Coagulation Tests and Platelet Economy in Atherosclerotic and Control Subjects

By E. A. Murphy, M.D., and J. F. Mustard, M.D.

EARLY investigations of atherosclerosis were largely based on the idea that the principal factor in the pathogenesis of the lesion and of the thrombotic complications was disease of the arterial wall and abnormalities in serum lipids. Attention has been rather belatedly directed to examination of differences in coagulation. In recent years, various investigators have provided evidence supporting the hypothesis of Rokitansky that atheromatous lesions can be caused by microthrombi formed from circulating blood.1-4 If this is indeed a mechanism, it is to be expected that atherogenesis will be affected by the coagulability of the blood. Furthermore, it has been shown in the normal animal that intravascular clotting is more prone to develop in the presence of hypercoagulability of the blood.5 Thus, it seems possible that an active clotting system may promote both the formation of atheromatous lesions and thrombotic complications.

Nevertheless, several studies comparing coagulation activity in atherosclerotic and control subjects by various test systems failed to show differences.6-8 In 1957, however, McDonald9 reported that subjects with coronary disease generate more thromboplastin and that their platelets exhibit a greater degree of adhesiveness than control subjects. Horlick,10 using a different method for measuring platelet adhesiveness, has confirmed McDonald's9 observations. Mustard11 demonstrated differences in thromboplastin generation not only between control and atherosclerotic subjects, but also between controls with positive and those with negative family histories for atherosclerosis. He showed further that an important source of these differences was an activity that corrected the defect in factor IX-deficient serum (possibly factor IX itself).

It is well established that the clumping and fusion of blood platelets is the earliest morphologic change in the formation of most kinds of thrombi.4,12-16 It is therefore of some importance to determine the relationship between the onset of platelet clumping and changes in factor IX activity and platelet adhesiveness. We have examined4-17 the order of events during in vitro clotting at 37°C by taking serial samples into silicone-coated tubes containing 3.8 per cent sodium citrate and kept at 4°C. Each sample specimen was subsequently assayed for the activity of various clotting factors. The time of onset of platelet clumping was determined retrospectively by examination of blood smears made serially as the blood clotted. The results of these studies are represented in figure 1. They show a steady rise in factor IX activity and platelet adhesiveness, culminating in the clumping of the platelets, which was followed by a precipitous fall in factor VIII and factor V activity, the onset of detectable thrombin formation, and finally fibrin formation. From this evidence, it appears that increased platelet stickiness and factor IX activity are antecedent to, and not consequences of, platelet clumping and are phenomena of the earliest stages of clotting. It is clear that the differences in clotting that have been described between atherosclerotic and control subjects occur in the earliest stages.
Studies of this kind are, however, open to the objection that the in vitro tests may not accurately reflect the in vivo state of the coagulation mechanism. It is therefore necessary to find some in vivo measure of the early stages of clotting that causes minimum disturbance of homeostasis. Since platelets are expended in the early stages of clotting, it seems reasonable to assume that measurement of platelet survival and turnover would be an index of the activity of this stage. Therefore, we have studied clotting in atherosclerotic and control subjects by appropriate in vitro and in vivo technics.

Materials and Methods

Patients

Seventy-five male subjects were studied. In 31 there was clinical evidence of complications of atherosclerosis, such as myocardial infarction, angina pectoris, carotid or basilar artery stenosis or occlusion, or intermittent claudication. None of these subjects had sustained a clinically apparent acute occlusive episode during the 3 months preceding the study, and none was receiving dietary or anticoagulant therapy. The control group was drawn from normal healthy subjects and from subjects suffering from disorders believed to be unrelated to atherosclerosis (e.g., prolapsed intervertebral disks, psoriasis, chronic bronchitis). No subject with cancer, collagen disorders, diabetes, gout, or venous thrombosis was used as a control.

Sixteen patients in each group gave a positive family history for atherosclerotic complications occurring below the age of 75 in siblings, parents, aunts, or uncles. In the remaining subjects, the family history was negative in this respect. It is apparent that subjects in whom the family history was incomplete tend to be misclassified as having a negative family history. This probably occurs to a similar extent in the two groups: if anything, rather more frequently in the control subjects. This will tend to underestimate rather than exaggerate the differences between the groups to be compared.
Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of subjects</th>
<th>Age</th>
<th>Mean plasma thromboplastin time (sec.)</th>
<th>Mean platelet count (No./mm.³)</th>
<th>Mean platelet clumping time (sec.)</th>
<th>Mean platelet adhesive index</th>
<th>Mean clotting time (min.)</th>
<th>Mean one-stage pro-thrombin time (sec.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atherosclerotic (A)</td>
<td>31</td>
<td>55.3 ± 2.0</td>
<td>11.61</td>
<td>229,500</td>
<td>308</td>
<td>1.250</td>
<td>12.65</td>
<td>14.1</td>
</tr>
<tr>
<td>Positive family history (A+)</td>
<td>16</td>
<td>54.5 ± 2.8</td>
<td>11.45</td>
<td>225,700</td>
<td>304</td>
<td>1.291</td>
<td>12.66</td>
<td>14.1</td>
</tr>
<tr>
<td>Negative family history (A-)</td>
<td>15</td>
<td>56.1 ± 2.9</td>
<td>11.78</td>
<td>219,100</td>
<td>313</td>
<td>1.207</td>
<td>12.65</td>
<td>14.1</td>
</tr>
<tr>
<td>Control (C)</td>
<td>44</td>
<td>46.4 ± 2.1</td>
<td>12.48</td>
<td>214,400</td>
<td>311</td>
<td>1.087</td>
<td>12.26</td>
<td>14.2</td>
</tr>
<tr>
<td>Positive family history (C+)</td>
<td>16</td>
<td>41.1 ± 2.6</td>
<td>12.18</td>
<td>225,900</td>
<td>299</td>
<td>1.174</td>
<td>11.87</td>
<td>13.9</td>
</tr>
<tr>
<td>Negative family history (C-)</td>
<td>28</td>
<td>49.5 ± 2.8</td>
<td>12.66</td>
<td>207,800</td>
<td>318</td>
<td>1.037</td>
<td>12.47</td>
<td>14.4</td>
</tr>
</tbody>
</table>

Comparison of age-adjusted mean t values

(≡ √F )

<table>
<thead>
<tr>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.95†</td>
<td>4.26†</td>
<td>1.71</td>
<td>0.41</td>
<td>2.08*</td>
<td>3.50†</td>
<td>1.17</td>
<td>2.44*</td>
<td>3.87†</td>
<td>1.51</td>
</tr>
</tbody>
</table>

* *p <0.05
†p <0.01
‡p <0.001

The groups were not matched according to age because we have found this to be a wasteful process. The age ranges, however, are not very different (table 1) and differences between groups have been corrected for age by covariance analysis. In no instance has this age correction substantially altered the results.*

In Vitro Coagulation Tests

Blood was collected from each subject with a paraffin-coated glass syringe and an 18-gage silicone-coated stainless-steel needle. One milliliter quantities were placed in each of three glass tubes of 10-mm. internal diameter; these were used for the clotting time determinations. Five milliliters of blood were placed in a silicone-coated glass centrifuge tube standing in a basin of melting ice ("native blood"). This sample was used for determining the platelet clumping time. Four and one half milliliters of blood were placed in a silicone-coated glass centrifuge tube containing 0.5 ml of 3.8 per cent trisodium citrate. Forty-five milliliters of blood were placed in a silicone-coated tube containing 5 ml of a 1 per cent disodium ethylene diamine tetraacetate solution. This sample was used for preparing the platelets for radioactive counts.

Whole Blood Clotting

This was determined by the method of Lee and White as described by Biggs and Macfarlane.19 The tilting of the tubes was not started until 5 minutes after the blood was placed in them. The range (the mean ± 2 standard deviations) for 110 normal subjects is 6.5 to 16 minutes, with a mean of 11.25 minutes.

The Prothrombin Time

This test was carried out as previously described,20 with tissue thromboplastin prepared from human brain. The range (the mean ± 2 standard deviations) for 110 normal subjects not receiving Dicumarol therapy is 11.7 to 15.1 seconds, with a mean of 13.4 seconds.

Platelet Adhesive Index

This was determined by the glass-wool filter method of Moolten et al.21 The experimental error...
of this test, based on 14 studies in triplicate, was found to be 0.37 or 30 per cent. The normal range is 0.82 to 1.55.

Platelet Count

This was carried out by a direct technic that has been previously described. The citrated blood sample was used for this test.

Platelet Clumping Time

This was determined by a technic similar to that described by Sharp.

Platelet Clumping Time

A sample of the citrated plasma was diluted 1:10 and used in place of serum in a thromboplastin generation-test system with use of brain extract and normal aluminum hydroxide-treated plasma. This test carried out as previously described gives a measure of plasma factor IX (Christmas factor, PTC) activity if it is assumed that the activity that corrects the defect in factor IX-deficient serum is factor IX. In the present study, this test was not done with factor IX-deficient serum. Therefore, the activity of dilute plasma in the thromboplastin generation test (plasma thromboplastin time) can be considered to represent in addition the activity of other factors such as factor X and Hageman factor. The maximum amount of thromboplastin generated in 6 minutes (as represented by the clotting time of the substrate) was taken as the test value. The thromboplastin generation test with use of normal serum, normal plasma treated with aluminum hydroxide, and a brain extract gives a peak value at 6 minutes in the range of 9.3 to 15.1 seconds, with a mean of 12.25 seconds. The standard error of this test, based on 15 studies in duplicate, was found to be 0.14 or ± 12.8 per cent.

Platelet Survival

Platelet survival was estimated by the use of di-isopropyl fluorophosphonate as P³² (DFP³²) according to the method of Leeksma and Cohen.

In this technic, the drug is given intravenously or intramuscularly, in doses not exceeding 2 mg. and containing from 75 to 125 μg. of P³². Certain modifications of the technic used have been described elsewhere. Blood samples were taken daily over a period of from 9 to 12 days following the injection.

The Evaluation of Mean Platelet Survival

The pattern of platelet destruction is unknown. It may be random, in which case the curve of destruction would be exponential whatever the population of platelets labeled. It may be dictated by a normally distributed lifespan, and if all the circulating platelets have an equal chance of being labeled (as is probably the case with DFP³²), then the survival curve should follow the second integral of the normal (Gaussian) distribution, which tends to a straight line as the coefficient of variation tends to zero. Finally, the pattern of destruction may be a compound of these two forms, or may be described by some other mathematical function. Many possible patterns are very similar, and sampling error introduced from various sources makes it impossible from these data to distinguish between them with confidence. Thus it is only possible at present to obtain values that are indices of platelet survival, and confident absolute estimates cannot be made. In this study, two mathematical treatments have been used:

1. Exponential. A half-life is computed from the slope of the best line fitted by the method of least squares to the plot of the logarithms of the counts against time. From this value the mean survival can be computed by dividing by log₂, which is 0.69315.

2. Gaussian. The intercept on the time axis of the best line fitted by the method of least squares to the plot of the counts against time, is a measure of mean survival from the date of labeling. This involves the simplifying assumption that variance of the lifespan is negligibly small. The problem of curve-fitting to so complex a function...
with two parameters and considerable experimental error is otherwise intractable.

The two methods of computing platelet survival assume homogeneity of variance from the regression line. More extensive and refined experimentation might show this assumption to be unjustified. In the absence of such evidence, this simple method has been used in preference to fitting lines by eye, because of the risk of systematic observer bias.

A plot of the Gaussian mean against the exponential mean in 161 miscellaneous subjects (fig. 2) gives a correlation coefficient of +0.872 and the Spearman non-parametric correlation coefficient is +0.818. These values provide evidence that while the two methods give different results, they are closely correlated and both probably provide good indices of true mean platelet survival.

Adelson, Rheingold, and Crosby\(^27\) have leveled various criticisms against the DFP\(^28\) method of estimating platelet survival, and by the use of an alternative method have arrived at an estimate of 1/2 to 3 days for the platelet half-life in dogs compared with estimates of 9 to 11 days found by other investigators using DFP. It must be pointed out, however, that they have calculated their results on an exponential pattern while others have used the Gaussian pattern. Since both estimates agree quite well with the results presented below, it seems probable that the discrepancies arise from differences in mathematical evaluation rather than from differences in laboratory technics. Furthermore, similar estimates have been obtained by the use of other methods—S\(^{35}\)-cohort tagging in pigs\(^28\) and Cr\(^{51}\) in man.\(^29\) Finally, Athens Mauer, Ashenbrucker, Cartwright, and Wintrobe\(^30\) have examined the criticisms of Adelson et al.\(^27\) and show them to be in practice of little consequence.

A series of studies in pigs and in dogs in which concurrent labeling of platelets in vivo with S\(^{35}\) and DFP\(^28\) has been done, provided mutually corroborative evidence of the reliability of these two methods.\(^31\)

### Statistical Considerations

In previous communications,\(^24, 26\) we have reported that the several variates are distributed as follows for the data we have collected: whole-blood clotting time and platelet clumping time are logarithmically normally distributed; plasma thromboplastin activity is harmonically distributed and the remaining clotting tests and the two evaluations of platelet survival and turnover are normally distributed. Appropriate transformations have been used for statistical calculations. Mean values have been decoded before insertion into the tables.

### Results

#### In Vitro Clotting Tests

All tests were performed some five times on every subject, and average values for each subject were calculated.

The comparison of the mean of the values for the atherosclerotic and control groups is shown in table 1. Despite the considerable

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**Table 2**

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean platelet count (No./mm.(^3))</th>
<th>Exponential treatment</th>
<th>Gaussian treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean platelet half-life (days)</td>
<td>Mean platelet turnover (days)</td>
<td>Mean platelet survival (No./mm.(^3)/day)</td>
</tr>
<tr>
<td>Atherosclerotic (A)</td>
<td>225,500</td>
<td>2.86</td>
<td>58.220</td>
</tr>
<tr>
<td>Positive family history (A+)</td>
<td>225,500</td>
<td>2.58</td>
<td>64.700</td>
</tr>
<tr>
<td>Negative family history (A-)</td>
<td>219,000</td>
<td>3.15</td>
<td>51.320</td>
</tr>
<tr>
<td>Control (C)</td>
<td>214,400</td>
<td>3.42</td>
<td>48.550</td>
</tr>
<tr>
<td>Positive family history (C+)</td>
<td>225,900</td>
<td>2.88</td>
<td>59.360</td>
</tr>
<tr>
<td>Negative family history (C-)</td>
<td>207,800</td>
<td>3.74</td>
<td>42.370</td>
</tr>
</tbody>
</table>

Comparison of age-adjusted mean \(t\) values

\(\equiv \sqrt{F}\)

| Group | \(A\) & \(C\) | \(A\) & \(C+\) | \(A\) & \(C-\) | \(A+\) & \(A-\) | \(A+\) & \(C\) | \(A+\) & \(C+\) | \(A+\) & \(C-\) | \(A-\) & \(C\) | \(A-\) & \(C+\) | \(A-\) & \(C-\) | \(C+\) & \(C-\) |
|-------|---------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|       | 2.58\(^*\)    | 2.32\(^*\)      | 2.11\(^*\)      | 1.70\(^*\)      | 0.52            | 0.83            | 0.55            | 0.21\(^*\)      | 3.37\(^\dagger\) | 3.39\(^\dagger\) | 2.88\(^\dagger\) | 2.40\(^*\)      | 2.09\(^*\)      | 1.93            | 2.49\(^*\)      | 1.85\(^*\)      | 3.14\(^\dagger\) | 2.94\(^\dagger\) | 2.86\(^\dagger\) | 2.31\(^*\)      | 0.90            | 0.50            | 0.72            | 0.55\(^*\)      |
|       | 4.14\(^\dagger\) | 3.86\(^\dagger\) | 3.40\(^\dagger\) | 2.77\(^*\)      | 1.03            | 0.76            | 0.52            | 0.36\(^*\)      | 2.06\(^*\)      | 1.59            | 2.09\(^*\)      | 1.31\(^*\)      | 1.66            | 1.94            | 1.20            | 1.13\(^*\)      | 2.04\(^*\)      | 2.75\(^*\)      | 2.13\(^*\)      | 2.26\(^*\)      |

\(^*\)See footnote table 1.
technical error involved in the platelet adhesive index test, there is a very highly significant difference between the two groups. There is also a highly significant difference between the mean values for the plasma thromboplastin time (activity of dilute plasma in the thromboplastin generation test). The mean platelet count is a little higher in the atherosclerotic group but not significantly so. The other three tests are identical.

No doubt the control group contains some subjects with advanced atherosclerosis not manifest clinically. These misclassified cases will probably be diminished in number if we restrict the control group to those with no family history for complications of atherosclerotic vessel disease. The result of comparing the residual group with the atherosclerotic subjects is shown in table 1. It will be seen that the results are substantially the same, but that in every instance, with the exception of the clotting time, the differences are enhanced.

The control group with the positive family history showed results similar to the atherosclerotic group (table 1). Both groups differ in the same respects from the control group with the negative family history.

It seemed to be of importance to study the effect of family history on the clotting tests in patients with complications of atherosclerotic vessel disease. It will be seen in table 1 that none of the differences between the mean values is significant though there are trends corresponding to the significant differences seen with other groups. The two "purest" groups that can be compared are the atherosclerotic patients with a positive family history and the control subjects with a negative family history. These show the greatest differences of all between the mean values.

In Vivo Clotting Tests

The comparison of the mean platelet half-life values for the atherosclerotic subjects and controls shows a significant difference, which is reflected to a slightly lesser degree in the mean platelet turnover values (exponential), table 2. When the Gaussian values are compared, the difference is significant for the mean platelet survival and, although present, is not quite significant for mean platelet turnover. As with the in vitro clotting tests, if the control subjects with the positive family history are excluded, all the differences are considerably enhanced, and the differences for the Gaussian values reach significance at the 1 and 2.5 per cent levels, respectively. The controls with the positive family history have mean values that are again similar to the atherosclerotic group (table 2). Platelet survival is shorter and platelet turnover greater even than in the atherosclerotic group with a negative family history, although these differences are not significant. Moreover, the differences between the control groups with positive and negative family histories are significant.

Comparison of the atherosclerotic groups with positive and negative family histories showed a trend in values similar to those seen with the in vitro clotting tests. The differences between the atherosclerotic group with the positive family history and the control group with the negative family history were highly significant.

The Relationship between In Vitro and In Vivo Clotting Tests

In view of the chronologic relationships between the clotting factors illustrated in figure 1, the statistical relationships for the data from the subjects in the present study have been evaluated and the results for the in vitro tests are shown in table 3. It may be said in general that the activity of dilute plasma in the thromboplastin generation test (plasma thromboplastin time) and the measurements that involve the platelets are interrelated one with another, but seem to be poorly related to those tests that measure the later stages of coagulation.

As to the relationship of these tests to the evaluations of platelet survival and turnover, the most impressive and uniform correlations are found with the adhesive index (table 4). Both the activity of dilute plasma in the thromboplastin generation test and the platelet clumping time show fairly good correlations.
Table 3

Correlation Coefficients between the Various in Vitro Tests

<table>
<thead>
<tr>
<th>Correlation between</th>
<th>Control group</th>
<th>Atherosclerotic group</th>
<th>Pooled results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+ve F.H.</td>
<td>-ve F.H.</td>
<td>+ve F.H.</td>
</tr>
<tr>
<td>Adhesive index (A.I.)</td>
<td>+0.19</td>
<td>+0.20</td>
<td>+0.27</td>
</tr>
<tr>
<td>and P.T.T.</td>
<td>+0.30</td>
<td>+0.22</td>
<td>+0.35*</td>
</tr>
<tr>
<td>Pl. count</td>
<td>-0.27</td>
<td>-0.20</td>
<td>-0.27</td>
</tr>
<tr>
<td>P.C.T.</td>
<td>-0.52*</td>
<td>+0.12</td>
<td>-0.27</td>
</tr>
<tr>
<td>O.S.P.T.</td>
<td>-0.49</td>
<td>-0.10</td>
<td>-0.27</td>
</tr>
<tr>
<td>C.T.</td>
<td>-0.51*</td>
<td>+0.34</td>
<td>+0.43†</td>
</tr>
<tr>
<td>Plasma thromboplastin time (PTT) and Pl. count</td>
<td>-0.06*</td>
<td>-0.37*</td>
<td>-0.46†</td>
</tr>
<tr>
<td></td>
<td>-0.16</td>
<td>+0.23</td>
<td>+0.05</td>
</tr>
<tr>
<td>O.S.P.T.</td>
<td>-0.24</td>
<td>-0.10</td>
<td>-0.17</td>
</tr>
<tr>
<td>Platelet count (Pl. count)</td>
<td>-0.52*</td>
<td>-0.21</td>
<td>-0.34*</td>
</tr>
<tr>
<td>and P.C.T.</td>
<td>-0.09</td>
<td>-0.03</td>
<td>-0.14</td>
</tr>
<tr>
<td>O.S.P.T.</td>
<td>-0.55*</td>
<td>+0.04</td>
<td>-0.18</td>
</tr>
<tr>
<td>C.T.</td>
<td>+0.08</td>
<td>-0.24</td>
<td>-0.07</td>
</tr>
<tr>
<td>Platelet clumping time (P.C.T.) and O.S.P.T.</td>
<td>+0.44</td>
<td>+0.48†</td>
<td>+0.47†</td>
</tr>
<tr>
<td>C.T.</td>
<td>+0.02</td>
<td>+0.18</td>
<td>+0.17</td>
</tr>
</tbody>
</table>

*See footnote table 1.
F.H. = Family history.

Discussion

In Vitro Clotting Tests

The results for the platelet adhesiveness and the activity of dilute plasma in the thromboplastin generation test (plasma thromboplastin time) confirm the previous reports of McDonald, Horlick, and Mustard. These two tests are measures of the early stages of clotting in contrast to the prothrombin and whole blood clotting times: these latter tests were similar in all the groups. Since the in vitro tests suggest that platelet clumping is an early morphologic change in clotting (fig. 1), it is perhaps surprising that average values for platelet clumping are so similar in the two groups. This may mean that the plasma thromboplastin time and platelet adhesiveness test are measuring an even earlier stage of clotting than that which is directly related to the onset of platelet clumping. Further, we have elsewhere raised the possibility that platelet adhesiveness and platelet cohesiveness may be separate phenomena, the former measured by the adhesive index, the latter by platelet clumping. Lastly, we would point out that in the test systems for assaying the activity of dilute plasma in the thromboplastin generation test and platelet adhesiveness contact of the test material with glass surfaces is kept to a minimum. This is not true in the platelet clumping-time test, which may, therefore, be more a reflection of the activating effect of glass surfaces on clotting, than of the activity of clotting in vivo. Any one or all of these three factors may have a bearing on the seeming lack of agreement between platelet adhesiveness and platelet clumping.

In Vivo Clotting Tests

Though there is some doubt as to how the duration of survival of the platelet should be estimated from the data, the two different mathematical patterns used (exponential and Gaussian) show similar results. From this, it is a reasonable assumption that they are at least indices of platelet survival. If it be further assumed—and this is borne out by data not presented here—that the venous platelet count while varying from time to time is unaffected by the study, we can estimate the platelet turnover per unit volume of blood per day. The results show that in atheroscle-
COAGULATION AND PLATELETS

The platelet survival is shorter than in the control subjects, and this is compensated for by an increased turnover of platelets. The differences are on the whole more striking if we assume that platelet destruction is random (exponential) and not primarily determined by senescence (Gaussian). There is evidence that arterial platelet counts are on the average higher than venous, so that calculations on the basis of venous counts (used in this study) may underestimate the true platelet turnover. This underestimate may be more marked in the presence of gross arterial disease, so that the differences noted in this study might be enhanced if arterial platelet counts had been used.

Relationship between In Vitro and In Vivo Clotting Tests

In view of the susceptibility of the coagulation mechanism to activation by foreign surfaces, there is a general uncertainty about the reliability with which in vitro clotting tests measure in vivo phenomena. The parallel differences revealed above suggest that at least two tests, the plasma thromboplastin time and the platelet adhesive index, are informative. More precise comparison of the results by correlation techniques shows a significant relationship between platelet survival and the adhesive index, and the platelet survival and plasma thromboplastin time—the correlations are better with the exponential than the Gaussian estimates. The correlations of these two clotting tests with platelet turnover are even better. These findings become even more impressive when it is recalled that all these tests, in vitro and in vivo, are subject to very large experimental error. It must be emphasized that both in correlations and in comparisons of means, experimental error tends to lead only to an underestimate of the significance of the results, if the collection and analysis of the data have been unbiased. Although the newer clotting tests and the studies of platelet survival are more sensitive than older technics, they have still grave deficiencies, and it seems probable that a more accurate method of evaluating platelet adhesiveness would prove a very valuable test. On the other hand, it is important to stress that though the results presented show differences for mean values between the various groups, they provide no information about the overlap of the respective distribution curves and hence about the discriminant value of the tests. The percentage misclassifications are too high for these measures to be of any real diagnostic value. A discriminant analysis between the two most distinct groups A+ and C− using the three tests that gave the most significant differences (platelet adhesive index, plasma thromboplastin time, and platelet exponential turnover) gave a misclassification of 20 per cent, i.e., the efficiency of the discriminant function is only two and one half times that of random guessing.

The net results indicate that there are differences in coagulation between atherosclerotic and control subjects and that these differences are probably centered in the earlier stages of clotting. Neither in vitro nor in vivo tests taken separately are unassailable evidence of this. The in vitro tests reflect in part distortions produced by change in environment while the in vivo tests involve the assumption that platelet turnover is a measure of the activity of autochthonous coagulation. The two lots of evidence, however, are mutually confirmatory and are further supported by the observation that parallel changes in both are produced by the use of Dicumarol. It is well established that Dicumarol depresses the level of factor IX. The extrinsic system of clotting (that part of blood coagulation that is activated by tissue thromboplastin) is principally measured by the one-stage prothrombin time, which is similar in the atherosclerotic and control groups. In contrast, the activity of dilute plasma in the thromboplastin generation test shows striking differences between the groups and also a close correlation with platelet turnover. As discussed in the section on materials and methods, we cannot be certain what factor or factors are responsible for
this increased activity. In an earlier study,\textsuperscript{10} it was shown that this activity would correct the factor IX defect in hemophilia B serum. It would seem reasonable to expect, therefore, that part of this difference is due to variations in the amount of factor IX activation. Wessler et al.\textsuperscript{5} have shown in a series of experiments in which normal and defective sera were infused into animals, that stasis thrombi could be consistently produced in the recipient animals unless the infused serum was deficient in factor IX or substances believed necessary for its activation. All this evidence suggests that factor IX and factors involved in its activation are intimately concerned with changes in platelet adhesiveness and thrombus formation. Although there is in vitro evidence that platelet phospholipid promotes factor IX activation,\textsuperscript{35, 36} it is as yet uncertain whether factor IX regulates platelet adhesiveness and clumping, whether viscous metamorphosis influences factor IX activity, whether both propositions are true, or whether both are independently related to some other factor or factors. Wessler and associates think that factor IX is a necessary component for activation of a serum thrombotic accelerator, but that this accelerator is distinct from factor IX. Recently, Spittel, Pascuzzi, Thompson, and Owen\textsuperscript{38} have shown acceleration of the early stages of coagulation in certain patients with occlusive arterial or venous disease. Although their evidence indicates that this is a factor involved in the early stages of clotting, it does not seem to be a known clotting factor. Further study may show these differences to be artifacts of the various test systems and that the factor responsible for the increased activity is a known clotting factor or group of factors.

\begin{table}
\centering
\caption{Correlation Coefficients between the Various in Vitro and in Vivo Tests}
\begin{tabular}{|l|ccc|ccc|cc|}
\hline
Correlation between & \multicolumn{3}{c|}{Control group} & \multicolumn{3}{c|}{Atherosclerotic group} & \multicolumn{2}{c|}{Pooled results} \\
& (+e F.H.) & (+e F.H.) & Total & (+e F.H.) & (+e F.H.) & Total & \\
\hline
Adhesive index & & & & & & & & \\
and P.H.I. & -0.21 & -0.47* & -0.48\dagger & -0.35 & -0.17 & -0.32 & -0.48\dagger & \\
P.M. & -0.27 & -0.41* & -0.47\dagger & -0.30 & -0.03 & -0.30 & -0.44\ddagger & \\
P.T.O. & +0.29 & +0.45* & +0.51\dagger & +0.73\dagger & +0.06 & +0.59\ddagger & +0.58\ddagger & \\
P.T.O. & +0.30 & +0.38* & +0.49\dagger & +0.74\dagger & +0.01 & +0.62\ddagger & +0.56\ddagger & \\
\hline
Plasma thromboplastin time & & & & & & & & \\
and P.H.I. & -0.39 & -0.02 & -0.18 & -0.17 & -0.39 & -0.38* & -0.32\ddagger & \\
P.M. & -0.42 & +0.19 & -0.06 & -0.29 & -0.28 & -0.32 & -0.22 & \\
P.T.O. & +0.55* & +0.23 & +0.41\dagger & +0.44 & +0.49 & +0.46\ddagger & +0.48\ddagger & \\
P.T.O. & +0.57* & +0.15 & +0.36* & +0.49 & +0.19 & +0.40* & +0.41\ddagger & \\
\hline
Platelet clumping time & & & & & & & & \\
and P.H.I. & +0.56* & +0.16 & +0.30* & +0.33 & +0.01 & +0.16 & +0.25* & \\
P.M. & +0.41 & +0.07 & +0.50 & +0.50 & -0.13 & +0.24 & +0.22 & \\
P.T.O. & -0.67† & -0.26 & -0.46† & -0.60* & -0.13 & -0.45* & -0.45† & \\
P.T.O. & -0.55* & -0.22† & -0.58* & & & & & \\
\hline
Prothrombin time & & & & & & & & \\
and P.H.I. & +0.52* & -0.05 & +0.20 & +0.54* & +0.16 & +0.26 & +0.22 & \\
P.M. & +0.58* & +0.01 & +0.26 & +0.58* & -0.00 & +0.24 & +0.26* & \\
P.T.O. & -0.28 & +0.07 & -0.22 & -0.49 & -0.06 & -0.23 & -0.23* & \\
P.T.O. & -0.31 & +0.05 & -0.19 & -0.46 & +0.24 & -0.19 & -0.20 & \\
\hline
Clotting time & & & & & & & & \\
and P.H.I. & +0.24 & -0.15 & -0.00 & +0.29 & -0.17 & +0.04 & -0.02 & \\
P.M. & +0.29 & -0.18 & -0.01 & +0.16 & -0.28 & -0.03 & -0.03 & \\
P.T.O. & -0.38 & +0.13 & -0.12 & -0.40 & +0.35 & -0.19 & -0.12 & \\
P.T.O. & -0.50* & +0.14 & -0.13 & -0.34 & +0.36 & -0.19 & -0.13 & \\
\hline
\end{tabular}
\end{table}

P.H.I. = Platelet half-life (exponential).
P.M. = Platelet survival (Gaussian).
P.T.O. = Platelet turnover (exponential).
P.T.O. = Platelet turnover (Gaussian).
*See footnote table 1.
F.H. = Family history.
Family History

There is now considerable evidence that atherosclerosis is familial.\textsuperscript{39, 40} Indeed, since atherosclerosis is a progressive disorder not usually manifest until the later years of life, family history may be of diagnostic value, especially since it is usually available before clinical manifestations appear. Any “control” population must, therefore, contain a certain number of subjects who will in due course develop complications of atherosclerosis. In an attempt to exclude these misclassified cases, Mustard\textsuperscript{11} divided his controls into those with a positive and those with a negative family history, and found that there was a difference between the two groups in certain clotting tests. In this study, the atherosclerotic subjects also are divided into two groups according to family history. In the main, it may be said that the atherosclerotic subjects with the positive family history exhibited the shortest platelet survivals, the highest platelet turnovers, and the greatest activities in the early stages of clotting, whereas the controls with the negative family history showed the converse. The other two groups that showed no significant differences from each other occupied an intermediate position. This raises interesting genetic possibilities.

It seems a mistake then to treat the “atherosclerotic” and the “control” subjects as homogeneous groups, and comparisons of them that do not take family history into account may fail to reveal differences between them. This fact together with the use of tests that do not specifically measure the early stages of clotting may explain why some authors have failed to detect differences between the groups.\textsuperscript{6-8}

Relationship of Platelet Economy to Atherosclerosis

While the evidence shows that the economy of the platelet is related to atherosclerosis, it is not clear what the nature of the relationship is. Advanced atheroma that has led to intimal ulceration may encourage the deposition of platelets, which will increase the rate of their consumption and possibly, by mechanisms already discussed, activate factors in the early stages of clotting such as factor IX. Such changes, therefore, could only be expected at an advanced stage of the disease. Changes may exist, however, from an early age—this could be readily decided at least for the in vitro tests—and they may be at least partly responsible for the development of atherosclerosis. This latter explanation would be in keeping with the encrustation hypothesis.\textsuperscript{1, 2, 4, 41}

The good correlations between platelet adhesiveness and platelet survival and turnover are a point of considerable theoretical importance. If the widely held view is correct, that platelet survival is normally distributed about a mean, then it must be supposed either that adhesiveness increases with the age of the platelet or that it has little to do with platelet survival. On the first point the scanty evidence available\textsuperscript{42} suggests that, if anything, the younger platelets are more adhesive. As for the second point, apart from the probability that the more adhesive platelets are more likely to be used up in maintaining the integrity of the vascular tree, administration of adequate Dicumarol causes both prolongation of platelet survival and reduction in platelet adhesiveness.\textsuperscript{26} It seems much more plausible to suppose that platelet destruction follows some pattern other than Gaussian at least in the atherosclerotic population.

Summary

Blood coagulation was studied in 31 atherosclerotic male subjects and in 44 control male subjects.

The mean platelet adhesive index was significantly greater in the atherosclerotic group and the plasma thromboplastin time was significantly shorter. These two tests are measures of the activity of the early stages of coagulation. The other in vitro tests of coagulation showed no significant differences.

At present, platelet consumption is probably the only available measure of the activity of the early stages of coagulation in vivo. Platelet survival and daily turnover was accordingly determined with DFP\textsuperscript{32}. Mean platelet survival was shorter and mean plate-
let turnover was greater in the atherosclerotic group.

Division of the groups according to family history of atherosclerosis proved important. The most clearly separated groups were the atherosclerotic group with a positive history and the controls with a negative history. The remaining two subgroups are much more alike. This was true of both in vitro and in vivo tests.

Despite the high experimental error, the in vivo tests correlate well with the in vitro tests of the early stages of clotting though poorly with tests of the later stages. It is concluded that differences exist between atherosclerotic and control subjects in the early stages of coagulation, and that in vitro tests are satisfactory indices of this difference. In their present form, none of these tests is suitable for diagnostic or prognostic purposes in the individual.

Acknowledgment
We are indebted to our technicians Mr. R. Kendall and M. Boroeski for their patience and industry.

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Circulation, Volume XXV, January 1962


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Coagulation Tests and Platelet Economy in Atherosclerotic and Control Subjects
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Circulation. 1962;25:114-125
doi: 10.1161/01.CIR.25.1.114

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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