Epidemiologic observations on outbreaks of hemorrhagic fever in urban and rural areas of Thailand are reported. These data are examined with respect to the question whether there are dengue viruses of a serious pathogenic nature and of recent origin prevalent in various parts of the world.

RECENT EPIDEMICS OF HEMORRHAGIC FEVER IN THAILAND

OBSERVATIONS RELATED TO PATHOGENESIS OF A "NEW" DENGUE DISEASE

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DENGUE virus in Southeast Asia is the cause of severe febrile disease characterized by hemorrhagic phenomena, shock, and frequently death. Nowhere has the problem of dengue hemorrhagic fever been so extensive nor so well studied as in Bangkok, Thailand. Since the explosive outbreak there in 1958, many virologic, clinical, and epidemiologic aspects of this disease have been described.[1-7] While of interest in themselves, Thai hemorrhagic fever studies have acquired additional importance in the intervening years, as similar disease has been reported from country after country in South and Southeast Asia.[1,8-10] The recent epidemic of dengue in the Americas has re-emphasized the disease potential of dengue viruses, and raised the specter of spread of a new and severe pandemic dengue disease throughout the world.

This paper presents in brief form selected epidemiologic observations on outbreaks of hemorrhagic fever occurring in a large urban area and a rural town in Thailand in the period 1962-1964. Data are discussed with particular reference to the most crucial question raised by this disease: "Are dengue viruses with serious pathogenic potential loose in the world?"

Method of Study

In November, 1961, the Virology and Entomology Departments of the US Army-SEATO Medical Research Laboratory, in collaboration with the Department of Microbiology of the Faculty of Public Health, Bangkok, began linear epidemiologic, clinical, ecologic, and virologic studies of hemorrhagic fever in Thailand and Southeast Asia. Details of these studies will be reported in subsequent publications. Technics for recovery and identification of dengue viruses have been reported elsewhere.[11,12] All serologic studies reported were done in microvolumes using Microtiter* equipment and basic technics described by

* Cooke Engineering Co., Alexandria, Va.

Casals[13] and Clarke and Casals.[14] In this paper viruses resembling dengue 1 and/or TH-Sman[6] are referred to as dengue type 1; viruses resembling dengue 2 and/or TH-36[6] are referred to as dengue 2. Dengue 1 (Hawaii), dengue 2 (New Guinea C), dengue 3 (H-87), and dengue 4 (H-241) described by Hammon,[1,6] and Japanese encephalitis (JE), Nakayama strain, were used as serologic antigens.

Results

Inquiry into the Historical Origin of Thai Hemorrhagic Fever

Despite the apparent novelty of hemorrhagic fever in 1958, examination of records at the Pediatric Service at Siriraj Hospital has uncovered cases clinically identical to Thai hemorrhagic fever in every year since 1950. The loss of clinical records from 1943 through 1948 has precluded further search during that period. However, detailed hospital records kept in English under the supervision of an American medical professional staff exist for the period 1932-1942. No case histories resembling hemorrhagic fever were found among charts of 572 children admitted to hospital in July or August during that period. Although it is impossible with assurance to fix a date for the beginning of endemic hemorrhagic fever in the country, present evidence indicates that this is a post-World War II phenomenon.

Epidemiologic Characteristics of Hemorrhagic Fever in Bangkok

Yearly Occurrence—Figure 1 shows the total disease experience for the Bangkok metropolitan area for 1958-

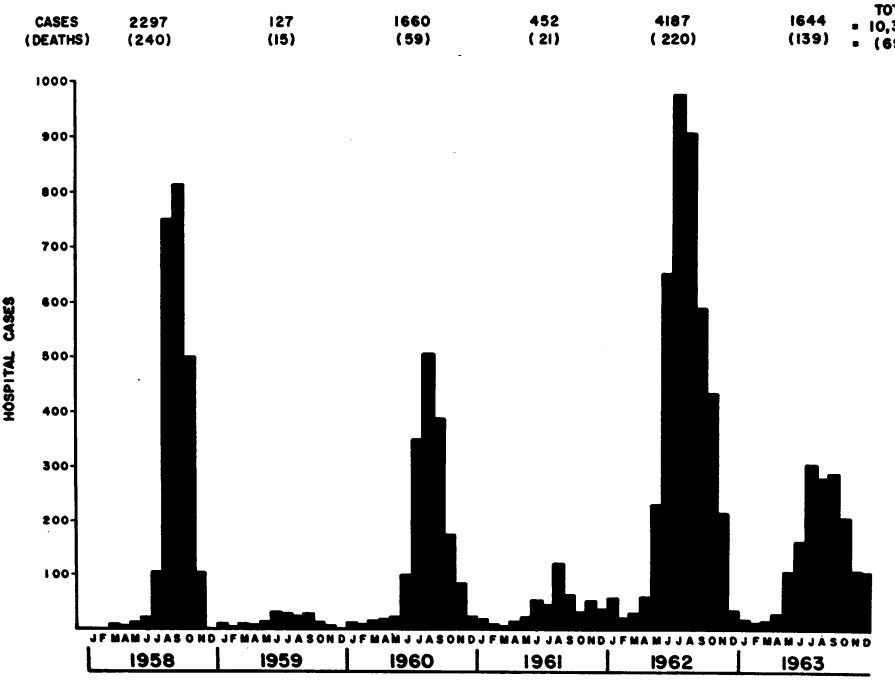


Figure 1—Monthly Hospitalizations for Thai Hemorrhagic Fever in Bangkok and Thonburi, 1958-1963.

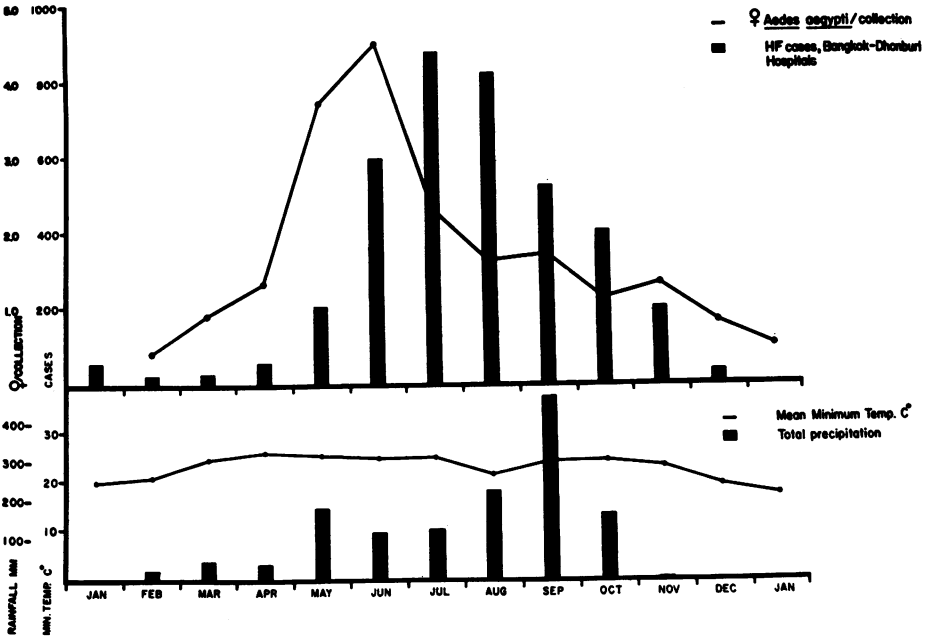


Figure 2—Aedes aegypti Collections and Hemorrhagic Fever Cases, Bangkok, 1962 (after Scanlon).[15]

1963. Biannual epidemic surges in 1958, 1960, and 1962 are obvious. It is of interest to note that during the six-year period, hospital diagnoses of hemorrhagic fever were made every month.

Seasonal Occurrence—Closer examination of Figure 1 shows the relationship of epidemic periods to seasonal rainfall in Thailand. The annual monsoon generally commences in late May or early June and ends in October or November. Figure 2 shows in more detail meteorological data for 1962 together with Aedes aegypti population data obtained from five collection sites in Bangkok by Scanlon.[15] Data are expressed in terms of a unit of collection, i.e., the number of female mosquitoes one man collected from his bare legs in one half hour.

Mosquito Vector—Figure 2 shows the relationship of hospitalized hemorrhagic fever to populations of Aedes aegypti in 1962. It is obvious that hospitalized hemorrhagic fever seasonally correlates with the Aedes aegypti population. Furthermore, both viruses have been recovered almost exclusively from this species in Bangkok.[1,5]

Etiologic Association with Multiple Viruses—Studies in Bangkok in 1962 and 1963 have reconfirmed observations of earlier investigators of the association of hospitalized hemorrhagic fever with dengue viruses of multiple types and with a group A virus, chikungunya.[1,2] Although chikungunya disease is included in hemorrhagic fever diagnoses, recent etiologic and clinical studies have shown that chikungunya infections are relatively mild and not associated with severe morbidity or mortality.[16] Table 1 details chikungunya and dengue virus isolations from humans with a clinical diagnosis of hemorrhagic fever in 1962. Representative complement-fixation antibody responses

Table 1—Viruses Recovered from 154 Hospitalized Hemorrhagic Fever Patients, Bangkok, 1962

	d1*	d2†	d3	d4	Chikungunya
Viruses recovered	9	10	3	0	9
Per cent recovery	5.8	6.5	1.9	—	5.8

* Dengue 1—TH-Sman complex.
† Dengue 2—TH-36 complex.

following dengue hemorrhagic fever in residents of Bangkok are shown in Table 2. Sera shown were chosen randomly from 46 paired sera from patients hospitalized in 1962. The high initial antibody levels and broadly reactive antibody patterns seen in many sera are consistent with the hypothesis that many or all of these patients have had at least one previous exposure to closely related group B agents, most likely other dengue viruses.

Age and Sex Distribution—Shown in Figure 3 are age specific hospitalization rates in 1962 and 1963 for males and females resident in Bangkok and Thonburi at the time of onset of hemorrhagic fever. Hospital data obtained for each outbreak from 1958 through 1961 also show similar age specific attack rates.[17]

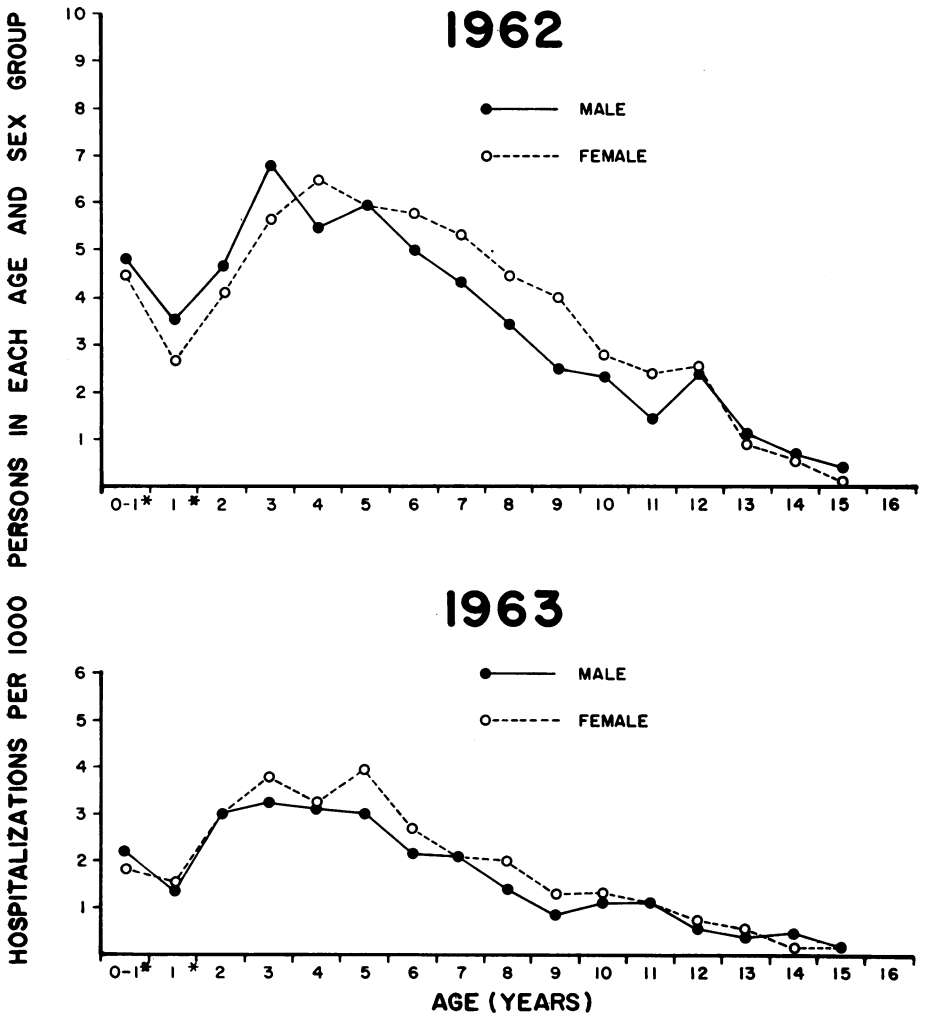
From Figure 3 it appears that hemorrhagic fever is only a disease of children. However, adult cases have been

Table 2—Representative Complement-Fixation Antibody Titers in Acute Convalescent Serum Pairs in Thai Hemorrhagic Fever Patients Resident in Bangkok, 1962

Patient No. (HFI)	Age (Years)	Day of Disease	Reciprocal Complement-Fixation Titers Against Indicated Antigens*				
			Dengue 1	Dengue 2	Dengue 3	Dengue 4	JE
37		2	0†	0	0	0	0
	5	8	512	32	64	4	32
56		4	0	0	0	0	0
	8	8	128	64	0	0	8
69		4	64	128	0	0	0
	4	8	1,024	32	0	16	64
76		8	64	8	8	8	4
	3	14	64	32	4	4	16
126		5	16	4	0	0	0
	10	22	64	64	32	4	16
147		5	0	0	0	0	0
	11	21	8	4	4	0	4
204		6	32	0	0	0	0
	6	12	32	16	0	64	64
93		6	512	1,024	32	8	64
	3	13	256	1,024	64	16	128
104		4	0	128	16	4	8
	7	13	16	1,024	64	32	64
109		5	64	64	0	4	16
	8	12	1,024	2,048	32	16	128

* Serial twofold dilutions of serum were tested against two units of antigen using two exact units of complement.
† 0 = <1:4.

Figure 3—Age-Specific Hospitalization Rates for Males and Females, Thai Hemorrhagic Fever, Bangkok-Thonburi, 1962-1963.



* Age-sex population estimated from registered live births and infant mortality figures, Ministry of Public Health. Demographic data for other age groups obtained from 1960 Thailand Population Census.

reported. Physicians on adult medicine wards are not as experienced with this disease as pediatricians. Since individuals older than 14 are not admitted to pediatric wards, absence of reported cases after this age may be influenced by observer variation. In the largest women's hospital in Bangkok in 1962 and 1963 there were 15 clinical diagnoses of hemorrhagic fever in young women between the age of 15 and 20. More than half of these were confirmed as dengue virus infection. There was one death in each year in 19- and 15-year-old females, respectively, whose clinical courses were strongly suggestive of hemorrhagic fever.

Age specific death rates in residents

of Bangkok and Thonburi for 1962 and 1963 are shown in Figure 4. No reasons are evident to explain the fluctuation in death rates for various age and sex groups or the apparent greater severity of disease in older girls. In the two years there were ten deaths attributed to hemorrhagic fever in 10-14-year-old girls while no deaths were reported in boys of this age group. The over-all death rate in 1963 was 8.3 per cent while in 1962 it was 5.0 per cent.

Character of Dengue Illnesses in Caucasians—In the period April, 1962, to August, 1964, 151 Americans and Europeans with dengue virus infection and 31 with chikungunya disease have been confirmed at our laboratory. The nonindigenous population accessible to study was disproportionately composed of young males of military age and, hence, the age and sex distribution of confirmed cases was skewed toward this age group. None of the studied patients and, in fact, no Caucasian of European descent living in Thailand since 1958 has developed an illness with signs and symptoms of the hemorrhagic fever syndrome (hepatomegaly, shock and/or abnormal bleeding). All patients had a disease compatible with the varied manifestations of classical dengue fever.

It is difficult to estimate the total number of persons in the Thailand Caucasian community infected with dengue since 1958 because this community is constantly changing composition. On the basis of discovered cases and serologic surveys conducted in 1962 and 1963, it can reasonably be guessed that the total number of such infections is in excess of 500. In Chinese or Thai children, 500 dengue infections would result in perhaps ten hemorrhagic fever cases severe enough to be hospitalized.[5] If Caucasians regardless of age can be regarded as "immunologic children," absence of disease in this large a group appears to be significant.

Epidemiologic Characteristics of an Outbreak of Hemorrhagic Fever in a Rural Town in Thailand

In 1964, an outbreak of hemorrhagic fever occurred in Ubon, a town of 27,000 located on the border between Laos and Thailand, 300 miles east of Bangkok. Fortuitously, this town had been visited at regular intervals since 1962 by members of the Virus Department. From these visits, it was known

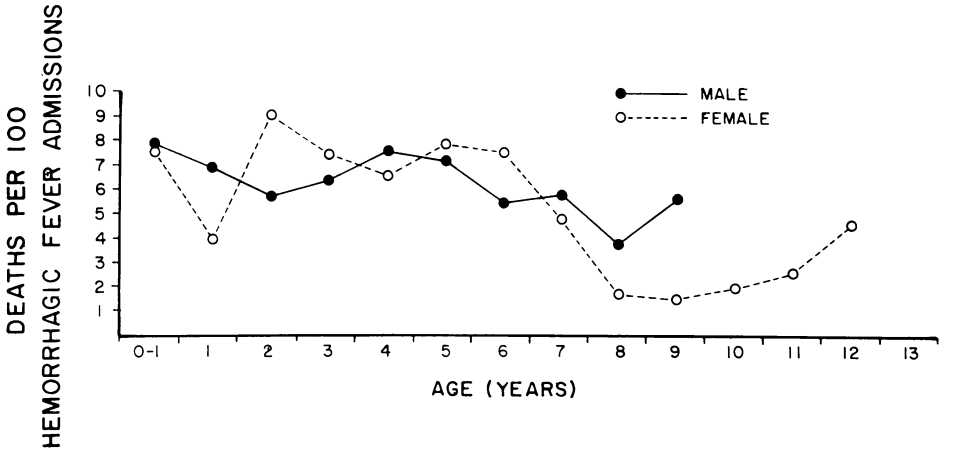


Figure 4—Death Rate by Age and Sex Among Patients Hospitalized with Thai Hemorrhagic Fever, 1962-1963.

Table 3—Age Distribution of Cases and Deaths and Estimated Age-Specific Hospitalization Rates of Clinically Diagnosed Hemorrhagic Fever, Ubon, Thailand. March-August, 1964. Estimate of Age Distribution of Ubon Population Made from 1960 Thailand Census.

Age (Years)	Deaths	Cases	Estimated Children in Age Group (Ubon)	Estimated Hospitalization Rate per 1,000 Age Group
0-1	1	4	1,025	3.9
1	1	5	975	5.1
2		7	925	7.6
3	3	14	900	15.6
4		16	910	17.6
5		15	900	16.7
6		5	850	5.9
7	2	9	800	11.3
8		4	750	5.3
9	1	4	700	5.7
10		2	740	2.7
11		4	680	5.9
12		1	630	1.6
13		2	600	3.3
14		1	550	1.8
Total	8	94		

that only sporadic patients with the diagnosis of hemorrhagic fever had been admitted to Ubon Provincial Hospital during the period, and it had been established that no outbreak of hemorrhagic fever had been recognized since the syndrome was described in Bangkok in 1958. In April, 1964, children with a severe illness diagnosed as Thai hemorrhagic fever were admitted to Ubon hospital. Admissions reached a peak during the first week of June. By August only sporadic admissions were recorded. Altogether, 94 patients were admitted; eight children died. The age distribution of cases and deaths are shown in Table 3. Despite the absence of apparent disease in preceding years, the resemblance of the age distribution of cases to that in Bangkok is striking.

To date dengue type 1 viruses have been recovered from 12 of 34 studied patients in this outbreak. Of 26 patients with paired sera and diagnostic HI antibody response, ten had CF antibody in convalescent sera obtained on or before the 18th day after illness. CF antibody titers in these patients are shown in Table 4. The relatively low titers and lack of heterologous response suggest a primary group B infection. Ten patients whose earlier convalescent sera were negative were bled again. These sera, obtained 60-80 days after illness, all contained low titered dengue 1 CF antibody and no JE CF antibody.

Discussion

Broadly speaking, two alternate hypotheses of the pathogenesis of hemorrhagic fever exist: (a) that hemorrhagic fever resides in the agent and (b) that hemorrhagic fever is a response peculiar to the host. Many of the epidemiologic observations cited above illustrate the difficulties confronting the investigator attempting to

choose between these hypotheses. The most important difficulty may be posed as two questions: What is hemorrhagic fever? What is the reliability of clinical diagnosis of this disease in Thailand? Answers to these questions must precede any epidemiologic study.

Recently, detailed clinical studies of virologically confirmed hemorrhagic fever patients have been completed by the U. S. Army-SEATO Medical Research Laboratory and Clinical Research Center. When these studies are published, the pathophysiologic disturbances distinguishing hemorrhagic fever syndrome from dengue fever or other acute viral illnesses should be better defined. Until all physicians and hospitals reporting this disease can employ similar patient study methods, however, the epidemiologist must fall back on cruder diagnostic criteria. For the purposes of this paper hemorrhagic fever is defined as a febrile disease of dengue etiology which occurs in outbreaks, and which is characterized in some instances by cardiovascular collapse, spontaneous bleeding phenomena, and death. Since the ratio of fatal cases to total hospital admissions has not changed significantly in Bangkok since 1958,[3,5] it seems probable that clinical diagnoses there are consistent and data obtained of value, at least for comparative purposes. Hemorrhagic fever in Ubon is less well-defined, but diagnoses were made by two pediatricians trained in Bangkok; some cases were observed by the senior author.

In outbreaks outside Thailand there has been extensive experience with dengue viruses. In epidemics proved by dengue virus isolation total human infections must number in the millions.

Table 4—Representative Complement-Fixation Antibody Response in Acute Convalescent Serum Pairs in Thai Hemorrhagic Fever Patients Resident in Ubon, Thailand, 1964

Patient No.	Age (Years)	Day of Disease	Reciprocal Complement-Fixation Titers Against Indicated Antigens*				
			Dengue 1	Dengue 2	Dengue 3	Dengue 4	JE
TH-733		4	64	0†	0	0	0
	7	18	256	256	256	0	16
TH-737		1	0	0	0	0	0
	4	15	64	32	0	0	0
TH-742		2	0	0	0	0	0
	6	13	128	32	8	8	8
TH-746		2	0	0	0	0	0
	10	15	128	0	0	0	0
TH-749		3	0	0	0	0	0
	4	16	256	0	0	0	0
TH-753		2	0	0	0	0	0
	6	14	64	0	0	0	0
TH-761		2	0	0	0	0	0
	1	15	32	0	0	0	0
TH-796		4	0	0	0	0	0
	7	15	64	0	0	0	0
TH-797		4	64	0	0	0	0
	7	17	256	0	0	0	0
TH-832		4	0	0	0	0	0
	3	16	32	0	0	0	0

* Serial twofold dilutions of serum were tested against two units of antigen using two exact units of complement.
† 0 = <1:4.

Yet these outbreaks have been reportedly benign. If hemorrhagic fever is due to the same agent or agents, what has happened in Southeast Asia? One possibility is that dengue virus or viruses have mutated to acquire new pathogenic properties. Such an hypothesis is supported by the previous association on at least five separate occasions since 1897 of disease resembling dengue hemorrhagic fever with dengue epidemics: North Queensland in 1897,[18] the southern United States in 1922,[19] Durban, South Africa, in 1927,[20] Greece in 1928,[21] and Formosa in 1933.[22] Unless factors as yet unappreciated operate, it would be expected that chance mutation would occur only in a single dengue virus type in a short span of time. The epidemiological association of different dengue viruses in different areas with hemorrhagic fever during the past decade (types 3 and 4 in the Philippines,[1] types 1, 2, and 3 in Thailand,[1] types 1 and 2 in Singapore,[8] and type 2 in Vietnam,[9] Malaysia,[23] and India)[10] weaken, but do not rule out this hypothesis.

A remarkable characteristic of outbreaks of hemorrhagic fever in Thailand has been the simultaneous epidemic dissemination of dengue viruses of different types and a group A virus, chikungunya. It may be speculated that this concurrence of multiple agents might be related to the pathogenesis of the disease. Perhaps hemorrhagic fever is the result of mixed simultaneous chikungunya and dengue infection, or mixed dengue infection. Attractive as these hypotheses may be, combinations of viruses have not as yet been recovered from patients studied virologically. Further, the relation of chikungunya virus to hemorrhagic fever must be excluded because typical outbreaks have occurred in the Philippines,[1] Malaysia,[23] and in small towns in Thailand without evidence of simultaneous chikungunya infection. Finally, it seems doubtful that mixed dengue virus infection may be a cause of hemorrhagic fever on the basis of outbreaks such as the one described in Ubon, where evidence of infection with only one dengue virus could be found. At present, it appears that the mixed arbovirus flora of large Southeast Asian cities merely reflects the ecologic opportunities for multiple agents to coexist and may have no bearing upon disease pathogenesis.

An important finding in studies in Thailand has been the failure to observe in any Caucasian of European descent typical hemorrhagic fever with dengue infection despite the exposure of this group to epidemics occurring over a seven-year period. This observation suggests that dengue or chikungunya viruses alone do not possess hemorrhagic fever pathogenetic properties, but that the syndrome is related to the host, in particular to the long-term resident of endemic areas. It may be that hemorrhagic fever is a response accompanying reinfection with a heterologous dengue virus, perhaps a kind of hyperimmune reaction. Rising attack rates of hemorrhagic fever observed during the first three to four years of life are consistent with such an hypothesis. Since seasonal dengue infection rates of Bangkok residents may be as high as 30-50 per cent,[5] reinfection must occur frequently and early in life. The shape of the age-specific hemorrhagic fever attack rate curves for Bangkok might then be explained if a second dengue infection caused hemorrhagic fever (in some individuals) but, thereafter, heterologous immunity was sufficiently broad to protect. If this hypothesis is correct, why should the age distribution of cases in Ubon be similar to that in Bangkok when the antibody responses of Ubon patients suggest the absence of previous dengue virus exposure?

This leads to the alternate possibility that factors unrelated to dengue virus

exposure within the Caucasian and Oriental host may govern the pathogenesis of dengue infection. Such factors may be inherited or acquired, as for example, nutritional status. While the genetic control of different disease manifestations with single agents has not been proved for viruses, such a mechanism has been postulated for human streptococcal syndromes. Until it is unequivocally proved that nonindigenous Caucasians are susceptible or not susceptible to the hemorrhagic fever syndrome, judgment as to whether or not mutant dengue viruses are the cause of hemorrhagic fever or if other mechanisms are involved, must be held in abeyance.

Meanwhile, responsible public health officials in countries infected with Aedes aegypti should prepare for the unhappy possibility that lethal dengue virus epidemics may occur outside Southeast Asia at any time in the future.

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REFERENCES

1. Hammon, W. McD.; Rudnick, A.; and Sather, G. E. Viruses Associated with Epidemic Hemorrhagic Fevers of the Philippines and Thailand. Science 131:1102–1103 (Apr.), 1960.
2. Dasaneyavaja, A., and Pongsupat, S. Observations on Thai Hemorrhagic Fever. J. Trop. Med. 64:310–314 (Dec.), 1961.
3. Proceedings of the Symposium on Hemorrhagic Fever. (Aug. 10-11), 1961. SEATO Medical Research Monograph No. 2. Bangkok, Thailand.
4. Nelson, E. R. Hemorrhagic Fever in Children in Thailand. J. Pediat. 56:101–108 (Jan.), 1960.
5. Halstead, S. B.; Yamarat, C.; and Scanlon, J. E. The Thai Hemorrhagic Fever Epidemic of 1962. A Preliminary Report. J. M. A. Thailand 46:449–465 (Aug.), 1963.
6. Hammon, W. McD., and Sather, G. E. Virologic Findings in the 1960 Hemorrhagic Fever Epidemic (dengue) in Thailand. Am. J. Trop. Med. 13: 629–641 (July), 1964.
7. Nelson, E. R.; Bierman, H. R.; and Chulajata, R. Hematologic Findings in the 1960 Hemorrhagic Fever Epidemic (dengue) in Thailand. Ibid. 13:642–649 (July), 1964.
8. Lim, K. A.; Rudnick, A.; and Chan, Y. C. Recent Studies of Hemorrhagic Fevers in Singapore. Singapore Med. J. 2:158–161 (Dec.), 1961.
9. Halstead, S. B.; Voulgaropoulos, E.; Tien, N. H.; and Udomsakdi, S. Dengue Hemorrhagic Fever in Vietnam. Report of the 1963 Outbreak. Am. J. Trop. Med. (In press.)
10. Sarkar, J. K.; Pavri, K. M.; Chatterjee, S. N.; Chakravarty, S. K.; and Anderson, C. R. Virological and Serological Studies of Cases of Hemorrhagic Fever in Calcutta. Indian J. M. Res. 52:684–691 (July), 1964.
11. Halstead, S. B.; Sukavachana, P.; and Nisalak, A. In vitro Recovery of Dengue Viruses from Naturally Infected Human Beings and Arthropods. Nature 202:931–932 (May), 1964.
12. ————. Assay of Mouse Adapted Dengue Viruses in Mammalian Cell Cultures by an Interference Method. Proc. Soc. Exper. Biol. & Med. 115:1062–1068 (Apr.), 1964.
13. Casals, J. Acetone-Ether Extracted Antigens for Complement-Fixation with Certain Neurotropic Viruses. Ibid. 70:339–343 (Feb.), 1949.
14. Clarke, D. H., and Casals, J. Techniques for Hemagglutination and Hemagglutination Inhibition with Arthropod-Borne Viruses. Am. J. Trop. Med. 7:561–573 (Sept.), 1958.
15. Scanlon, J. E. Annual Progress Report SEATO Medical Research Laboratory, Bangkok, Thailand. (Apr.), 1964, p. 200.
16. Dasaneyavaja, A.; Robin, Y.; and Yenbutra, D. Laboratory Observations Related to Prognosis in Thai Hemorrhagic Fever. J. Trop. Med. 66:35–41 (Feb.), 1963.
17. Jatanasen, S.; Sakuntanaga, P.; and Dhanasiri, C. Some Aspects of the Epidemiology of Thai Hemorrhagic Fever. SEATO Medical Research Monog. No. 2:6–18, 1962.
18. Hare, F. E. The 1897 Epidemic of Dengue in North Queensland. Australasian M. Gaz. 17:98–107 (Mar.), 1898.
19. Scott, L. C. Dengue Fever in Louisiana. J.A.M.A. 80:387–393 (Feb.), 1923.
20. Anon. The Dengue Epidemic in Greece. League of Nations Monthly Epidemiological Report. 7:334–338 (Sept.), 1928.
21. Copanaris, P. L'epidémie de dengue en Grèce au cours de l'ete 1928. Off. Internat. d'Hyg. Pub. Bull. 20:1590–1601 (Oct.), 1928.
22. Nomura, S., and Akashi, K. On Fatal Cases with Hemorrhage Caused by Dengue Fever (in Japanese). Taiwan Igakkai Zasshi. 30:1154–1157 (Nov.), 1931.
23. Rudnick, A. Personal communication.

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