

## BODY OF REPORT

SEATO Medic Study No. 95                      Studies on the Pathology of Thai Hemorrhagic  
Fever. Pathological Studies of the Super-  
ficial 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:                              US Army-SEATO Medical Research Laboratory  
APO San Francisco 96346

    Division of Medical Research Laboratories

    Department of Geographic Pathology

Period Covered by Report:                              1 April 1964 to 31 March 1965

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Reports Control Symbol:                              MEDDH-288

Security Classification:                              UNCLASSIFIED

## FINAL REPORT

Objective: The objective of this study is to describe the morphological and functional changes of the blood vessels of the skin obtained by biopsy from patients who are suffering from Dengue hemorrhagic fever. It has been shown by

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clinical and laboratory examination that there is a widespread capillary damage in the patients resulting in a leakage of plasma protein, water and electrolytes into the extra-vascular component leading to a marked reduction in plasma volume, and to shock. In some cases, diapedesis of red cells is also present which is due to some intrinsic changes in the vessel wall alone or in combination with thrombocytopenia and coagulation abnormalities which are not infrequently observed. Morphological studies on autopsy tissue reveal only non-specific alterations of the small blood vessels, i.e., edema of the wall and perivascular tissue, perivascular diapedesis of red cells, and rare infiltration by mononuclear cells. It is realized that the changes seen in autopsy material represent the states of the tissue during or after several hours of shock. It is thus desirable to know the changes during the febrile or preshock period as well as during the convalescent period. Since some vascular changes have been observed in the skin (petechiae, positive tourniquet test), and the fact that the skin is the most accessible tissue for biopsy with a minimum degree of risk and discomfort to the patients, this study is initiated.

Description: Biopsies of the skin were performed in patients who suffered from hemorrhagic fever at Siriraj Hospital Medical College. The biopsy was obtained from the anterior surface of the thigh in all cases regardless of the presence or absence of skin manifestations in that particular area. The biopsy was done without the application of local anesthetics, using rotary punch biopsy instrument. This yields a circular piece of skin 3 mm. in diameter within 10-15 seconds. The biopsies were fixed in 10% neutral formalin, embedded in paraffin, sectioned and stained with hematoxylin-eosin, PAS and Giemsa. Portions of the skin biopsy were divided in some cases. These were quick frozen and sectioned in a cryostat and stained for activity of certain hydrolytic and oxidative enzymes, as well as with fluorescent antidengue and antihuman gammaglobulin antibodies.

Result: Twenty-three biopsies of the skin were obtain from proven cases of dengue hemorrhagic fever and twelve from cases with clinical picture resembling hemorrhagic fever but showed inconclusive serologic evidence for the infection. In addition biopsies were obtained from 2 cases of cerebral palsy, one case of enteric fever, 3 cases of nephrotic syndrome, and one case of measles with hemorrhagic rash. These were used for controls. The age of these cases varied from 6 months to 12 years.

Among the twenty-three biopsies from proven cases of hemorrhagic fever, five were obtained during the febrile period, five from the threatened or pre-shock period, six during the shock period and seven during the convalescent period.

The findings of the skin are summarized as follows:

Control Cases: The skin in control cases shows essentially normal epidermis. The connective tissue in the upper part of the corium is homogenous and dense while the lower part of dermis, bundles of collagen fibers are seen. In cases of nephrotic syndrome there is some edema of the connective tissue with separation

of the collagen bundles. The small arterioles in the upper and lower part of the corium and the papillae show wide lumen and occasionally, red cells are seen inside. Alongside the media and adventitia, mast cells with closely packed dense granules are observed. For vessels which are cut at cross section, usually 1-2 mast cells are present, while vessels which are cut tangentially, there are up to 3-4 mast cells depending upon the length of the vessels. Mast cells which lie along the cross cut vessels usually are round in appearance while those that lie along the tangential cut vessels may be round or spindle shaped. In cerebral palsy and 2 nephrotic syndrome cases, the number of mast cells around the blood vessels is minimal while in one case of nephrotic syndrome, and in the case of typhoid, there is a slight increase in number of mast cells (average 2-3 for one arteriole cut in cross section). The granules of mast cells in these cases are still packed tight in the cytoplasm. Around the vessels in the upper dermis and papillae occasional lymphatics are noted. Mast cells are also present in the tissue close to the basement membrane of the peripheral nerves. Occasionally individual mast cells with stellate appearances and contain metachromatic granules are seen lying free in the connective tissue of the dermis.

Biopsies during the febrile period: Biopsies were obtained from five patients. These patients had had fever from 4 to 7 days before the biopsy was done. The tourniquet test was positive in 3 cases, thrombocytopenia present in 4 cases, and petechiae over the body and extremities were observed in 2 cases. These patients received only symptomatic treatments. Microscopic examination reveals intact epidermis and skin appendages in all cases. The wall of the blood vessels show no significant changes and the lumen are dilated and occasionally filled with red blood cells. Diapedesis of red cells is noted in 3 instances. The erythrocytes are seen lying around the wall of the vessels. Slight perivascular edema was seen in 2 cases. The number of cells around the blood vessels was increased in 2 cases. The cells are mononuclear cells possibly lymphocytes, and some mast cells. Loosening of the granules and slight vacuolation of the cytoplasm of the mast cells is noted in 4 cases. In three of these the changes occur around both upper and lower dermal vessels while in one case, only upper dermal vessels are involved. The number and appearance of free mast cells in the connective tissue is not unusual.

Threatened Shock: Biopsy was obtained from five patients. They had been in fever for 5 to 6 days and were showing some evidence of impending peripheral vascular collapse at the time of biopsy. Positive tourniquet test and thrombocytopenia were present in all of these cases, while petechiae were noted in three cases. Microscopic examination revealed that in 2 cases, epidermal blebs containing erythrocytes are seen; and in 4 cases, diapedesis of red cells was observed. Some acidophilia of the cytoplasm and slight pyknosis of the nuclei of the endothelial cells is noted and slight edema around the vessels is seen in 2 cases. The lumen of the blood vessels is seen to be patent in 2 cases but erythrocytes are not visible inside. In one case the lumen is congested with erythrocytes while in 2 other cases the vessels are constricted. Increase in the number of cells around the blood vessels is noted in 2 cases, the cells being both

lymphocytoid and mast cells. In two cases, a moderate to severe degree of degranulation of mast cells is observed. The cytoplasm contains smaller number of granules, and occasional granules are seen lying outside the cells. In one of these two cases, degranulation is more prominent around the upper dermal vessels including the papillar vessels. Slight vacuolation of the cytoplasm of the mast cells was noted in two cases while in the last case of this series the granules cannot be evaluated due to technical artifacts. It is also noted that in the 2 cases where significant degranulation is noted there is an increase in number of mast cells which lie freely in the dermal connective tissue. Some of these cells are bipolar and could not be differentiated with any certainty from fibroblasts, which may have taken up the discharged mast cell granules (Tephocytotic fibroblast).

Shock Period: Biopsy was obtained from six cases who were in the stage of shock for a period from 5 hours up to 24 hours. All but one had fever for 4-5 days before the stage of shock while the last case had fever for 10 days. Positive tourniquet test and thrombocytopenia were present in 5 cases, and petechiae in 3 cases. These patients were treated with fluid therapy and in one case with high dosage of hydrocortisone 48 hours before the stage of perivascular collapse.

Microscopic examination reveals swelling and acidophilia of the endothelial cells of the blood vessels in 3 cases, a moderate degree of perivascular edema in 3 cases, and collapse of blood vessels in all 6 cases. Diapedesis of erythrocytes was seen in only 2 cases. In 2 of these cases, a slight increase in the number of cells around the blood vessels is observed. A marked degree of degranulation of mast cells is seen in 3 cases, while in 2 cases there is a moderate degree of degranulation with more severe degranulation in the upper dermis. In one case only slight degranulation is observed. The number of free mast cells was increased in 2 cases.

Convalescent Period: Biopsy was obtained from seven cases during the convalescent period, every one of which went through a period of peripheral vascular collapse. The biopsy was performed between 2-3 days after recovering from shock in 5 cases, and 10 and 15 days after recovering in two instances. At the time of biopsy the tourniquet test was positive in 5 cases, and petechiae were seen in two cases. Six patients received hydrocortisone during the course of illness.

Microscopic examination reveals that in cases which were biopsied within 3 days after recovery, the blood vessels show no significant changes and a moderate degree of perivascular edema is noted in only 1 case. The lumen of the vessels contain red blood cells in 4 instances, while in one the lumen is dilated but red blood cells are not seen and in other one the vessels appear collapsed. In two cases in which biopsy was performed 10 and 15 days after shock respectively, hypertrophy of endothelial cells and some stratification of these cells is noted. A mild degree of diapedesis of erythrocytes is noted in one case, 10 days after shock. In three cases there appears to be an increase in the number of cells

around the vessels. The cells are both lymphoid cells and mast cells. The mast cells seen during the convalescent stage are smaller and the granules firm and densely packed. There appear to be an increase in number of the free mast cells in the dermal connective tissue.

Fluorescent Antibody: Staining of the sections with antidengue mouse antiserum shows no specific staining of the epidermis and dermis in 18 cases tested by this method. Staining of the sections with fluorescent antihuman globulin on these 18 cases showed positive staining in 6 cases (2 febrile, 3 shock, 1 convalescent). The staining is seen on the endothelial surfaces, in the wall of the arterioles, and also in the immediate perivascular tissue in both upper and lower dermis. A comparison between the histologic findings and the positive fluorescent antibody staining shows no significant correlations except that there seems to be more perivascular edema in cases with positive staining.

Conclusions: From the findings it can be said that the nature of the vascular damage in Thai hemorrhagic fever remains elusive. If the findings we observe in the skin can be assumed to represent the findings elsewhere in the body of the patients with Thai hemorrhagic fever we can conclude that there is no evidence of vasculitis, platelet or fibrin thrombosis, or necrosis of the vessel walls, to explain the diapedesis of the red cells or loss of plasma protein. The changes of the endothelial cells consist of some pyknosis of the nuclei and acidophilia of the cytoplasm during the threatened shock and shock period. No necrosis is observed during the convalescent period. In two cases which were biopsied up to 10 and 15 days after the period of shock, some hypertrophy and stratification of the endothelial cells was observed. This latter finding may indicate that some damage of the endothelial cells of the blood vessels occurred during the active clinical stage. The change in appearance of the mast cells are of interest even though there is some overlapping in the pattern of changes during the various clinical stages. There seems to be some disturbances in the granulation of mast cells, beginning in some cases, during the febrile period, while during the threatened shock and shock period, degranulation of mast cells is observed which reached the point of complete degranulation in some cases. This process of degranulation is seen to be associated with perivascular edema and with the increase in perivascular lymphoid and mast cells and free cells containing metachromatic granules in the dermal connective tissue. During the convalescent period some cases show an increase in lymphoid cells around the small blood vessels. Apparently there is either regranulation or regeneration or both of mast cells during the convalescent period since mast cells in the biopsy of the skin obtained during this period are somewhat small and contain fine granules. The overlapping pattern of alterations of mast cells may be due to the fact that the changes in the clinical stages were gradual, and that recycling of mast cell granules may be rather rapid.

The significance of these findings in relationships to the pathogenesis of dengue hemorrhagic fever can be outlined as follows:

It is rather unlikely that the damage of the wall of the vessels resulting in in-

creased permeability can be explained on the basis of direct multiplication of the virus in the endothelial cells, with consequent damage of the cell membrane after the release of virus into the blood. The negative staining of the tissue with fluorescent antidengue antisera during the febrile stages supports this hypothesis. If we compare the effect of certain viruses which are known to attack the endothelial cells directly, e.g. measles or cytomegalic inclusion virus, the morphological changes of the endothelial cells in those instances are striking and rather different from what has been observed here. The mobilization and degranulation of mast cells may be a consequence of some forms of injury on the walls of the vessels. This form of injury may represent a cytotoxic effect of the antigen antibody complex which among other factors, has been shown in both in vivo and vitro experiments to cause mobilization and degranulation of mast cells. It is quite possible that the alterations observed here may be unspecific or unrelated to the basic pathogenetic phenomenon in this disease and may be caused by some local factors or artifacts. The correlation between the changes with the clinical course and the fact that mast cell granules are retained very well in control cases including cases with nephrotic syndrome where a large amount of edema fluid is present in the tissue, is against this explanation.

The finding of the globulin on the endothelial surface, in the wall, and around the vessels, in some cases is of interest. This may indicate the presence of concentrated globulin in antigen antibody complex settling on the vessel walls. It may also represent the staining of globulin which may leak out through the vessel wall due to the increase in capillary permeability. (Analysis of serous effusion obtained from autopsy cases has shown that some high molecular weight globulin may leak into the effusion in addition to the albumin). Proof of the immunological fixation of the globulin on the vessel (due to the localization of antigen antibody complex) must await further study. If the mobilization and degranulation of mast cells is related to the basic pathogenesis of this condition, the mechanism of shock may be related to mast cell-histamine chain reaction. Even though 5 hydroxy tryptamine (serotonin) is a component of rat and mouse mast cells, it is still not certain whether this is a component of human mast cell or not. Therefore serotonin will still have to be considered as a probable chemical mediator.

These findings suggest that a study of the chemical mediator in the phenomenon of shock in Thai hemorrhagic fever, as well as a well controlled clinical study of the therapeutic trials of various forms of antihistamines and steroids should be initiated. To be effective, the therapeutic agents may have to be given relatively early in the course of illness, since some alterations of the mast cells system have been observed during the febrile period, well before the stage of peripheral vascular collapse.