Update
Le point

Articles in the ***Update*** *series give a concise, authoritative, and up-to-date survey of the present position in the selected fields, and, over a period of years, will cover many different aspects of the biomedical sciences and public health. Most of the articles will be written, by invitation, by acknowledged experts on the subject.*

Les articles de la rubrique ***Le point*** *fournissent un bilan concis et fiable de la situation actuelle dans le domaine considéré. Des experts couvriront ainsi successivement de nombreux aspects des sciences biomédicales et de la santé publique. La plupart de ces articles auront donc été rédigés sur demande par les spécialistes les plus autorisés.*

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Dengue haemorrhagic fever—a public health problem and a field for research*

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Dengue haemorrhagic fever/dengue shock syndrome (DHF/DSS) is an enigmatic and growing public health problem which is confined at present to countries of South-East Asia. Since 1956, over 350 000 patients have been hospitalized and nearly 12 000 deaths have been reported. Dengue viruses, a group of four flaviviruses, are transmitted to man by Aedes aegypti. *Currently, dengue viruses are actively transmitted in 61 countries which circle the globe in the tropical zone and have a combined population of 1500 million. Because the precise antecedents to DHF/DSS are unknown, the public health hazard posed by this syndrome is potentially worldwide. Epidemiological studies in South-East Asia clearly link DHF/DSS to individuals who have had a previous dengue infection or who have acquired maternal dengue antibody. Such antibody may serve as an opsonin, enhancing dengue virus infection of mononuclear phagocytes—the type of cell in man to which dengue infection may be confined. Antibody-mediated infection of these cells is the central concept in the hypothesis of immune infection enhancement. This hypothesis provides a conceptual framework for design of future research. There is an urgent need for a comprehensive identification of "risk factors" in DHF/DSS. This research could be approached by undertaking comparative prospective epidemiological studies in dengue-endemic areas with and without DHF/DSS. Although important progress is being made in the development of attenuated dengue vaccines for each dengue type, a clearer understanding of the pathogenesis of DHF/DSS may be required to provide guidelines for safe and lasting immunoprophylaxis in man.*

Of the "great neglected diseases of mankind", perhaps no infection commands so vast a domain as do the dengue viruses. The enormous territory occupied by this group of flaviviruses, much of it newly won, is a tribute to the resourcefulness of its principal vector, the yellow fever mosquito, *Aedes aegypti*. While yellow fever virus has been confined to sylvan foci in Africa and South America, dengue viruses have kept pace with the urban encroachments of *A. aegypti*. In 1979, dengue viruses girdle the globe in the tropical zone (Fig. 1).

* A French version of this article will be published in a later issue of the *Bulletin*.

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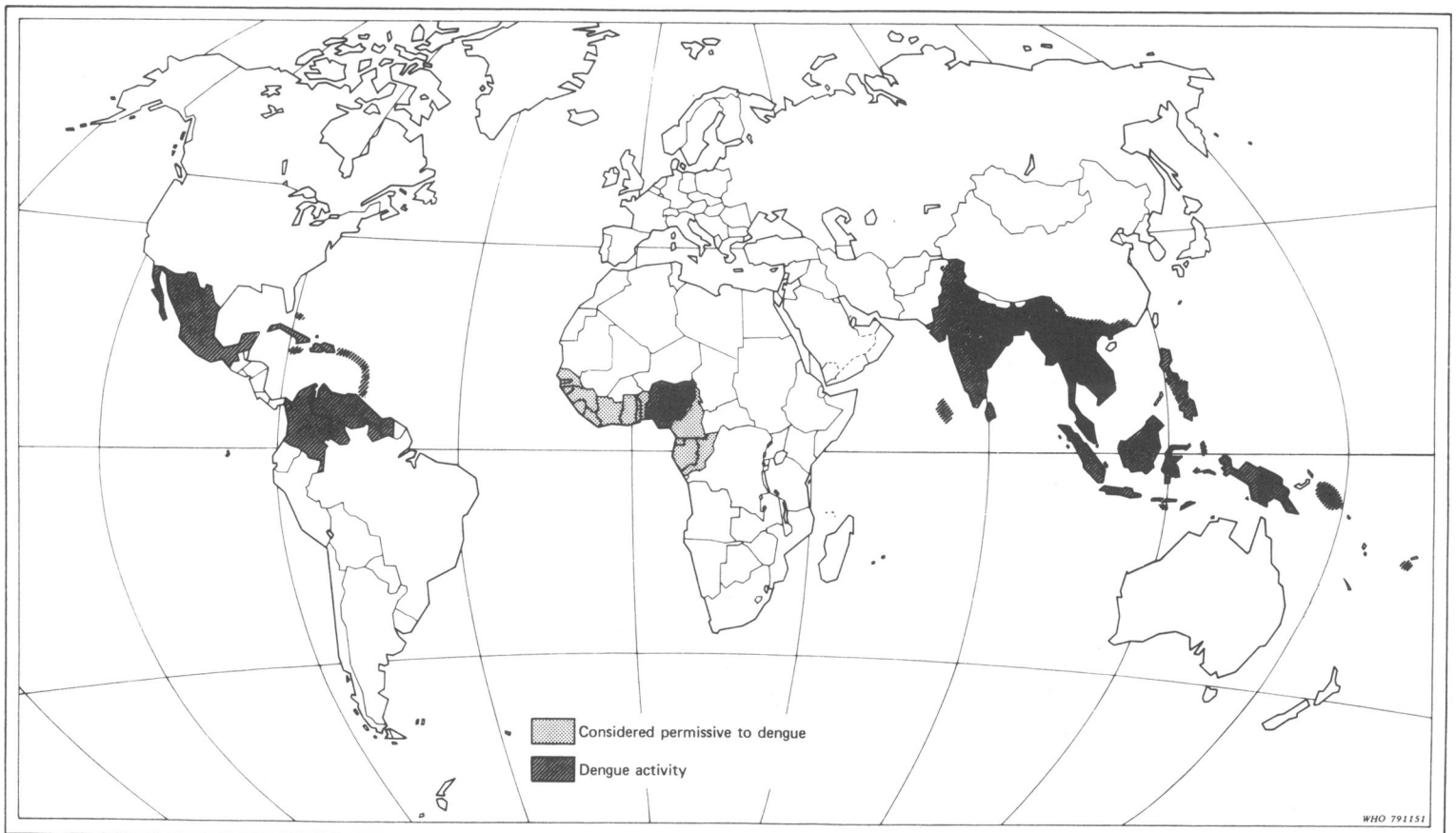

Fig. 1. World distribution of dengue infections based upon virus isolation from man within the past decade or areas considered permissive to dengue because of the prevalence of urban *Aedes aegypti*.

In a sense dengue is not "neglected", it is merely undiscovered. For nearly two centuries, dengue was classified along with grippe or diarrhoea as a minor incident in acclimatization to tropical life. It is not widely appreciated that since the 1950s, dengue viruses have caused a shock syndrome that strikes with dramatic swiftness, and is often complicated by gastro-intestinal haemorrhage and followed by death. Dengue viruses are among the leading causes of paediatric morbidity and mortality, and a cause of panic of epidemic proportions, in countries with populations totalling 342 million; further, at least 1500 million people throughout the world live in areas with recent dengue activity. Morbidity patterns for severe dengue disease are illustrated by reports to the World Health Organization of children hospitalized or dying of dengue haemorrhagic fever/dengue shock syndrome (DHF/DDS) in tropical Asia (Table 1). In Thailand in 1977, DHF/DSS was the second leading cause of hospitalization of children and the leading cause of death due to communicable diseases at any age. The potential severity of the dengue problem is underlined by the fact that there is not yet any comprehensive understanding of why dengue viruses produce a disease with fatal outcome. There is no model for predicting DHF/DSS outbreaks. It is not known why dengue viruses have become lethal and why DHF/DSS outbreaks are so far restricted to South-East Asia. These compelling research questions prompt this review. Answers should be sought vigorously, for they have profound implications on the timetable for eventual control of dengue.

Table 1. Number of cases of dengue haemorrhagic fever (and in parentheses dengue shock syndrome) reported to the World Health Organization, 1956–78

	Country							
Year	Philippines	Thailand	South Viet Nam[a]	Malaysia	Indonesia	Burma	Sri Lanka	Singapore
1956	1207 (72)							
1958	94 (34)	2706 (296)						
1959	40 (11)	160 (21)						
1960	551 (40)	1851 (65)	100					
1961	1459 (33)	561 (36)						
1962	134 (62)	5947 (308)	283					42 (12)
1963	189 (74)	2215 (173)	374 (127)	41 (1)				
1964	759 (169)	7763 (385)	559 (177)					
1965	652 (109)	4094 (193)	171 (39)				4 (2)	
1966	9384 (250)	5816 (137)	53				19 (5)	630
1967	1371 (105)	2060 (65)					29 (8)	826
1968	1116 (115)	6430 (71)					9 (2)	848
1969	1336 (103)	8670 (109)			198 (50)		1	189
1970	922 (83)	2767 (47)			400 (69)	1654 (81)	2	71
1971	438 (34)	11540 (299)			174 (13)	691 (34)	3	116
1972	1570 (83)	23786 (682)	763 (215)		970 (25)	1013 (32)	8	64
1973	591 (62)	8280 (315)	14320 (986)	969 (54)	9947 (454)	349 (15)		1324 (27)
1974	1665 (153)	8160 (328)	4261 (438)	1482 (104)	3667 (188)	2477 (159)		229 (4)
1975	603 (42)	17771 (441)		735 (57)	4160 (259)	6750 (363)		59 (2)
1976	460	9561 (359)	21361	773 (71)	2620 (109)	3153 (98)		30 (0)
1977	376	38768 (756)	45011 (736)	341	7388 (301)	5364 (236)	4	92 (1)
1978	—	12547 (308)			6395 (283)	2029 (82)		352 (2)

[a] The figures for 1976 and 1977 are for the Socialist Republic of Viet Nam.

HISTORY

The term "dengue" was introduced into the English medical literature from the Spanish West Indies during the 1827–28 Caribbean epidemic of an exanthem with arthralgia. Dengue is a Spanish homonym for the Swahili "Ki denga pepo" (a sudden cramp-like seizure caused by an evil spirit). Strictly speaking, the terms "Knokkelkoorts" from Jakarta in 1779 and "Breakbone fever" from Philadelphia in 1780 have precedence. We now know that these two 18th century febrile exanthems were not the same disease. A comparison of clinical features suggests that knokkelkoorts and West Indian dengue were epidemics of chikungunya fever (an *A. aegypti*-borne alphavirus), while breakbone fever was modern dengue fever. During much of the 19th century these alphavirus and flavivirus exanthems were reported interchangeably as "dengue fever", contributing to the notion that dengue was a disease of little clinical consequence. This was so firmly rooted in conventional medical wisdom that dengue outbreaks which may have included DHF/DSS were ignored. Shock cases and deaths accompanied a dengue epidemic in Queensland, Australia, in 1897, while nearly 1250 persons died during the explosive Greek dengue epidemic of 1928. It is typical of dengue epidemiology that this latter outbreak was directly related to substandard living conditions among refugees repatriated from Turkey following the Greco–Turkish War of 1922. The recognition of DHF/DSS had to await more rigorous proof of a dengue viral etiology. This was forthcoming after dengue viruses were adapted to laboratory animals in the 1940s (types 1 and 2) and 1950s (types 3 and 4). In 1954, Filipino paediatricians, and shortly thereafter, physicians in other South-East Asian countries, described the DHF/DDS syndrome and it was associated with dengue virus infection by Hammon et al. in 1956.

DENGUE DISEASES

The benign form of dengue, *classical dengue fever*, is seen in syndromes that are age-dependent. Infants and children may have undifferentiated febrile illness or mild febrile disease with maculopapular rash. Older children and adults usually have an overt illness characterized by fever, headache, myalgia, and gastrointestinal symptoms, often terminating with a maculopapular rash. The characteristic features and the evolution of signs and symptoms are schematically represented in Fig. 2.

In contrast to classical dengue fever, severe dengue disease has largely been studied in Asian children. DHF/DSS proceeds through two stages. The illness begins with abrupt onset of fever accompanied by dengue-like symptoms; during or shortly after the fall in temperature the condition of the patient suddenly deteriorates, the skin becoming cold, the pulse rapid, and the patient lethargic and restless. In some children the range of pulse pressure progressively narrows, the patient becomes hypotensive and if not treated, may expire in as little as 4–6 hours. The presence of shock distinguishes dengue shock syndrome from dengue haemorrhagic fever without shock. DHF is the inclusive term—DSS occupies the severe end of the spectrum of DHF.

Minor haemorrhagic phenomena may be seen during the febrile phase, such as a positive tourniquet test, petechiae, epistaxis, or liability to bruising. A maculopapular rash or confluent petechial eruption may be seen after the temperature falls. Enlargement of the liver is found in many, but not all cases. The clinical signs and symptoms described are

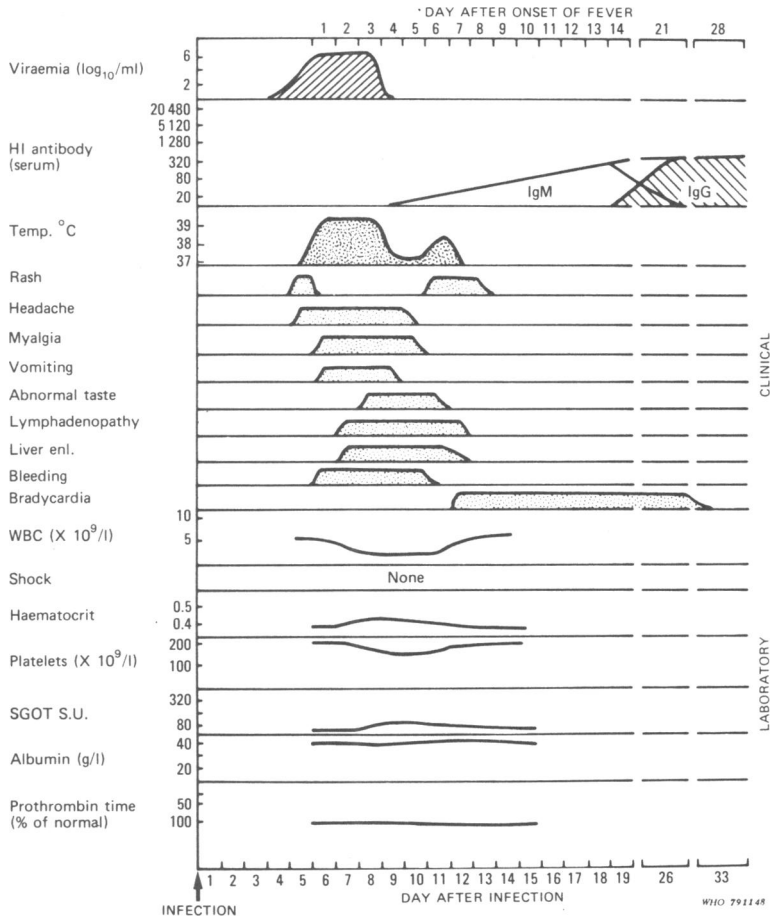

Fig. 2. Clinical and laboratory features of classical dengue fever syndrome.

accompanied by laboratory abnormalities that evolve characteristically (Fig. 3). Thrombocytopenia and haemoconcentration are constant findings; a platelet count of less than 100 x 10^9/litre is usually seen between the third and eighth day. The severity of haemoconcentration is directly related to the degree of shock. Other common findings are hypoalbuminaemia, hypovolaemia, and elevated serum transaminases and blood urea nitrogen. Many patients have a prolonged prothrombin time with reductions in serum levels of factors II, V, VI, IX, and XII. Hypofibrinogenaemia and elevated levels of the products of fibrin splitting are found during the hypovolaemic stage. Early in the acute stage of DHF/DSS, blood levels of C1q, C4, C5–8, and C3 proactivator are depressed and C3 catabolic rates are elevated. In most cases, complement is activated by both the classical and alternative pathways. The kinin system apparently is not involved. Complement depletion and hypofibrinogenaemia correlate with the onset of shock and, in degree, with the severity of disease.

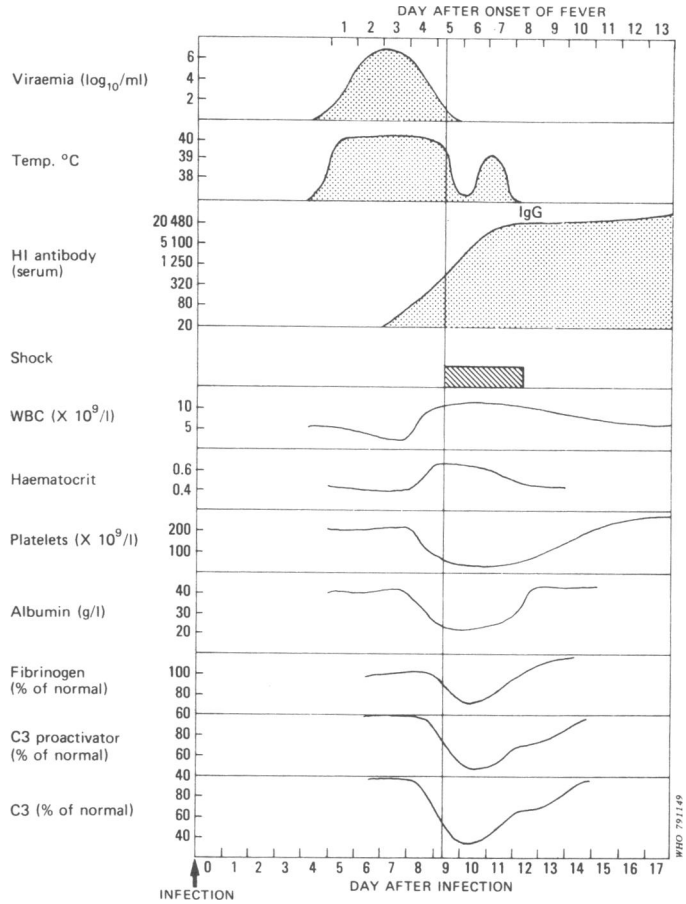

Fig. 3. Selected clinical and laboratory features of dengue shock syndrome.

From these findings, DHF/DSS can be defined as an acute vascular permeability syndrome accompanied by activation of the blood clotting and complement systems. Diagnostic criteria have been proposed by the World Health Organization.[a]

[a] The following criteria based on the clinical manifestations (*vide supra*) have been selected for the clinical diagnosis of DHF and in 90% of cases dengue infection has been confirmed by etiological diagnosis in the laboratory. Their use will avoid an overdiagnosis of the disease.

Clinical

(*a*) Fever—acute onset, high, continuous, and lasting for 2–7 days.

(*b*) Haemorrhagic manifestations including at least a positive tourniquet test and any of the following:
- petechiae, purpura, ecchymosis
- epistaxis, gum bleeding
- haematemesis and/or melaena

(*c*) Enlargement of liver (observed at some stage of illness in 90–96% of Thai children and 60% of adults).

(*d*) Shock—manifested by rapid and weak pulse with narrowing of the range of pulse pressure (20 mmHg/2.7 kPa or less) or hypotension, with the presence of cold, clammy skin and restlessness.

Laboratory

(*a*) Thrombocytopenia (100 x 10^9/litre or less).

(*b*) Haemoconcentration—haematocrit increased by 20% or more.

The presence of the first two or three clinical criteria with thrombocytopenia and haemoconcentration is sufficient to establish a clinical diagnosis of DHF. When shock occurs with high haematocrit levels (except in patients with severe bleeding and marked thrombocytopenia) the diagnosis of DHF/DSS is highly likely.

These criteria are discussed in more detail in: *Technical guides for diagnosis, treatment, surveillance, prevention, and control of dengue haemorrhagic fever.* Prepared by the Technical Advisory Committee on Dengue Haemorrhagic Fever for the South-East Asia and Western Pacific Regions, World Health Organization, 1975.

Other severe outcomes have been described with dengue infection, but these differ from DSS. The most common are episodes of meno- or metrorrhagia in adult women or acute gastrointestinal haemorrhage usually seen in adults of either sex. These bleeding episodes are usually not accompanied by shock; but severe bleeding may be followed by hypotension. In DHF/DSS there is a period of uncorrected metabolic acidosis and shock which *precedes* gastrointestinal haemorrhage. Such patients may be diagnosed as DHF. Misidentification of DHF/DSS cases can be a source of confusion in epidemiological and pathogenetic studies on dengue disease.

EPIDEMIOLOGY

Dengue viruses can be included among the malign pathogens of man which thrive during troubled times. Almost every major disturbance in human ecology during the past 40 years has served to amplify populations of *A. aegypti* and dengue viruses. The Second World War introduced large numbers of susceptible persons into the dengue-endemic Asian war zone, resulting in what may have been the largest dengue outbreaks in history. After the war, the settlement of refugees, the rapid growth of cities, the population explosion, the steady deterioration in urban environments and in the standards of urban sanitation, the recent conflicts in South-East Asia and, now, the large-scale migration of refugees in that area have enlarged the territory of *A. aegypti* in tropical Asia, and increased the densities of both the vector and the human host to the point where they support the endemic transmission of several types of dengue viruses. While the interaction of all these factors has not been studied prospectively, there can be little doubt that the past three decades have witnessed the stable establishment of endemic DHF/DSS in eight South-East Asian countries. The pattern of recognition of DHF has been invariable. Scattered cases are recognized first in the major towns; progressively, the number of cases increases and there is centrifugal spread of disease to smaller urban and rural communities.

Certain epidemiological features of endemic DHF/DSS are relatively unusual. These phenomena make an important contribution to our present concepts of the pathogenesis of dengue haemorrhagic fever.

Residence

When DHF/DSS was discovered, an epidemiological oddity was recognized: dengue infection in persons of limited residence in DHF/DSS endemic areas resulted in dengue fever but *not* DHF/DSS. With the growth of foreign business communities, the posting of large numbers of voluntary workers throughout tropical Asia, and the introduction of millions of military personnel from dengue-free countries, dengue infections have occurred in untold thousands of non-indigenous persons. Despite this large experience, only two cases that meet the clinical, physiological, and virological criteria of dengue shock syndrome have been reported. Both of these were young children of North American parentage who had been born in South-East Asia.

Age

In the vast majority of instances, DHF/DSS has been a children's disease. Fig. 4 shows the age-specific hospitalization rates for the Thai cities of Bangkok and Thonburi for 1962 and 1978. The 1962 curve has an epidemiologically important feature. It is bimodal. One mode is composed of infants less than 1 year of age; the larger number of cases are in children older than 1 year, with a modal age of 3 years. When antibody responses to dengue infections were analysed, most infants aged less than 1 year had primary-type responses, while children 1 year and older had secondary-type immunological responses. In contrast to most diseases caused by viruses that are transmitted in the home, DHF/DSS attack (hospitalization) rates are lower in 1-year-olds than in any other group of infants (as shown in Fig. 4). The DHF/DSS age-specific hospitalization rate curve bears no resemblance to the curve for prevalence of dengue haemagglutination-inhibition antibody by age in Bangkok in 1962 (shown in Fig. 5). If there were no confounding host factors, dengue illness rates would be expected to describe a monotonically decreasing curve with the highest attack rates in the age group with the largest number of susceptibles, roughly the inverse of the antibody prevalence curve (Fig. 5).

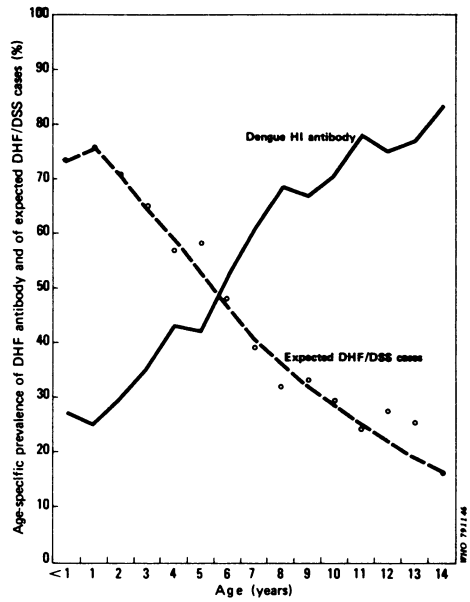

Fig. 5. Prevalence of dengue 1 haemagglutination-inhibition antibody in 2230 randomly selected residents of Bangkok sampled prior to the DHF/DSS outbreak of 1962 and expected age-distribution of DHF/DSS cases if DHF/DSS occurs during primary dengue infections. Data for antibody prevalence from: HALSTEAD, S. B. *American journal of tropical medicine and hygiene,* **18**: 997-1021 (1969).

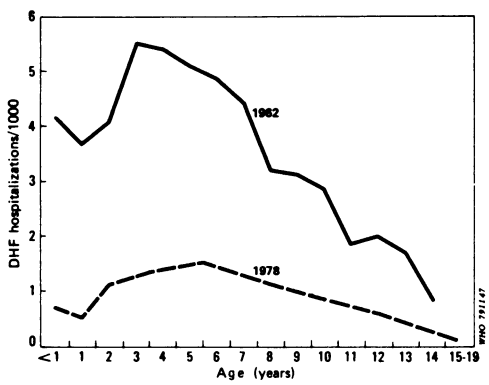

Fig. 4. Age-specific DHF/DSS hospitalization rates for metropolitan Bangkok in 1962 and 1978. Data from: HALSTEAD, S. B. *American journal of tropical medicine and hygiene,* **18**: 997-1021 (1969).

For reasons not well understood, DHF/DSS hospitalization rates in Bangkok have declined over the past 10 years. Accompanying this decline, there has been a shift in modal age of hospital admission to older children but no change in the bimodal shape, as illustrated in data for 1978 (Fig. 4). This "age shift" is a useful indicator of movement in communicable disease, indicating decreasing virus transmission rates in populations supporting endemic infection.

Sex

Although crude haemorrhagic fever hospitalization statistics may fail to show sig-

nificant differences in attack rates for males and females, analysis of fatal cases and studies on etiologically and physiologically defined shock cases show a striking age-dependent increase in the number of girls with shock syndrome compared with boys (Table 2). This is strictly host-related, since age-stratified serological surveys in Bangkok showed no difference in the infection rates between boys and girls.

Table 2. Relationship between age and sex and the severity of secondary infection DHF/DSS

	< 1–3 years			4–14 years		
	DHF[a]	DSS[a]	Deaths[b]	DHF[a]	DSS[a]	Deaths[b]
Males	39	24	228	84	45	951
Females	35	29	239	104	92	1237
M : F	1 : 0.9	1 : 1.2	1 : 1	1 : 1.2	1 : 2	1 : 1.3

[a] Data from 1962–64 Bangkok Children's Hospital Study (*8*).

[b] Data from 1968–77 Epidemiology Division, Ministry of Health of Thailand (1972 data omitted).

Nutritional status

A large body of anecdotal evidence from every country in which DHF/DSS has been recorded indicates a strong association between good nutritional status in the child and an *increased* risk of developing dengue shock syndrome. DSS is rarely seen in the clinically malnourished child.

Disease severity and preinfection immune status

The most unusual feature of the epidemiology of DHF/DSS is that the syndrome usually occurs in persons with preinfection dengue antibody, actively or passively acquired. As established by Halstead et al. in 1969, infants less than 1 year old frequently have primary-type dengue infections accompanying their episode of DHF/DSS. Since virtually all adults in Bangkok are immune to dengue, infants with this primary infection DHF/DSS presumably have received dengue antibody transplacentally from their mothers. Disease in infants and older children is pathophysiologically identical, including activation of complement and blood clotting mechanisms.

In children 1 year old or older, there is a striking relationship between disease severity and a secondary-type dengue antibody response. The immunological relationship can be demonstrated only if cases are classified physiologically. Mild DHF and dengue fever are often indistinguishable, making overdiagnosis a confounding variable, particularly in epidemiological studies. The immunopathological nature of DHF/DSS has been demonstrated in two field investigations that included prospective studies and in the Bangkok Children's Hospital Study, now in its 18th year. Results of prospective studies are summarized in Table 3. The prospective studies in Bangkok in 1962 and Ko Samui in 1966 showed that DHF/DSS occurred exclusively in children possessing dengue antibody before the epidemic. Using serological survey data, and demographic and hospitalization statistics, both the Bangkok and the Ko Samui studies were projected to the whole populations at risk. Calculation of DSS attack rates per 1000 secondary dengue infections in these independent estimates differed only twofold (Table 3). DHF/DSS occurred on Ko Samui again in 1967 and cases were studied in villages of Na Muang township. All of the 15 cases of DHF/DSS admitted to hospital had secondary infections.

Hospital data can be used to document the relationship between disease severity and immune response (Table 4). The data for 1962 alone and grouped for 1962–64 show increasing association of secondary dengue immune response with increasing severity of disease. The differences between the frequency of secondary dengue infections associated with various syndromes in 1962–64 are highly significant.

Table 3. Prospective studies on the relation between dengue immunity status and the occurrence of dengue shock syndrome

Study	No. of children observed	No. with antibody	No. of primary infections	No. of cases of primary DSS	No. of cases of primary DSS per 1000	No. of secondary infections	No. of cases of secondary DSS	No. of cases of secondary DSS per 1000
Bangkok, 1962[a,b]	1 253	655	205	0	0	150	5[c]	33
Ko Samui, 1966[d]	336	268	26	0	0	83	3	36
Bangkok, 1962, projected to whole city[e]	842 451	438 075	165 794	12	0.07	125 728	1 428	11.4
Ko Samui, 1962, projected to whole island[f]	13 975	9 200	1 900	0	0	2 700	14	5.5

[a] HALSTEAD, S.B. ET AL. Observations related to pathogenesis of dengue hemorrhagic fever. IV. Relation of disease severity to antibody response and virus recovered. *Yale journal of biology and medicine,* **42**: 311–328 (1970).

[b] HALSTEAD, S.B. ET AL. Dengue and chikungunya virus infection in man in Thailand, 1962–1964. II. Observations on disease in outpatients. *American journal of tropical medicine and hygiene,* **18**: 972–983 (1969).

[c] Hospitalized. Shock not ascertained.

[d] WINTER, P.E. ET AL. An insular outbreak of dengue hemorrhagic fever: I. Epidemiological observations. *American journal of tropical medicine and hygiene,* **17**: 590–599 (1968).

[e] HALSTEAD, S.B. Immunological parameters of Togavirus syndromes. In: Schlesinger, R.W., ed., *Togaviruses*, New York, Academic Press, 1980 (In press).

[f] RUSSELL, P.K. ET AL. An insular outbreak of dengue hemorrhagic fever. II. Virologic and serologic studies. *American journal of tropical medicine and hygiene,* **17**: 600–608 (1968).

Table 4. Association between secondary-type antibody response and dengue disease syndromes of increasing severity, children, ages < 1–14 years. Children's Hospital Study, Bangkok 1962–64

	1962 (ages 1–14 years)		1962–64 (ages < 1–14 years)		
Group	No. of cases of secondary infection	Secondary cases as % of total no. of infections	No. of cases of primary infection	No. of cases of secondary infection	Secondary cases as % of total no. of infections
All children	125 728	43.1[a]			
Fever of unknown origin, outpatients			33	61	64.9[b]
Fever of unknown origin, inpatients			13	23	63.9[c]
DHF, non-shock	46	71.8[c]	65	262	80.1[d]
DSS	55	96.4[c]	6[e]	190	96.9[d]

[a] HALSTEAD, S.B. Immunological parameters of Togavirus syndromes. In: Schlesinger, R.W., ed., *Togaviruses*, New York, Academic Press, 1980 (In press).

[b] HALSTEAD, S.B. ET AL. Dengue and chikungunya virus infection in man in Thailand, 1962–1964. II. Observations on disease in outpatients. *American journal of tropical medicine and hygiene,* **18**: 972–983 (1969).

[c] NIMMANNITYA, S. ET AL. Dengue and chikungunya virus infection in man in Thailand, 1962–1964. I. Observations on hospitalized patients with hemorrhagic fever. *American journal of tropical medicine and hygiene,* **18**: 954–971 (1969).

[d] HALSTEAD, S.B. ET AL. Observations related to pathogenesis of dengue hemorrhagic fever. IV. Relation of disease severity to antibody response and virus recovered. *Yale journal of biology and medicine,* **42**: 311–328 (1970).

[e] Includes 4 infants < 1 year of age.

Sequence of infection

Clinical–virological studies in Bangkok in 1962–64 have shown a consistently high frequency of isolation of dengue 2 viruses from shock syndrome cases. During the same period, the frequencies of transmission of dengue viruses types 1 and 3 were similar to that of dengue 2. The association between DSS and dengue 2 might be explained if one or more of the infection sequences, 1–2, 3–2, or 4–2, were more pathogenic than other sequences of dengue infection.

Association between secondary dengue infections and DHF/DSS

Although DHF/DSS cases are positively associated with secondary-type infections, not all secondary-type infections result in DHF/DSS. This is illustrated in a summary of recent dengue outbreaks outside South-East Asia (Table 5). Secondary dengue infections *not* associated with outbreaks of DHF/DSS have occurred under the following circumstances: (*a*) sequential infection with two dengue types at relatively long intervals (5 years or more); (*b*) sequential infections not ending with dengue type 2; and (*c*) in several areas (including India, West Africa, and Puerto Rico—in 1977) with two or more dengue viruses endemic, including dengue 2.

STUDIES ON IMMUNOPATHOGENESIS

Pathological and pathogenetic studies on dengue infection in man and in the rhesus monkey model show that dengue viruses have a marked predilection for lymphoid tissue. Viral antigen has been visualized in macrophages, histiocytes, and Kupffer cells. An exciting recent development is that dengue virus can be recovered from circulating peripheral blood leukocytes during the acute phase of DHF/DSS (R. McN. Scott, personal communication, 1979). These cells have been provisionally identified as monocytes.

The possibility that mononuclear phagocytes (monocytes, macrophages, histiocytes, and Kupffer cells) may be principal sites of dengue infection in man is important in relation to

Table 5. Simultaneous or sequential epidemics involving two or more dengue virus types (D1, D2, D3, or D4) that have not been associated with epidemic DSS[a]

Place	Date	Virus	Date	Virus	Date	Virus	Date	Virus
Puerto Rico	1963	D3	1969–77	D2	1977	D1, 2, 3		
Colombia	1971–72	D2	1975–77	D3				
Dominican Republic	1972–73	D2, 3						
Jamaica	1963	D3?	1968–69	D2, D3	1977	D1		
Panama	1941–54	D2, D3						
Tahiti	1963–69	D3	1971–73	D2	1975	D1	1979	D4
Fiji	1971–73	D2	1974–75	D1				
Samoa	1940s	D1	1972	D2				
Tonga	1930	D1	1972–74	D2	1974–75	D1		
Nigeria	1964–67	D2	1967–70	D1, D2				
India	1957–66	D1, 2, 4						

[a] Data taken from: HALSTEAD, S.B. Immunological parameters of Togavirus syndromes. In: Schlesinger, R.W., ed., *Togaviruses*, New York, Academic Press, 1980 (In press).

fundamental observations on dengue virus–monocyte interactions. Dengue viruses replicate readily in human peripheral blood monocyte cultures if the donor is dengue-immune.[b] Dengue replication also occurs in cultures of monocytes from susceptible persons when subneutralizing concentrations of dengue antibodies are added to the culture medium.[c] Without antibody, monocyte suspension cultures from susceptible persons are relatively nonpermissive to dengue infection. This immune enhancement phenomenon requires the attachment of anti-dengue IgG–dengue virus complexes to cellular Fc receptors. These mediate the internalization of the immune complex resulting in infection of the monocyte.

The immune enhancement of dengue infection in monocyte cultures, *in vitro*, is paralleled by similar *in vivo* phenomena. Monkeys individually infected with dengue viruses types 1, 3, or 4 and then challenged with dengue 2, circulated virus at higher levels than did susceptible controls infected with the same strain and dose of dengue 2.[d] In other experiments, non-immune animals were injected intravenously with small amounts of dengue-immune human cord blood serum and then infected with dengue 2 virus. These animals circulated up to 51-fold more virus than monkeys injected with normal cord blood serum and then given dengue 2.[e]

To understand how shock syndrome is produced in man, it may be important to study the relationship between the infection of mononuclear phagocytes and the generation of vascular permeability factors, and the activation of the complement and blood clotting systems. It is of interest that the known biological properties of activated human mononuclear phagocytes include the release of enzymes that cleave C3, the release of leukocyte thromboplastin, and the generation of one or more vascular permeability factor(s). These effector phenomena generated by this cell system can account for all the major physiological changes in DSS.

But an additional mechanism in the pathogenesis of shock remains to be identified. If mononuclear phagocytes are activated during dengue infection, how does this happen? Of endogenous stimuli, C3b, immune complexes, and lymphokines are known to activate mononuclear phagocytes. A current working hypothesis of dengue shock is that the immune elimination response directed against dengue-infected mononuclear phagocytes is the event that activates these cells. In support of this possibility, it is known that the competence of the immune response is under genetic and nutritional control. With respect to their ability to combat infectious diseases, females are generally considered to be more immunologically competent than males and well nourished individuals more immunologically competent than malnourished ones. It has been noted above that DHF/DSS occurs more frequently in females than males and in well nourished than malnourished individuals.

On the basis of the preceding experimental and clinical studies a simple molecular and cellular model for DHF/DSS has been developed (Fig. 6A, B, C). This model has important implications for the design of epidemiological and pathophysiological studies on DHF/DSS.

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[b] MARCHETTE, N. J. ET AL. Replication of dengue viruses in cultures of peripheral blood leukocytes from dengue-immune rhesus monkeys. *Journal of infectious diseases,* **133**: 274-282 (1976).

[c] HALSTEAD, S. B. & O'ROURKE, E. J. Dengue viruses and mononuclear phagocytes. I. Infection enhancement by non-neutralizing antibody. *Journal of experimental medicine,* **146**: 201–217 (1977).

[d] HALSTEAD, S. B. ET AL. Studies on the pathogenesis of dengue infection in monkeys. II. Clinical laboratory responses to heterologous infection. *Journal of infectious diseases,* **128**: 15-22 (1973).

[e] HALSTEAD, S. B. *In vivo* enhancement of dengue infection by passively transferred antibody. *Journal of infectious diseases,* **140**: 527-533 (1979).

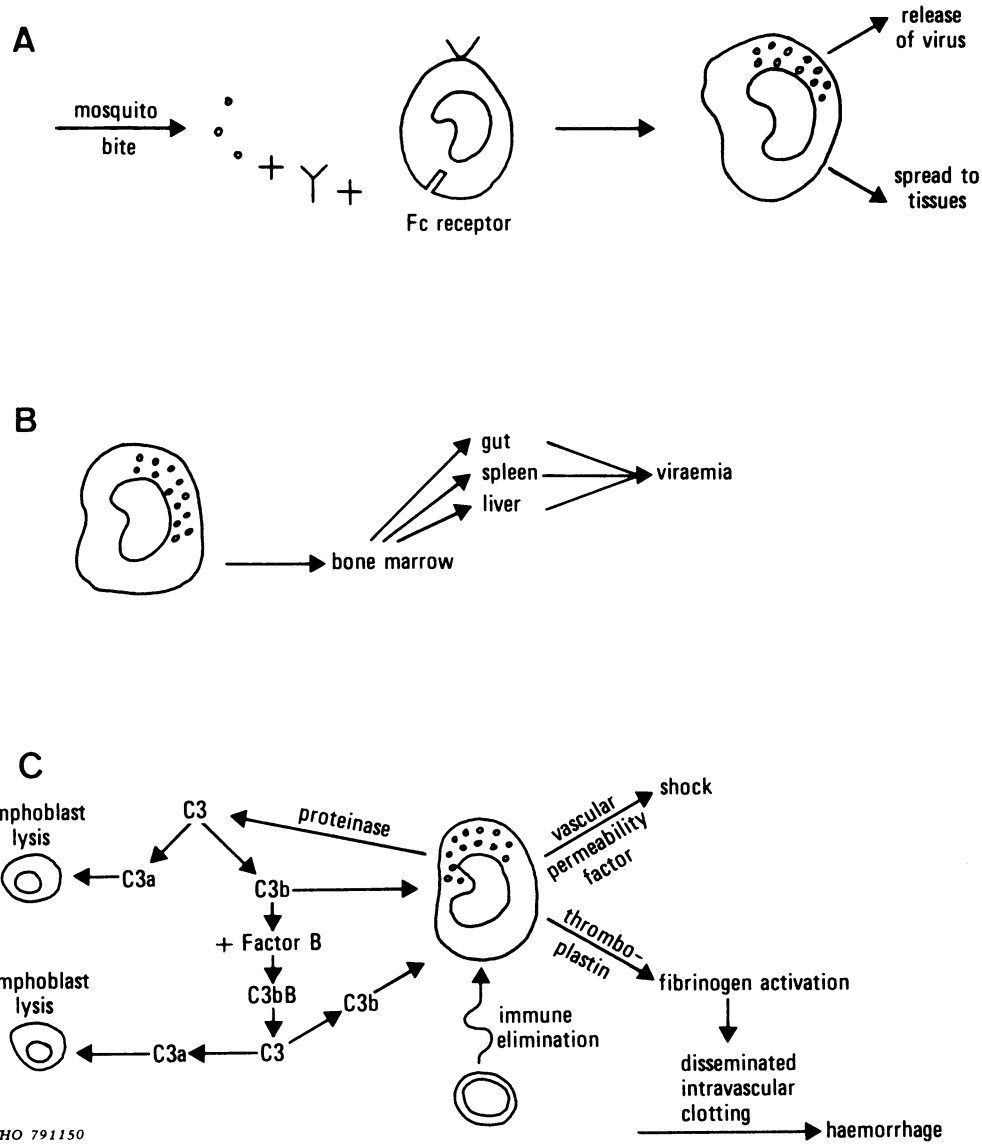

Fig. 6. Schema for "immunological enhancement of infection" hypothesis of DHF/DSS pathogenesis. (A) *Afferent mechanism*. In the presence of enhancing antibody (antibody which complexes with virus but does not kill) immune complex attaches to an Fc receptor triggering phagocytosis followed by replication of virus. Because mononuclear phagocytes may be motile, they may spread infection as well as provide a site for replication. (B) *Afferent mechanism.* Dengue virus grows in bone marrow, liver, spleen, gut lymphoid tissue, and histiocytes in skin. A central tenet of the immune-enhancement hypothesis is that enhancing antibody regulates the number of cells infected; the more cells infected, the more severe the disease. (C) *Effector mechanisms*. It is believed that the major pathophysiological abnormalities are caused by factors released from activated mononuclear phagocytes that are infected with dengue. The immune response, possibly T lymphocytes, may be important in activating mononuclear phagocytes. Nutrition, sex, and genetic factors may be important in modulating individual infections through their regulatory effect on T lymphocytes or mononuclear phagocyte function.

CONTROL OF DHF/DSS

Mosquito control

It is an unhappy commentary on modern sanitary engineering that we must look back 50 years to major triumphs in the control of *Aedes aegypti*-borne disease. The virtual eradication of urban yellow fever from the Americas and portions of Africa in the two decades before the Second World War ranks as one of the most remarkable public health accomplishments of this century. Only modern Singapore has combined its legal and administrative skills to effect a similar result with DHF.

The almost ubiquitous distribution of *A. aegypti* in some of the most densely populated areas of the world demands mosquito abatement on a scale that exceeds realistic budgeting unless innovative methods are used. Reasonably cost-effective control of *A. aegypti* and of dengue transmission might be achieved if public education and legal sanctions were combined with an ecologically sound mosquito abatement programme. Dengue transmission can be aborted most effectively by attacking *A. aegypti* populations at their seasonal minimum. This occurs regularly during the cool season in the subtropics. Over-reliance on adulticides, such as malathion, which can be delivered by ultra-low-volume spray machines mounted on trucks or aircraft, has, to some measure, diverted public health programmes from serious evaluation of the mosquito control methods that were successful in the 1920s and 1930s. These centred on the reduction of *A. aegypti* breeding sites, enlisting the aid of the citizenry by use of legal sanctions. A WHO technical guide provides detailed instructions for organizing community and country-wide abatement schemes.[f]

The reintroduction of *A. aegypti* into many Caribbean and Central American countries within the past two decades is sobering evidence that country-level eradication of *A. aegypti* will demand endless vigilance unless eradication of the species is carried out on a global basis. For this reason a dengue vaccine may provide the best opportunity for realistic control of dengue diseases.

Immunization

Natural infection of humans with dengue viruses confers long-lasting protection against the same antigenic type. From the precedent of other live vaccines it is assumed that dengue vaccine will also produce lifelong immunity. Since cross-protection between strains is of limited duration, a vaccine for each dengue serotype is required.

Investigations into development of live, attenuated dengue type 2 and 4 vaccines began in 1971 at the Walter Reed Army Institute of Research and the University of Hawaii, respectively. Precautions were taken from the outset to ensure that any vaccine developed could ultimately be used in humans. This required working in laboratory suites devoted solely to dengue experiments, using only primary or diploid cells for propagation of virus, historical documentation of the original virus isolate and subsequent passages of virus, and testing to ensure the safety and purity of the final product.

Dengue 2 virus isolated from man in Puerto Rico in 1969 was inoculated into primary green-monkey kidney (PGMK) cells and plaque isolates were cloned. One of these clones, S-1, produced small plaques on LLC-MK2 cells, had reduced mouse and monkey virulence, was temperature sensitive,[g] and showed reduced growth in human monocyte cultures. After

[f] *Technical guides for diagnosis, treatment, surveillance, prevention and control of dengue haemorrhagic fever*. Prepared by the Technical Advisory Committee on Dengue Haemorrhagic Fever for the South-East Asia and Western Pacific Regions, World Health Organization, 1975, Chap. 2, pp. 3-4.

[g] ECKELS, K. H. ET AL. Isolation of a temperature-sensitive dengue-2 virus under conditions suitable for vaccine development. *Infection and Immunity*, **14**: 1221-1227 (1976).

further plaque purification, three passages were made in fetal rhesus lung diploid cells (DBS-FRhL-2). After safety tests were completed, a group of six human volunteers received a single dose of vaccine containing approximately 10^5 PFU of virus. All volunteers had received yellow fever immunizations in the past. Five recipients developed temperature sensitive, small-plaque viraemia 8 or 9 days after vaccination which lasted from 1 to 10 days. No virus could be isolated from circulating monocytes of the vaccinees. Two recipients developed fever of short duration (oral temperature $\geqslant 38$ °C) accompanied by leukopenia. One of these also developed headache, myalgia, and photophobia. Another recipient developed an erythematous rash of the chest and abdomen. All six volunteers seroconverted by HI test and five by CF and neutralization tests.

A second study was carried out involving 19 volunteers non-immune to flaviviruses. These volunteers were divided into groups that received vaccine doses ranging from 3×10^5 PFU to 3×10^2 PFU. Eight of the 19 vaccinees seroconverted by HI, CF, or neutralization tests. Five had detectable viraemia, and virus isolates from plasma retained the characteristics of the vaccine virus. Only 1 of the 8 developed a mild dengue-like illness, while 3 others had mild clinical changes. *A. aegypti* mosquitos were fed on 3 viraemic volunteers. Virus with characteristics of the vaccine virus was isolated from 2 of 72 engorged mosquitos. Future trials will examine responses to booster immunization and different routes of inoculation.

Development of attenuated dengue types 1, 3, and 4 is in progress. Work on type 1, isolated in the South Pacific in 1974, is being performed at the US Army Medical Research Institute of Infectious Diseases, Frederick, MD, and a type 3 vaccine is being developed at the Walter Reed Army Institute of Research. This virus was isolated from man in Bangkok in 1973. With dengue 1 and 3 viruses, original seed containing mixed populations of virus was used to clone naturally-occurring variants. Attenuated clones were found that appear to be suitable for further testing. For each dengue type, small-plaque strains are available that are temperature sensitive, and of reduced mouse and monkey virulence.

Dengue 4 virus, the prototype isolated from man in 1956, has been serially passaged 50 times in primary dog kidney cells (PDK). These cells were used in the United States of America for commercial production of rubella and measles vaccines. At passage level 15, reduced plaque size was observed; as passage levels rose, plaque size grew smaller until at the 50th passage plaques were barely detectable. Viruses at arbitrary passage levels (15, 30, 50) were cloned and are now being adapted to DBS-FRhL cells in an attempt to make it possible to produce dengue vaccine for all types in a single tissue substrate. Dengue 4 PDK-passaged strains are temperature sensitive, have reduced mouse neurovirulence, fail to produce viraemia in monkeys and do not grow in human monocytes. Current efforts are focussed on producing a candidate vaccine with PDK 24-passaged, dengue 4 virus.

RESEARCH

As the foregoing brief review suggests, we are in a period of rapidly changing concepts as regards the cause of severe dengue disease. It is also a period that has brought us to the threshold of the development of a tetravalent dengue vaccine. Important questions need answering if we are to gain the necessary measure of understanding about the physiological and epidemiological antecedents that result in DHF/DSS. Some of these research questions are:

1. Why does DHF/DSS occur with secondary dengue infections in some but not all areas of the world?
2. Can risk factors in a population be identified and epidemics of DHF/DSS predicted?

3. Can risk factors for DHF/DSS be identified in individuals?
4. What are the mechanisms that generate shock and haemorrhage in dengue?
5. Can therapeutic agents for DHF/DSS be found?
6. Do dengue viruses differ in virulence?
7. Will natural immunity to one or more dengue types prevent adequate immunization with a multivalent dengue vaccine?
8. Will a dengue vaccine protect and not sensitize recipients?

Epidemiological research

Many of the fundamental research questions in dengue can be answered only by well designed prospective epidemiological studies. It appears essential to document the chronology of, and virus types associated with, both first and second dengue infections and to have available representative virus strains from different periods of transmission. Epidemiological studies are particularly appropriate for studying secondary-infection DHF/DSS because of factors that severely limit the possibilities of studying retrospective events in individuals. These are: (*a*) The secondary-type antibody response in dengue is broadly reactive, obliterating the monospecific neutralizing antibody generated after the first infection; usually this has happened by the time a DHF/DSS patient is hospitalized. (*b*) The secondary antibody response also markedly reduces the rate of recovery of the virus that is responsible for the second illness. (*c*) Two months or more following primary dengue infection it is no longer technically possible to estimate the time of onset of such infection, as dengue specific IgM has usually disappeared during that time. (*d*) No methods are available for the recovery of dengue virus from man after the acute stage of the illness.

Much valuable information would be obtained if studies could be carried out, according to a carefully designed and standardized protocol, in several areas with endemic DHF/DSS and several areas without DHF/DSS. Elements of an acceptable prospective study are: (*a*) longitudinal measurement of dengue infection rates for a period of at least five years; (*b*) collection of representative dengue viruses from infected humans; and (*c*) active surveillance and documentation of DHF/DSS cases. These are discussed in more detail below.

Longitudinal measurement of dengue infection rates

Longitudinal observations on dengue infection in a representative population sample would provide a denominator for the number of cases of shock syndrome. The method of detecting dengue antibody allows this to be done on a large scale. Serum from finger-tip blood can be kaolin-treated in microvolumes for use in the haemagglutination-inhibition (HI) test. Since, in man, HI antibody titres following a dengue infection do not deteriorate to undetectable levels, individuals without dengue types 1–4 HI antibody can reliably be considered to be susceptible.

In the initial year of any study, a representative age-stratified serological survey should be done, sampling, at least, children aged 1–15 years. The simplest method of calculating seasonal dengue transmission rates is to measure antibody conversions in a cohort of dengue-susceptible young children. The rate of dengue infection is then calculated from seroconversions over a discrete period, usually 1 year. A microplaque reduction neutralization test can be used to characterize the type-specific dengue antibodies in HI positive serums.

Seroepidemiology is potentially quite powerful. For example, if neutralizing antibody is measured in HI positive serums from children aged 1–5 years, it may be possible to reconstruct the dengue transmission events during the previous 5 years in their community. Antibody in children 1 year old reflects the virological experience during the preceding year, and so forth.

Collection of dengue viruses

It is important to have available in permanent storage a representative collection of viruses that have been associated with human illnesses in each year of the study. These may be used to test the ability of strains to produce enhancing antibody, studied for virulence factors, or used to test vector competence. A recent study in Bangkok provides suggestive evidence that dengue viruses can be recovered at the highest possible frequency from secondary dengue infections using washed, glass-adherent peripheral blood leukocytes overlayered with monolayers of LLC-MK2 cells (R. McN. Scott, personal communication, 1979). Dengue viruses can also be isolated efficiently from acute phase serum using the delayed-plaque technique or by intrathoracic inoculation of *Aedes* or *Toxorhynchites* mosquitos. Dengue antigen can be detected in mosquito head squashes using a fluorescent-antibody technique. Viruses in the mosquito body can be typed by complement-fixation. Each of these latter methods detects non-structural as well as structural antigens. The accuracy of the discriminative typing of these serological methods requires careful standardization and parallel virus typing with one of the plaque-reduction neutralization tests that measures virion surface antigenic determinants, presumably relevant to human immunity.

Surveillance and documentation of DHF/DSS cases

A clinical programme that studies a representative sample of DHF/DSS cases in endemic areas or one that provides active surveillance for DHF/DSS in areas where the syndrome is not recognized, would not only make available patient materials for virus isolation, but would make it possible to calculate the ratio of overt disease to infection. This ratio is a composite measure of the virulence of dengue infections and knowledge of such ratios in different situations is essential for consideration of the several hypotheses of the pathogenesis of DHF/DSS. Studies in Thailand have estimated the frequency of shock syndrome at a median value of 11 cases per 1000 secondary dengue infections. Using census data and type-specific infection rates and an estimate of the proportion of susceptibles and immunes derived from the age-stratified serological survey, it should be possible to calculate the total number of primary and secondary dengue infections in the entire population. These data could then be related to the number of DSS cases that are categorized as having primary or secondary-type antibody responses. It is important to distinguish the cases of shock syndrome; this considerably improves diagnostic accuracy. When the ratios are calculated, they will provide an answer to the questions: Is DSS occurring among secondary dengue infections at rates similar to those measured in Thailand? Is DSS occurring among primary dengue infections? Ratios above those estimated in Thailand (Table 4) would constitute evidence of an outbreak of increased virulence. The failure to observe DSS would be evidence that the pre-conditions for shock syndrome have not been met.

Another important dividend to be gained from virological documentation of hospitalized cases of DHF/DSS would be to test the usefulness of the modal age of admission as a predictor of degree of endemicity.

While it is impossible to predict the outcome of longitudinal studies on dengue infection, if conducted conscientiously, such studies would provide the first detailed comparisons of dengue epidemiology in areas with and without DHF/DSS. It may also be possible to make observations on which sequences and intervals of dengue infection are associated with DHF/DSS and which are not. Countries of the South-East Asia Region of the World Health Organization are organizing just such a collaborative study. Additional studies outside the region are needed to provide a global perspective.

Other studies

Most of the rest of the epidemiological research questions outlined in this paper relate to "risk factors" or more broadly, to the question, why does DHF/DSS accompany only certain secondary dengue infections?

Nutrition. It has been noted above that malnutrition appears to exercise a profound sparing effect on DHF/DSS. This phenomenon has not been adequately documented. It should be. In such a study, it would be appropriate to record the height, weight, and birth date of clinically and serologically documented DHF/DSS patients at the time of hospital admission and compare these data with anthropometric data for children representative of the population which contributes DHF/DSS patients to hospital. Alternatively, as a control group, height- and weight-for-age could be documented for randomly selected well children who have serological evidence of two or more dengue infections, but no history of DHF/DSS. Greater discrimination could be obtained by measuring height, weight, skinfold thickness, arm and head circumference, and by examining hair-roots in patients with serologically confirmed dengue shock syndrome and in controls selected from the community at large, using only those with serological evidence of two or more dengue infections but no history of DHF/DSS. If there is a significant effect of nutritional status, it would be expected that DHF, especially DSS patients, would cluster in high-normal values of height- and weight-for-age, while controls would show a broader spectrum of values.

Genetic factors. Antibody response, cell mediated immune response, and complement production are under genetic control. Although considerable individual variation has been described in immune responsiveness, major heterogeneities between different ethnic groups in immune responses are not well described. Since immune response genes are closely adjacent to genes controlling HLA antigens, and human ethnic groups differ in HLA antigen distribution, it is possible that differences in immune responsiveness exist in human subpopulations as well as between individuals. An appropriate study of genetic factors in DHF/DSS might be approached by several avenues: (*a*) An analysis of the immune response to dengue virus by measuring residual antibody levels in dengue shock syndrome cases 1 or more years after illness in comparison with age, sex, and ethnic-group matched controls. (*b*) An analysis of sib pairs; one recently hospitalized subject with DSS who has a sibling who previously had DSS. Both affected sibs and their parents would be extensively tested for marker genotypes to test the hypothesis of linkage of a critical gene for DHF/DSS with a marker gene. (*c*) Linkage analysis to determine whether there are discernable associations, other than linkage, between marker phenotypes or genotypes and level of immune response or clinical severity of DSS. These analyses should be based on a comparison of data from affected probands and their non-affected sibs.

Specific studies should be designed in consultation with geneticists experienced in field studies, while data analyses would require access to population genetics computer programs.

Enhancing antibody. Until the present time, no attempt has been made to characterize and compare dengue infection-enhancing antibodies in populations with and without DHF/DSS. In our laboratory, cord blood serums from infants born to dengue-immune South-East Asian mothers have demonstrated enhancement antibody at titres of more than one million times![h] The ability of naturally acquired monotypic dengue antibody to enhance dengue infection in monocytes, using viruses isolated from the area sampled, is an important subject for future study. The enhancement assay can be performed most easily in a laboratory that has access to large numbers of dengue-susceptible blood leukocyte

[h] HALSTEAD, S. B. *In vivo* enhancement of dengue infection by passively transferred antibody. *Journal of infectious diseases,* **140**: 527-533 (1979).

donors. After an initial survey, a prospective study might be undertaken. It would be necessary to collect enough serums to include some children who subsequently develop DSS, but it would not be necessary to test all serums for enhancing antibody. Pre-illness serums from children developing DSS could be tested retrospectively, along with suitable controls.

Laboratory studies

The relationship between virion determinants and infection enhancement

The antigenic determinants and immunoglobulin subtypes that mediate enhanced dengue infection have not yet been studied. Since the enhancing power and enhancing titres of individual serums differ markedly, there is a real possibility that there may be a family of enhancing antibodies existing at different concentrations. The enhancement phenomenon predicts that the larger the number of the enhancing determinants shared between sequential dengue infections, the greater the enhancing power of the antibody generated by the first infecting virus on the second infection. Use of monoclonal antibodies developed in hybridoma cells could make available batteries of determinant-specific antisera which might make it possible to map antigenic determinants on dengue viruses and to shed light on this question.

Virus virulence

Although it has been asserted that there are differences in the intrinsic ability of dengue viruses to produce DSS, laboratory markers to study virulence have not been available to test this hypothesis. The immune-enhancement hypothesis provides a self-evident corollary: *any* event that increases infection of mononuclear phagocytes may result in enhanced disease. A simple one-step mutation could result in a dengue virus that differs in its ability to survive phagocytosis or to show improved replication in or release from mononuclear phagocytes (or other cells if these are found important in pathogenesis). Alternatively, viruses may differ in their ability to alter the surface structure of virus-infected cells. Such an attribute might be related to the strength or efficiency of the immune-elimination response which, in turn, should be related to disease severity. These biological properties of dengue viruses can be studied in the laboratory.

Clinical studies

This review has been directed mainly at epidemiological rather than clinical approaches to DHF/DSS research. It is important, however, to note several extremely important problems that challenge the physician-physiologist and the pathologist. The evidence available at present that histamine contributes to vascular permeability in DSS is far from conclusive. Can other mediators of vascular permeability be identified? There is no evidence in the published literature of attempts to detect non-kinin and non-histamine factors from DSS patients.

Can the degree to which mononuclear phagocytes serve as host cells for viral infection be defined in the living patient? Is there any evidence that activation, then destruction of mononuclear phagocytes accompanies the shock syndrome? There is one published report of a marked reduction in clearance of colloidal gold by the reticuloendothelial system during the acute phase of DHF/DSS. Might the blood levels of specific monocyte lysosomal enzymes provide another index to involvement of this system? Is there evidence at autopsy that dengue viruses replicate in mononuclear phagocytes or other cells? Why is there a

generalized destruction of lymphocytes in T lymphocyte-dependent areas of the spleen, lymph nodes, and thymus? And does this have prognostic significance for the outcome of infection? Is there a human equivalent to our unpublished monkey experiments in which dengue 2 viraemia was enhanced by pretreating animals with *Corynebacterium parvum* or pertussis vaccine? To investigate this possibility, it will be necessary to obtain a history of infections that have preceded an episode of shock syndrome. Of interest is the report from Thailand that α-thalassaemia patients do not develop DHF/DSS. Since α-thalassaemia occurs in 1% of the Thai population, the absence of such cases among DHF/DSS cases was readily noted. This observation requires further investigation. Chronic activation of the mononuclear phagocyte system may in someway reduce the size of the dengue-permissive cell population.

Implications of immune-enhancement for immunoprophylaxis of DHF/DSS

A matter of current interest and concern to persons who must face annual epidemics of DHF/DSS is the development of a dengue vaccine. For practical as well as sound immunological reasons, it seems necessary to develop a vaccine, one dose of which would evoke tetravalent immunity. If four monovalent vaccines have to be given separately, not only would sequential immunization be tedious, but cross-protection between dengue types might make it difficult to advise a suitable immunization regimen. If it were shown that DSS is caused by a single dengue type, vaccine production would be simplified and many scientific and administrative problems would be eased. This is one of many possible dividends that may be gained from prospective epidemiological studies on DHF/DSS.

One vaccine incorporating four dengue types has been used in rhesus monkeys to produce solid immunity to all four viruses. However, in dengue endemic areas many vaccinated subjects will already be immune to one or two dengue viruses. The efficacy of multivalent vaccines in a partially immune population will require careful evaluation. Another problem requiring direct and vigorous confrontation is the possibility that one of the vaccine viruses will produce DHF/DSS in persons with dengue immunity or that the vaccine will sensitize recipients to enhanced infection with wild dengue viruses. Nature has shown us that sequential dengue infections, probably with any combination of two dengue viruses, effectively removes a human from being "at risk" to DHF/DSS. DHF/DSS is rarely seen in adults in South-East Asia. Although there may be intrinsic differences between children and adults in susceptibility to shock syndrome, recent as well as historical occurrences of DHF/DSS suggest that adults, in fact, are more prone to severe dengue disease, particularly disease complicated by severe disseminated intravascular coagulation. It is the putative protective effect of two or more dengue virus infections that may provide a margin of safety for vaccination. Alternatively, booster immunizations may be needed, much as with trivalent oral poliomyelitis vaccine.

DHF/DSS is an important cause of human morbidity and mortality. This disease threatens millions of persons around the globe. It will be evident to the reader that important questions remain to be answered, particularly, with regard to defining risk factors for DHF/DSS, in understanding the pathogenesis of the disease, and in gauging the relative contribution of the virus strain and host in the production of shock syndrome. It is difficult to envisage embarking upon a serious effort to evaluate attenuated dengue vaccines in man before fundamental studies of the kind described here have been completed.

More than 25 years have passed since epidemic DHF/DSS was first recognized. Thirteen years have passed since an immunological role was postulated in the causation of DHF/DSS. Although there is not complete agreement about hypotheses of the immunopathogenesis of the shock syndrome, the immune-enhancement hypothesis provides a large

number of opportunities for direct testing in the laboratory, in the field, and in man. Dengue virology is a difficult and demanding scientific discipline, but the challenges posed do not exceed the capacity of a purposeful and organized international scientific community. The investment made by developing countries in modern acute care facilities to save the lives of critically ill DSS cases and in launching nationwide mosquito abatement programmes is already very substantial. The time has come for an investment in research.

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