

Catabolic Rates of C3 and C1q of Patients With Dengue Hemorrhagic Fever

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BACKGROUND: A small but important proportion of children experiencing secondary dengue virus infections develop dengue hemorrhagic fever (DHF) and its most severe form, dengue shock syndrome (DSS). Principal manifestations of DSS are hypovolemic shock, increased vascular permeability, mild to moderate disseminated intravascular coagulation, and hemorrhage. Previous observations of marked depressions of C3 levels during acute DSS suggested complement activation as an immunopathogenic mechanism. In 1971 a study to define the role of the complement system in the immunopathogenesis of DHF was carried out in Bangkok (SMRL Annual Report, 1972 p. 75-87). Studied were 55 patients with DHF, 36 of whom had DSS. Serum concentrations of 9 complement proteins, transferrin, fibrinogen and fibrin-split products were measured by radial immunodiffusion. The concentration of all measured complement components with the exception of C9 were depressed during shock. Concomitant but lesser reductions of the non-complement protein transferrin, however, suggested that depressions of complement proteins were at least in part due to extravasation from the vascular compartment. There was an inverse correlation between serum complement levels and grade of disease. C3 and C5 concentrations were most affected and were reduced to 20-40% of normal in severe DSS cases. Activation of both known complement pathways was indicated by simultaneous depression of C4 and C3 proactivator levels. Decrease of plasma fibrinogen, appearance of fibrin-split products, and severe thrombocytopenia during shock indicated occurrence of disseminated intravascular coagulation.

Low levels of serum complement components in DSS could be due to 1) increased consumption, 2) decreased synthesis and/or 3) sequestration of protein extravascularly due to increased vascular permeability. In order to confirm the strong but circumstantial evidence of complement component consumption in DSS found in the 1971 study, catabolic studies of 2 complement proteins, C3 and C1q, were undertaken in DHF patients in 1972.

METHODS: Metabolic studies using ^{125}I -C3 or ^{125}I -labeled C1q were performed on 23 DHF patients. Patients with non-acute, non-infectious diseases served as controls. The degree of extravasation of serum proteins during shock was assessed by injecting ^{131}I -IgG as a marker simultaneously with ^{125}I -C3 or ^{125}I -C1q. In both studies, thyroid uptake of radioactive I was blocked by preliminary and continuing administration of saturated potassium iodide.

In the C3 metabolic studies 15 μc of ^{125}I -C3 and 15 μc of ^{131}I -IgG were inoculated intravenously. Eight blood specimens were obtained during the day of inoculation and two blood specimens were obtained on days 2-8 post inoculation. Determinations of total radioactivity for ^{125}I and ^{131}I , immunochemical quantitation of all complement proteins, transferrin, fibrinogen, and hemopexin were performed on all samples.

In C1q metabolic studies, 15 μc of ^{125}I -C1q and 15 μc of ^{131}I -IgG were inoculated intravenously. Blood was drawn at 1 minute, 10 minutes, 30 minutes, 1 hour, 3 hours, 6 hours, 24 hours, 36 hours, 48 hours and 60 hours after injection for determination of ^{125}I and ^{131}I radioactivity and immunochemical quantitation of serum components as in the C3 studies.

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Serum samples obtained daily during the study from patients were tested for HI antibody to dengue 1-4, JEV, and Chikungunya antigens. Patients with 4-fold or greater titer rises to at least one dengue antigen or fixed titers $\geq 1:640$ to two or more dengue antigens were considered to have dengue infections.

RESULTS: Sequential levels of C3, C5, and transferrin and the platelet counts of one DSS patient studied are shown in Figure 1. In this patient (as with most studied), transferrin was lowered concomitantly with levels of C3 and C5, although to a much lesser degree. This example demonstrates the difficulty in assessing qualitatively or quantitatively complement activation by determining sequential serum levels of complement proteins.

In the catabolic studies, extravasation was assessed by injecting radio-labeled IgG simultaneously with C3. The left half of Figure 2 shows the elimination of IgG and C3 in a normal individual; the right half of Figure 2 shows the elimination of these proteins in patients with severe (grade IV) DSS. Within the first 20 hours of study, approximately 85% of the injected IgG was eliminated. That a sizeable proportion of IgG eliminated was pooled extravascularly is indicated by the increase of percentage of injected IgG recovered from 20-110 hours after injection (mobilization of extravasated IgG back into the vascular compartment after the shock period). By comparing the measured values of IgG of each patient with the expected normal values, a factor was obtained which served to correct C3 values for the amount of extravasation.

Figure 3 compares the elimination of C3 in the same DSS and control patient after correction for extravasation. The difference in the rate of elimination occurred within the first 20-30 hours. Thereafter both individuals eliminated C3 at the same rate. The initial rapid phase of C3 elimination coincided with shock in this patient.

Figure 4 compares the elimination of radio-labeled C1q in a patient with grade II DHF with that of a normal control. For the C1q values depicted, similar corrections as for C3 were made; radio-labeled IgG was injected simultaneously with C1q and IgG values were used to correct for extravasation. A vast difference in C1q elimination between these individuals is evident with a more rapid catabolic rate in the DHF patient.

Turnover studies of C3 were performed on 17 patients and 6 controls. Studies of C1q catabolism were performed on 7 patients and 3 controls. Figure 5 summarizes results of the C3 study. The fractional catabolic rate of C3 correlated well with serum C3 levels and the grade of illness. Eleven patients with DSS (grade III-IV) eliminated 2.6-3.5% of the C3 plasma pool per hour during a seven day observation period, whereas 5 patients with DHF without shock eliminated only 1.9-2.6%. The average of 5 controls was 1.9%. The greatest increase in catabolic rate of C3 was observed during the initial 24-48 hour period of study which coincided with shock.

The catabolic rate of C1q in the 7 dengue patients studied was increased over that of control individuals (Figure 6). During the 3 day observation period, the DHF patients eliminated 3.8-8.3% of their C1q plasma pool per hour, while control individuals eliminated 2.8-3.5% per hour. There was no apparent correlation between catabolic rate and grade of disease in the 7 patients; this was expected since both complement pathways (classical, involving C1q; and the bypass mechanism, not involving C1q) are activated in DHF.

DISCUSSION: The results lend support to the concept that activation of complement constitutes an essential part of the pathogenic mechanism of DSS and DHF. Complement-dependent release of vasoactive amines and generation of platelet procoagulant activity are envisaged as major pathogenic factors of the dengue shock syndrome.

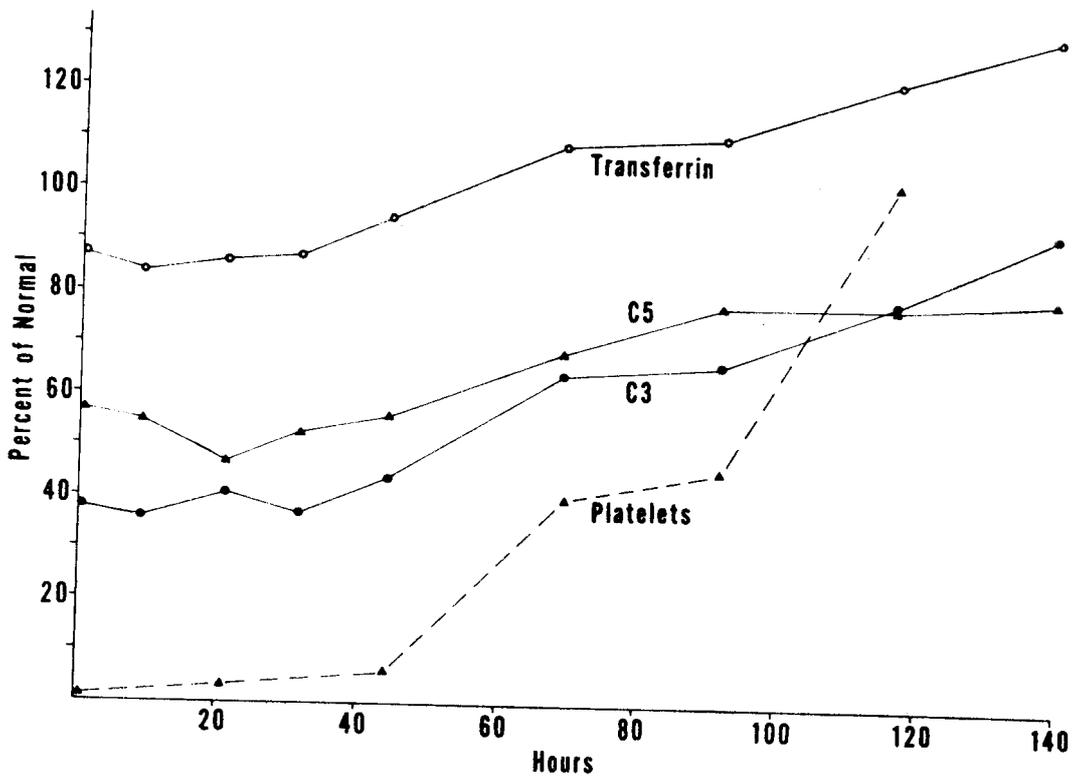


Figure 1. Sequential Plasma Concentrations of C3, C5, and Transferrin and Platelet Counts in a Patient with DSS

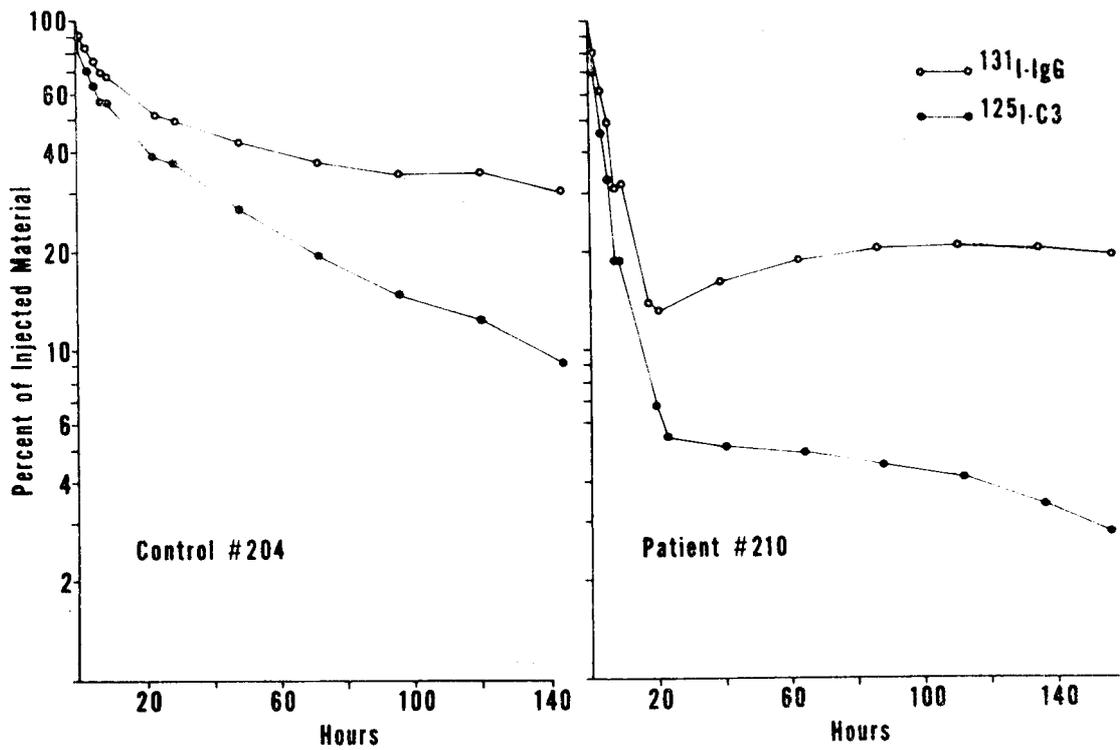


Figure 2. Disappearance of Injected ^{125}I C3 and ^{131}I IgG In a Normal Control and DSS Patient

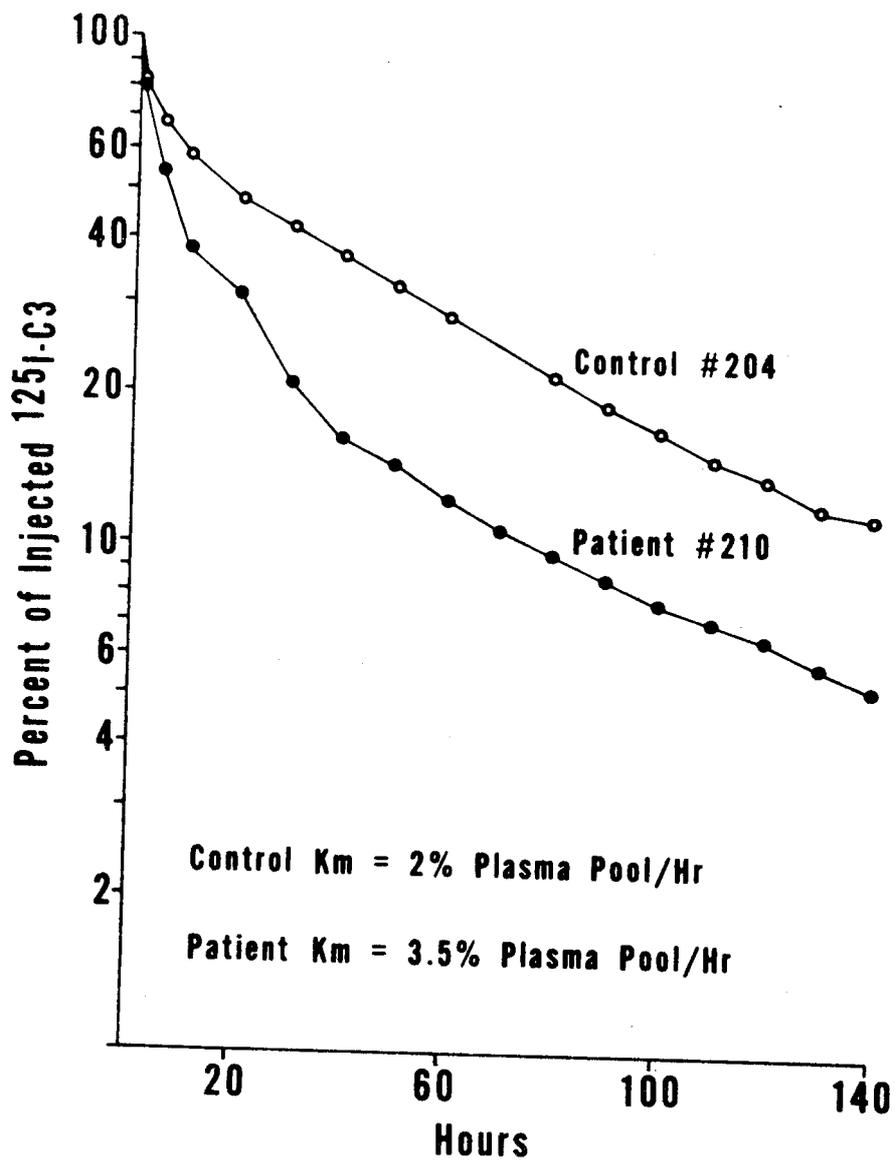


Figure 3. Corrected Elimination Rates of ^{125}I C3 in a Normal Control and DSS Patient

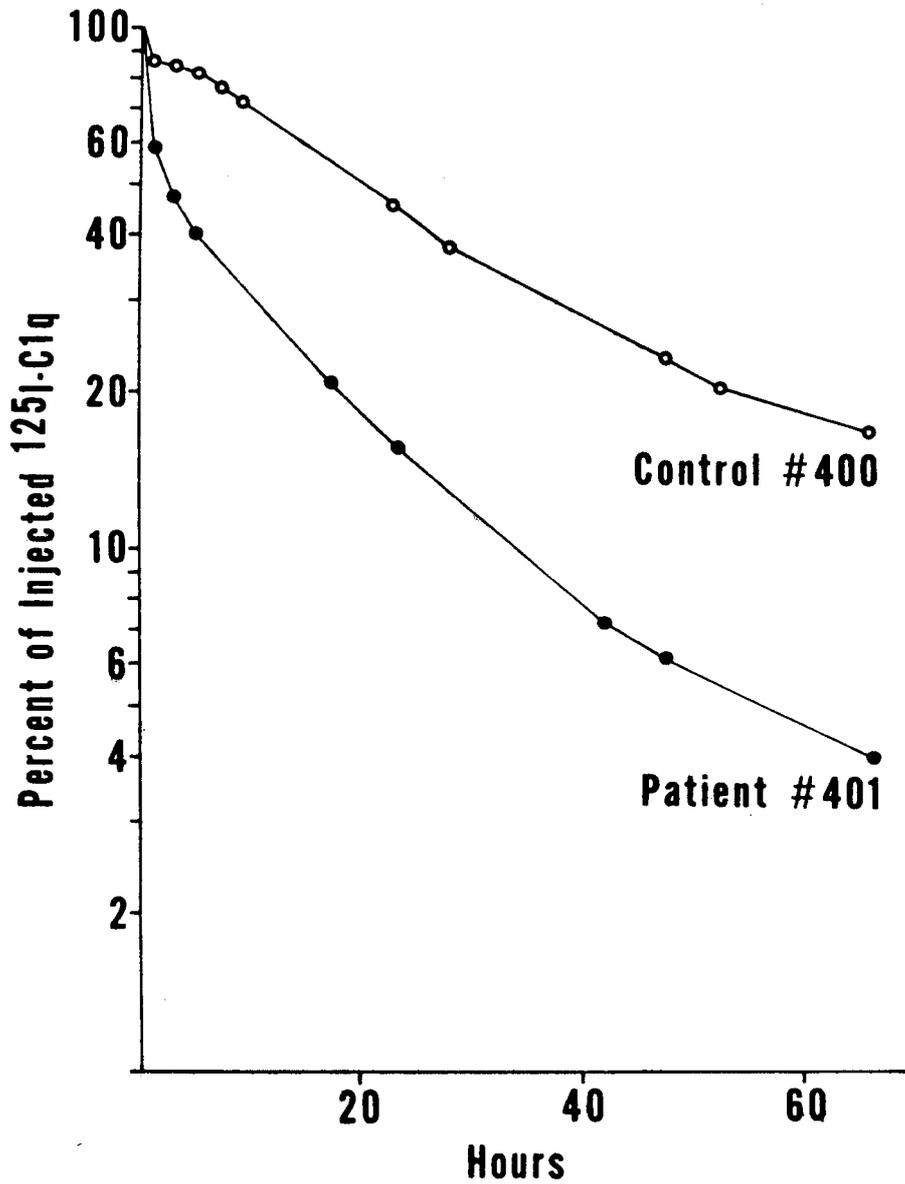


Figure 4. Corrected Elimination Rates of $^{125}\text{I C1q}$ in a Normal Control and DHF Patient

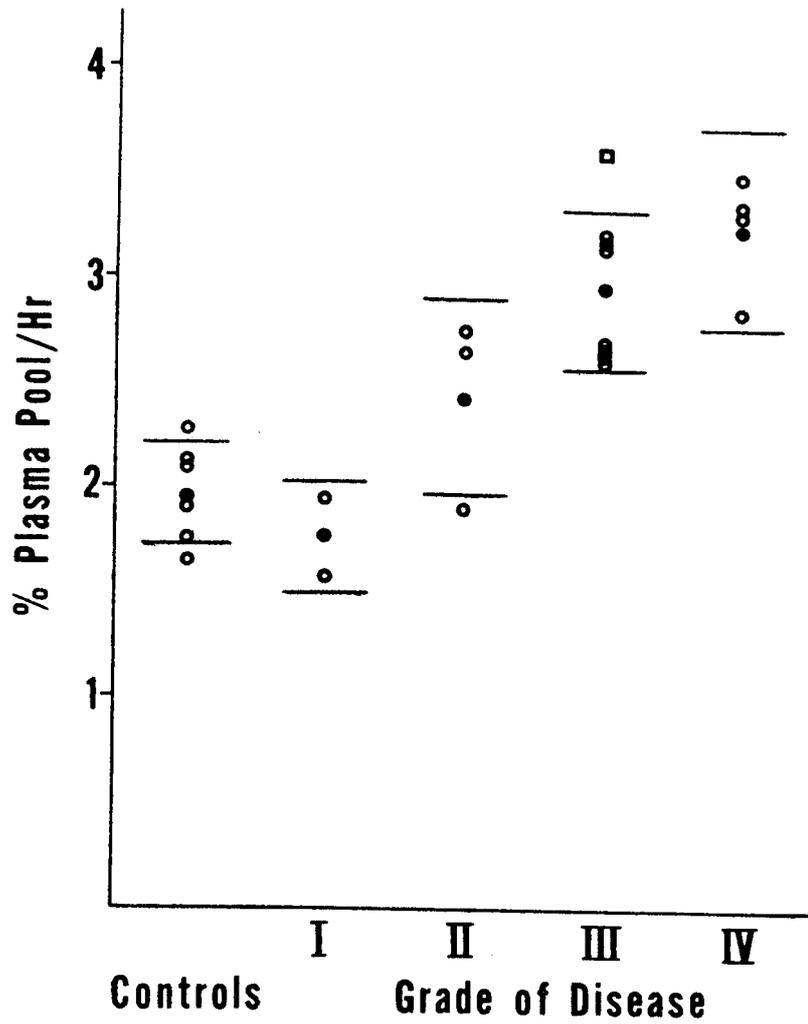


Figure 5. Corrected Elimination Rates of ^{125}I C3 in 17 DHF Patients and 6 Control Patients

● = Mean values of group
 — = \pm 1 S.D. of mean

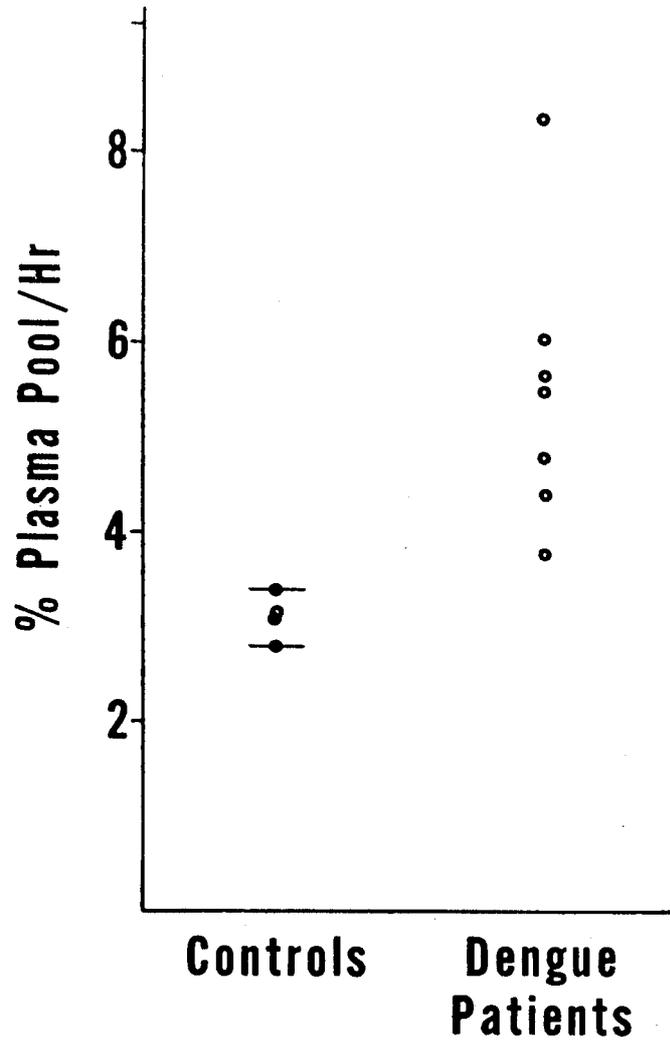


Figure 6. Corrected Elimination Rates of ¹²⁵I Clq in 7 DHF Patients and 3 Control Patients

○ = Mean values of group
 — = ± 1 S.D. of mean