Effect on Blood Coagulation of Massive Intravascular Haemolysis

By P. M. MANNUCCI, G. F. LOBINA, L. CAOCCI AND N. DIOGUARDI

There have been several reports in recent years on the effect of intravascular haemolysis on blood coagulation. Varied results have been obtained.

Increased coagulant activity has been demonstrated in plasma from patients with paroxysmal nocturnal haemoglobinuria. Egeberg studied five patients with chronic hemolytic diseases and found slightly increased levels of fibrinogen and factor VIII, the other clotting factors being within the normal range. In 1967 Abilgaard and his associates found elevated factor VIII without significant increase of other clotting factors in patients with sickle-cell anemia.

On the other hand, a bleeding syndrome accompanying hemolysis after incompatible blood transfusions has been described in man. It was characterized by hypofibrinogenemia and thrombocytopenia without evidence of hyperfibrinolysis, and was thought to be produced by intravascular coagulation through the release of thrombo plastic material from the lysed red cells. These findings were confirmed by experimentation in dogs: it was shown that incompatible blood transfusions lead to intravascular coagulation and consumption coagulopathy.

Very recently, Rabiner and Friedman found that infusion of hemolysate into dogs produced hypercoagulability, frequently followed by consumption coagulopathy. The latter phenomenon was much more prominent when depression of reticulo-endothelial (RE) system was induced by carbon or splenectomy.

The acute and massive destruction of red cells which may follow ingestion of fava beans or inhalation of their pollen by sensitive subjects deficient in glucose-6-phosphate dehydrogenase seemed to offer a suitable naturally-occurring experimental model in which to investigate in man the effect of massive intravascular hemolysis on blood coagulation.

In the present study, fibrinogen, factor V and factor VIII were found to be markedly high in the days following the hemolytic crisis. Other clotting factors and platelets were normal, and no evidence of consumption coagulopathy was detected.

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Fig. 1.—Highest levels of fibrinogen, factors V and VIII and lowest levels of hemoglobin recorded after the hemolytic crisis. They are compared with "control" levels as found two months after the hemolytic episode.

MATERIALS AND METHODS

The investigation was carried out in April 1967 on 28 Sardinian subjects deficient in glucose-6-phosphate dehydrogenase who developed acute hemolytic anemia of variable severity after ingestion of fava beans or the inhalation of their pollens. In each subject, coagulation tests were performed on admission during the hemoglobinuric phase and then on alternate days over the first two weeks after admission. "Control" tests were carried out on 23 patients two months after the crisis, when the hemoglobin concentration had returned to normal.

The prothrombin time, kaolin partial thromboplastin time and thrombin time were performed as screening tests.9 Specific assays of clotting factors were carried out for fibrinogen (by the clot-weight method of Ingram10), prothrombin (a one-stage method using Tiger Snake Venom11), factor V and complex VII-X,12 factor VIII and factor IX (one-stage methods based on partial thromboplastin time where maximal contact activation was obtained with activation product9), factor XI and factor XII,13 platelet count.14 Factor VIII-assay was also carried out with the two-stage method of Biggs as described by Denson,12 in a few samples whose potencies recorded by the one-stage assay were particularly high. Potencies were calculated against pools of at least five normal plasmas whose values were taken as 100 per cent.

Studies on fibrinogen degradation products (FDP) were carried out in April 1968 on 9 patients with very severe hemolytic crisis who were studied from the early onset of red cell destruction. Blood coagulation screening tests carried out in these patients showed a pattern very similar to those obtained in the first series. FDP were assayed in serum obtained from blood to which Kunitz inhibitor (100 U./ml.) had been added. After incubation with thrombin (100 U./ml.) for one hour at 37 C., serum was tested in an immunodiffusion system on cellulose acetate strips, using a commercial antifibrinogen serum.*

RESULTS

Prothrombin time and thrombin time were found to be slightly shortened in the days following the hemolytic crisis; kaolin partial thromboplastin time showed a more marked shortening, ranging from 25 to 31 seconds, with normal

*Supplied by Behringwerke, A. G. Marburg-Lahn, Germany.
controls ranging from 39 to 46 seconds. The assays of clotting factors showed very high values of fibrinogen and factor VIII, and less pronounced increases of factor V. Other clotting factors and platelets were within normal ranges. Fibrinogen degradation products were not detectable in the sera of the second series of nine patients studied in the early phases of hemolysis.

Figure 1 shows the highest levels of fibrinogen, factor V, factor VIII and the lowest values of hemoglobin observed in our patients as well as the levels found two months after the hemolytic episode. The observed differences were highly significant in each case (P < 0.001). It may be seen that the two month levels are very close to those observed in a random population of normal individuals.

A significant inverse correlation (r = −0.58, P < 0.01) was observed between the highest factor VIII-levels and the lowest hemoglobin levels recorded after the hemolytic episode in our patients (Fig. 2). An inverse correlation (r = −0.36) was also found between fibrinogen and haemoglobin levels but was not significant (P < 0.1). No correlation was found between factor V and hemoglobin. Factor VIII was positively correlated with both fibrinogen and factor V (r = 0.40; r = 0.43; P < 0.05), whereas no correlation was found between fibrinogen and factor V.

Figure 3 shows changes in fibrinogen, factor V and factor VIII in our patients in the days following the hemolytic crisis. It may be seen that the highest values were recorded in the first four days and that the clotting factors then fell as the hemoglobin levels rose.

While the assay of fibrinogen used in the present study is relatively straightforward and measures clottable protein, factor V and factor VIII assays are based on the corrective effect of the tested plasma on a clotting system lacking only the specific factor under investigation. Therefore, when increased levels of clotting factors are recorded by these bio-assays, the possibility exists that
Fig. 3.—Changes in fibrinogen, factor V, factor VIII, and hemoglobin levels in the days following the hemolytic crisis. The mean values and the ranges as observed in 24 patients are represented.

a nonspecific activation of the clotting system may be mimicking a true increase in concentration. In the present context, circulating red cell material might invalidate the assay, for hemolysate is known to activate the clotting system. However since other factors which are assayed by the same clotting systems as factor V and factor VIII were not concomitantly elevated, it is unlikely that there was general nonspecific activation of the assay system. Moreover, the increase in clotting factors was still present after red cell material had been cleared from the circulation.

Nevertheless, because of the extremely high levels of factor VIII found in some of our patients, additional tests have been performed in order to rule out the possibility of “activation” of this factor indistinguishable from true increase. The activity recorded in the one-stage factor VIII assay was not absorbed by A1 (OH)₃, could be activated by thrombin, survived incubation at 37 C. for 60 minutes, and storage at −20 C. with repeated freezing and thawing. It was non-dialyzable and it was inactivated in 30 minutes heating at 50 C.

Since the two-stage assay of factor VIII is thought to be less influenced by nonspecific activation, some samples were also tested with the method of Biggs which is based on a modification of the thromboplastin generation test. A
marked increase was also recorded with this system, and the values were even somewhat higher than those found with the one-stage assay. This is in agreement with the previous work of Ingram\textsuperscript{21} who compared the two methods and observed higher values with the two-stage method in the range of the highest potencies.

**Discussion**

The observed pattern of changes distinguishes the present phenomenon from two others: the very rapid rise in factor VIII alone due to strenuous exercise\textsuperscript{22} and adrenalin,\textsuperscript{23} and the fall in factors V and VIII and fibrinogen due to defibrination which has followed massive intravascular haemolysis caused, for instance, by the transfusion of incompatible blood.

The hyperacute adrenalin response appears so rapidly that it is difficult to imagine that the synthesis of factor VIII could be sufficiently accelerated to account for it. On the other hand, in the many acute and subacute clinical situations\textsuperscript{24,26} where a combined increase of factors V, VIII and fibrinogen has been observed over a longer period increased synthesis is a much more plausible explanation.

However, the rates of hemolysis in the acute attack of favism and in incompatible blood transfusion may not greatly differ. It is therefore difficult to explain why a simple rise in the clotting factors was observed in the former situation and a fall due to consumption coagulopathy in the latter.

A possible explanation would be that consumption coagulopathy occurs in favism in the earliest phases of the haemolytic crisis and is then followed by a secondary increase of the clotting factors. No evidence substantiating this hypothesis was given by the continuous observation of a few patients admitted at a time when most of the red cells showed the presence of Heinz bodies and hemolysis was not yet present. A progressive rise of fibrinogen, factor V and VIII was seen beginning after the onset of hemolysis. Moreover, the absence of fibrinogen degradation products in the sera from the second series of nine patients studied in the early phases of haemolysis seems to rule out definitely this possibility.

It has been shown by Spaet and his associates\textsuperscript{27} that thromboplastic material is removed from the circulation by the R.E. system through phagocytosis. One might tentatively assume that the consequences of hemolysis depend on the state of the R.E. system. R.E. blockade would prevent the removal from the circulation of thromboplastic material which might in turn trigger the coagulation sequence and lead to consumption coagulopathy. On the other hand, an intact R.E. system would rapidly remove the red cell debris before defibrination could occur.

It has been suggested that the synthesis of fibrinogen, factor V and factor VIII is dependent on the R.E. system.\textsuperscript{28,30} It is therefore conceivable that the red cell debris may stimulate an intact R.E. system to an increased production of these clotting factors. While most subjects in whom hemolysis has led to defibrination have been acutely ill and probably in some degree of shock,\textsuperscript{5,6,31} the hemolytic attack of favism produces little constitutional disturbances.
SUMMARY

This study was carried out on twenty-eight Sardinian subjects undergoing massive intravascular hemolysis after ingestion or inhalation of fava beans. The patients were all deficient in glucose-6-phosphate dehydrogenase but otherwise hematologically normal when investigated after the acute hemolytic episode.

Prothrombin time, thrombin time and activated partial thromboplastin time were found to be shortened in the days following the hemolytic crisis. Marked increase of factor VIII (antihemophilic factor) and fibrinogen was also observed, together with a less pronounced rise of factor V. The other clotting factors were within the normal range, and fibrinogen degradation products were not present in serum. The observed rise was found to be generally proportional to the degree of red cell destruction. Progressive normalization of the abnormal parameters followed the recovery from acute hemolysis. These observations are discussed in relation to previous findings and to the occurrence of intravascular coagulation following massive red cell destruction.

SUMMARIO IN INTERLINGUA

Le presente studio esseva effectuate in vinti-octo subjectos sardinian qui habeva experi-entiate massive hemolyse intravascular post le ingestion o inhalation (Ic 

Le patientes habeva carentia de dehydrogenase de glucosa-6-phosphato sed esseva altere-mente normal in lor parametros hematologic quando illes esseva studiate post le acute epi-

Esseva trovate durante le dies post le crise hemolytic que le tempore prothrombinic, le tempore thrombinic, e le tempore de activate de thromboplastina partial esseva reducite. Esseva etiam observate marcate augmentos de factor VIII (factor antihemophilic) e de fibrinogeno, insimul con un minus pronunciate augmento de factor V. Le alte factores coagulatori esseva intra le limites del norma, e nulle productos de degradation de fibrino-

esseva presente in le sero. Le observate augmento esseva, a generalmente parlar, pro-

Un progressive normalisation del parametros anormal sequeva le restablimento ab le episodio de acute hemolyse. Iste observa-

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