

ANNUAL PROGRESS REPORT

SEATO Medic Study No. 95: Studies on the Pathology of Thai Hemorrhagic Fever: Pathological Studies of the Superficial Vessels of the Skin

Project No. 3A 025601 A 811: Military Medical Research Program S. E. Asia

Task 01: Military Medical Research Program S. E. Asia

Subtask 01: Military Medical Research Program SEASIA (Thailand)

Reporting Installation: U.S. Army-SEATO Medical Research Laboratory, APO 146, San Francisco, California

Division of Medical Research Laboratories

Department of Geographic Pathology

Period Covered by Report: 1 April 1963 to 31 March 1964

Principal Investigator: Natth Bhamarapravati, M.D. *

Associate Investigators: Captain Sylvanus W. Nye, USAF, MC **
Sompote Bukkhavesa, M.D. **
Nivat Chandarakula, M.D. **
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Reports Control Symbol: MEDDH-288

Security Classification: UNCLASSIFIED

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ABSTRACT

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The objective of this study is to describe the morphological and functional aspects of the blood vessels in Thai Hemorrhagic Fever in an attempt to elucidate the changes which occur before peripheral vascular

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collapse. In order to do this, biopsies of skin will be performed on patients who have been admitted to the Pediatric Department, Siriraj Hospital. Biopsies will be obtained from infants and children of the same age group. Clinical records will be prepared. Blood will be drawn for laboratory studies to include a hemogram, liver function test, coagulation status, and paired antibody titers for dengue virus and chikungunya viruses. Results of the urinalysis, stool examination, bone marrow examinations, will be recorded. The biopsies of the skin will be taken with a skin punch and will be fixed in formalin and osmic acid and quenched in either dry ice acetone or isopentane liquid nitrogen. Routine and special stains will be done on formalin fixed tissue and the frozen tissue will be used for enzyme studies. Cryostat sections will be stained with fluorescent dengue antibody. No biopsies have been collected and no results or conclusions are available.

BODY OF REPORT

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Objective: Studies on the Pathology of Thai Hemorrhagic Fever conducted on fatal cases have shown three basic things. Namely: 1. vascular changes, 2. reticulo-endothelial changes and 3. hepatic changes. The objective of this study is to further elucidate the vascular changes which include diapedesis of red cells into perivascular tissue, edema of the wall of small blood vessels, perivascular infiltration by round cells, and effusion into serous cavities.

Description: Physiological studies have revealed that some 12 to 24 hours before the peripheral vascular collapse in Thai hemorrhagic fever, there are evidences pointing towards a generalized capillary damage. The tourniquet test becomes positive, petechiae appear and a gradual hemoconcentration takes place while the plasma volumes decrease. The packed cell volume is slightly reduced while there is a minimal evidence of cardiac disfunction. Thus, it seems that a decrease in plasma volume rather than a loss of blood, or cardiac failure is probably the most important cause of the peripheral vascular collapse. In fatal cases, additional evidences supporting this hypothesis of vascular disfunction have been obtained through the study of adrenal glands which show overwhelming changes in the zona glomerulosa which may occur as a result of the lowering of blood volume augmenting the effects of aldehyde.

In a study of the changes in lymph nodes obtained from three cases of hemorrhagic fever done elsewhere, during the beginning of the recovery period proliferation of endothelial cells of the vessels in lymph nodes has been described and may reflect the recovery of the vessels from previous injury.

Clinical and virological studies have shown that dengue and chikungunya viruses are related to the disease in Thailand, however, serious cases of hemorrhagic fever with peripheral vascular collapse are probably caused only by dengue virus, while mild cases may be caused by chikungunya virus. Thus, it appears that there may be a difference either in the degree or the character or both of the vascular injury between the two viruses.

The pathogenesis of the vascular injury is uncertain. The possibility that the vascular injury may occur as a direct effect of viral multiplication in the endothelial cells and a viral release from these cells has to be considered. Another possibility is that the damage to the walls of the vessels may be due to the deposit of antigen and antibody complex from the general capillary bed. This hypothesis is supported by the fact that the vascular disfunction occurs toward the end of viremia at the time when evidence of active antibody formation are apparent.

As it is not possible to study the blood vessels of the visceral organs, the skin offers the most accessible tissue for study. The changes in the skin may reflect the pattern elsewhere in the body. Biopsies of the skin will be performed on patients who are admitted at the Department of Pediatrics, Siriraj Hospital, with a clinical diagnosis of Thai hemorrhagic fever. The biopsies will be obtained in the suspected cases during the various stages of the disease, namely: pyrexia, shock and recovery. If possible, repeated biopsies will be performed on the same individuals at intervals in order to observe the changes at different clinical stages.

In addition, clinical and virological studies will be performed and recorded in a central registry for correlative analysis. Control biopsies of the skin will be obtained from infant children of the same age groups. The biopsies will be taken by a punch biopsy method. Blood for laboratory studies will be drawn on the day the biopsy is done. Serum will be separated and frozen for paired antibody studies. The last specimen for the antibody studies will be obtained on the day the patient leaves the hospital. The laboratory studies will include: hemogram, transaminases, prothrombin time, bleeding time, coagulation time, paired antibody titers for dengue viruses, and chikungunya viruses, urinalysis stool examination, bone marrow examination and platelet counts.

Pathologic studies will be done in four areas, a portion of biopsy will be fixed in neutral formalin for routine sectioning and embedding. This tissue will be stained with hemotoxylin and eosin. Alcian blue periodic acid Schiff, phosphotungstic acid hemotoxylin, Giemsa and Fielgen or Methyl green pylonine stains. A second portion of the biopsies will be quenched either in dry ice acetone or in isopentane cooled with liquid nitrogen. Sections will be cut in the cryostat and stained for alkaline phosphatase, adenosine triphosphatase, DNP-diaphorase and succinic dehydrogenase. Third, a tiny portion of the biopsy will be fixed in osmic acid for electron microscopic study. In connection with this portion of the study, it is hoped to send one of the investigators to Armed Forces Institute of Pathology for additional training in electron microscope techniques. Finally, cryostat sections will be stained with fluorescent anti-dengue or fluorescent anti-chikungunya antibodies.

In the analysis of the material, attention will be paid to pathophysiologic features of the blood vessels; constriction or dilatation at different stages of the disease, changes in endothelial cells, trapping of leukocytes, sludging or erythrocytes, diapedesis of erythrocytes and infiltrates of leukocytes into the tissue. A semi-quantitative estimation of the nucleic acid in endothelial cells will be made. Alterations in the ground substances in the skin, number of mast cells, and degranulation of mast cells will be noted. Alterations in the pattern of enzymatic staining of vessels walls will be searched for. A concentration of, absence of gamma globulin or complement and viral antigen on vessel walls will be looked for.

Progress: No biopsies have been collected at present, and the materials for the study are being collected.

Conclusion: None