Changes in the Hemostatic Mechanism After Myocardial Infarction

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SUMMARY
Plasma coagulation factors, adenosine diphosphate-induced platelet aggregation, and fibrinolytic activity were studied in male survivors of myocardial infarction and in healthy normal men. Infarction survivors had significant elevations of factors VIII and X and of adenosine diphosphate-induced platelet aggregation. The fibrinolytic system was altered toward reduced plasminogen activation, increased antiurokinase activity, and elevated antiplasmin activity. These findings suggest that some men with prior myocardial infarction have a heightened tendency to thrombogenesis. This tendency may represent a response to previous cardiac insult or may contribute to its pathogenesis.

Additional Indexing Words:
Myocardial infarction  Platelet aggregation  Hemostasis, alterations in
Fibrinolysis  Hypercoagulability  Thrombogenesis  Blood coagulation

During the 19th century, Karl von Rokitansky postulated that fibrin deposition on the arterial intima was the primary pathological process in atherogenesis.1 This idea has stimulated considerable 20th century interest in the relationship between blood coagulation and coronary atherosclerosis.2-4 For the most part, attention has been directed to the detection in vitro of the so-called hypercoagulable state, an in vivo condition wherein the postulated balance between clot-promoting and clot-reducing tendencies is shifted toward thrombogenesis.5, 6 In some investigations, excessive amounts of thromboplastin generation, coagulation factor activity, platelet stickiness, and platelet numbers have been found.7-10 In addition, diminished blood fibrinolytic activity has been noted in certain patients.11 However, most studies have examined just one or a few aspects of the hemostatic mechanism, and no overall picture of the coagulation status in patients with ischemic heart disease has arisen.

This paper describes the results of a broad coagulation survey performed in normal persons and in patients with prior myocardial infarction. The work began as an ancillary study to the National Coronary Drug Project, a longterm study of the effects of cholesterol-lowering drugs on male patients with documented myocardial infarction.12, 13 Results of the present interim data analysis suggest that statistically significant differences in certain coagulation factors, in platelet aggregation,
and in fibrinolytic activity exist between normal men and male patients with ischemic heart disease (before treatment with cholesterol-lowering drugs).

**Materials and Methods**

Two groups were studied in this investigation. The cardiac disease (CD) group consisted of 41 ambulatory men with one or more previous myocardial infarctions diagnosed according to specified electrocardiographic, clinical, and biochemical criteria. These men attended the Project's cardiac clinic at Grady Memorial Hospital, Atlanta, Georgia.

The group consisted of 32 white and nine black patients; their ages ranged from 31 to 64 years (median 52 years). These patients were selected for the coagulation study in the order of their recruitment to the clinic between February and July, 1969. At the time of recruitment, all patients belonged to functional class I or class II of the New York Heart Association cardiac classification, and had suffered their most recent infarctions at least 1 month prior to the first clinic visit. In all cases, coagulation testing was initiated 2 or more months after myocardial infarction. When recruited, CD group members were free of chronic renal and hepatic disease, and had no pulmonary insufficiency, malignancy, thyroid dysfunction, or ulcer disease. At the time of coagulation testing, none of the patients was under treatment with anticoagulants, estrogen, or insulin, and none was taking cholesterol-lowering drugs.

The normal group included one black and 33 white male volunteers selected from the technical, professional, and administrative staffs of the Center for Disease Control, Atlanta, Georgia. These men were recruited from February, 1969 to February, 1970. Their ages ranged from 38 to 55 years (median 48.5 years).

Participants were selected from among 150 volunteers, according to the following criteria: age between 30 and 70 years; negative personal history of cardiovascular disease, liver disease, blood dyscrasia, and diabetes mellitus; normal physical examination, chest X-ray, and electrocardiogram; normal fasting cholesterol, triglycerides, glucose, and blood urea nitrogen; negative history of symptomatic cardiovascular disease before the age of 60 in parents, grandparents, and siblings, and no history of diabetes mellitus or blood dyscrasia in this family group. Patients in the normal group were symptomatically free of infection at the time of coagulation testing and were taking no medications except for occasional aspirin.

Whole blood for coagulation analysis was drawn monthly for 2 months from each subject. Collections were usually made between 8 a.m. and 10:30 a.m., 12 to 14 hours after the patient's last meal.

Blood was drawn into plastic syringes with minimal venous stasis, by the two-syringe technique; it was transferred immediately to polycarbonate tubes and mixed with sodium citrate-citric acid anticoagulant in a ratio of 9 parts blood to 1 part anticoagulant. The tubes were either chilled on wet ice (for coagulation and fibrinolysis studies) or were kept at room temperature (for platelet aggregation studies). Additional blood was added to disodium-EDTA for platelet counts.

For coagulation and fibrinolysis testing, platelet-poor plasma was prepared by centrifuging whole blood at 1500 × g for 20 min at 4°C. The plasma was separated, rapidly frozen, and stored at −20°C in capped plastic tubes.

To test platelet aggregation, platelet-rich plasma was prepared by centrifuging whole blood at 150 × g for 10 min at room temperature. Plasma so prepared was separated immediately and was distributed into plastic tubes.

Frozen plasma was thawed at 37°C for the estimation of fibrinogen and of the activities of coagulation factors II, VII, VIII, and X. Thromboplastins used for the latter four coagulation assays included Simplastin, human brain, Platein, and Russell viper venom with horse cephalin.

For estimation of plasminogen activator, anti-urokinase activity, and antiplasmin activity, euglobulin suspensions or plasma mixed with urokinase or plasmin were tested on heated and unheated fibrin plates by Kwaan's modification of the method of Astrup and Mullertz. Plasminogen was measured by using a caseinolytic assay. Platelets were counted by phase microscopy with the Unopette system. Platelet aggregation induced by the addition of adenosine diphosphate (ADP) to platelet-rich plasma was measured in a Chronolog Aggregometer at 37°C. In each case, a monophasic response curve was
obtained by using a final ADP concentration of 1 × 10⁻⁶ M. The magnitude of maximum aggregation was expressed in per cent as the relative change in light transmission induced by ADP as compared with that produced by high speed centrifugation of platelet-rich plasma. The details of this method are presented in a separate publication.

Table 1

<table>
<thead>
<tr>
<th>Clinical Characteristics of Study Groups*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
</tr>
<tr>
<td>-------</td>
</tr>
<tr>
<td>Normal</td>
</tr>
<tr>
<td>Cardiac disease</td>
</tr>
<tr>
<td>Significance level**</td>
</tr>
</tbody>
</table>

*Mean ± 1 sp for all characteristics except skin color.
†Results based on a tripalmitin standard.
‡Two outliers omitted (62, 78 inches).
§One outlier omitted (cholesterol 387 mg/100 ml; triglycerides 1294 mg/100 ml).
**Based on the Mann-Whitney U-test.
††Not significant at the 5% level.

Cardiac patients were taking a variety of medications at the time of coagulation testing. The drugs most frequently used included digitalis preparations, thiazide diuretics, aspirin-containing compounds, and nitroglycerin.
HEMOSTATIC MECHANISM AFTER MI

Table 2

Month-to-Month Test Correlations in Study Groups

<table>
<thead>
<tr>
<th>Test</th>
<th>Normal group (r)* (Level of significance)</th>
<th>Cardiac disease group (r)* (Level of significance)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrinogen</td>
<td>0.09 (NS†)</td>
<td>0.44‡ (0.05)</td>
</tr>
<tr>
<td>Factor II</td>
<td>0.70 (0.01§)</td>
<td>0.23 (NS†)</td>
</tr>
<tr>
<td>Factor VII</td>
<td>0.52 (0.01)</td>
<td>0.14** (0.01)</td>
</tr>
<tr>
<td>Factor VIII</td>
<td>0.24 (NS†)</td>
<td>0.45 (0.05)</td>
</tr>
<tr>
<td>Factor X</td>
<td>0.02 (NS†)</td>
<td>0.39 (0.01)</td>
</tr>
<tr>
<td>Plasminogen</td>
<td>0.66 (0.01)</td>
<td>0.66** (0.01)</td>
</tr>
<tr>
<td>Euglobulin lysis</td>
<td>0.53 (0.01)</td>
<td>0.13 (NS†)</td>
</tr>
<tr>
<td>Antiurokinase activity</td>
<td>0.16 (NS†)</td>
<td>0.39 (0.05)</td>
</tr>
<tr>
<td>Antiplasmin activity</td>
<td>0.41 (0.05)</td>
<td>0.02 (NS†)</td>
</tr>
<tr>
<td>Platelets</td>
<td>0.42 (0.05)</td>
<td>0.29 (0.05)</td>
</tr>
<tr>
<td>ADP aggregation</td>
<td>0.42 (0.05**)</td>
<td>0.35 (0.05)</td>
</tr>
</tbody>
</table>

*Correlation coefficient.
†Not significant at the 5% level.
‡2 outlier pairs omitted for calculation of r.
§4 outlier pairs omitted for calculation of r.
**1 outlier pair omitted for calculation of r.

Intergroup Comparisons

To derive representative data for each study group, individual month 1 and month 2 test values were averaged for each test, and the group mean was taken as the average of individual means (table 3).

To determine the validity of comparing coagulation data between study groups of different ages, the relationship of age to coagulation test results was examined in each group by plotting scatter diagrams of test values against age. In both the cardiac and normal groups, results from all 11 coagulation tests varied independently of plotted ages.

To determine racial effects, test means from 32 white patients and nine black patients in the cardiac group were compared. Black patients had greater euglobulin lysis activity (81 mm² vs. 51 mm²) and less ADP-induced platelet aggregation (27% vs. 43%) than white patients (P < 0.05). By use of the Mann-Whitney U-test, distributions of test results were compared between the cardiac and normal groups including and excluding black patients.

Results of these comparisons are shown in table 3, which includes values derived from black patients, since these values did not change the results of intergroup comparisons at the 5% confidence level. Cardiac patients had significantly higher plasma levels of coagulation factors VIII and X, reduced euglobulin lysis activity, elevated antiurokinase and antiplasmin activity, and increased platelet aggregation in response to ADP.

Histograms comparing the distributions of these test results in the two groups are presented in figures 1–3. The apparent departure of many results from a Gaussian distribution was the basis for using the Mann-Whitney U-test, a powerful nonparametric alternative to the t-test which avoids the assumptions inherent in the latter test. For comparative purposes, the same intergroup comparisons were calculated by using the Student t-test; results were the same.

Additional Relationships

Significant positive correlations at the 5% level were found between the following hematological data and characteristics of the normal group: fibrinogen vs. cholesterol (r = 0.38), factor II vs. cholesterol (r = 0.55), factor II vs. triglycerides (r = 0.39), factor X vs. triglycerides (r = 0.36), and antiurokinase activity vs. height (r = 0.36). Coagulation and fibrinolysis test results varied independently of age and weight.
### Table 3

**Comparison of Normal and Cardiac Disease Groups**

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of patients</th>
<th>Fibrinogen (mg/100 ml)</th>
<th>Factor II (%)</th>
<th>Factor VII (%)</th>
<th>Factor VIII (%)</th>
<th>Factor X (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>34</td>
<td>316.0 ± 56.20</td>
<td>153.8 ± 29.22†</td>
<td>130.8 ± 38.48</td>
<td>113.2 ± 25.86</td>
<td>101.9 ± 10.46</td>
</tr>
<tr>
<td>Cardiac disease</td>
<td>41</td>
<td>339.5 ± 76.74‡</td>
<td>153.8 ± 24.71</td>
<td>137.3 ± 37.49§</td>
<td>148.4 ± 52.78</td>
<td>123.3 ± 22.42§</td>
</tr>
</tbody>
</table>

Significance level**

NS††

NS††

NS††

0.01

0.01

*Mean of month 1 plus month 2 visits in each group ± 1 sd.
†Excludes 4 outlier pairs.
‡Excludes 2 outlier pairs.
§Excludes 1 outlier pair.
**Mann-Whitney U-test.
††Not significant at 5% level.

Abbreviations: u = units.

Among cardiac patients, the following test results and patient characteristics were correlated: factor II vs. cholesterol (r = 0.32), factor VIII vs. cholesterol (r = 0.32), factor X vs. cholesterol (r = 0.45), plasminogen vs. triglycerides (r = 0.40), antiurokinase activity vs. triglycerides (r = 0.35), and antiurokinase activity vs. height (r = 0.38). Coagulation and fibrinolysis test results varied independently of age and weight.

### Discussion

Current concepts of the hemostatic mechanism suggest that thrombosis occurring in response to vascular injury begins with local vessel contraction, platelet-to-vessel adhesion, and platelet-to-platelet aggregation. Subsequently, the enzymatic coagulation cascade is activated, and clotting proceeds by way of the "intrinsic" or "extrinsic" pathways to the deposition of a fibrin clot at the site of injury.
HEMOSTATIC MECHANISM AFTER MI

Any tendency to excessive fibrin accumulation is checked in part by a complex fibrinolytic system consisting of plasminogen (profibrin-olysin) and various activators of the plasminogen-plasmin conversion reaction. In turn, excessive fibrinolytic activity is normally opposed by circulating anti-activators and antiplasmins.

In the present study, we have examined several components of the coagulation-fibrinolysis system in normal men and in male survivors of myocardial infarction. Test results indicate that in comparison with normal men, cardiac patients have increased activity of coagulation factors VIII and X, increased ADP-induced platelet aggregation, reduced plasminogen activator activity, and elevated circulating antitrypsinase and antiplasmin activity.

These findings agree in part with those of

<table>
<thead>
<tr>
<th>Plasminogen (µg/0.5 ml/hr)</th>
<th>Euglobulin lysis (mm²)</th>
<th>Antiurokinase activity (u/ml)</th>
<th>Antiplasmin activity (u/ml)</th>
<th>Platelets (X10⁵/mm³)</th>
<th>ADP-platelet aggregation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>56.6 ± 14.88</td>
<td>73.9 ± 31.62</td>
<td>1.35 ± 0.270</td>
<td>0.230 ± 0.0507</td>
<td>277.0 ± 65.86</td>
<td>25.7 ± 10.46</td>
</tr>
<tr>
<td>56.0 ± 12.34§</td>
<td>57.4 ± 32.32</td>
<td>1.49 ± 0.331</td>
<td>0.274 ± 0.0498</td>
<td>286.0 ± 64.12</td>
<td>39.3 ± 18.45</td>
</tr>
<tr>
<td>NS†† 0.05</td>
<td>0.05</td>
<td>0.01</td>
<td></td>
<td>NS†† 0.01</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Figure 2

Plasma euglobulin lysis, antiurokinase, and antiplasmin activity in the two study groups.

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Cooperberg and Teitelbaum,27 who demonstrated elevated factor VIII concentrations in recent and remote male survivors of myocardial infarction. McDonald and Edgill28 studied platelet adhesiveness in eight men with acute myocardial infarction and found increased stickiness in comparison with healthy controls. Using the Chandler apparatus, Ardlie, Kinlough, and Schwartz29 demonstrated that thrombi formed from the blood of patients with recent myocardial infarction were longer and heavier than the thrombi of control subjects. Zahavi and Dreyfus30 measured the extent of ADP-induced platelet aggregation in male patients with acute myocardial infarction and found that the magnitude of response was greater in the infarction patients than in normal controls.

These and similar investigations have focused primarily on patients with acute disease and may have demonstrated a nonspecific reaction to acute illness rather than a phenomenon contributing to thrombogenesis.

More recently, attention has been directed to the role of defective fibrinolysis in the genesis of myocardial infarction. Chakrabarti, Hocking, and Fearnley31 measured whole blood fibrinolytic activity in 107 males 2 to 9 months after myocardial infarction. Comparison of test results between infarction patients and 90 age-matched healthy control patients showed that coronary patients below 60 years

**Figure 3**

*ADP-induced platelet aggregation in normal men and cardiac patients.*
HEMOSTATIC MECHANISM AFTER MI

of age had significantly reduced fibrinolytic activity. In a study of serum inhibitors of plasmin and plasminogen activator within 4 days of myocardial infarction, Tsitouris and co-workers found elevated antiplasmin and antiurokinase activity in most patients.

The meaning of these abnormalities in survivors of myocardial infarction is still unclear because the changes in coagulation and fibrinolysis observed during periods of acute physical stress such as myocardial infarction may be nonspecific, and because there is inadequate information about the hemostatic mechanism in affected individuals before infarction.

The pattern of abnormalities demonstrated in the present study suggests that a tendency to excessive thrombus formation may exist in male patients with prior myocardial infarction. By in vitro testing, platelet-to-platelet binding is greater than normal, the capacity for activation of plasminogen is reduced, and the inhibition of plasminogen activator and plasmin is increased. The platelet response to ADP in the cardiac group probably would have been even greater if nitroglycerin, rawolfia drugs, aspirin, and propranolol had not been used. Given an appropriate vascular insult, the hemostatic mechanism in post-infarction patients might readily respond by forming a platelet plug and triggering the coagulation sequence. Enzymatic clotting reactions could proceed through elevated factor VIII and X activities and lead to thrombin generation with fibrin clot formation. In the presence of a disordered fibrinolytic mechanism, the normal system of checks and balances would be shifted toward the permanent deposition of fibrin on the arterial wall or in the arterial lumen.

These hematological data were collected primarily to provide baseline information for later use in evaluating the action of lipid-lowering drugs on the hemostatic mechanism. Thus far, the data have provided information useful in formulating a concept of the hemostatic mechanism in male survivors of myocardial infarction. The question of pathogenesis in ischemic heart disease, however, remains as elusive as ever. Follow-up studies of the normal and CD groups are underway. These studies should be helpful in determining the prognostic significance of coagulation testing in these patients and also in demonstrating the effects of certain lipid-lowering drugs on the coagulation mechanism.

Acknowledgment

The authors are indebted to Dr. Robert Schlant and the staff of the Grady Memorial Hospital Cardiac Clinic for their help in this undertaking. Effie Brosious, M.T.(A.S.C.P.), and Maureen Mahoney, M.T.(A.S.C.P.), provided technical assistance.

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