

BODY OF REPORT

SEATO Medic Study No. 2 Clinical Disease Accompanying Thai Hemorrhagic Fever Virus Infections

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 Department of Virology

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Objectives: To determine the etiology of Thai hemorrhagic fever and describe the nature and relative frequency of inapparent, mild and atypical syndromes which accompany infection with Thai hemorrhagic fever viruses in Asians.

Description: Four categories of patients are studied at Children's Hospital by similiar clinical and virologic methods:

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1. Out-patients with febrile syndromes (PUO).
2. In-patients with admission diagnosis of hemorrhagic fever (HFI).
3. In-patients with febrile disease other than hemorrhagic fever (NH).
4. Surgical patients.

All patients, residents of Bangkok and Thonburi, are selected randomly within 24 hours of admission to Out-patient Department or hospital. Virologic materials include acute and convalescent sera, throat and rectal swabs.

Autopsy materials and other specimens from fatal cases of Thai hemorrhagic fever are collected whenever possible. Infectious and serologic specimens submitted voluntarily from other hospitals in Bangkok are included in certain aspects of this study.

Progress: Etiologic and Virologic Studies of Hemorrhagic Fever. At least 4 and perhaps 6 different types of dengue viruses have been isolated from patients hospitalized with a diagnosis of hemorrhagic fever in Thailand. Since pathophysiologic evidence is lacking that dengue infection without shock in Asian children differs significantly from other undifferentiated viral illnesses or from classical dengue fever and since outbreaks are mixtures of disease syndromes, it is not sufficient to merely associate virus and disease epidemiologically to establish the etiology of the hemorrhagic fever. For this reason careful etiologic studies of hemorrhagic fever are particularly important.

Table 1 summarizes virus isolation by severity of disease in Thailand from 1958-1962. As shown in the table, dengue type 2 viruses have thus far been most frequently associated with severe disease and deaths. During this same period dengue types 1, 2, 3 and 4 viruses were circulating in Bangkok and causing human disease. Virus isolations made during 1962-3 from mosquitoes and mildly ill patients are shown in Table 2. Although the numbers are small, there is a suggestion that types 1 and 2 dengue are more frequently associated with hospitalized patients while dengue 1, 2, 3 were recovered with equal frequency from mosquitoes and dengue infections. It is of interest that the only dengue virus isolated from the Penang, South Vietnam and Calcutta outbreaks was dengue type 2. Dengue 2, it seems, stands implicated in the etiology of severe dengue hemorrhagic fever. The status of others dengue types is less clear.

A presentation of data on the association of virus type and disease severity in hemorrhagic fever is not complete without the mention of 2 additional observations.

First, the inability to recover any virus from the tissues of patients dying of hemorrhagic fever. During the past 3 years homogenates of 198 tissues from 46 patients dying of hemorrhagic fever have been tested in suckling mice (Table 3). A blind passage technique was employed. This technique has been used successfully to recover nearly 300 dengue viruses from mosquito suspensions or human sera during the same period. Not one dengue virus was recovered from

these tissues, although 3 ether resistant viral agents, tentatively classified as Coxsackie A viruses were recovered.

Recently, additional techniques have been used to try to detect dengue antigen or to "unmask" dengue virus in tissues. These have been:

1. Tissue homogenates have been used as immunizing antigens, animals are then bled and tested for dengue antibody or challenged intracerebrally with a lethal dose of weanling mouse adapted dengue virus
2. Cell cultures have been made of fresh liver tissue and bone marrow cells
3. Tissue suspensions have been treated with genetron to attempt to break hypothetical antigen antibody bonds and,
4. Fresh frozen autopsy tissue has been stained with fluorescein conjugated dengue antibody.

Only 1 of these techniques has been successful. Three dengue viruses were recovered from 21 bone marrow culture attempts. These viruses, however, were recovered from surviving patients. The rate of virus recovery did not exceed the 20% recovery rate of dengue virus from venous blood obtained in other hemorrhagic fever survivors. These studies suggest 1 of 3 possibilities; either the correct tissue has not been examined for dengue virus; or dengue virus does not multiply in tissues which show pathologic damage or dengue virus has largely disappeared at the time of death.

A second observation suggests that dengue virus disappears more rapidly from the blood of fatal cases than from milder dengue infections. Three groups of patients were studied: out-patients with a variety of mild undifferentiated febrile diseases; patients hospitalized with the diagnosis of hemorrhagic fever including shock and non-shock cases and patients dying of hemorrhagic fever. The virus recovery rates from blood obtained on various days of illness are shown in Table 4. If isolations made on the 4th day of fever are compared in each group, it will be noted that the rate of virus recovery is inverse to the disease severity.

Virus recoveries are directly related to HI and CF antibody titers in acute phase serum. The scatter diagram in Figure 1 shows the distribution of HI antibody in an unselected group of dengue hemorrhagic fever patients in 1962. Extremely high antibody titer in the acute phase specimen is characteristic of 30-40% of patients with hospitalized hemorrhagic fever of varying severity and 70-80% of patients with the dengue shock syndrome.

Summary and Conclusions: Preliminary typing of dengue viruses from the 1962 outbreak has been accomplished. Nearly 37% of viruses recovered were dengue 1-TH Sman complex, 35% were dengue 2-TH-36 complex and 26% were dengue 3. Dengue 4 was also recovered. Multiple serologic types, predominantly dengue 1

and 2, were recovered from milder disease. Thus, dengue 3 may result in milder disease than dengue 2 or 1. In any event, evidence obtained does not support the concept that there is a single hemorrhagic dengue type. Of serologically confirmed dengue hemorrhagic fever infection, virus was recovered in 20%. In contrast, in 46 cases with fatal outcome virus was recovered in only 4% even though the day of collection of acute specimen was the same in both groups. Dengue viruses apparently disappear from the blood of mortally ill patients more rapidly than from patients who survive milder illness. At the time of autopsy, virus has disappeared.

Publications:

1. Bhamarapavati, N., Halstead, S. B., Sukhavachana, P., Boonyapetnevik, V. Studies on dengue virus infection. I. Immunofluorescent localization virus in mouse tissue. A.M.A. Archives of Path. 77:538-543, 1964.

Table 1

DENGUE VIRUSES ASSOCIATED WITH SEVERE HEMORRHAGIC FEVER OR DEATH
THAILAND, 1958-62

Disease severity	Investigator	Isolation of indicated virus			
		*	**	***	
		D1	D2	D3	D4
Hemorrhagic fever with shock and/or gastrointestinal hemorrhage	Hammon		3/?		
	Dasaneyavaja		3/61		
	Halstead	2/74	8/74		
Death	Dasaneyavaja				1/?
	Halstead	1/72	3/72		

* D1 or TH-Sman

** D2 or TH-36

*** Numerator indicates No. isolation. Denominator indicates total patients studied in disease category.

Table 2

DENGUE VIRUSES RECOVERED FROM Aedes aegypti AND MILD AND SEVERE
HUMAN ILLNESS, BANGKOK, 1962-3

Host	Number of viruses isolated			
	Dengue 1	Dengue 2	Dengue 3	Dengue 4
<u>Aedes aegypti</u>	5	6	7	1
Undifferentiated fevers	10	7	7	0
Hospitalized hemorrhagic fever	9	10	3	0

Table 3

RESULTS OF VIROLOGIC STUDY OF AUTOPSY MATERIALS FROM
46 FATAL CASES OF THF.

Organs	Numbers	Result
Brain	21	Neg.
Heart	24	Neg.
Liver	45	Neg.
Lung	23	Neg.
Spleen	40	Neg.
Kidney	35	Neg.
Pancreas	4	Neg.
Thymus	3	Neg.
Adrenal gland	2	Neg.
Stomach	1	Neg.

Table 4

RATE OF RECOVERY OF DENGUE VIRUSES FROM PERIPHERAL BLOOD OF PATIENTS WITH UNDIFFERENTIATED FEVER AND HOSPITALIZED HEMORRHAGIC FEVER PATIENTS SEROLOGICALLY CONFIRMED AS DENGUE INFECTION AND PATIENTS DYING OF HEMORRHAGIC FEVER.

Day of disease	PATIENT CATEGORY		
	Mild Dengue (35 cases)	Hemorrhagic fever (151 cases)	Death (46 cases)
1	8/14 (57.1%)	0/3 (0.0%)	1/3 (33.3%)
2	2/5 (40.0%)	7/16 (43.8%)	0/1
3	4/6 (66.6%)	11/42 (26.2%)	1/10 (10.0%)
4	5/8 (62.5%)	9/40 (21.4%)	0/14
5	0/2 0	3/25 (12.0%)	0/13
6	0	1/21 (4.7%)	0/3
7		0/4 0	0/2
Total	19/35 (49.1%)	31/151 (20.5%)	2/46 (4.3%)

HI ANTIBODY RESPONSE IN DENGUE HEMORRHAGIC FEVER
(60 CASES, AGES 1-3)

