THE AMERICAN JOURNAL OF CLINICAL NUTRITION
Vol. 22, No. 11, November, 1969, pp. 1423-1425
Printed in U.S.A.

Labile Factor Depression in Scorbutic Guinea Pigs[1]

M. BERNADETTE GARVEY, M.D., LEWIS H. DENNIS, M.D., AND MARCEL E. CONRAD, M.D.

HEMORRHAGE IS A PROMINENT manifestation of severe scurvy and was attributed to an inability to maintain capillary integrity. In recent years, other coagulation abnormalities have been reported that might worsen the bleeding tendency observed in this disease. A human with scurvy was reported with a slightly prolonged prothrombin time and reduced blood levels of factors II, V, VII, and X (1). Studies in scorbutic guinea pigs showed a prolonged prothrombin time (2), a deficiency of factors XI and XII (3), and a decrease in prothrombin, thrombin, and thrombokinase concentrations in plasma specimens (4). In addition, abnormal platelet adhesiveness has been reported in humans and guinea pigs with vitamin C deficiency (5, 6). The multiplicity of coagulation abnormalities suggested that disseminated intravascular coagulation might contribute to the hemorrhagic manifestations of scurvy (7). To investigate this possibility, we performed coagulation studies in normal guinea pigs and animals made scorbutic by a vitamin C-deficient diet.

METHODS

Scurvy was induced in 400- to 500-g guinea pigs by feeding them a scorbutogenic diet for 1 month. Control animals were fed the same diet supplemented with green leafy vegetables. Twenty-eight days after the initiation of the special diets, blood was collected from each animal into a polypropylene syringe by cardiac puncture with an 18-gauge hypodermic needle. Citrated and oxalated blood specimens were prepared by mixing 9 volumes of whole blood with 1 volume of anticoagulant (3.8% Na citrate or 0.1 M Na oxalate) in polypropylene test tubes. The blood was cooled to 4 C and platelet-poor plasma prepared by centrifuging the anticoagulated blood at 3,500 rpm for 5 min and pipetting the plasma into polypropylene test tubes with disposable polystyrene pipettes. Anticoagulated blood was used to measure the prothrombin time, partial thromboplastin time, fibrinogen concentration, euglobulin lysis time, serial thrombin time, one-stage thrombin time, and assays of factors II, V, VII, VIII, X, XI, and XII. Platelets were counted by the direct method of Brecher and Cronkite in blood anticoagulated with dry ethylenediaminetetraacetate (EDTA). All determinations were performed in duplicate by previously referenced standard methods (8) and compared to observations made on blood obtained from 20 normal guinea pigs. Assays and studies requiring clot formation as an end point were performed with a "fibrometer" clot-timer (Baltimore Biologicals Div. of Bioquest, Baltimore, Md.). Liver specimens were obtained from both control and scorbutic guinea pigs and prepared for examination by light microscopy; they were fixed in 10% buffered formalin and embedded in paraffin.

RESULTS

During the 4th week of a scorbutogenic diet, guinea pigs developed clinical scurvy manifested by weight loss, alopecia, and bleeding into the skin and subcutaneous tissues. A 25% decrease in the hematocrit occurred. In contrast, the control animals fed leafy green vegetables with the test diet remained healthy.

[1] From the Department of Hematology, Walter Reed Army Institute of Research, Washington, D. C. 20012.

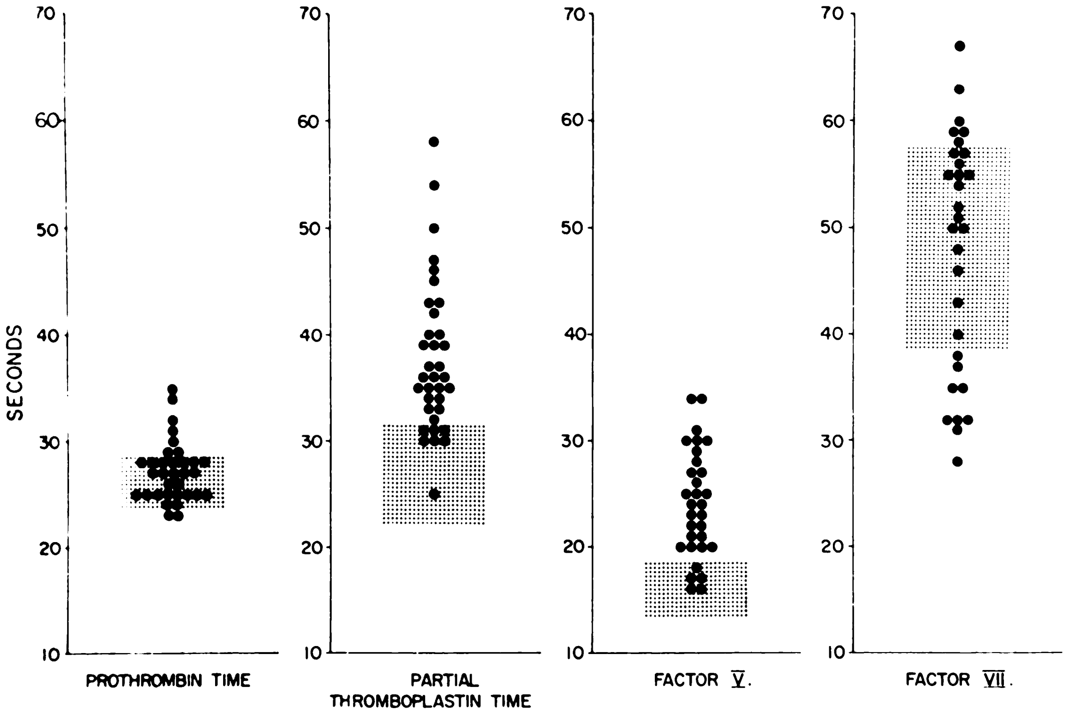

FIG. 1. Determinations are reported in seconds. The shaded areas are the range of values observed in blood from 20 normal guinea pigs. The dots are measurements in the scorbutic animals.

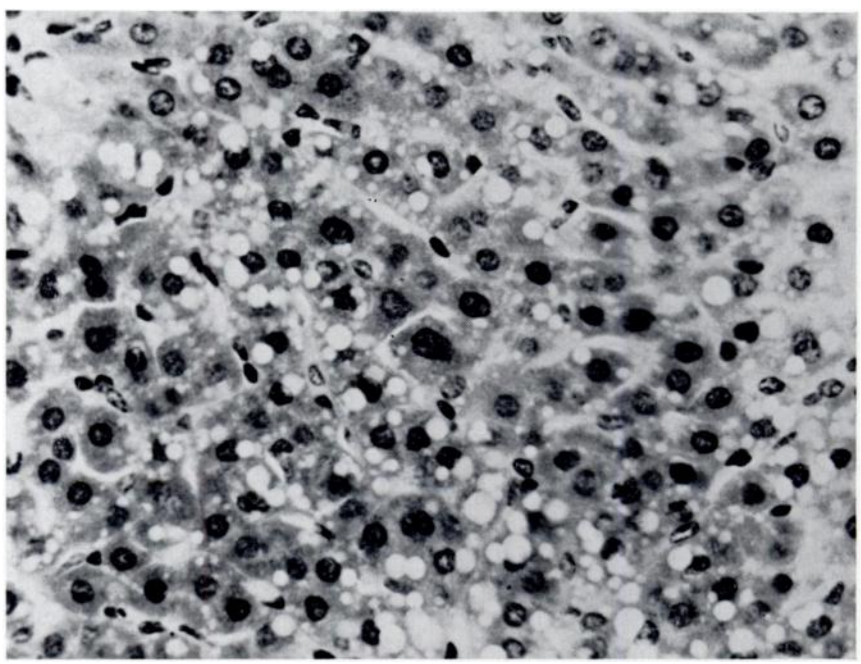

FIG. 2. Section of liver from a scorbutic guinea pig showing fatty infiltration. Hematoxylin and eosin stain. × 100.

The control animals had coagulation characteristics similar to other normal guinea pigs. Quantitative assays were similar to human blood except that factor VII was 20–30% of human values and the normal euglobulin lysis time for guinea pig blood was 10–15 min rather than 3–5 hr.

Coagulation studies in guinea pigs with scurvy showed slight prolongation of the one-stage prothrombin time and partial thromboplastin time in blood from some animals. Assays of multiple coagulation factors indicated that these abnormalities were caused by decreased labile factor activity (V) (Fig. 1). The platelet count and plasma concentration of other clotting factors (II, VII, VIII, IX, X, XI, and XII) remained normal. The euglobulin lysis time showed no evidence of fibrinolytic activity, and the fibrinogen concentration remained normal. Marked fatty infiltration was observed in liver sections from the scorbutic guinea pigs (Fig. 2).

DISCUSSION

The hemorrhagic manifestations of scurvy have been attributed to vascular fragility caused by defective formation of intracellular substances such as collagen and the cement substances of vascular endothelium. Recent investigation indicated that abnormalities of blood coagulation contributed to the hemorrhagic manifestation of scurvy. Our studies in scorbutic guinea pigs showed a moderately severe deficiency of labile factor (V) without other coagulation abnormalities. Demonstration of a decreased concentration of a single coagulation protein in circulating blood made it likely that the etiology was caused by defective synthesis rather than accelerated utilization of clotting factors. The demonstration of severe fatty metamorphosis of the liver in scorbutic guinea pigs provides additional support for this hypothesis because labile factor (V) is synthesized in the liver (9). Hemorrhagic manifestations of scurvy are probably worse in guinea pigs than humans because of the normal occurrence of a low concentration of stable factor (VII) in blood from these animals.

SUMMARY

The hemorrhagic manifestations of scurvy are caused by both increased capillary fragility and abnormal blood coagulation. Guinea pigs fed a vitamin C-deficient diet developed a moderately severe deficiency of labile factor (V) and fatty metamorphoses of the liver. The isolated deficiency of labile factor suggested that it was caused by diminished synthesis in the damaged liver. Hemorrhagic manifestations of scurvy are probably worse in guinea pigs than humans because of the normal occurrence of a low concentration of stable factor (VII) in blood from these animals.

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