

Detection of Infectious Virus and Viral Antigens
in Tissue Specimens from Fatal Cases
of Dengue Hemorrhagic Fever

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OBJECTIVE : To detect viruses and viral antigens in tissue specimens from fatal cases of dengue hemorrhagic fever (DHF) and to identify the infected cell types.

BACKGROUND : Dengue viruses are rarely recovered from autopsy materials from patients dying of DHF. In 1964 SEATO Lab workers were able to isolate dengue virus from tissues of only two of 169 fatal cases of DHF examined (1). However, techniques for isolation of dengue viruses from clinical materials have improved considerably since earlier studies.

A body of evidence is growing to suggest that dengue viruses replicate, in primates, primarily within the reticuloendothelial system, predominantly within macrophages or other phagocytic cells. Virus and viral antigens have been detected circulating in the blood in a variety of mononuclear cells, but to date identification of viral antigen in human tissues has not been achieved, and the cell types involved have not been identified.

METHODS :

Fatal Cases : Four fatal cases of DHF at Children's Hospital and Ramathibodi Hospital during 1978 were studied. In three of the four cases the clinical diagnosis was DHF, and in one the clinical diagnosis was unclear, with Reye's syndrome and DHF being considered. The age distribution of fatal cases was quite different from the age distribution of non-fatal cases seen during 1978 at Children's Hospital; 3 cases were in infants less than one year old and one was in a 17 years old.

Case 477-78, on the basis of a high fixed hemagglutination inhibition (HI) antibody titer, was serologically assigned a presumptive diagnosis of DHF. In case 78-110 a clear four fold HAI titer rise was not observed, but the

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detection of anti-dengue-2 IgM antibodies by sucrose density gradient fractionation proved the occurrence of a recent dengue infection. No serum was obtained from case 318-78; the anti-dengue HAI titer in case 319-78 was too low to permit sucrose density fractionation for detection of IgM.

In cases 318-78, 319-78, and 477-78, autopsy findings were consistent with dengue hemorrhagic fever, with diffuse cutaneous and visceral petechia and hemorrhages, pericardial and pleural effusions, and ascites. In addition, in case 477-78, jaundice was present and the liver showed "central necrosis;" a test for HBsAg in the blood was negative. In case D78-110, pathological changes consistent with Reye's syndrome predominated with severe fatty metamorphosis of the liver and kidneys and cerebral edema; visceral hemorrhagic involvement was confined to focal subarachnoid hemorrhages and hemorrhage of the left lung; no serosal effusions were noticed.

Virus Isolation from Tissues : Fresh, unfrozen autopsy specimens were minced, washed three times with chilled RPMI 1640 media to remove blood, and then assayed for virus by the direct and delayed plaque method on LLC-Mk2 cells and by the direct and delayed plaque method on LLC-Mk2 cells and by the *Toxorhynchites splendens* mosquito inoculation technique.

Fluorescent Antibody Staining of Tissues : Fresh autopsy specimens were frozen in liquid nitrogen, sectioned into 4-6 micron sections, mounted and air dried on glass slides, fixed in 2-octanol at -30°C for 30 minutes, dried at 4°C for one hour, and stained with fluorescein-conjugated globulin from a pool of high titered sera from convalescent dengue hemorrhagic fever patients.

RESULTS : Results of virus isolation and fluorescent antibody staining assays of tissues from the autopsied cases are presented in Table 2. In cases D78-110 and 477-78 neither was virus isolated nor antigen visualized. In case 318-78, a plaque forming agent was found in the original cultures of LLC-Mk2 cells inoculated with minced mesenteric lymph node, lung, and spleen, but the agent was unable to be passed and efforts to reisolate the agent later from frozen tissue specimens were unsuccessful. Fluorescent cells were found in the liver, mesenteric lymph node and spleen of this case.

In the case 319-78 both virus and viral antigens were readily detected. Dengue-2 virus was isolated from heart blood, lung, kidney, and jejunum, while fluorescent cells were detected in every tissue examined except brain. A sample positive field from the liver is shown in Figure 1. The fluorescent cells in the liver appear to be sinusoidal lining (Kupfer) cells.

Specimens from the fatal cases are also being studied by electron microscopy.

REFERENCES

1. Nisalak, A., Halstead, S.B., Singharaj, P., Udomsakdi, S., Nye, S.W., Vinijchaikul, K. Observations Related to the Pathogenesis of Dengue Hemorrhagic Fever III. Virologic Studies of Fatal Diseases Yale J. Biol Med. 42:293-310, 1970.

Table 1. Fatal Cases of DHF at Children's Hospital and Ramathibodi Hospital, 1978.

Case No.	Age/Sex	Clinical Diagnosis	Day of Illness	HAI Serologies				Autopsy Diagnosis
				D1	D2	D3	D4	
318-78	7 mo F	DHF	-----	None available				DHF
319-78	5 mo M	DHF	"Acute"	10	20	10	10	DHF
D78-110	9 mo F	DHF+	3	10	80*	10	10	Reye's syndrome +/- DHF
		Reye's syndrome	5	10	160*	20	10	
477-78	17 yr F	DHF	8	2560	2560	2560	2560	DHF+ liver necrosis
		+ jaundice ? etiology	11	5120	5120	5120	5120	

* Anti-dengue-2 IgM shown to be present as 2-mercaptoethanol labile 19S HAI antibody in sucrose density gradient fractions of serum.

Figure 1. Liver Tissue from a Fatal Case of DHF Stained with Fluorescence in Labeled Pooled Convalescent DHF IgG.

