The fact that exposure to one virus of the dengue subgroup may profoundly modify the immunopathological response to subsequent infection with another one has, in recent years, forced us dramatically to change our earlier view of dengue fever as a benign disease. The more alarming aspect of dengue viruses has emerged by virtue of their documented association with "new" and often fatal illnesses, *i.e.*, hemorrhagic fever and the dengue shock syndrome (Hammon, 1969; Hammon *et al.*, 1957, 1958, 1960a, b; cf. Halstead, 1965, 1966). Current hypotheses explain the pathogenesis of these syndromes by taking into account known or surmised aspects of the structural and antigenic nature of the viruses, the mode of their replication and release from infected cells, and the host's immune response.

A major difficulty in summarizing information about on "old" virus disease lies in the fact that many classical studies have been rendered obsolete or questionable by more recent findings. The monumental review of dengue by Siler, Hall, and Hitchens (1926) contains nearly 600 literature references, many of

them of fascinating historical interest. It would go beyond the scope of this monograph to sort out those which seem relevant in light of current ideas.

More recent epidemiological data on dengue are represented in a vast literature reporting the results of mass surveys of human and animal populations or of potential arthropod vectors for serological or virological evidence of infection with many different kinds of arboviruses (cf. THEILER and DOWNS, 1973). The main contribution of this approach is the elucidation of the overall prevalence of arboviruses and, specifically, of ecological factors which may be responsible for the emergence of a seemingly endless variety of species among these agents. Its value has been underlined by the recent recognition that a great majority of arboviruses not previously accommodated in the togavirus groups can in fact be fitted into a third large family, the bunyaviruses (MURPHY *et al.*, 1973; PORTERFIELD *et al.*, 1974; BERGE, 1975). Thus, it seems that the biological feature of arthropod-vertebrate-arthropod- ... transmission cycles is frequently associated with viruses belonging to two major structural families, the toga- and bunyaviruses.

Table 1. *Selected fever epidemics by year,*

Present-day chikungunya (? original dengue)
(Fever, joint pain, rash, residual arthralgias)

Year	Location	Designation
1779	Batavia	Knuckle fever
1779	Cairo	Knee trouble
1823	Zanzibar	Dinga, dyenga
1824—25	Calcutta; Madras; Gujarat	Scarletina rheumatica
1827—28	West Indies, New Orleans; Charleston, S.C.	Dandy fever, eruptive articular fever, exanthesis arthrosia, dengue
1870	Zanzibar	Dinga, dyenga, dengue
1871—72	Calcutta; Madras	Dengue
1901—02	Hong Kong; Burma; Madras	Dengue
1923	Calcutta	Dengue
1952	Tanganyika	Chikungunya (prototype virus isolated)
1964	Vellore, India	Chinkungunya (virus isolated)

From CAREY (1971). With permission of the author and the Journal of the History of Medicine.

This circumstance alone carries fascinating implications regarding possible parallels in their evolutionary history (cf. SCHLESINGER, 1971). Within the broad spectrum of the 200-odd viruses so far assigned to these families, the dengue virus subgroup presents unique features.

In this monograph we shall concentrate on knowledge about dengue viruses gained since the second World War, when they first became amenable to intensive study in the laboratory.

II. Definitions and Nomenclature

Dengue is the accepted name of an acute infectious disease of man characterized by fever, aches and pains in various parts of the body which may range from mild to excruciatingly severe, generalized rash, lymphadenopathy and leukopenia. Its effects may be debilitating to the point of prostration, but uncomplicated classical

location, symptomatology, and designation

Present-day dengue (? original breakbone)
(Diphasic fever, body and muscle pain, rash, post-illness asthenia)

Year	Location	Designation
1780	Philadelphia	Breakbone fever
1826—27	Charleston, S.C.; Savannah, Ga.	Breakbone fever
1850	Charleston, S.C.	Breakbone fever
1853—54	Calcutta	(loin and limb pain, languor, prostration. Only one case with severe articular pain)
1897	Queensland, Australia	Dengue
1905	Queensland, Australia	Dengue (transmission via *A. aegypti* shown)
1905—07	Calcutta	Seven day fever
1907	Philippines	Dengue (transmitted via filtered plasma)
1907	Brownsville, Tex.	Dengue
1911—12	Calcutta; Poona	Seven-day fever, dengue
1923	Galveston, Tex.	Dengue
1944—45	Hawaii, New Guinea; Calcutta	Dengue (prototype viruses isolated)
1961—63	Vellore, India	Dengue (viruses isolated)

(primary) dengue is rarely, if ever, fatal. It is caused by at least four antigenically distinct viruses constituting the dengue subgroup of group B togaviruses (see Section IV on Classification). By definition, they are transmitted to man by mosquitoes of the genus *Aedes* (Stegomyia). It follows that the occurrence of the disease (and the viruses) is restricted to those geographic areas in which suitable vector species are prevalent or in which, once imported, they can maintain themselves.

Virus can be isolated from the peripheral blood of naturally infected patients during the febrile phase of the acute disease (cf. SABIN, 1952a). In the past, it had to be identified by its ability to produce typical dengue in human subjects inoculated by a variety of routes or bitten by *Aedes* mosquitoes which had engorged on virus-containing source material (SILER *et al.*, 1926). Since the first successful experimental transmissions of dengue viruses to mice (KIMURA and HOTTA, 1944; SABIN and SCHLESINGER, 1945; MEIKLEJOHN *et al.*, 1952a; SCHLESINGER and FRANKEL, 1952a), this method and the use of various *in vitro* cell cultures, in combination with serological and physical-chemical criteria, have been applied to their primary isolation and identification (see subsequent sections).

The origin of the name dengue has been puzzling. According to HALSTEAD (1971), the word may have its roots in the Swahili term "ki-denga Pepo" or "a disease characterized by the sudden cramp-like seizure caused by an evil spirit", which was applied to an outbreak in Zanzibar in 1870. "The term 'denga' or 'dyenga' was used to designate the disease on the East Coast of Africa in 1823 and at least until 1870. Early authors have assumed that 'dengue' spread with the slave trade from East Africa to the Caribbean, where in 1827—28 an extensive outbreak occurred in the West Indies . . . It was here that the word dengue was first used", perhaps as a Spanish adaptation of the imported word. Although it now appears that the illness to which African natives referred as "denga" etc. may actually have been *chikungunya* (CAREY, 1971; HALSTEAD, 1971a; see next Section), it is now of course possible to distinguish these two diseases by virological and serological means. As might be expected for a disease as painful, wide-spread, and old as dengue, many exotic and picturesque synonyms have been in circulation at various times and places (fully discussed by SILER *et al.*, 1926, and by CAREY, 1971, see Table 1). The colloquial "breakbone fever" is a very apt and descriptive term.

III. History

The early history of "dengue", going back to epidemics in Java and in Egypt in 1779, was admirably reviewed by SILER *et al.* (1926). These authors quoted extensively from old documents to support their hypothesis that dengue had occurred in the Western hemisphere as early as the mid-17th century and had its origin in "tropical America" (l. c., p. 21). On the other hand, MATTINGLY (1960) summarized evidence which led him to believe that it originated in Southeast Asia.

A recent scholarly treatise by CAREY (1971) analyzed critically the historical accounts of "dengue" outbreaks during the 18th and 19th century. CAREY furnished persuasive arguments in favor of the belief that the earliest recorded

epidemics outside the Western hemisphere (including those in Java, Egypt and India) were probably due to chikungunya virus (a group A togavirus) rather than dengue viruses. Table 1, taken from CAREY's paper, summarizes the key differences in clinical characteristics and assigns various epidemics to one or the other of the two agents.

We owe to BENJAMIN RUSH the first accurate clinical description of true dengue fever as it occurred during the Philadelphia epidemic of 1780 (quoted in SILER *et al.*, 1926, and CAREY, 1971). It fits, in many details, the features of the disease seen in well-documented contemporary outbreaks (see Section X).

During the 19th and 20th centuries, extensive outbreaks were reported from tropical and subtropical areas on all continents and from many subcontinents and islands in the South Pacific and in the Caribbean. This extensive geographic spread has continued to the present day. For example, during this century major epidemics were recorded in the Southern United States (1920, 1922), Australia (1925—26, 1942, 1954—55), South Africa (1926—27), Greece (1927—28), Japan (1942—45), and the Caribbean (1963—69). The occurrence of dengue in the Caribbean during the past decade is illustrated in Figs. 1—4. Its relationship to the prevalence of *Aedes aegypti* is clearly shown in Fig. 4.

Each of these epidemics affected thousands or millions of people, with disease rates in some areas attaining 80% of the population (*e.g.*, COPANARIS, 1928, who reported that 80—90% of the populace of Athens and Piraeus contracted dengue during the Greek epidemic). Such massive outbreaks, even those occurring at a time when methods for virus isolation or serological diagnosis were not available, have aided greatly in the delineation of clinical and epidemiological features of the disease. This was particularly true for the outbreaks in South Africa, the United States, Greece and Japan because these areas have experienced no major subsequent occurrence. As a result, it has been possible, through retrospective serological surveys, to support the view that these epidemics were indeed caused by dengue viruses (KOKERNOT *et al.*, 1956; SIGEL and BEASLEY, 1959; HAMMON *et al.*, 1966; EHRENKRANZ *et al.*, 1971; THEILER *et al.*, 1960; PAVLATOS and SMITH, 1964; HOTTA, 1968; HOTTA *et al.*, 1968). As discussed in detail by WISSEMAN and SWEET (1961) and WISSEMAN (1961), this type of circumstantial confirmation is extremely important in view of the fact that the dengue syndrome is mimicked by a variety of other agents, including several other arthropod-transmitted viruses. Moreover, not all epidemics of verified dengue display the disease in its full-blown incapacitating form; occasionally relatively mild, easily confused variations predominate. Mild forms are particularly characteristic in childhood when most people are likely to acquire their first infection in heavily endemic areas (*e.g.*, Southeast Asia) (cf. HALSTEAD *et al.*, 1969b) (see Section X). In "hyperendemic" areas (Haiti and Dominican Republic), clinical illness may be completely absent (VENTURA *et al.*, 1975). Therefore, estimates of the geographic distribution of the disease or its viruses strictly on the basis of clinical evaluation of epidemic or sporadic occurrences of "dengue" or "dengue-like" disease are somewhat precarious. Again, the situation has been clarified to some extent by CAREY (1971). His searching review of the earlier literature leads to the conclusion that the distinction between "dengue" (*i.e.*, chikungunya) and "seven-day fever" or "breakbone fever" (*i.e.*, dengue by current designation) was indeed made by some astute

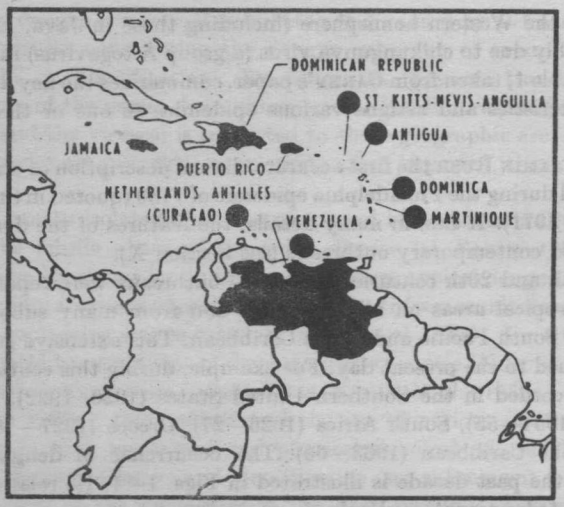

Fig. 1

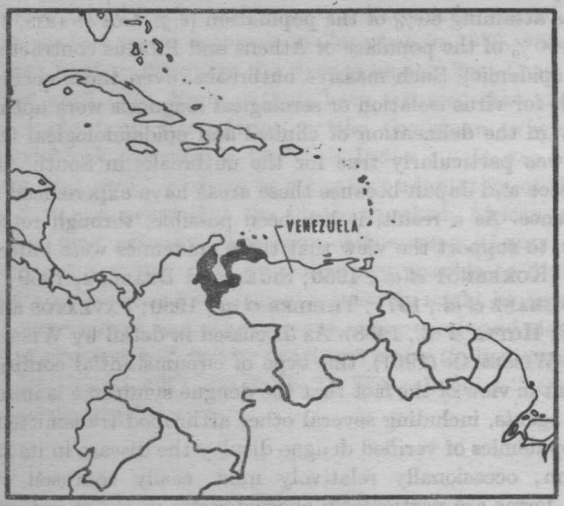

Fig. 2

Fig. 1. Occurrence of dengue in the Caribbean, 1963—1965
Fig. 2. Occurrence of dengue in the Caribbean, 1966—1967
Fig. 3. Occurrence of dengue in the Caribbean, 1968—1969
Fig. 4. Status of the aedes aegypti eradication campaign in the Americas, December 1969

Figs. 1—4. From "Surveillance of Dengue in the Americas: A Report to the Director", Pan American Health Organization, Report of the Scientific Advisory Committee on Dengue, First Meeting 15—16 January 1970
With permission of The Pan American Health Organization

Fig. 3

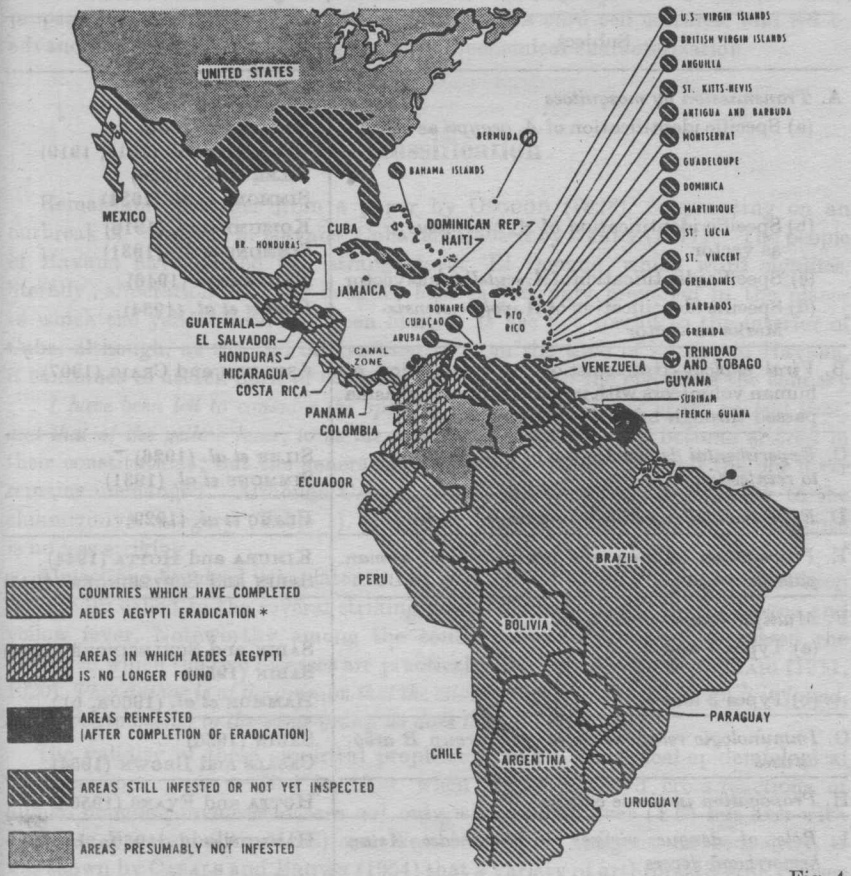

Fig. 4

* ERADICATION CARRIED OUT ACCORDING TO THE STANDARDS ESTABLISHED BY THE PAN AMERICAN HEALTH ORGANIZATION

early clinicians (see Table 1). The differences between the two diseases, as they have been observed in recent outbreaks, have been summarized by DELLER and RUSSELL (1967).

In the evolution of knowledge about dengue and similar illnesses, military exigencies have traditionally provided a major spur. One obvious reason for this interest is, of course, their debilitating nature which can render combat forces ineffective (cf. McCOY, 1964). Another factor is the massive transfer, in war or peace, of previously unexposed military personnel into endemic areas. Thus, an early entry of the United States Army into dengue research was forced by the experience that troops shipped to the Philippine Islands inevitably contracted the disease even though native Scouts, garrisoned alongside, did not. This remarkable contrast led to the first controlled studies on immunity to dengue (SILER *et al.*, 1926; SIMMONS *et al.*, 1931; see also the recent follow-up on their studies by HALSTEAD, 1975). Again, the Pacific phase of World War II confronted both

Table 2. *Summary of "classical" studies of dengue and its viruses*

Subject	References
A. *Transmission by mosquitoes*	GRAHAM (1903)
(a) Specific identification of *A. aegypti* as vector	BANCROFT (1906) CLELAND *et al.* (1916, 1919) SILER *et al.* (1926) SIMMONS *et al.* (1931)
(b) Specific identification of *A. albopictus* as vector	KOIZUMI *et al.* (1916) SIMMONS *et al.* (1931)
(c) Specific identification of *A. scutellaris* as vector	MACKERRAS (1946)
(d) Specific identification of *A. polynesiensis* Marks as vector	ROSEN *et al.* (1954)
B. *Viral etiology* (transmission by inoculation of human volunteers with infectious human plasma passed through bacteria-retaining filters)	ASHBURN and CRAIG (1907)
C. *Experimental demonstration of immunity to reinfection*	SILER *et al.* (1926) SIMMONS *et al.* (1931)
D. *Experimental infection of monkeys*	BLANC *et al.* (1929)
E. *Propagation in mice and attenuation of human pathogenicity*	KIMURA and HOTTA (1944) SABIN and SCHLESINGER (1945)
F. *Multiple immunologic types*	
(a) Types 1 and 2	SABIN and SCHLESINGER (1945) SABIN (1950)
(b) Types 3 and 4	HAMMON *et al.* (1960a, b)
G. *Immunologic relationship to other group B arboviruses*	SABIN (1950) CASALS and BROWN (1954)
H. *Propagation in tissue culture*	HOTTA and EVANS (1956a, b)
I. *Role of dengue viruses in Southeast Asian hemorrhagic fevers*	HAMMON *et al.* (1960a, b)

Japan and the United States with the potential hazards of combat in dengue endemic areas and stimulated renewed efforts in both countries to investigate the disease and its causative agent (cf. SABIN, 1952a; HOTTA, 1965, 1969).

The success of these studies was directly responsible for the specific identification of epidemic and endemic dengue in various parts of the world and for the unequivocal demonstration that dengue viruses are causally related to hemorrhagic fevers and the shock syndrome in South and Southeast Asia (HAMMON *et al.*, 1960a, b). The latter alarming development has provided renewed stimulus to dissect the virion physically and biochemically, to study its antigenic components, and to understand the pathogenesis of primary and secondary disease in terms of current pathophysiological and immunological concepts.

Some initial contributions to major phases of dengue virus research are summarized in Table 2. From the point of view of facilitating accumulation of knowledge about the basic properties of dengue viruses, their "adaptation" to the laboratory mouse was the crucial step. It permitted, for the first time, the circumvention of man as the only available experimental host, facilitated the eventual propagation and quantitation of the viruses in *in vitro* cell cultures, and led to advances in their physical, antigenic, and biochemical characterization.

IV. Classification

Remarkable passages from a paper by OSGOOD (1828), commenting on an outbreak of "dengue" in Havana, Cuba, are quoted by CAREY (1971): "The people of Havana have named this strange fever 'El Dengue' which word signifies, literally, affectation ... The dengues has, as yet, only prevailed in the places to which the yellow fever has been limited. It has not spread to the interior of Cuba, although, at the end of five months from the time of its rise in Havana, it continues to attack most of the persons who come to the city from the country ... *I have been led to consider the specific cause of the disease of the present time, and that of the yellow fever, to be the same.* The subjects have become altered in their constitutions; but the generating cause both of the new and the old fever remains unchanged." Although CAREY assigns the West Indian outbreak to the chikungunya category (Table 1), this early hint at epidemiological coincidence is no less striking.

Almost one-hundred years later, SILER *et al.* (1926) wrote: "Attention has been repeatedly called to the several striking points of similarity between dengue and yellow fever. Noteworthy among the contributions which have discussed the factors in which the two diseases are practically identical are those of CRAIG (1911, 1920). *This author is of the opinion that the etiologic agent of dengue, when it is found, will prove to belong to the same group as does that of yellow fever.*"

The validity of these empirical prophecies, based on clinical-epidemiological considerations, was verified in 1950, when SABIN reported cross-reactions of human dengue-convalescent sera not only with yellow fever (YF) but also with Japanese B encephalitis (JBE) and West Nile (WN) antigens. Subsequently, it was shown by CASALS and BROWN (1954) that a variety of arthropod-borne viruses

could be categorized into two major groups (A and B) on the basis of (a) optimal conditions required for hemagglutination (HA) (see Section V.B.2.), and (b) cross-reactivity in hemagglutination-inhibition (HI) tests (see Section VI, E). According to both criteria, dengue viruses belonged to group B, along with JE, Ilheus, Ntaya, Russian spring-summer encephalitis (RSSE), St. Louis encephalitis (SLE), WN, and YF viruses. Later studies by CASALS and coworkers led to the establishment of several other groups of arthropod-borne viruses on the basis of major or minor serological cross-reactions. The list of those assigned to group B has grown to 57 (BERGE, 1975), making it the second largest of the arbovirus groups [the largest being the Bunyavirus family (BERGE, 1975)] and the one containing the largest number (29) of proved human pathogens.

According to the recommendations of a WHO Study Group (WHO, 1967), the criteria for acceptance of an arthropod-borne virus should be as follows: "Arboviruses are viruses which are maintained in nature principally, or to an important extent, through biological transmission between susceptible vertebrate hosts by haematophagous arthropods; they multiply and produce viraemia in the vertebrates, multiply in the tissues of arthropods, and are passed on to new vertebrates by the bites of arthropods after a period of extrinsic incubation."

In recent years, the group designations (A, B, C, etc.) were amplified by generic names, e.g., "alphavirus" for group A, "flavivirus" (*flavus* for yellow fever) for group B (cf. WILDY, 1971). Various other amplifying suggestions for the refinement of a classification of arboviruses on purely biological grounds have been debated (SCHERER, 1968; CASALS, 1968a; JOHNSON, 1968; TAURASO and SHELOKOV, 1967). We shall follow SCHERER's suggestion (1968) to regard the four generally accepted, serologically distinct, dengue viruses as types 1, 2, 3, and 4 (hereafter referred to as dengue-1, -2, -3, -4) of the dengue subgroup of group B arboviruses (flaviviruses). This designation takes account of the fact that these 4 types (a) share the biological characteristics generally associated with dengue viruses (clinical features, epidemiology, natural and experimental host range, vector specificity), (b) possess type-specific antigens, (c) carry, in addition, some as yet unexplained subgroup-specific antigenic relationships, but (d) share antigenic determinant(s) with all group B arboviruses.

The term "arbovirus", by etymological derivation as well as by definition, implies nothing more than a convenient collection bag for viruses meeting the biological criteria cited above. The need for a more discriminating assignment of the different groups and members to a rational system of viruses based on physical-chemical properties (LWOFF and TOURNIER, 1966; GIBBS and HARRISON, 1968; WILDY, 1971) has been stressed by JOHNSON (1968). It is underlined by the confusing (but understandable) proposal to classify rubella virus as an arbovirus (HOLMES and WARBURTON, 1967; HOLMES *et al.*, 1969) on the grounds that it resembles certain group A arboviruses in physical-chemical characteristics and in features of its mode of replication and release. While the absence of serological cross reactions between rubella or other structurally similar viruses (cf. HORZINEK, 1973a, b) and a large number of arboviruses (METTLER *et al.*, 1968) does not seem an adequate basis for rejecting a systematic relationship (after all, arthropod transmission *could* be an ecologic accident and lack of it *could* be an evolutionary adaptation; moreover, different *groups* of arboviruses also are completely unrelated