The Coagulant and Thrombogenic Properties of Human Atheroma

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SUMMARY
Suspensions of gruel from severely atherosclerotic human aortas and coronary arteries were tested in several coagulation systems. The atheromatous material shortened the clotting time of normal whole blood and plasma and accelerated thrombus formation in the Chandler apparatus. Normal human platelets in platelet-rich plasma were aggregated by the atheromatous gruel. Aggregation did not occur when the gruel was added to platelet-rich plasma from a patient with a severe factor XII (Hageman) deficiency. The intravenous injection of atheromatous plaque suspensions into rats caused thrombocytopenia, shortening of the whole blood clotting time, and thrombosis.

Coagulation of blood from patients with classical hemophilia, with Hageman factor deficiency, and with coumarin-induced anticoagulant effect was accelerated by the addition of the atheromatous gruel. Blood from patients given heparin, however, largely retained its anticoagulant activity.

Atheromatous material from both aortic and coronary arteries behaved similarly in the systems tested. The coagulant and platelet clumping properties of atheromatous plaques may be related to their content of fatty acids, phospholipids, collagen, or thromboplastin.

Additional Indexing Words:
Coronary artery Aorta Atheromatous thromboplastin
Blood coagulation Mural thrombosis Hypercoagulability

Thrombosis is a complication of atherosclerosis that frequently leads to arterial occlusion and subsequent organ infarction.2,3 This complication is more likely to occur in an atherosclerotic artery when the endothelial surface has been disrupted.4,5 When an atherosclerotic plaque ulcerates, its tissue components are exposed to the circulating blood and may cause thrombosis.

While tissue extracts in general are believed to be thrombogenic, conflicting results have been reported concerning the coagulant properties of atheromatous plaque material. Kirk6 reported that the ulcerated areas of arteriosclerotic arteries had only low concentrations of thromboplastin. Byers and Friedman7 found that native gruel from human coronary artery plaques did not have thrombogenic properties. In contrast to these reports, Stevenson and associates8 observed that the thromboplastic activity of the atherosclerotic intima was greater in the thromboplastin generation test than that of normal intima.

In view of these divergent reports, the present study was performed to test the coagulant and platelet aggregating properties of human atheromatous plaque suspensions.
in several different systems. In addition, atheromatous plaque material was injected intravenously into animals to estimate its thrombogenic potential.

**Methods**

**Preparation of Atheromatous Material**

Aortas and coronary arteries with severe atherosclerosis were obtained from 33 human autopsy cases within 24 hours after death. Abdominal aortas and coronary arteries were isolated, washed, frozen, and stored in plastic bags at -20°C. These arteries were later thawed and the intimal layer was stripped from the atheromatous plaques. The gruel-like material was separated from the underlying tissue. The atheromatous material was then homogenized. A 2% suspension (w/v) was prepared in a 0.9% sodium chloride solution. One-milliliter aliquots of this suspension were stored at -20°C for later use.

**Blood Coagulation Tests**

Venous blood was obtained from normal human subjects. Special care was taken in handling the blood to avoid activation of the Hageman factor (factor XII). Each venipuncture was made with a new 18-gauge, silicone-coated needle,* and the blood was channeled through a 5-inch length of new plastic tubing.† If the venipuncture was difficult or if the blood did not flow freely, blood was obtained from a different vein. The first 3 ml of blood was discarded to dispose of any tissue juice introduced by the venipuncture. Blood for the thrombus formation determination was collected in chilled, silicone-coated glass tubes with 4.5 ml of blood to 0.5 ml of 3.8% trisodium citrate solution. The tubes were capped with Parafilm and inverted for mixing. The blood was then kept in melting ice until testing when it was warmed to 37°C. Blood stored in this way gave consistent results when tested from 10 minutes to 2 hours after venipuncture.

Studies of the effects of atheromatous suspension were performed on blood taken from subjects given 5,000 units of heparin sodium intravenously 5 minutes previously. Studies were done using the blood from patients receiving therapeutic doses of coumarin drugs. Blood from patients with the Hageman trait and classical hemophilia was tested also with the atheromatous suspensions.

The test for the whole blood clotting time was carried out by collecting 1.0 ml of whole blood into each of three silicone-coated 10 by 75 mm test tubes containing either 0.1 ml of atheromatous suspension or 0.9% sodium chloride as a control solution. The test for the thrombus formation time was performed with the rotating plastic disc apparatus modified from Chandler's original description.† One milliliter of citrated blood was pipetted with a silicone-coated pipette into a 12.5-inch polyvinyl chloride tubing of 0.118-inch inside diameter. One-tenth milliliter of either 2% atheroma suspension or 0.9% NaCl was then placed in the tubing whose ends were joined closely by a tightly fitting coupling of polyethylene.§ This was placed on the plastic disc and mixed at 12 rpm for 1 minute. The blood was then recalcified with 0.1 ml of 0.25 M CaCl₂, timing begun and rotation of the tubing started. The thrombus formation time was defined as the time at which the column of blood shifted 4° or more due to an increased friction between the inner wall of the tube and the column of blood which had changed its physical state.

The clotting time of recalcified plasma was determined by mixing 0.1 ml of oxalated human plasma (from blood centrifuged at 4,000 rpm for 10 minutes) and 0.1 ml of either the 2% atheroma suspension or 0.9% sodium chloride control solution in a plain 75 by 10-mm glass tube. This mixture was allowed to incubate at 37°C for 1 minute. One-tenth milliliter of 0.025 M CaCl₂ was then added, and the tube was slowly tilted and observed for the formation of a fibrin web.

**In Vivo Rat Studies**

Atheromatous suspension was injected into a tail vein of male rats, weighing approximately 300 g. The rats were anesthetized with ether, and the abdominal aorta was exposed. Control platelet counts were obtained from small leg artery cutdowns. The 1 ml of the 2% atheroma suspension was injected intravenously into 13 rats, and the 1 ml of 0.9% NaCl solution was given intravenously into 11 other control rats. Each animal was observed for 5 minutes. Blood was then collected from the aorta through a length of polyethylene tubing in the same manner as for the human blood studies. Whole blood clotting times, thrombus formation times, and direct platelet counts using phase microscopy were performed on this blood. All animals were examined for gross and microscopic thrombi after death.

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* *Cutter Laboratories, Berkeley, California.
†Mayon Plastics, Hopkins, Minnesota.

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In other in vivo experiments, 10 rats were given intravenously atheroma suspension for observation of the effects of the material upon the cardiopulmonary system. These animals were killed 20 minutes later in the event that they had not already died and were examined for thrombi. Five other rats were injected intravenously with 1,000 units of heparin and then 1 minute later with 1.0 ml of atheromatous suspension. After a 12-hour period of observation, they were killed and examined for thrombosis.

Platelet Studies

Platelet aggregation was determined by three methods: observation for gross clumping, direct microscopic examination, and a turbidimetric method. Washed platelet suspensions were prepared by collecting 9.25 ml of blood from normal human subjects into silicone-coated tubes containing 0.75 ml of 0.077 M sodium EDTA (ethylene diamine tetraacetic acid), pH 7.4. The blood was spun at 300 g for 16 minutes at 4°C, and the platelet-rich plasma separated from the lower layer of cells. The platelets were then separated from the plasma by spinning the suspension at 750 g for 15 minutes. The plasma was then pipetted from the platelets and an equivalent amount of medium was added. It was composed of 0.154 M sodium chloride, 0.154 M trishydrochloride buffer, 0.077 M EDTA mixed in the proportion of 90:8:2 by volume. The platelets were then spun for 12 minutes at 250 g. The solution was again withdrawn and replaced with a medium containing 9 parts of 0.154 M sodium chloride and one part tris buffer, pH 7.4, to the original plasma volume and the platelets were resuspended. All glassware was silicone-coated. A sample of this suspension was then observed under a phase microscope in order to rule out platelet clumping which might have occurred as a result of the collection procedures.

A 1-ml aliquot of the platelet suspension was then placed into a silicone-coated 10 by 75-mm test tube which was inserted into a photoelectric apparatus over a magnetic stirrer. A silicone-coated wire rod was put into the suspension, the magnetic stirrer was started, and the base line of the photoelectric apparatus was taken. A 0.1-ml aliquot of 2% atheromatous suspension was added, the base line was re-established, and the turbidimetric curve recorded.

Platelet-rich plasma was obtained by collecting 9 ml of blood from normal human subjects into a tube containing 1 ml of 3.8% trisodium citrate. This blood was spun at 300 g for 16 minutes, and the plasma was carefully separated from the underlying cells. This platelet-rich plasma was used in the same fashion as the washed platelet suspension, except that no calcium was added.

Figure 1

The effects of atheromatous suspension from 18 different aortas upon the thrombus formation time of blood from 12 healthy subjects. Each combination of atheroma and blood was studied only once. Each black dot represents the individual result from the addition of aortic atheroma to the test system. The open circle above each black dot indicates the corresponding control 0.9% sodium chloride determination. Mean values with standard deviations for both of the "atheroma" control tests are depicted on the right. "Atheroma" differed from control, P < 0.001.

Fatty Acid Studies

Measurement of the free fatty acid in the atheromatous plaque gruel was done by the method described by Bierman and associates. The free fatty acid composition of atheromatous
plaques from two aortas was ascertained by gas chromatography.*

Results

The atheromatous suspensions prepared from aortic plaques invariably accelerated the thrombus formation time of blood (fig. 1). The addition of aortic atheroma shortened the control thrombus formation mean time from 12.4 to 4.4 minutes. Suspension of coronary atheroma caused a similar degree of acceleration; the control mean time of 11.0 minutes was shortened to 3.9 minutes in a number of tests (fig. 2).

Acceleration of blood coagulation occurred from the addition of the atheromatous suspension to whole blood in silicone-coated glassware (fig. 3). The 2% suspension of aortic atheroma shortened the clotting time of whole blood from a mean control time of 43.1 to 5.2 minutes. Coronary atheroma shortened the clotting time of whole blood from a mean control of 44.8 to 5.9 minutes (fig. 4). As with the thrombus formation test, no differ-

*Studies were performed by Dr. F. M. Mattson, Miami Valley Research Laboratory, The Proctor and Gamble Co., Cincinnati, Ohio.

![Figure 3](image)

*Figure 3*

Atheromatous suspensions from 16 different aortas were tested for activity in the whole blood clotting test of 16 healthy subjects. Each black dot represents an individual test, and the clear circle above, the corresponding control test. The mean values with standard deviation for the "atheroma" and control 0.9% sodium chloride tests are depicted on the right. "Atheroma" differed from control, P < 0.001.

![Figure 4](image)

*Figure 4*

The effects of atheromatous suspension from nine different coronary arteries upon the whole blood clotting time of blood obtained from six subjects. The result of each trial using atheroma is indicated by a dark bar and each control test by a light bar. Mean values with standard deviations for both the "atheroma" and control 0.9% sodium chloride tests are depicted on the right. "Atheroma" differed from control, P < 0.001.

ence was found between the effects of aortic and coronary atheroma. The effects of various dilutions of atheromatous plaque material upon the thrombus formation time and upon the whole blood clotting time in silicone-coated tubes are indicated in figure 5. The clotting time of recalcified oxalated plasma was reduced from a mean control time of 2.6 to 1.5 minutes (fig. 6).

The results of clotting tests with blood from a patient with classical hemophilia (factor VIII deficiency) and from a patient with Hageman trait (factor XII deficiency) are given in table 1. The atheromatous gruel

![Figure 5](image)

*Figure 5*

The effect of various dilutions of atheromatous suspension upon the thrombus formation time and the whole blood clotting time.
CLOTTING TIME OF RECALCIFIED PLASMA WITH AORTICATHEROMA

Figure 6

Clotting time of plasma from seven different subjects after addition of a suspension of atheroma from 11 different human aortas. The result of each trial using atheroma is indicated by a dark bar and each control test by a light bar. Mean values with standard deviations for the “atheroma” and control 0.9% sodium chloride tests are depicted on the right. “Atheroma” differed from control, P < 0.001.

partially corrected the delayed clotting of each patient's blood. It is of interest that the patient with Hageman trait previously had sustained a myocardial infarction. Atheromatous gruel decreased the greatly prolonged whole blood clotting times in glass tubes and silicone-coated tubes of blood from each of these patients. The thrombus formation time of both blood samples was likewise accelerated by the atheromatous suspension, especially in the Hageman deficient blood.

Further studies were carried out on the blood drawn from subjects who had been given therapeutic doses of anticoagulants (table 2). The thrombus formation time of blood drawn from subjects receiving heparin was reduced from greater than 30 minutes to 12.8 and 14.7 minutes by the atheromatous suspension. This blood failed to clot after 5 hours in the whole blood clotting test; the addition of atheromatous suspension produced clotting in 35 to 180 minutes. The thrombus formation time of blood drawn from subjects receiving coumarin drugs was shortened from a mean control time of 19.0 to

Table 1

<table>
<thead>
<tr>
<th></th>
<th>Whole blood clotting time*</th>
<th>Thrombus formation time*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glass tubes</td>
<td>Silicone-coated tubes</td>
</tr>
<tr>
<td>Atheroma</td>
<td>13</td>
<td>18</td>
</tr>
<tr>
<td>0.9% NaCl</td>
<td>140</td>
<td>200</td>
</tr>
<tr>
<td>Hageman (factor XII)</td>
<td>9</td>
<td>15</td>
</tr>
<tr>
<td>deficient blood</td>
<td>75</td>
<td>145</td>
</tr>
</tbody>
</table>

*All values in minutes.
Table 2
Effects of Atheromatous Suspension on Blood from Patients Receiving Anticoagulant Drugs

<table>
<thead>
<tr>
<th>Patient and drug given</th>
<th>Prothrombin time (sec)</th>
<th>Thrombus formation time (min)</th>
<th>Whole blood clotting time (min)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Atheroma NaCl</td>
<td>Atheroma NaCl</td>
</tr>
<tr>
<td>Heparin*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>12.8</td>
<td>30+</td>
<td>35</td>
</tr>
<tr>
<td>2</td>
<td>14.7</td>
<td>30+</td>
<td>180</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>Coumarin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>18.6</td>
<td>6.0</td>
<td>8.0</td>
</tr>
<tr>
<td>2</td>
<td>21.5</td>
<td>6.8</td>
<td>6.0</td>
</tr>
<tr>
<td>3</td>
<td>26.6</td>
<td>7.0</td>
<td>8.0</td>
</tr>
<tr>
<td>4</td>
<td>39.9</td>
<td>5.5</td>
<td>8.0</td>
</tr>
<tr>
<td>5</td>
<td>57.0</td>
<td>8.0</td>
<td>—</td>
</tr>
<tr>
<td>6</td>
<td>24.0</td>
<td>7.0</td>
<td>—</td>
</tr>
<tr>
<td>Mean</td>
<td>31.2</td>
<td>6.7</td>
<td>7.5</td>
</tr>
</tbody>
</table>

*Subjects were given 5,000 units of sodium heparin intravenously 5 minutes before blood was drawn.

Table 3
Effects Produced by the Addition of Atheromatous Suspensions to Platelet-Rich Plasma or Washed Platelets

<table>
<thead>
<tr>
<th></th>
<th>Observations</th>
<th>Tests with turbidimetric change</th>
<th>Tests with platelet aggregation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet-rich plasma</td>
<td>13</td>
<td>12</td>
<td>9</td>
</tr>
<tr>
<td>Washed platelets</td>
<td>7</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

6.7 minutes. The whole blood clotting time of this same blood was shortened from a mean control time of 111 minutes to 7.5 minutes by the atheromatous material.

Platelet Studies
The platelets of platelet-rich plasma were aggregated by the atheromatous suspension (fig. 7). On the other hand, platelets first washed with EDTA and then suspended in tris buffered saline did not aggregate after the addition of atheromatous suspension (table 3). Platelets suspended in native plasma drawn from the patient deficient in Hageman trait failed to aggregate when the atheromatous suspension was added (fig. 8).

In Vivo Rat Studies
The atheromatous suspensions injected intravenously into rats produced thrombocytopenia in association with accelerated clotting
Table 4

<table>
<thead>
<tr>
<th>Atheroma infusion (13 rats)</th>
<th>Thrombus formation time (min)</th>
<th>Whole blood clotting time (min)</th>
<th>Platelets before (thousands)</th>
<th>Platelets after (thousands)</th>
<th>Gross heart thrombi</th>
<th>Gross pulmonary thrombi</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.79 ± 0.37*</td>
<td>5.45 ± 1.75*</td>
<td>931 ± 203</td>
<td>598 ± 247*</td>
<td>8</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>0.9% NaCl infusion (11 rats)</td>
<td>3.43 ± 0.32</td>
<td>10.00 ± 1.35</td>
<td>914 ± 178</td>
<td>949 ± 114</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Difference from the control NaCl infusion (P < 0.001); ± standard deviation.

Table 5

<table>
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<tr>
<th>Fatty acids</th>
<th>Plaque 1</th>
<th>Plaque 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>C12:0</td>
<td>trace</td>
<td>trace</td>
</tr>
<tr>
<td>C14:0</td>
<td>0.9</td>
<td>0.8</td>
</tr>
<tr>
<td>C16:0</td>
<td>18.6</td>
<td>18.1</td>
</tr>
<tr>
<td>C16:1</td>
<td>5.4</td>
<td>3.4</td>
</tr>
<tr>
<td>C18:0</td>
<td>3.7</td>
<td>5.4</td>
</tr>
<tr>
<td>C18:1</td>
<td>28.3</td>
<td>26.8</td>
</tr>
<tr>
<td>C18:2</td>
<td>22.6</td>
<td>31.5</td>
</tr>
<tr>
<td>C18:3</td>
<td>0.5</td>
<td>0.9</td>
</tr>
<tr>
<td>C20:0</td>
<td>0.5</td>
<td>0.3</td>
</tr>
<tr>
<td>C20:1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>C22:0</td>
<td>1.8</td>
<td>1.8</td>
</tr>
<tr>
<td>C22:1</td>
<td>8.1</td>
<td>6.0</td>
</tr>
<tr>
<td>Unknown</td>
<td>9.1</td>
<td>3.6</td>
</tr>
</tbody>
</table>

Gas Chromatographic Analysis of Free Fatty Acids in Aortic Atheromatous Plaques (Percentage of Total)

and caused the formation of thrombi in the heart and lungs (table 4). No thrombi were found in rats which had received saline injection.

An additional 10 rats were also given 1.0 ml of atheromatous suspension. Six rats died within a few minutes after injection. Nine had pulmonary thrombi, and four had cardiac thrombi. Five other rats, which had been given heparin and then given intravenous injections of atheromatous suspension, were allowed to live for 12 hours before they were killed. No gross thrombi were found in the hearts in these rats, but four of the five had gross pulmonary thrombi.

Lipid Studies

The mean free fatty acid content of 12 aortic plaques was 736 μEq/g wet weight of tissue, with a range of 350 to 1,630 μEq and a standard deviation of 343. The gas chromatographic analysis of the gruel from two plaques is listed in table 5. These results are similar to those reported by Bottcher and associates. With the use of results in these studies, it can be estimated that the 2% atheromatous suspensions contained approximately 0.02 to 0.03% stearic acid (C18:0) and 0.06 to 0.07% palmitic acid (C16:0), concentrations similar to those previously reported by Connor which accelerated blood coagulation in in vitro systems.

Discussion

Thirty years ago, Leary emphasized that the ulcerated atheromatous plaque was the critical etiological factor in coronary thrombosis. Our results support and strengthen this opinion. Many investigators have suggested that the atheromatous plaque has an important role in the formation of arterial thrombi. It is likely that the contact of luminal blood with the plaque material exposed through intimal fissures often precedes arterial occlusion by thrombosis.

In contrast to the idea that atheromatous plaque material has coagulant properties, Byers and Friedman reported that atherosclerotic plaque gruel showed an insignificant amount of clotting activity when tested on the Chandler wheel in the thrombus formation determination. These investigators tested non-homogenized plaque gruel from human coronary arteries. It must be emphasized that the methods of our study differ in that human
plaque material was homogenized and thus provided a greater surface area for contact with blood. This well may explain the much greater activity of the material in the thrombus formation test in our experiments.

The coagulant and thrombogenic properties of atherosclerotic plaque material may be derived from one or more of the constituents of the plaque. Lipids compose a large portion of the plaque, the major lipids being cholesterol, cholesterol esters, phospholipids, triglycerides, and free fatty acids. Our studies indicate that free fatty acids are present in the atheromatous plaque, in particular the saturated fatty acids 14 to 24 carbon atoms in length. Saturated fatty acids of these lengths have been shown to accelerate blood coagulation in vitro and it has been suggested that these substances act upon the surface sensitive factors in a manner similar to glass. Thus, some of the acceleration of clotting by atheromatous suspensions may have occurred in a manner similar to the acceleration of clotting produced by glass or kaolin.

The atheromatous plaque contains considerable phospholipids. Connor reported previously that soybean phosphatide added to whole blood did not shorten the thrombus formation time. Phospholipid from soybeans can be used to substitute for platelets in the thromboplastin generation test when performed with platelet-poor plasma but has no effect upon the clotting of platelet-rich plasma. In the present study, atheromatous gruel added to frozen platelet-rich plasma accelerated the clotting of this plasma. It is therefore unlikely that the phospholipid constituents of the atheroma were chiefly responsible for its coagulant and thrombogenic properties.

Among the other major lipid constituents of the atheromatous plaque are cholesterol, cholesterol esters, and triglycerides. There is no evidence that any of these constituents affect blood coagulation.

Platelet aggregation may well be one of the earliest events in the formation of a thrombus over an ulcerated atheromatous plaque. The formation of the white head of the thrombus in the Chandler wheel is the first event to be seen during the process of thrombus formation. The main constituent of this head is platelets. In addition, platelets seemed to be affected in some way by the atheroma in the in vivo studies as evidenced by the thrombocytopenia of the atheroma-injected rats. The platelets have evidently clumped since portions of the thrombi found in the hearts and lungs of rats were composed of platelets.

The free fatty acids of the atheromatous plaque conceivably might cause platelet aggregation. Haslam first reported that washed platelets suspended in tris buffered saline clumped rapidly in the presence of long-chain saturated fatty acids and calcium chloride. He suggested that activation of an adsorbed protease such as Hageman factor may be a mechanism involved in platelet aggregation. Kerr and co-workers also found that saturated fatty acids in solution with lecithin caused aggregation of platelets suspended in plasma. Others have also reported a similar finding, that washed pig platelets were aggregated by the long-chain saturated fatty acids such as behenic, arachidic, stearic, palmitic, myristic, and lauric, and the unsaturated oleic acid. Hoak and associates found that linoleic and linolenic fatty acids (as well as stearic and oleic acids) aggregated platelets suspended in citrated plasma and washed platelets suspended in tris-buffered saline. In the present study, atheromatous suspension did not cause clumping of washed platelets. This suggests that the free fatty acids in the atheromatous gruel did not lead to platelet aggregation because fatty acids act without the presence of plasma. The atheromatous material may first act on a substance in plasma, with platelet aggregation later occurring. The failure of platelets to aggregate in Hageman trait deficient plasma further suggests that the plasma substance might well be Hageman factor as earlier proposed by Sharp. Another important platelet-aggregating factor is thrombin, a substance that might be present in atherosclerotic plaque material. We have no information.

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about trace quantities of thrombin in atheroma. Certainly, the failure of atheromatous gruel to clot fibrinogen would rule out the presence of very much thrombin.

Collagen, a major constituent of the atheromatous plaque, aggregated platelets suspended in plasma but did not aggregate EDTA-washed platelets. This finding is similar to that of this study in that atheromatous material did not affect EDTA-washed platelets but did cause clumping of the platelets in plasma. Prentice and associates have described two platelet-aggregating factors in aortic atheromatous material, one heat-labile substance and one heat-resistant substance. It is their belief that platelet aggregation occurs through the activity of collagen present in the plaque material. The observations of Friedman and Van den Bovenkamp suggest that platelet adherence to the edge of endothelial ulceration over the plaque is possibly the first event in the formation of the arterial thrombus. In addition, it has been reported that collagen may adsorb and activate factor XII which is then followed by activation of factor X.

Thromboplastin-like activity was noted in this study by a shortening of the clotting time of recalcified plasma. This activity might be due to fatty acids or phospholipids, or it might result from the rather poorly defined and somewhat nonspecific action of tissue juice. Astrup first reported that such activity was present in arterial and venous tissues. Kirk later observed that ulcerated portions of atherosclerotic tissue had a low thromboplastin activity. In our study, atheromatous suspension added to blood taken from a subject deficient in antihemophilic factor (factor VIII) caused acceleration of coagulation. Similar results were obtained from studies on blood taken from a subject deficient in the Hageman trait (factor XII). These results suggest that the plaque constituents can accelerate clotting in the absence of either factor VIII or factor XII. It would appear that the atheroma activates a process of coagulation which requires factors VII, X, and V.

Nossel has suggested that dilute tissue extract may activate blood clotting in the same manner as do activated contact factors. In our studies, high dilutions of atheromatous suspension maintained their propensity to activate clotting of normal blood. It is possible that ulceration of endothelium does expose luminal blood to dilute tissue juice which in turn activates factors XI or XII.

In recent years, patients with thrombosis have received anticoagulant drugs to prevent further thrombosis. In our study, though the number of subjects is small, the atheromatous gruel accelerated the coagulation of blood from patients receiving coumarin agents at therapeutic dosage levels. Though numerous in vitro studies indicate that coumarin drugs delay the coagulation of blood, this may not always be the case of blood exposed to the components of the atheromatous plaque. Tissue juice provides a thromboplastin which may act directly on blood exposed to the ulcerated plaque. Decreased prothrombin from treatment with coumarin drugs may provide little antithrombotic effect under such circumstances. The action of collagen in clumping platelets is probably not affected by coumarin drugs either. Under the conditions of our study, heparin appeared to block the coagulant activity of atheromatous gruel more effectively than did coumarin drugs.

References

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COAGULANT PROPERTIES OF ATEROMA


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