

DENGUE HEMORRHAGIC FEVER IN THAI INFANTS: IMMUNE
ENHANCEMENT OF INFECTION BY MATERNAL
ANTIBODIES

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BACKGROUND : A high incidence of DHF/DSS in infants age 6 to 10 months was first noted in Bangkok during the early 1960's. However, this important epidemiological observation was largely ignored during the late 1960's and early 1970's because (1) the phenomenon virtually disappeared and (2) the phenomenon was difficult to reconcile with the main working hypothesis that vigorous fixation of complement was important in the pathogenesis of DHF.

The discovery of "immune enhancement" or "antibody dependent enhancement, (ADE)" of dengue virus growth in cells with Fc receptors forced a re-examination of the possible mechanisms whereby partial (heterologous or sub-neutralizing) immunity could predispose to severe diseases in dengue virus infections.

One hypothesis which can reconcile the experimental *in vitro* observation of ADE with the epidemiological observation of infant DHF is that passively acquired maternal (transplacental) antibodies could cause *in vivo* ADE of dengue virus growth.

We therefore established studies (A) to determine if infant DHF did appear to be a distinct form of DHF on epidemiological grounds and (B) to investigate the role that maternal sera could have in infant DHF.

MATERIALS AND METHODS :

DHF patients : As detailed in another section of this annual report, all patients admitted to Bangkok Children's Hospital are routinely studied with (1) a complete clinical evaluation (S.N.) (2) paired acute and convalescent dengue HAI serologies and (3) virus isolation from acute blood by leukocyte cocultivation with LLC-Mk2 cells and/or intrathoracic inoculation of *Toxorhynchites* mosquitoes. Techniques used are listed in another section of this annual report.

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ADE study patients : Beginning in the latter half of 1979 and continuing through the 1980 calendar year, an acute blood sample was routinely obtained from the mother of all DHF infants (12 months old or less) at the time the child was admitted to the hospital. In most cases a convalescent blood sample was also obtained from the mother at the same time that a convalescent sample was obtained from the infant. Virus was isolated and typed from the acute infant blood specimen. Infant and mother acute and convalescent sera were tested for anti-dengue antibodies by HAI and by an "antibody capture" radio-immunoassay for IgM dengue antibodies. Thirteen well documented cases were selected for more intensive study which met the following criteria (Group I) :

- (1) Age \leq 12 months
- (2) Clinical diagnosis DHF grade II or more severe
- (3) A definite HAI seroresponse
- (4) A definite serum RIA IgM anti-dengue response
- (5) No evidence of recent dengue infection of the infant's mother either by HAI or IgM anti-dengue RIA.

Two additional groups (Group II "PUO infants," Group III "DHF toddlers" were chosen as controls, as defined in Table 1. Sera from all mothers in all three groups were tested for plaque reduction neutralizing antibodies to DEN 2 on LLC-Mk2 cells by routine laboratory methods. All maternal sera were also tested for their ability to "enhance" prototype DEN-2 virus (New Guinea-C, 33rd suckling mouse brain passage) growth on culture of P-388 D1 cells (a continues mouse macrophage-like cell line returning Fc and C3 receptors) as shown in Figure 1. Results were expressed as PFU/ml in the P-388 D1 cell culture fluids or as the ratio of PFU/ml in test versus control (virus plus diluent, no serum added) controls. As shown in Figure 2, normal (non-immune) human sera typically gave some non-specific increase in virus yield when tested undilute or diluted 1:10; at these dilutions immune sera invariably neutralized most virus.

We tested the hypotheses that :

- (1) Maternal anti-dengue 2 antibody titer should be directly related to infant age at onset of DHF.
- (2) Maternal sera should show *in vitro* ADE of growth of the dengue virus type which affected the infant (in these patients DEN-2).
- (3) Perhaps sera from mothers of DHF infants has greater ADE than sera from control mothers.

All maternal sera from groups I, II, and III were randomized and coded before being tested blindly for neutralizing and enhancing antibodies.

RESULTS : Figure 3 shows the age distribution of all confirmed DHF, DSS, and fatal DHF cases at Bangkok Children's Hospital from 1 January 1979 through 31 December 1980. An excess of disease of all degrees of severity is seen in infants less than 1 year old.

Figure 4 shows the proportion of primary and secondary HAI seroresponse, by age, of all seroproven DHF cases at Bangkok Children's Hospital, 1 January 1979 - 31 December 1980.

Figure 5 shows the proportion of primary and secondary HAI seroresponses by age, of all seroproven DSS cases, Bangkok Children's Hospital, 1 January 1979 - 31 December 1980.

Figure 6 shows the same data as in Figure 3 above, except only for ages less than 36 months, and broken down by 3 month intervals rather than yearly intervals.

Figure 7 shows the same data as in Figures 5 and 6 above, except only for ages less than 36 months, and broken down by 3 month intervals rather than by yearly intervals.

Figure 8 shows the differences in the convalescent HAI titers of infant DHF cases from the convalescent HAI titers in older children (toddlers).

Figure 9 is a summary of the hospital-based epidemiologic studies of DHF in Thai children.

Table 2 shows the correlation of the infant age at onset of DHF with his mother's Den-2 PRNT₅₀ titer.

Table 3 shows that although mothers of the three study groups had similar mean Den-2 PRNT₅₀ antibody titers, only in group I (infant DHF) did the maternal antibody significantly correlate with the age of the infant at the time of hospital presentation.

Figure 10 shows a schematic diagram of the mechanisms of dengue virus entry into Fc receptor bearing cells.

Figure 11 shows the ADE of dengue 2 virus growth in a representative experiment with three separate test sera. Typically maximum enhancement of virus growth *in vitro* is found at a dilution of serum at or just beyond the PRNT₅₀ titers. This association is demonstrated graphically for the 13 group I maternal sera in Figure 12.

Figure 13 is a schematic representation of the role of passively acquired (transplacental) antibodies in infant DHF.

Table 1.

		GROUP		
		I (N=13) DHF INFANTS	II (N=12) PUO INFANTS	III (N=12) DHF TODDLERS
AGE		< 12 MOS	< 12 MOS	13-60 MOS
CHILD	CLINICAL DX = DHF	+	+/-	+
	HAI DENGUE SERORESPONSE	+	0	+
	IgM ANTI-DENGUE RIA	+	0	ND
	DEN-2 VIRUS ISOLATED	+	0	+/-
MOTHER	HAI DENGUE SERORESPONSE	0	0	0
	IgM ANTI-DENGUE RIA	0	0	0

Table 2. Correlation of Infant Age at Onset of DHF with Mother's DEN-PRNT₅₀ Titer.

Group I	Mothers DEN-2 PRNT ₅₀	Calculated* Time for Infant Antibody to Fall to < 1:10 (Months)	Infant Age at Onset of DHF (Months)	Age Onset DHF - Age Antibody Disappearance (Months)
1	30	2	4	2
2	50	3	3	0
3	80	4	6	2
4	90	4	8	4
5	200	5	7	2
6	290	5	7	2
7	350	6	8	2
8	360	6	4	-2
9	420	6	8	2
10	500	6	6	0
11	720	7	8	1
12	2000	8	11	3
13	8200	10	12	2

* Assumes T $\frac{1}{2}$ Passive Antibody = 30 days.

Table 3. Correlation Between DEN-2 PRNT₅₀ Titers in Mothers and the Age of DHF Infants.

Group	Number (N)	Child Age (Months)	Mother DEN-2 PRNT ₅₀ (LOG ₁₀)	Child Age Vs. Mother PRNT ₅₀	
				r	p
I (INFANT DHF)	13	7.2 ± 2.6	2.5 ± 0.6	0.841	0.0003
II (INFANT PUO)	12	6.0 ± 3.7	2.3 ± 0.8	0.525	NS
III (TODDLER DHF)	12	36.0 ± 11.3	2.8 ± 0.5	-0.230	NS

Fig. 1

EXPERIMENTAL PROCEDURE FOR DEN-2 ENHANCEMENT TEST

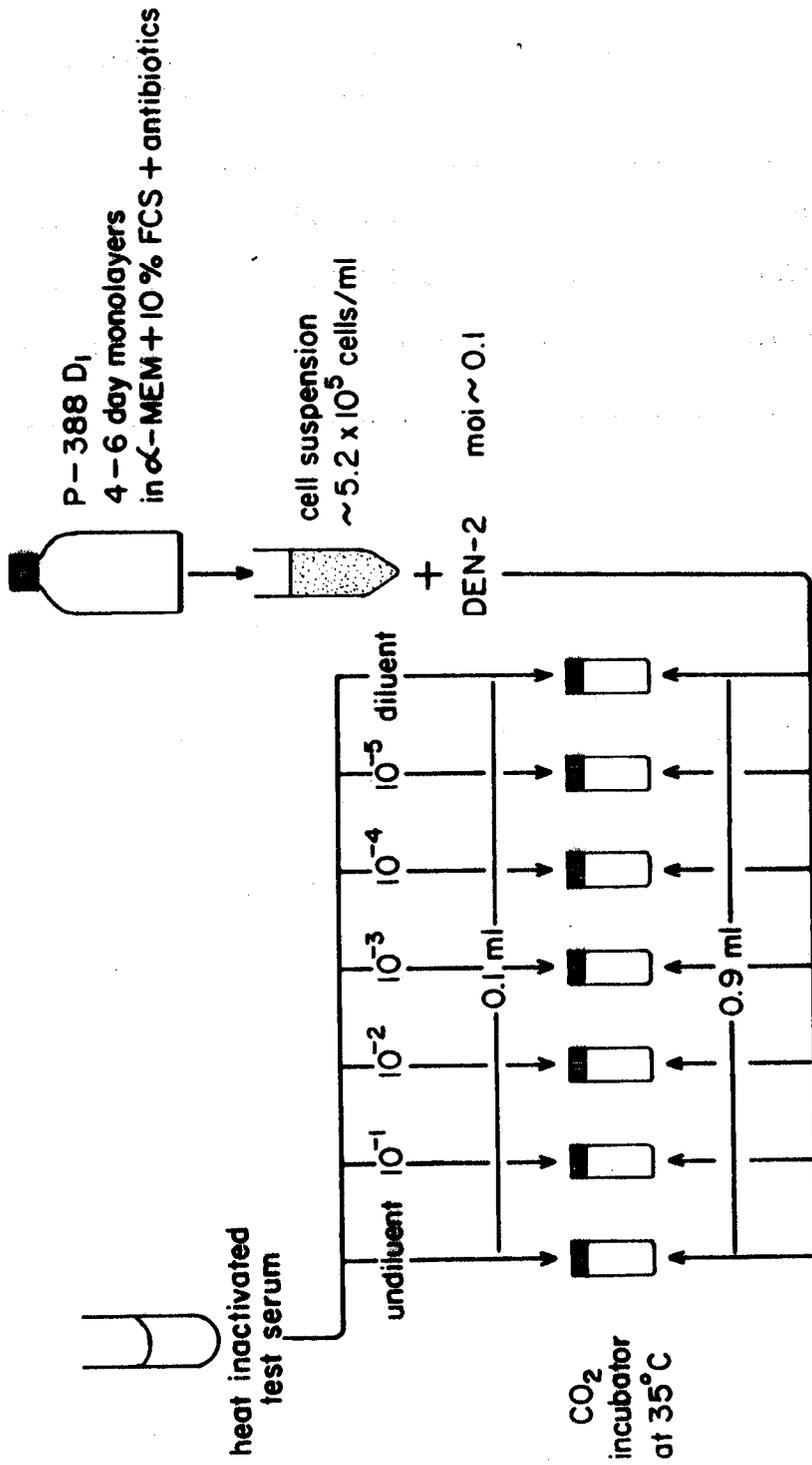


Fig. 2

Non specific enhancement of DEN 2 growth
by normal human serum.

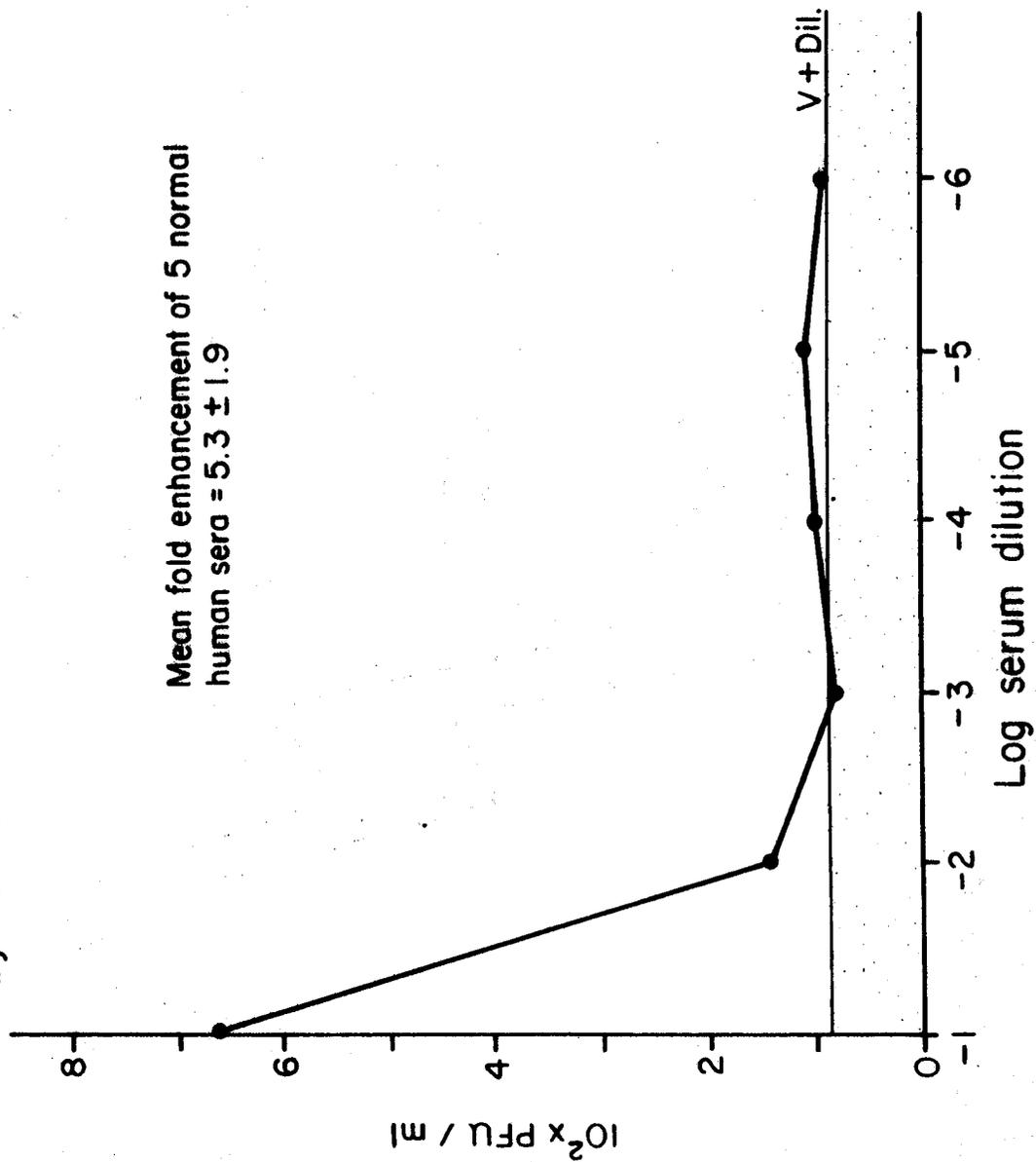


Fig. 3

Age distribution of all confirmed DHF, DSS, and fatal DHF cases, Bangkok, Children's Hospital, 1 Jan 1979 - 31 Dec 1980.

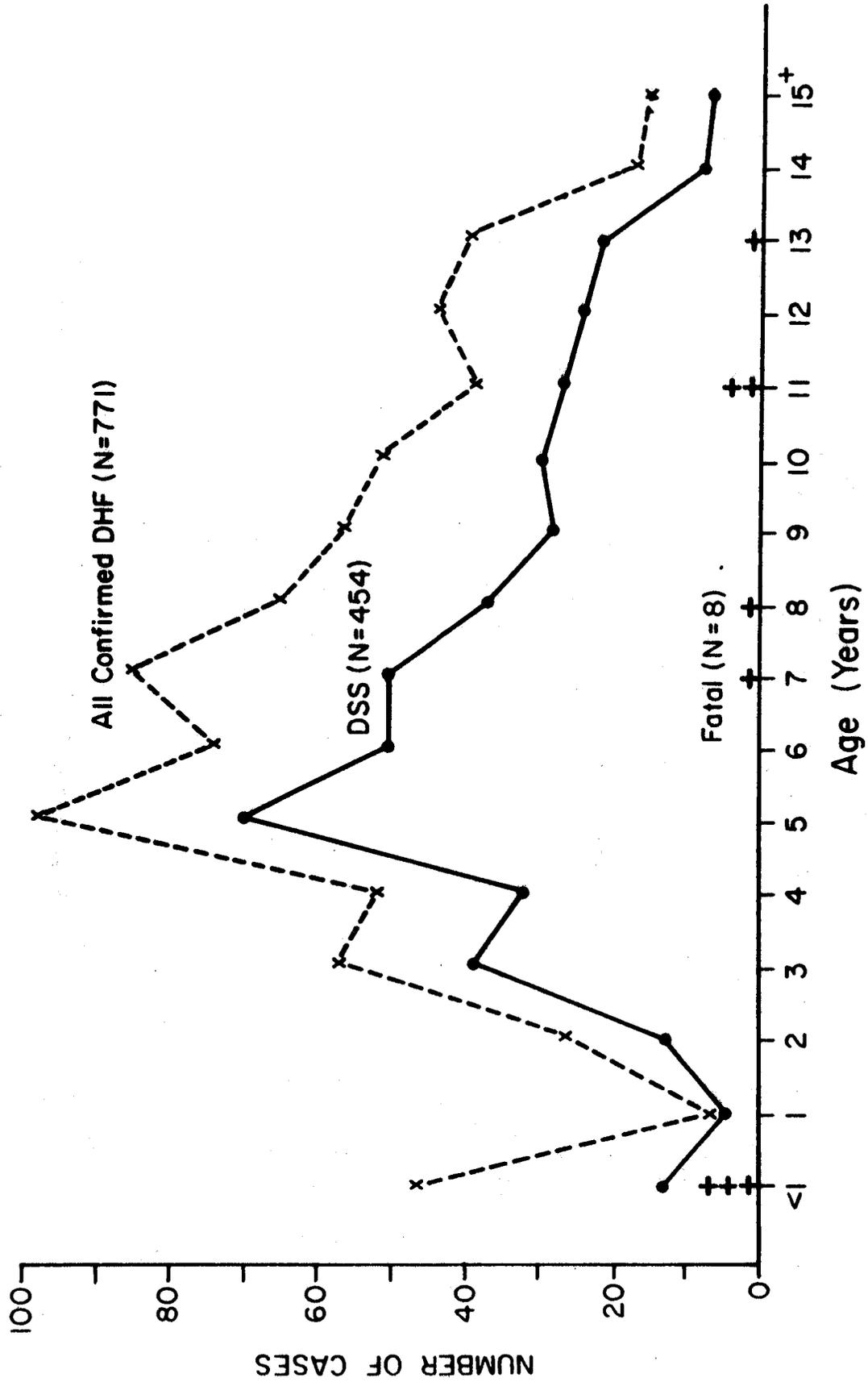


Fig. 4

Age distribution of all sero proven DHF cases, Bangkok Children's Hospital,
1 Jan 1979 - 31 Dec 1980

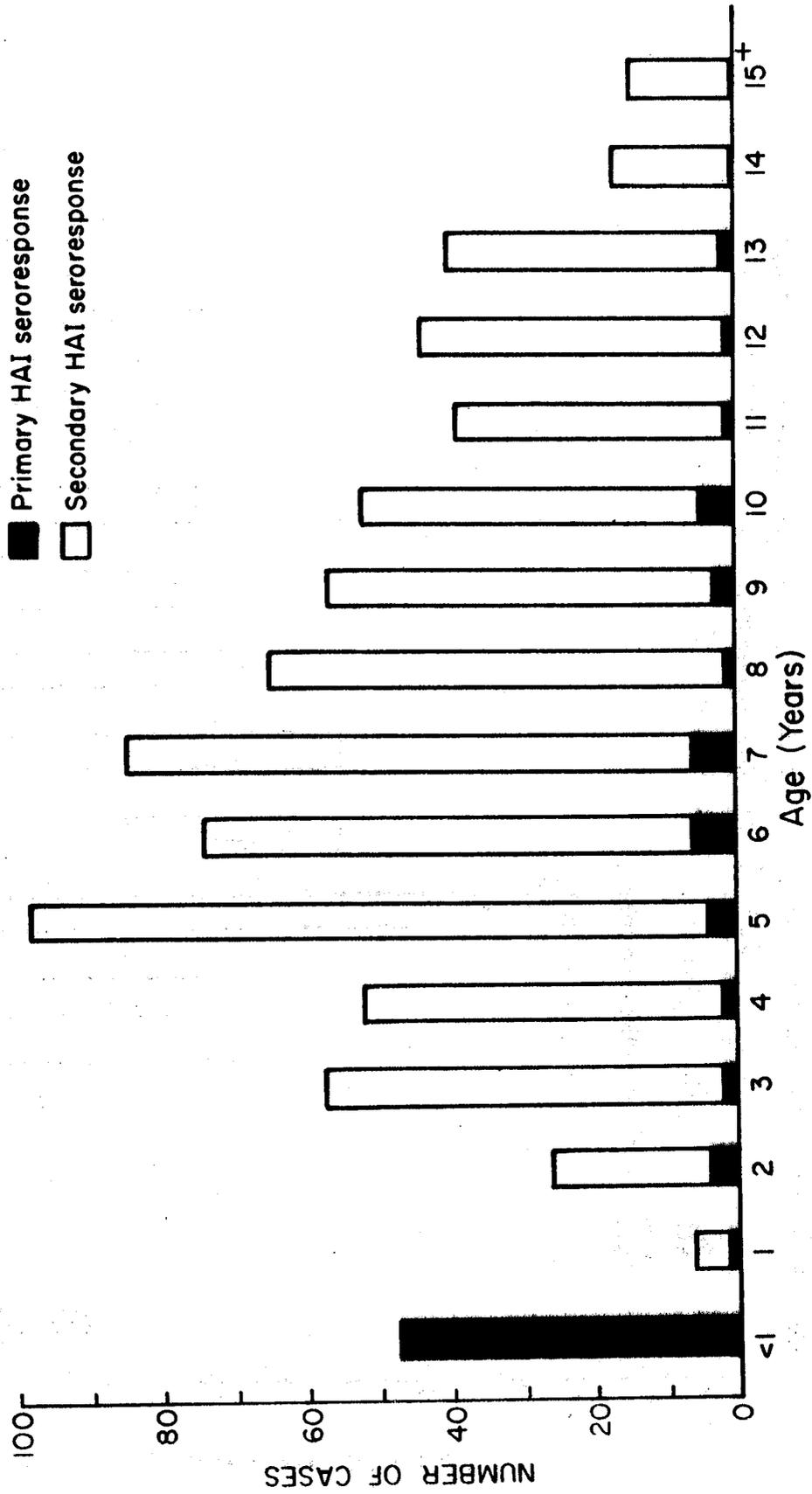


Fig. 5

Age distribution of all dengue shock syndrome cases, Bangkok Children's Hospital,
1 Jan. 1979 - 31 Dec. 1980

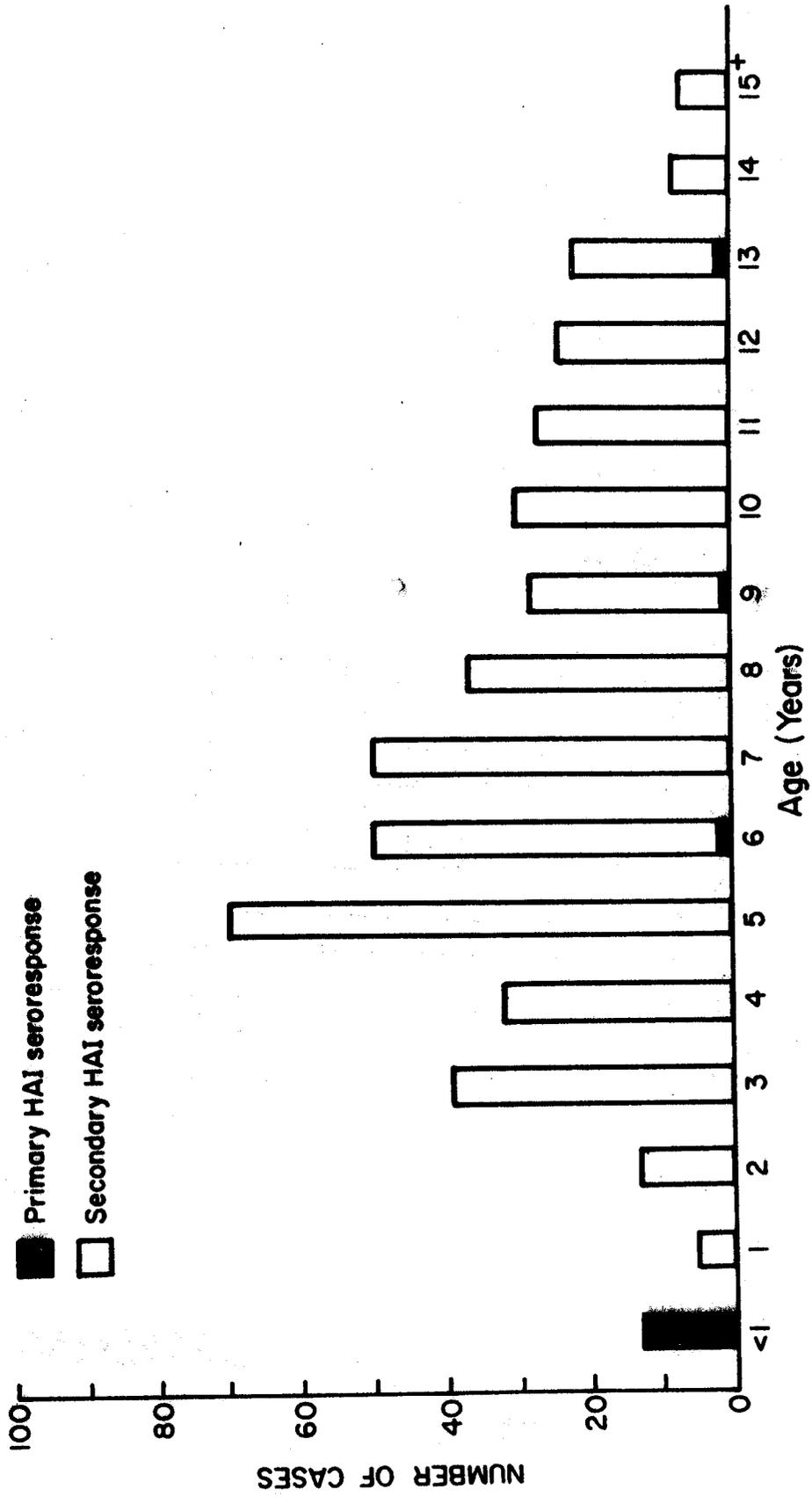


Fig. 6

Age distribution of all DHF, DSS, and Fatal DHF cases less than 36 months old, Bangkok Children's Hospital, 1979-1980.

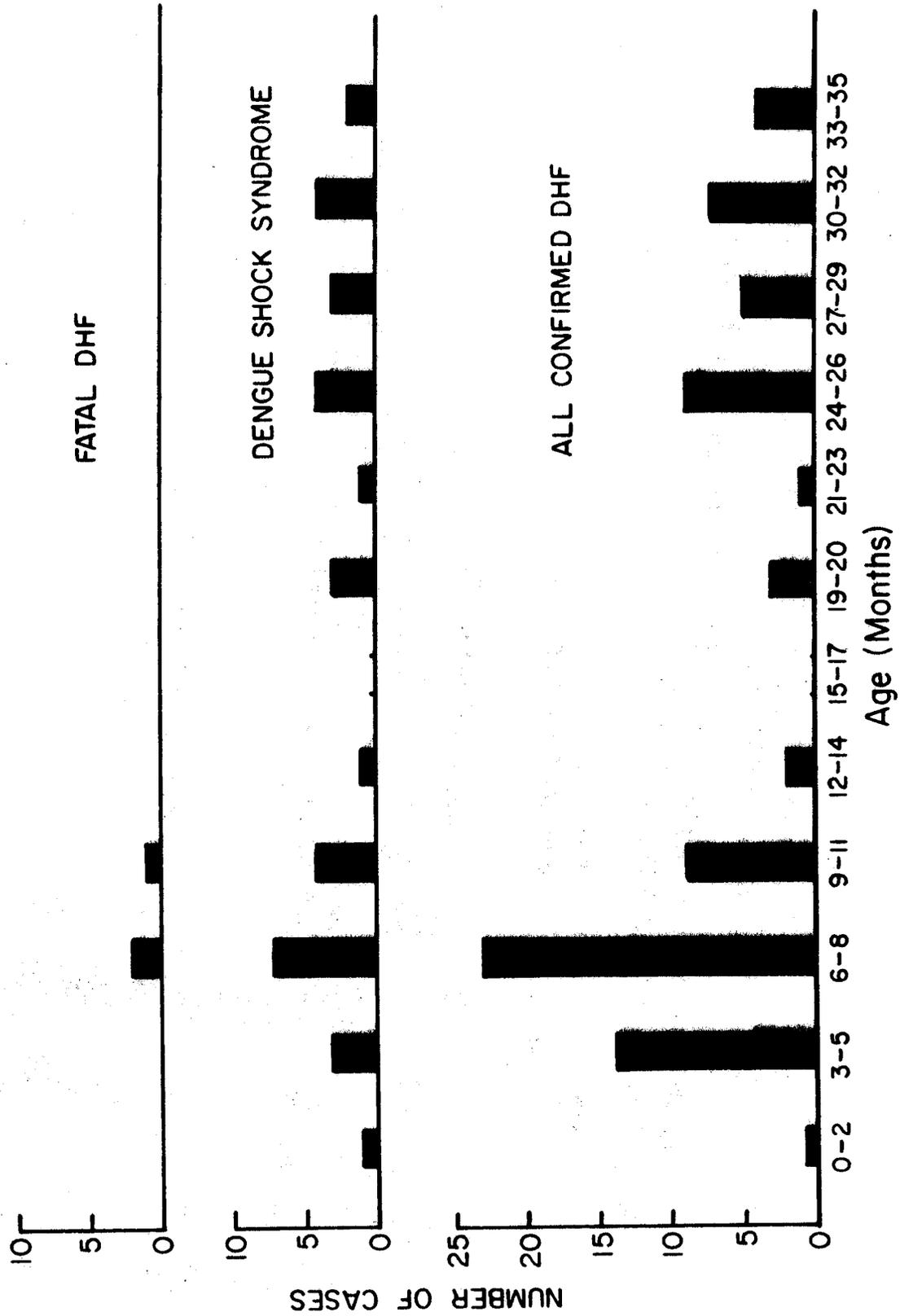


Fig. 7

Age distribution of all confirmed DHF and DSS cases less than 36 months old, Bangkok Children's Hospital, 1979 - 1980

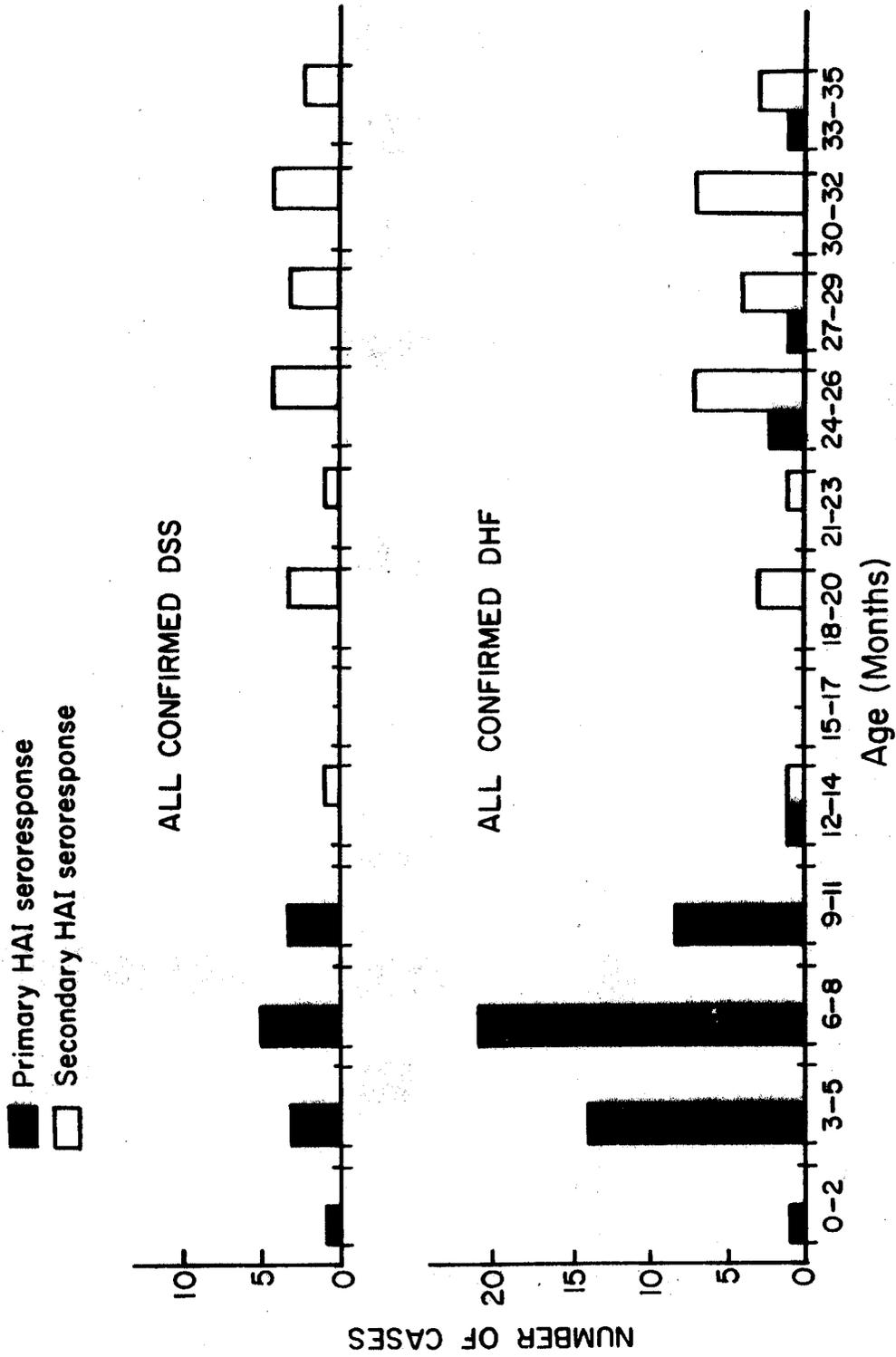


Figure 9

DHF IN THAI CHILDREN

- (1) AGE DISTRIBUTION IS BIPHASIC :

PEAKS AT AGES 6 - 8 MONTHS AND 5 - 6 YEARS.

- (2) SEVERE DISEASE OCCURS IN CHILDREN WITH
PRE-EXISTING IMMUNITY :

ACTIVE IMMUNITY IN OLDER CHILDREN → AMNESTIC
(2^o) ANTIBODY RESPONSE.

PASSIVE IMMUNITY IN INFANTS → PRIMARY (1^o)
ANTIBODY RESPONSE.

- (3) PEAK OF INFANT DHF AT AGE WHEN MATERNAL ANTIBODIES
ARE DECLINING TO UNDETECTABLE LEVELS.

Fig. 10

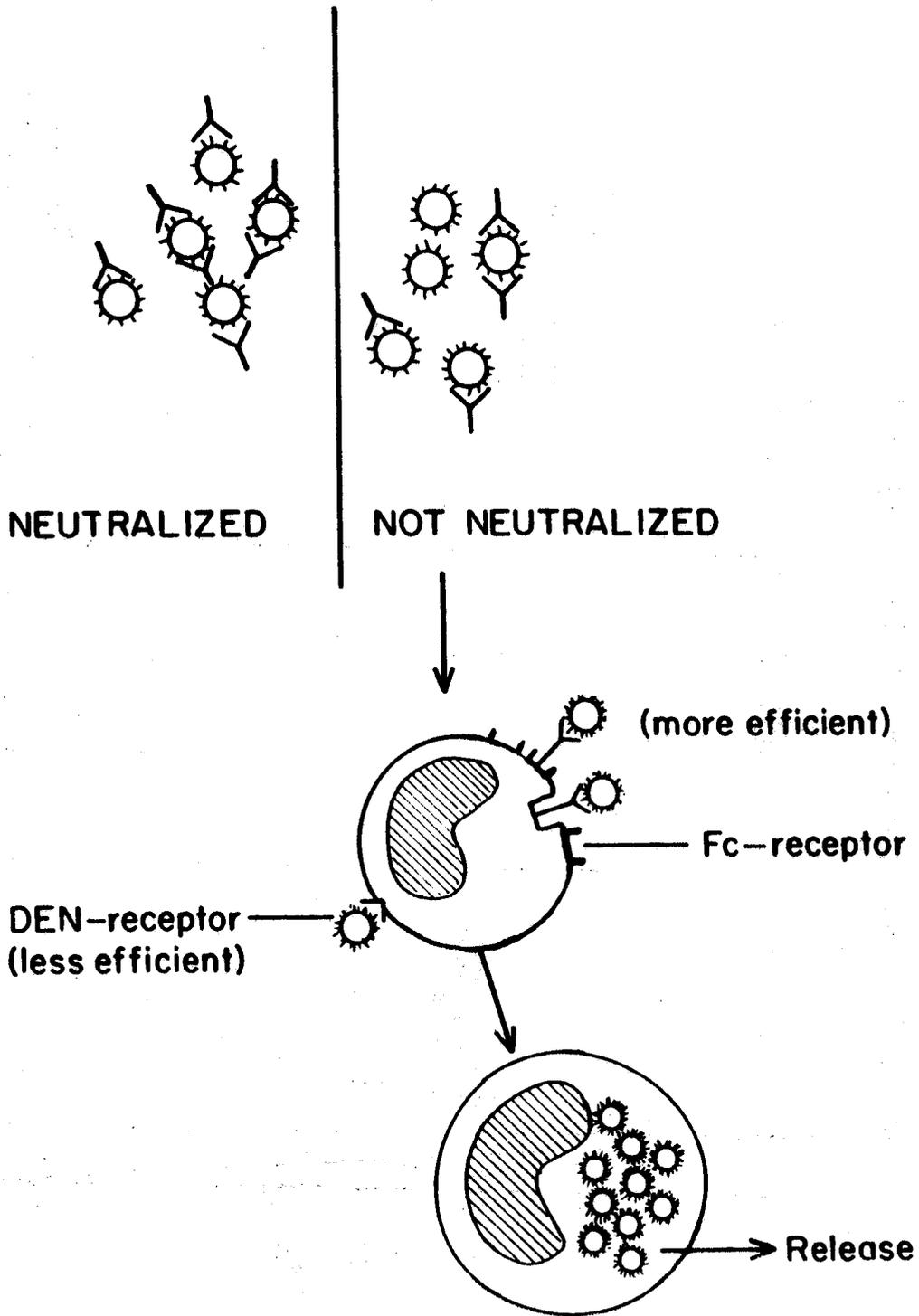


Fig. 11

Antibody dependent enhancement of DEN-2 virus growth

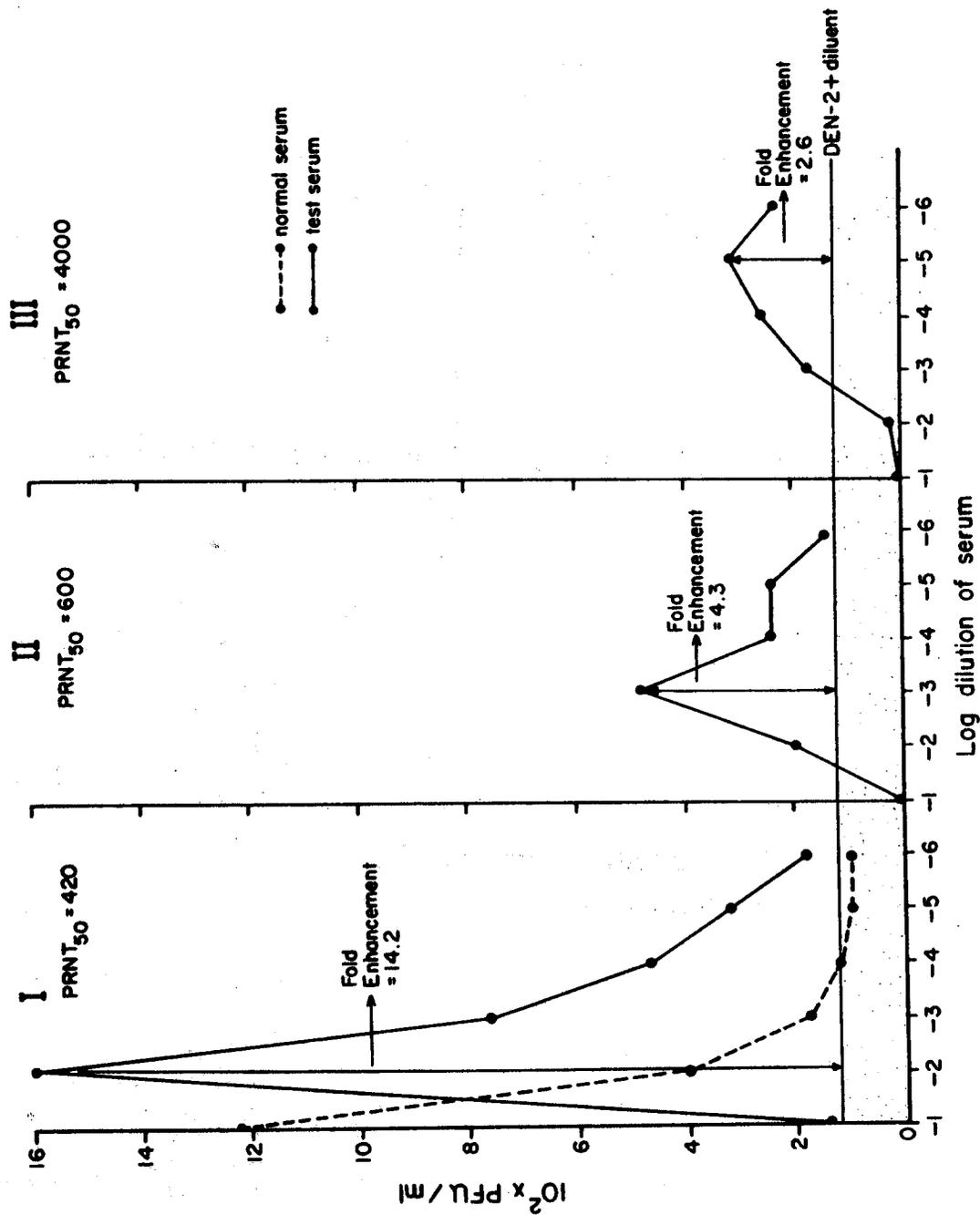


Fig. 12

Correlation between peak DEN 2 enhancement titer and DEN 2 PRNT₅₀

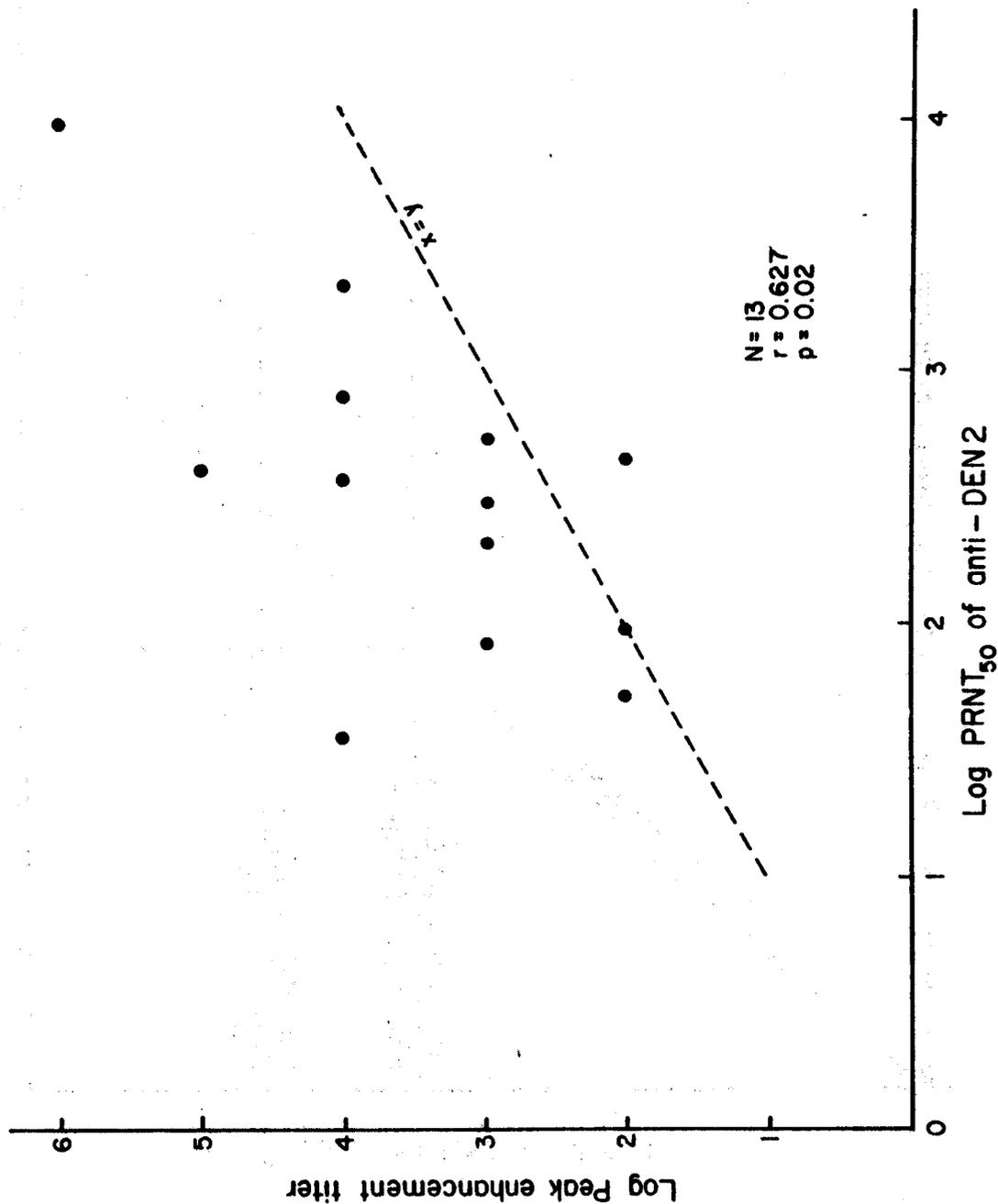


Fig. 13

