

The Pathogenesis of Dengue Hemorrhagic Fever. The Role of Biological Mediators: Histamine and Serotonin

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OBJECTIVE: A study of biological mediators of shock in dengue hemorrhagic fever.

BACKGROUND: Dengue hemorrhagic fever (DHF) differs from "normal" or "unmodified" dengue in the development of hemorrhagic phenomenon (a positive tourniquet test), "hypotension", (a low blood pressure and/or a narrowed pulse pressure), a decrease in plasma volume, (a rising hematocrit) and thrombocytopenia (a rapid drop in platelet count). The differences appear to be related to the formation of antigen antibody complexes (1), and it has been suggested that this is related to an individual experiencing a second dengue infection (2). These changes have a rapid onset suggesting the involvement of short-lived biochemical mediators.

At least two phenomena have been observed during the development of this illness which may be the source of these mediators. Observations suggest that there is activation of the complement system by antigen-antibody complexes with liberation of pharmacologically active components C3A and C5A (3). These low molecular weight polypeptides are potently vasoactive and their release leads to a marked increase in vascular permeability (4). They may act directly on the vasculature or by liberating histamine, slow reacting substance (SRS-A) and/or heparin from mast cells and white blood cells.

The other phenomenon is the decrease in platelets (5). Platelet counts often fall in a matter of hours from a normal level of approximately 250,000/mm³ to as low as 11,000/mm³. Platelets also return rapidly to supra-normal levels during the recovery phase (6). As the half life of the platelet is only two to three days this suggests that there is acute lysis of many platelets with supra-normal replacement from a hyperactive bone marrow. A similar but less marked phenomenon has also been noted with white blood cells (5).

The reason for the acute decrease in platelets is not clearly understood and requires further study. The lysis of platelets may lead to the sudden release of several potent vasoactive agents. Histamine, serotonin and heparin are all found in platelets and could be released into the circulation.

C3A and C5A are labile polypeptides and can not yet be accurately measured (7). SRS-A which is released from mast cells, has not been characterized and can only be measured inaccurately by a biological assay (8). Heparin is stored in both platelets and mast cells; it also is difficult to measure in serum.

Serotonin is manufactured in fairly large amounts in the chromaffin cells of the gastrointestinal tract. This vasoactive amine has a rapid turnover time. Most of the serotonin manufactured appears as metabolites in the urine within 24 hours; however, a small amount of it is taken up and stored in platelets (9). In man no serotonin is found in mast cells (10). The major metabolite of serotonin, 5 Hydroxy-indolacetic/acid (5 HIAA), can be measured in the urine by colorimetric analysis.

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Histamine is a major storage product of mast cells with a small amount being stored in platelets (9). In the past it could be measured only by biological assay but recently a sensitive radioenzymic assay has been developed (11). The purpose of this study was to determine whether the excretion of histamine or 5 HIAA increased in the urine during the development or the course of DHF as compared to other febrile diseases and normal controls.

DESCRIPTION: This study was an integral part of other dengue studies performed in collaboration with the Children's Hospital of Bangkok during 1974. During the dengue epidemic season from 24 June to 26 August 1974 all patients admitted to the ward service with an admission diagnosis of DHF and appropriate febrile and afebrile controls were accepted for study. Blood samples were taken daily in heparinized syringes for the first five days in hospital and then approximately 15 and 30 days following hospitalization. Plasma was submitted for virus isolation using a direct and delayed plaque technique previously described and a mosquito isolation technique (see elsewhere this report). Plasma was also used for the detection of antibodies to dengue types 1-4 and Japanese Encephalitis virus using a hemagglutination inhibition (HI) technique. Urines, collected over either 8 or 12 hour periods, were obtained from each patient in plastic bottles and stored on wet ice. At the conclusion of the collection period these were divided into aliquots in plastic containers using toluene as a preservative and frozen at -70°C . A white blood count, differential count, platelet count and hematocrit were performed on each blood obtained. The clinical status of each patient was assessed by a physician at least once every 12 hours and usually more often. All clinical and laboratory details were recorded on a flow sheet, which was kept on each case.

Patients to be further studied for the presence of biological mediators were selected retrospectively after all clinical and laboratory information was evaluated.

PROGRESS: During this two month period at the height of the dengue season only 64 patients with an admission diagnosis of DHF were collected. This, however, represented almost 50% of the dengue patients collected through the entire year, as there was a low incidence of disease during 1974. Of these 64 patients, 11 or 18% did not have laboratory evidence of dengue virus infection, one patient was not adequately followed and three patients died. From the three patients who died no convalescent sera was obtained and therefore no judgement could be made on the patient's prior experience with dengue. Table 1 shows a breakdown on the clinical grading and type of convalescent antibody response seen in the 49 patients who were studied.

Of these patients five were selected who exhibited DHF grades 1 or 2 and five were selected who exhibited DHF grades 3 or 4. Three patients with bacterial infections and five non-infected children were selected as controls. All urine samples collect on these children from the time of admission until two days after the period of shock were submitted for biochemical analysis of creatinine, 5 HIAA and histamine. The 5 HIAA and creatinine assays were done at SEATO Medical Research Laboratory. Urine for histamine analysis was forwarded through Walter Reed Army Institute of Research to Dr. Michael Beaven at the NIH in Bethesda, Maryland to be tested for histamine by radioenzymic assay. When all of the assays are completed the data will be examined to determine whether the excretion of histamine or serotonin breakdown products was related to the development or severity of DHF.

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Table 1. Clinical Grading and Type of Convalescent HI Antibody Responses seen in 49 Dengue Patients Collected Between June 24 and August 26, 1974

Grade of Disease	Primary Infection ≤ 1:640	Secondary Infection > 1:640
UF*	1	3
1 & 2	5	22
.3	2	12
4		4
TOTAL	8	41

* Undifferentiated fever