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OBSERVATIONS RELATED TO PATHOGENESIS OF DENGUE HEMORRHAGIC FEVER. IV. RELATION OF DISEASE SEVERITY TO ANTIBODY RESPONSE AND VIRUS RECOVERED

We have previously shown that disease differing widely in severity and prognosis is included in the entity, dengue hemorrhagic fever (DHF) in Thailand.[1,2] Nearly 40 percent of 523 children hospitalized with DHF had a syndrome characterized by shock following a fever of several days duration. The rest had less severe febrile illnesses with various mild hemorrhagic manifestations. When sera from these children were studied, a significant correlation between shocked patients and a secondary-type antibody response to dengue virus was found.[3] This report considers in greater detail the various manifestations of dengue disease and the antibody response in the host. Observations are included that suggest that severity of host response to dengue infection is influenced by an interaction between immune status and the age and sex of the patient. Associations between severity of illness and the rate of virus recovery, the quantity of antibody produced and the type of dengue virus recovered are described. A synthesis of these data and their relevance to the pathogenesis of human dengue infection are presented in the final paper in this series.[4]

MATERIALS AND METHODS

Patient selection, serologic methods, virus isolation and identification techniques and definition of primary and secondary dengue antibody responses have been described.[1,2,5]

This report is based upon experience with 604 dengue hemorrhagic fever patients: 400 admitted to Children's Hospital between April 19, 1962 and January 1, 1965 (CH Study); 123 admitted to the Thai Hemorrhagic Fever Ward in July through September, 1964 (CRC Study), and 81 fatal hemorrhagic fever cases admitted to other Bangkok hospitals in 1962-1964. All DHF patients who survived their infection had serologic evidence of recent dengue infection according to established criteria[2] and a discharge diagnosis of hemorrhagic fever. Included for some evaluations are 35 out-

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patients with dengue viruses isolated from plasma during an episode of illness,[6] 29 patients with virologically proven chikungunya illnesses, and 76 CH and CRC patients admitted to the hospital with a febrile illness, specimens adequate for serologic study, but no diagnosis established.

In this paper the term "infant" refers to an individual less than one year of age and "children" is used to denote individuals older than 12 months and under 15 years.

RESULTS

Illness characteristics and the type of antibody response in the patient

We have published a scatter diagram showing the hemagglutination-inhibition (HI) antibody titers by day after onset of disease in one half of CH and CRC patients.[2] The HI responses from these patient series fell into two groups:

I. Patients who had no detectable HI antibody at a 1:20 dilution of serum collected through the fourth day after onset of illness and who subsequently developed HI antibody to a titer *no greater* than 1:1280 at 14 to 28 days after onset of disease and who had relatively monotypic complement-fixing (CF) antibody to dengue 1-4 antigens in serum specimens taken 14 or more days after onset of illness. These patients were considered to have had a primary-type dengue antibody response ("primary dengue").

II. Patients who either had HI antibody at 1:640 or higher in sera obtained through the fourth day after onset of illness, or developed HI antibody to titers of 1:1280 or greater 14-28 days after onset of illness, *and* had CF antibody to all four dengue antigens in serum obtained during the convalescent phase. These patients were considered to have had a secondary-type dengue antibody response ("secondary dengue").

Since we were unable to characterize antibody to immunoglobulin-type in our patients, a further description of the attributes of antibody responses classified as secondary dengue is included. Among 452 patients with "secondary dengue," 288 (62%) had a fourfold or greater HI antibody response in two sera spaced a minimum of 14 days apart. The remainder had no change in HI antibody titers at 1:640 or greater in two sera. Thirty-six patients (8%) had no HI antibody at 1:20 in the acute phase serum specimen (obtained within seven days of onset of illness). Two hundred fifty-six patients (56.6%) had HI titers of 1:20, 480 or greater in convalescent specimens (serum obtained 14 or more days after onset of illness). Of 240 representative serum pairs studied by CF, 207 had titers of 1:4 or greater against one or more dengue types 1-4 antigens in the acute specimen and 239 showed two-fold or greater titer rises in paired sera against two or more of the following antigens: dengue types 1, 2, 3, 4, Japanese encephalitis.
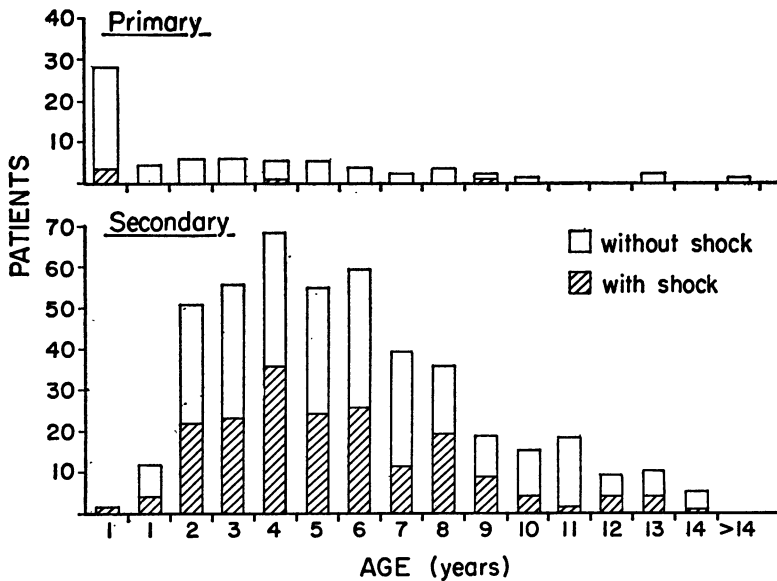
FIG. 1. Age distribution and occurrence of shock in 71 DHF patients with primary dengue antibody response and in 452 DHF patients with a secondary dengue antibody response.

It was of interest to determine the age distribution of patients with "primary" and "secondary" dengue antibody responses. These are shown in Figure 1. As expected, patients with primary dengue were younger than those with serologic evidence of previous dengue infection. Primary infections occurred most frequently in infants, while secondary dengue cases were distributed around a mode of four years.

Irrespective of age, shock was significantly related to secondary antibody response. There were 452 patients with secondary dengue and 71 with primary dengue; 190 secondary dengue patients, but only 6 primary dengue patients developed shock during their dengue infection. The difference was highly significant ·(p<.001).

Since antibody response and occurrence of shock were related, antibody titers in patients with secondary dengue were examined to see if there was a further correlation between the amount of antibody produced and the severity of illness. The distribution and geometric mean HI antibody titers during the first 10 days of disease in patients with secondary dengue and illness of varying severity are shown in Table 1. Geometric mean titers are not shown for groups with very small numbers. Distribution of log titers were compared in a one-way analysis of variance with two orthogonal contrasts. No significant differences in antibody titers were observed in the

TABLE 1. DISTRIBUTION OF HI ANTIBODY TO DENGUE TYPE 1 ANTIGEN IN 604 DHF PATIENTS WITH SECONDARY DENGUE ANTIBODY RESPONSES BY DAY AFTER ONSET OF FEVER AND SEVERITY OF ILLNESS.

Reciprocal HI titer[a]	Non-Shock										Shock										Death[c]									
Day of illness	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
<20	10	3	4	2		1					3			2	1	1									1	2				
20	3	2	2	1		1																			1					
40		1	5	1							1		3	1									1		1	1				
80	1	2	5	4		1								4	2					1			2	5	1	1	1			
160	1	1	8	8	1	4	1		1			2	3	5		1	1	1				1	2	1	2	1				
320			2	2	2			1				1	2	4	3	1						5	3	3	4	1	1			
640	3	5	9			3						1		5	5	1		1	1	2		1	1	3	3	2				
1280	3	3	4	6	4	4	1	1	2	2	1		5	4	5	2	1		1	1		1	2	4	4	1				
2560	1		5	5	7	4	3	1	1	3		1	4	2	10		1	1	2	2			1	5	3	3				
5120	1	1	3	4	6	7	4	3	5	3	1	2	1	4	5	6	6	1	1	2	1		1	4	3	1				
10240	1	3	2	4	5	4	3	3	3	9	1	2	1	4	5	6	1	1	2	5		1	1	6	5	1	1			
≥20480	2	5	16	18	15	17	12	12	6	9	2	3	9	13	15	7	6	3	3											1
Geometric mean titer	83	773	1002	1897	4129	4669	11691	14524	14310	13250	450	3073	1859	2614	6548	6552	14813	10119	8709	13752	453	783	2200[d]	2383	928•					
95% Confidence limits[b]	21-327	238-2512	489-2053	1026-3500	2411-7072	2251-9784	6359-21493	7871-26803	5783-35411	6045-29044	30-6723	812-11631	742-4661	1398-4887	3458-12398	2416-17770	4286-51205	2144-47763	3607-21025	5558-34022	163-1252	311-1977	1062-4560	898-6327	225-3825					

[a] For statistical purposes <1:20 and ≥1:20,480 assumed to be 1:10 and 1:40,000, respectively.
[b] Calculated from log mean titer.
[c] Serum titers in patients dying of disease clinically and pathologically resembling DHF.
[d] Different from log titers on day 4 in shock group, .05> P>.01.
• Different from log titers on day 6 in non-shock and shock group, .05>P>.01.

shock and non-shock groups. Geometric mean titers in the death group, with a single exception, were *lower* than mean titers on the same day after onset of fever in the other two groups. Significant differences were noted between titers in the fatal outcome and other groups on days 4 and 6 after onset of illness.

Tables 2 through 6 and Figures 2 and 3 compare various features of host response to dengue infection by the type of antibody response in the patient, and his age and sex. Not included in these analyses are signs and symptoms that did not differ (p>.05) between primary and secondary dengue in children: positive tourniquet test, petechiae, epistaxis, palpable liver, maculopapular rash, lymphadenopathy, duration of fever, post-infection bradycardia, myalgia, nausea, vomiting and headache. The frequency of these findings in DHF has been published.[1,2] Purpura (18/99) and gastrointestinal hemorrhage (11/91) were noted only in patients with secondary dengue infection.

In the analyses that follow, it became evident that patients with primary dengue fell into two groups: those who were less than one year old and whose illness characteristics resembled those observed in patients with secondary dengue and children older than one year whose illnesses resembled disease in infants or children of non-dengue etiology. Data have been arranged to facilitate these comparisons.

Table 2 compares the time in the course of the disease at which symptoms became sufficiently severe to warrant hospitalization. Children with primary dengue and patients without dengue were hospitalized earlier in the course of their illnesses than were infants with primary dengue or children with secondary dengue. Differences in the distribution of day of hospitalization between infants and children with primary dengue were significant (p=.05); the difference between day of hospitalization of children with primary dengue and children with secondary dengue was highly significant (p<.001).

The distribution of clinical syndromes in various sub-groups is compared in Table 3. Of six shock cases in the primary dengue group, four were infants. The two children with primary dengue who were in shock had brief episodes of mild hypotension. The incidence of "shock" in this group did not differ from that in patients in the non-dengue group. While the rate of occurrence of shock in infants with primary dengue was not as high as in secondary dengue, infants with primary dengue had significantly more hemorrhagic manifestations than children with primary dengue (.05>p> .01) or patients without dengue (.01>p>.001).

A further index of similarity or distinctness of host response in dengue in the designated antibody and age sub-groups is provided by physiological

TABLE 2. CLINICAL FEATURES OF HEMORRHAGIC FEVER BY ETIOLOGY, IMMUNOLOGICAL RESPONSE AND AGE GROUP. DAY OF HOSPITALIZATION AFTER ONSET OF ILLNESS IN 599 PATIENTS, CH AND CRC STUDIES. BANGKOK, 1962-1964

Day of hospitalization	Dengue etiology								
		Antibody response			Type of virus isolated				
	Not dengue	Primary		Secondary	d1	d2	d3	d4	none*
		>1 Yr.	<1 Yr.						
Cases	76	43	28	452	20	45	22	2	434
	%	%	%	%	%	%	%	%	%
0	2.6	4.7	0	0.2	0.0	2.2	0.0	0.0	0.5
1	18.4	11.6	3.6	7.7	5.0	6.7	4.5	0.0	8.3
2	19.8	25.6	3.6	12.2	40.0	13.3	18.2	0.0	11.3
3	18.4	32.5	28.5	27.0	20.0	22.2	36.4	50.0	27.9
4	18.4	16.3	32.1	29.2	10.0	24.4	27.3	50.0	29.5
5	15.8	7.0	14.3	16.8	10.0	26.7	9.1	0.0	15.4
6	2.6	2.3	10.7	4.0	10.0	4.4	4.5	0.0	3.9
7	2.6	0	3.6	2.2	5.0	0.0	0.0	0.0	2.3
>7	1.3	0	3.6	0.7	0.0	0.0	0.0	0.0	0.9

* Includes patients with unidentified viruses isolated.

TABLE 3. CLINICAL FEATURES OF HEMORRHAGIC FEVER BY ETIOLOGY, IMMUNOLOGICAL RESPONSE AND AGE GROUP. CLINICAL SYNDROME IN 599 PATIENTS, CH AND CRC STUDIES. BANGKOK, 1962-64

Syndrome*		Dengue etiology								
			Antibody response			Type of virus isolated				
		Not dengue	Primary		Secondary	d1	d2	d3	d4	none**
			>1 Yr.	<1 Yr.						
Cases		76	43	28	452	20	45	22	2	434
		%	%	%	%	%	%	%	%	%
I		55.3	51.2	17.8	21.5	30.0	24.4	31.8	50.0	22.8
II		39.5	44.2	67.9	36.6	45.0	40.0	54.6	—	37.8
III		3.9	4.6	7.1	31.4	20.0	26.7	9.1	50.0	29.2
IV		1.3	0	7.1	10.6	5.0	8.9	4.5	—	10.2

* I = fever, positive tourniquet test.
II = fever, hemorrhagic manifestations (petechiae, purpura, epistaxis, gum bleeding) with or without positive tourniquet test.
III = fever, hypotension or narrow pulse pressure (<20 mm Hg) with or without I or II.
IV = no detectable blood pressure or pulse.
** = Includes patients with unidentified viruses isolated.

TABLE 4. HEMATOLOGIC AND OTHER LABORATORY ABNORMALITIES IN HEMORRHAGIC FEVER PATIENTS BY ETIOLOGY, IMMUNOLOGIC RESPONSE AND AGE GROUP. CH AND CRC STUDIES. MODIFIED FROM WEISS AND HALSTEAD[7] AND COHEN AND HALSTEAD.[1] BANGKOK, 1962 AND 1964

		Abnormal finding by antibody response and age group		
	Not dengue	Dengue		
Finding		Primary, >1 year	Primary, <1 year	Secondary
	26	12	8	103
	%	%	%	%
CRC Study				
Adm.* Na<135mEq/L	15.4	0	50.0	26.2
Adm. CO_2≤15mM/L	11.5	0	50.0	27.7(101)
SGOT>150S.F. units	3.9	16.6	37.5	23.3
SGPT>100S.F. units	0.0	16.6	25.0	10.7
Adm. SUN >20 mg%	11.5	0	25.0	33.0
Total Protein ≤5.5 gm%	7.7	25.0	50.0	40.8
Hemoconc.**	11.8(17)†	12.5(8)	33.3(6)	52.2
CH Study				
Bleeding time >7 min.	—	0 (3)	66.6(3)	60.0(20)
Tourniquet test. pos.	—	0 (3)	100.0(3)	52.6(19)
Platelet count 100,000/mm³	—	33.3(3)	100.0(3)	95.2(21)
Silicone clotting time >45 min.	—	0 (3)	66.6(3)	63.2(19)
Prothrombin time >14.0 sec.	—	0 (8)	0 (3)	25.0(20)
Fibrinogen <180 mg%	—	0 (3)	100.0(2)	27.3(11)

* Admission.
** 20% higher hematocrit on admission than after recovery.
† ( ) = number tested if different from that at head of column.

TABLE 5. Frequency of Selected Hematologic and Other Laboratory Abnormalities in Patients With and Without Shock and With a Secondary Dengue Antibody Response, or a Primary Antibody Response in Infants Less Than 1 Year Old. CH and CRC Studies. Bangkok, 1962 and 1964. Modified from Weiss and Halstead[7] and Cohen and Halstead.[1]

| | | Abnormal finding by indicated antibody response or disease severity | | | |
| | Finding | Primary (<1 yr.) | | Secondary | |
		Shock 1 %	Non-shock 7 %	Shock 22 %	Non-shock 81 %
CRC Study[7]	Adm.* Na<135 mEq/L	0	57.1	68.2	14.8
	Adm. CO$_2$ ≤ 15 mM/L	100	42.9	60.0(20)	19.8
	SGOT > 150 S.F. units	0	42.9	59.1	13.6
	SGPT > 100 S.F. units	0	28.6	31.8	4.9
	Adm. SUN > 20 mg%	0	28.6	63.6	24.7
	Total Protein ≤ 5.5 gm%	100	42.9	77.3	30.9
	Hemoconc.**	100	20.0(5)†	94.7(19)	36.0(50)
CH Study[7]	Bleeding time > 7min.	100(2)	0 (1)	64.3(14)	50.0(6)‡
	Tourniquet test positive	100(2)	100 (1)	57.1(14)	50.0(4)‡
	Platelet count <100,000/mm³	100(2)	100 (1)	100.0(15)	83.3(6)
	Silicone clotting time >45 min.	100(2)	0 (1)	69.2(13)	50.6(6)‡
	Prothrombin time >14.0 sec.	0(2)	0 (1)	28.6(14)	16.7(6)‡
	Fibrinogen <180 mg%	100(1)	100 (1)	25.0(8)	33.3(3)

* Admission.
** 20% higher hematocrit on admission than after recovery.
† ( ) = number tested if different from figure at head of column.
‡ One patient with abnormal findings admitted in coma on 4th day after onset of fever. History suggests hypotensive episode prior to admission.

TABLE 6. ETIOLOGY, IMMUNE RESPONSE, DISEASE SEVERITY AND SEX IN
DENGUE HEMORRHAGIC FEVER AND CONTROLS. BANGKOK, 1962-1964

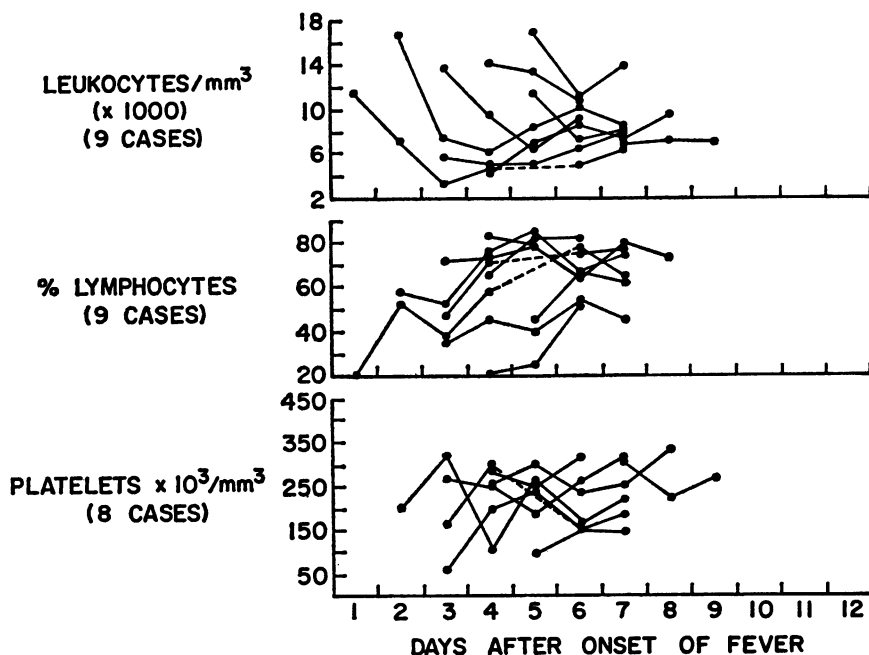|	Category	Male	Female	Total
Control	chikungunya	18	11	29
	no diagnosis	42	34	76
Dengue	primary, age <1 year	15	13	28
	primary, age ⩾1 year	25	18	43
	secondary, non-shock <1-3 years	39	35	74
	secondary, shock <1-3 years	24	29	53
	secondary, non-shock ⩾ 4 years	84	104	188
	secondary, shock ⩾ 4 years	45	92	137
	Totals	292	336	628

FIG. 2. Sequential leukocyte counts, percent lymphocytes and platelet counts in 9
patients hospitalized for hemorrhagic fever without shock, 1 year and older with a
primary dengue antibody response.

SECONDARY

LEUKOCYTES/mm³
(x 1000)
(12 CASES)

% LYMPHOCYTES
(9 CASES)

PLATELETS x 10³/mm³
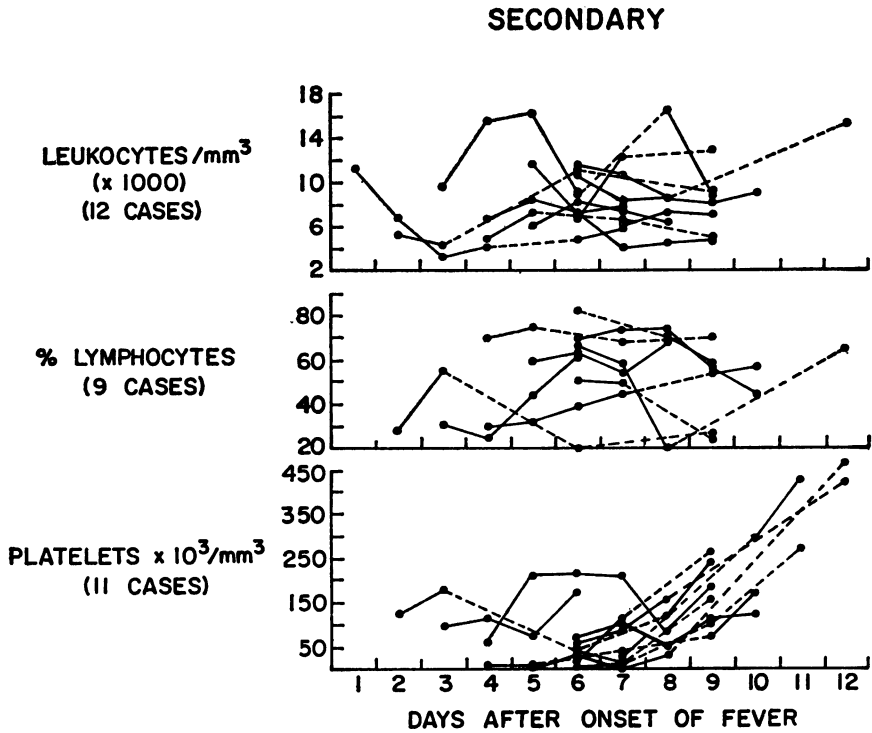(11 CASES)

DAYS AFTER ONSET OF FEVER

FIG. 3. Sequential leukocyte counts, percent lymphocytes and platelet counts in 11 patients hospitalized for hemorrhagic fever without shock and with a secondary dengue antibody response.

studies made during the acute phase of infection (Table 4). Many of these data have been published and arranged in a different form.[1,3,6] Because the number of observations is small, the large differences are not significant.

These data suggest that primary dengue in children and non-dengue illnesses resemble one another in having a similar low frequency of metabolic acidosis, elevated transaminases, azotemia, hypoproteinemia and hemoconcentration as compared with a higher frequency of such abnormalities in primary dengue in infants and secondary dengue cases. One third to one half of patients in the latter groups had the mentioned abnormalities, and in addition, a high rate of hemostatic abnormalities.

Differences in hematological response in primary and secondary dengue are illustrated in a somewhat larger group of patients in Figures 2 and 3. These data compare sequential white blood cell counts (WBC), percent lymphocytes and platelet counts in patients studied on three or more days.

The two groups include 9 children with primary dengue and 11 children with secondary dengue, who were *not in shock*. All but one of 9 primary dengue patients had a depression of WBC *or* sustained leucopenia ($<$5000/ mm^3) during the first six days of fever. All primary dengue patients had increases in percent of lymphocytes or relative lymphocytosis (lymphocytes $>$ 50% of total WBC). Although several primary dengue patients had a single platelet count below 100,000/mm^3, only one had a low platelet count on two or more days and these were above 100,000/mm^3. Patients with secondary dengue tended to have slightly higher total WBC and somewhat lower percent of lymphocytes than in the primary dengue group at the equivalent day of disease. The direction of responses was similar in both groups, i.e., decrease in WBC and increase in percent lymphocytes during days 1-6 after onset of fever. A marked difference in platelet counts was noted between the groups. All but 1 of 11 secondary dengue patients had pronounced thrombocytopenia on two or more days.

Although, as expected, the frequency of physiologic abnormalities increased with severity of clinical manifestations, *some* patients with secondary dengue and not in shock had thrombocytopenia, hypoproteinemia, elevated transaminases and hemoconcentration and these occurred more frequently than in controls (Table 5).

It has been noted that females predominated among patients hospitalized or dying of hemorrhagic fever in Thailand during the period 1962 through 1964,[8,9] and also among patients whose illnesses were virologically confirmed as dengue.[2] The predominance of females over males in hemorrhagic fever hospital statistics begins only in children older than three years.[8] Table 6 documents this same inversion in the normal male-female ratio at age four, but shows that this phenomenon occurs only in patients with secondary dengue. It is notable that in the shock group there were twice as many females as males. Although differences in the male : female ratio within the secondary infection groups are not significant, the sex ratio in children older than three years with secondary dengue with and without shock differed significantly from the sex ratio in patients with chikungunya combined with nondengue illnesses (p$<$.001 and .05$>$p$>$.01, respectively).

We were interested to know whether the antibody response differed with sex of the patient as a possible explanation for sex differences in severity of disease. HI antibody titers for the 192 males and 260 females with secondary dengue were tabulated from day 1 through day 22 after onset of illness. Values for each day were compared by t test. The only differences observed were that HI antibody titers were significantly higher in females on days 18 and 20. No trends were noted in data at other intervals after infection.

TABLE 7. ISOLATION OF DENGUE VIRUSES BY ANTIBODY RESPONSE AND DAY OF ILLNESS FROM PATIENTS WITH CONFIRMED DENGUE INFECTIONS*

		Primary response						
		Day of illness						
Virus type		*1*	*2*	*3*	*4*	*5*	*6*	*7+*
1		3	4	7	2	2		2
2		3	4	7	3	2		
3		1	2	7	4			
4				1				
Unident.		1	1		2			
Total isol.	no. sera tested	8/15	11/16	22/34	11/33	4/10	0/3	2/2

		Secondary response						
		Day of illness						
Virus type		*1*	*2*	*3*	*4*	*5*	*6*	*7+*
1		4	3	2				1
2		12	6	13	11	3	2	
3		7	3	7	4			
4					1		1	
Unident.				1		1		
Total isol.	no. sera tested	23/51	12/56	23/119	16/133	4/98	3/55	1/24

* Includes out-patients (6) and patients hospitalized for hemorrhagic fever (2) and other diagnoses (2), Bangkok, 1962-4.

Disease characteristics and dengue virus type recovered

As a measure of the sensitivity of the virus recovery systems employed, the virus isolation experience in hospitalized patients with primary dengue is summarized in Table 7. We were able successfully to recover dengue viruses in about two thirds of cases from plasmas obtained before the fourth illness day.

Virus recoveries from patients with secondary dengue were consistently lower than from primary dengue at every interval, although viruses could be isolated for the same length of time after onset of illness. Although virus isolation rates were lower in secondary as compared with primary dengue infections, no differences were noted in virus isolation rates in patients who had secondary dengue with or without shock who survived illness. Virus isolation rates in patients dying of hemorrhagic fever (Table 8) were lower than in the other two groups at every interval after onset of illness.

TABLE 8. DENGUE VIRUS ISOLATIONS FROM SERUM OR PLASMA OF 642 PATIENTS WITH SECONDARY ANTIBODY RESPONSES ARRANGED BY SEVERITY OF DISEASE*

Disease severity	Day of illness						
	1	*2*	*3*	*4*	*5*	*6*	*7+*
No shock	19/43	9/44	17/84	8/79	3/55	3/37	0/10
Shock, survival	4/8	3/12	6/35	8/54	1/43	0/19	0/8
Death	0/1	2/11	1/17	1/35	0/25	0/16	1/5

* Includes out-patients (6) and patients hospitalized for hemorrhagic fever (2) and other diagnoses (2), Bangkok, 1962-4.

The association between virus recovered and disease patterns in the host have been summarized in Tables 2 and 3. The day of hospitalization after onset of illness differed in patients with dengue type 1 and dengue type 2 infections. The modal day of admission of patients with dengue type 1 infection was day 2 while for patients with dengue type 2, it was day 5. This difference was significant ($.05 > p > .01$).

Finally, dengue type 2 virus was significantly associated with secondary dengue cases. Dengue viruses isolated from mosquitoes and from persons with primary dengue in Thailand, 1962-1964, are summarized in Table 9. Dengue viruses isolated from patients with secondary dengue at exactly the same time and in the same population are shown in Table 10. Distribution of viruses isolated in the two groups differed ($p < .001$).

TABLE 9. IDENTIFICATION OF 108 DENGUE VIRUSES RECOVERED FROM MOSQUITOES AND PATIENTS WITH PRIMARY DENGUE INFECTIONS. BANGKOK, 1962-1964

Patient group	Ref.	Dengue virus type				
		1	*2*	*3*	*4*	*Unident.*
Non-hospitalized*	(6)	4	5	6	1	2
Hospitalized-no shock†	(2)	13	14	10		1
Hospitalized-shock	(2)	3				1
Caucasians	(10)	8	9	9		
Mosquitoes	(8)	6	8	8	2	
Totals		34	36	33	3	2

* Children with virologically confirmed dengue infection studied in Children's Hospital Out-Patient Department, Bangkok, 1962-4.

† Children with virologically confirmed dengue infection admitted to Children's Hospital with diagnosis of hemorrhagic fever and other syndromes, Bangkok, 1962-4.

TABLE 10. IDENTIFICATION OF 84 DENGUE VIRUSES RECOVERED FROM PATIENTS WITH SECONDARY ANTIBODY RESPONSE BY DISEASE SEVERITY. BANGKOK, 1962-4

Patient group	Ref.	Dengue virus type				
		1	2	3	4	Unident.
Non-hospitalized*	(6)	3	11	5		
Hospitalized-no shock†	(2)	5	22	14	1	1
Hospitalized-shock	(2)	2	15	3	1	1
Totals		10	48	22	2	2

* Children with virologically confirmed dengue infections studied at the Children's Hospital Out-Patient Department, Bangkok, Thailand, 1962-4.

† Children with virologically confirmed dengue infections admitted to Children's Hospital with diagnoses of hemorrhagic fever or other syndromes, Bangkok, 1962-4.

DISCUSSION

This paper has presented several new observations on dengue hemorrhagic fever:

1) The bimodal age specific hospitalization rate curve, a regular feature of disease in Thailand,[8] is comprised of patients with different immunological response. The mode of infants under one year old was predominantly composed of primary dengue cases, while children with secondary dengue are grouped around a mode at four years.

2) Illness characteristics in infants with primary dengue and children with secondary dengue are similar; both differ from illnesses in children with primary dengue. The former group differs significantly from the latter in that illness was more severe with a higher incidence of either shock or hemorrhage. Our data suggest that patients in the former group were also alike in having a relatively higher incidence of hemoconcentration, elevated transaminases, hypoproteinemia, metabolic acidosis, azotemia, thrombocytopenia, elevated prothrombin time and prolonged silicone clotting time than children with primary dengue.

3) Severe secondary dengue illness occurs with significantly greater frequency in females than males, but only in children older than three years.

4) The dengue HI antibody response accompanying secondary dengue infection did not differ in females and males.

5) Dengue virus recovery rates did not differ with disease severity in patients with secondary dengue who survived infection, but were lower at every infection interval in patients fatally ill. Paradoxically, antibody levels were lower in fatal cases than in matched specimens from patients who survived infection.

6) The rate of recovery of dengue type 2 viruses from patients with secondary dengue was greater than from patients with primary dengue. Patients in both groups were studied at the same time and place.

Some of the observations above have received fuller discussion elsewhere.[4] Here we concern ourselves with an examination of the limitations imposed by methodology on the conclusions drawn from this material.

Perhaps the most important limitation to interpretation of our data is the degree of certainty that patients were correctly categorized as having a first dengue infection or a second dengue infection and further, whether persons with secondary dengue had antibody derived only from sequential dengue infection or other group B arboviruses.

In the absence of measurements of the rate of production of antibody by specific immunoglobulin type in the first instance, and prospective demonstration of sequential dengue infections in the second, direct confirmation of our assumptions cannot be achieved. It should be noted that our criteria for primary and secondary dengue classified responses observed in foreigners experiencing their first dengue infection as "primary."[10] Further, the age distribution of primary and secondary dengue in this study as well as in out-patients of the Children's Hospital conformed to the expected.[6] Finally, our criteria for primary and secondary dengue infections are closely similar to those used at the SEATO Laboratory by Russell and colleagues who found dengue antibody associated only with IgG in Thai DHF patients with secondary antibody responses.[11]

A large amount of data have already been presented and discussed that suggest that dengue viruses were the only group B arboviruses causing human infection in urban Thailand during the time of this study.[8,9]

The high rate of occurrence of severe secondary dengue in females older than three years is not related to the frequency of exposure to *Aedes aegypti* in persons of this age and sex group since we have shown that the prevalence of dengue antibody among children in Bangkok did not differ by sex.[8] Further, chikungunya viruses, also transmitted by *Aedes aegypti*, produced disease at a sex distribution which conforms to that in the total population. In out-patients, chikungunya disease and primary dengue infections also occurred more frequently in males than in females.[6]

We routinely tested for HI antibody only to a serum dilution of 1 :20,480. In the secondary dengue group, over one half of convalescent specimens had no end-point titer measured. For this reason, there is some uncertainty whether our comparative analyses of quantitative antibody response by sex of the patient or by severity of the disease are correct. The question asked is an important one and should be the subject of further study.

The high frequency of recovery of dengue type 2 viruses from secondary as compared with primary dengue infections is of great interest. Since this predominance of dengue 2 in secondary dengue was noted each year of the study, this observation suggests that *different* sequences of dengue infection result in variations in the ratio, shock/total secondary infections. If this were the case, the hitherto unexplained variations in annual DHF mortality ratio in Thailand would be understood.[8] Data currently available do not allow us to rule out completely the possibility that infected humans may produce larger quantities of dengue 2 than of other dengues resulting in more virus remaining unneutralized by heterologous antibody or early appearing secondary dengue antibodies, resulting in an apparent increase in dengue 2 disease.

SUMMARY

Host responses to dengue infection were investigated with respect to type of antibody response, age and sex of the patient, and type of virus recovered. A syndrome characterized by worsening of disease following two or more days of fever, shock, hemoconcentration, elevated transaminase, hypoproteinemia, acidosis, azotemia and abnormalities of hemostasis was seen in patients with secondary dengue infection *and* in infants less than one year old with primary dengue antibody response but infrequently in primary dengue infections after the first year of life. More girls than boys over the age of three years with secondary antibody response had severe responses to dengue infection. Dengue 2 viruses were more frequently isolated from patients with a secondary antibody response than were other dengue viruses, while the proportions of dengue 1, 2 and 3 viruses recovered from mosquitoes and patients with primary dengue infection were nearly equal. Both virus recovery rates as well as geometric mean HI antibody titers were lower at each illness day in patients dying of dengue infection than in those who were in shock but survived.

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