Dicumarol Therapy
Some Effects on Platelets and Their Relationship to Clotting Tests

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

The clumping of platelets is probably the fundamental stage in the formation of thrombi. It was early demonstrated in experimental animals that administration of Dicumarol (bishydroxycoumarin; Dicoumarol) in doses adequate to prevent thrombus formation was associated with delay or inhibition of platelet clumping. Although it may not be necessary to delay platelet clumping in order to prevent thrombus formation, this evidence suggests that it is a relevant factor. In view of this, it would seem important to know the effect in man of Dicumarol and related drugs on platelet behavior. Furthermore, it would be worthwhile to know the relationships between these changes and various clotting tests, in particular those commonly used to control Dicumarol therapy. Quick drew attention to this problem when he wrote, "Obviously, one of the essential needs for the rational use of Dicumarol clinically is an exact study correlating the coagulation time and level of prothrombin with the inhibition of platelet agglutination." Although this point was made 15 years ago and Dicumarol and related drugs have been used extensively, it has received scant attention.

The evidence of the effect of Dicumarol and related drugs on platelets is conflicting. Some investigators have found that administration of these drugs to patients and animals makes platelets less adhesive. Since platelet adhesiveness appears to be related to the onset of platelet clumping, one might expect that the formation of platelet clumps would be delayed during Dicumarol therapy. Although this has been observed in animal experiments, a study of human subjects receiving "Dindevan" therapy, showed no such delay. Furthermore, Fulton, Akers and Lutz observed that the administration of Dicumarol to hamsters actually enhanced platelet adhesiveness and clumping. Factors which might account for the divergent results are differences in anticoagulant dosage and methods of controlling the dose. The latter is probably important, since it is now recognized that the prothrombin time does not measure all the clotting changes, notably those which influence platelet function.

An attempt has, therefore, been made to investigate the changes in platelet clumping and adhesiveness during Dicumarol therapy in a group of male subjects followed over a period of 6 months. In addition, the prothrombin time, whole blood clotting time and plasma activity in the thromboplastin generation test were determined. The results have been evaluated in order to assess 3 things: (1) effect of Dicumarol therapy on platelet adhesiveness and clumping; (2) correlations between the changes in platelet behavior and the change in the prothrombin time, clotting time and plasma thromboplastin activity; (3) clinical consequences of these findings.

Methods

Statistical Considerations

The plasma thromboplastin time, platelet clumping time, prothrombin time and whole blood clotting time were measured on 350 samples of blood from 21 patients, the majority of the readings being made during Dicumarol therapy. On 160 of these samples from 14 of the subjects, the adhesive index was also measured.

As a necessary preliminary to the statistical evaluation of the relationships between the various measurements, distribution was examined with the aid of probit paper.

Platelet Clumping Time

The distribution of this measurement was positively skewed in both the pooled 350 readings and in 88 values in untreated normal subjects. The logarithms of the values were found to be normally distributed in both samples.
Whole Blood Clotting Time

Like the platelet-clumping time, this was also logarithmic normally distributed when Diemarol was being given. It is of interest to note that the distribution of values in the untreated normal patients is approximately normal, unlike that of the treated patients. It can be shown that where the standard deviation is small (as in this situation), an exponential transformation (i.e., using the actual readings instead of their logarithms) has little effect on symmetry.

Adhesive Index

This was normally distributed in the treated series and (with the exception of 2 inordinately high readings) in an untreated series of 56 patients.

Plasma Thromboplastin Time

This was fairly normally distributed with some positive skewing which was over-corrected by logarithmic transformation. The most satisfactory distribution was obtained by taking the reciprocals of the values.

Prothrombin Time

This measurement gave a good probit curve up to the 95 percentile, but there were 9 readings below 150 seconds which distorted the upper end of the distribution. If these values were excluded, the remaining 341 values were normally distributed. From these considerations and other evidence, it seems that the validity of the test breaks down for readings above 50 seconds. For this reason and because correlation coefficients are sensitive to non-normalcy of distribution, these 9 results have been set aside. In all future calculations, results will be presented for comparison with and without the results for the 9 samples in which these readings were obtained, and designated by the terms "untrimmed" and "trimmed," respectively. These 9 readings, constituting only 2.6 per cent of the whole, produce a marked difference in the results.

Coagulation Tests

Blood Collection

From each subject, 20 ml of blood were collected with paraffin-coated glass syringe and a gage 18 silicone-coated stainless steel needle. One-milliliter quantities of blood were placed in each of 3 glass tubes of 10 mm. internal diameter. These were used for the clotting time determinations. Five ml. of blood were placed into 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. The remaining blood (9 ml.) was placed in a silicone-coated centrifuge tube in the proportion of 9 parts of blood to 1 part of 3.8 per cent trisodium citrate. Only those blood samples obtained with a clean venepuncture and free flow of blood were used.

Platelet Clumping Time (P.C.T.)

The technic used here is a modification of those described by Mills, Necheles, Chu,11 and Sharp.8 Platelet-rich plasma was prepared by centrifuging the native blood at 1,000 r.p.m. for 10 minutes in a (M.S.E. major) refrigerated centrifuge at 4 C. Following this, the blood sample was maintained at 4 C. in melting ice. A 1.0 ml. sample of the supernatant platelet-rich plasma was placed in a glass tube 10 mm. in internal diameter previously warmed to 37 C. The contents were agitated manually for 10 seconds every 30 seconds. Samples of plasma were removed at intervals, placed on a glass slide and immediately examined under the high dry objective of a microscope for evidence of platelet clumping. As a retrospective check, from a part of each sample, a smear was made, stained with Wright's stain, and later examined. Microscopic evidence of platelet clumping by either technic was found to occur about 10 to 20 seconds earlier than macroscopic evidence. The onset of this reaction was taken as the point when the clump was visible to the naked eye.

"Normal" Range. The platelet clumping time was measured in 58 untreated control patients attending the laboratory for other reasons. This means of selection may be biased and the results, therefore, may not be representative of the population as a whole. In 7 subjects repeated tests were done and the geometric mean of the readings for each subject was used.

The 58 results were logarithmic normally distributed and when the values were decoded, the mean (that is, the geometric mean) was 274 seconds with 95 per cent range of 160 and 470 seconds. Variability of results is in part derived from differences between subjects, in part from other sources. The latter moieties would be reduced if technical error were eliminated and the condition of the test well standardized. Analysis of variance makes possible comparison of the relative importance of any 2 sources of variation.

Seven untreated subjects had from 4 to 15 tests done, 59 readings in all. Logarithms of the values were examined. Bartlett's test for heterogeneity of variance was not significant \( (\chi^2 = 10.16, p < 0.2) \) and an analysis of variance was set up as follows:

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Degrees of freedom</th>
<th>Sum of squares</th>
<th>Mean square</th>
</tr>
</thead>
<tbody>
<tr>
<td>Within subjects</td>
<td>52</td>
<td>0.7454</td>
<td>0.01433</td>
</tr>
<tr>
<td>Between subjects</td>
<td>6</td>
<td>0.1627</td>
<td>0.02711</td>
</tr>
<tr>
<td>Total</td>
<td>58</td>
<td>0.7454</td>
<td>0.01433</td>
</tr>
</tbody>
</table>

\[ F = \frac{0.02711}{0.01433} = 1.891 \]

This suggests that variation within individuals and
in the technic is relatively large compared with variation between one individual and another and that there is room for improvement in the standardization of the method.

**Platelet Adhesive Index (A.I.)**

This was determined by the glass wool filter method of Moolten and Vroman. The citrated blood sample was used for this test.

"Normal" Range. The adhesive index was measured in 56 subjects, many of whom were included in the control series for platelet clumping time. Where repeated tests were performed on the one subject, the mean of the readings was used.

The values were normally distributed except for 2 inordinately high readings (1.87 and 1.88, respectively). The mean and standard deviations obtained after these values were excluded agreed well with those estimated by drawing the best line through the probit points up to the 90 percentile. With these points removed, the mean was found to be 1.18 and the "normal range" (the mean ± 2 standard deviations) 0.82 and 1.55.

Repeated measurements of the adhesive index were made in 7 subjects, the smallest number being 4, the highest 15, the total 56. Bartlett's test for heterogeneity of variance was again negative (x² = 4.7, p <1.0) and an analysis of variance set up as before. The overlap was even greater than for the platelet clumping time (F = 1.123, p <1.0).

**Whole Blood Clotting Time (W.B.C.T.)**

This was determined by the method of Lee and White as described by Biggs and Macfarlane. The range (the mean ± 2 standard deviations) for 110 normal subjects not receiving Dicumarol therapy is 6.5 to 16 minutes with a mean of 11.25 minutes.

**One-Stage Prothrombin Time (P.T.)**

The tests were carried out with tissue thromboplastin prepared from human brain as previously described. 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.

**Plasma Thromboplastin Activity in the Thromboplastin Generation Test (P.T.T.)**

A sample of the citrated plasma was diluted 1:10 and used in place of serum in a thromboplastin generation test system employing brain extract and normal Al(OH)₃-treated plasma. This test carried out as previously described gives a measure of plasma Factor IX (Christmas factor, P.T.C.) activity and possibly other factors, such as Stuart and Hageman Factor. The maximum amount of thromboplastin generated within the first 6 minutes is taken as the text value. The range in 150 normal untreated subjects (the mean ± 2 standard deviations) was 9.3 to 15.1 seconds with a mean of 12.2 seconds. The test is referred to as the plasma thromboplastin time in the text.

**Results**

**Part I**

**Platelet Clumping Time and Dicumarol Therapy**

The results of this study are shown in table 1. The paired differences between the mean values for the platelet clumping time before and during Dicumarol therapy are statistically highly significant.

Comparison of these results for the Dicumarol group with the normal standardized range showed that none of 24 untreated and 56 of 98 treated values fell above the upper limits of the normal range. From Fisher's method of exact probability the odds against this difference being due to sampling error are greater than 27,000,000 to 1.

**Platelet Adhesive Index and Dicumarol Therapy**

The results for this study are shown in table 1. There was a significant decrease in platelet adhesiveness when the subjects were receiving Dicumarol therapy.

Comparison of these results for the Dicumarol group with the range for the untreated controls showed that none of the untreated and 36 of 144 treated values fell below the lower limit of normal. From Fisher's method of exact probability the odds against this difference being due to sampling error are 75 to 1.

**Correlation Between Platelet Clumping Time and Platelet Adhesive Index**

In view of the possibility that the stickiness of platelets and the onset of clumping may be related, it would not be unreasonable to expect some degree of correlation between the results for these 2 tests. The coefficient of correlation for 160 tests done on 14 subjects while receiving Dicumarol therapy was -0.591 (untrimmed). The trimmed value was -0.589. Both of these values are highly significant (p <<0.001).

Estimates of the correlation coefficient between 2 variables are subject to sampling...
Table 1

<table>
<thead>
<tr>
<th>Test</th>
<th>No Dicumarol therapy</th>
<th>Dicumarol therapy</th>
<th>Paired differences between means</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of subjects</td>
<td>No. of tests</td>
<td>Mean of mean values</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>24</td>
<td>255 sec.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>98</td>
<td>517 sec.</td>
<td>5.472</td>
</tr>
<tr>
<td>Platelet clumping time</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>16</td>
<td>1.200</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>0.973</td>
<td>8.093</td>
</tr>
<tr>
<td>Platelet adhesive index</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1) The p values in this and subsequent tables refer to the probabilities that the values observed are artifacts of sampling. Asterisks are employed to call attention to significant values: the less likely the value is due to sampling error and the more likely the difference or relationship is real, the more asterisks are added.

error and for samples of finite size are distributed around the parameter or true value. The extent of the scatter noted in this series can be compared with the expected scatter, and the probability that all the subjects have the same parameter can be estimated. If this probability is greater than 0.05, the coefficients may be called homogeneous.

Tests for heterogeneity showed that the differences in the coefficients of correlation from subject to subject between the platelet adhesive index and clumping time are attributable to sampling error rather than individual differences. ($\chi^2$ untrimmed = 6.662, $p < 0.05$; $\chi^2$ trimmed = 7.841, $p < 0.05$).

Correlation Between Platelet Clumping Time and Other Tests

The results of this study pooling all the data are shown in table 2. The clotting time gives a correlation with the platelet clumping time which is significantly better than either of the other tests whether the untrimmed or trimmed values are used ($p < 0.001$). The prothrombin time gave the poorest correlation with the platelet clumping time and the plasma thromboplastin time gave an intermediate correlation. If the trimmed values only are used, these latter 2 correlations are approximately equal.

Significance of the Differences Between Subjects. The results for the $\chi^2$ test (table 3) indicate that the variation in the coefficients of correlation for individual subjects between the clotting time and platelet clumping time are attributable to sampling error. This also holds true for the differences observed between subjects in the correlation coefficients between the plasma thromboplastin time and the platelet clumping time. In the case of prothrombin time and platelet clumping time, the odds are 1,000 to 1 (untrimmed) and 200 to 1 (trimmed) against the differences being due to sampling error. Therefore, this is strong evidence that there are significantly different coefficients of correlation between the prothrombin time and the platelet clumping time from subject to subject, whereas the clotting time appears to have a consistent degree of correlation with platelet clumping time.

Distribution of Coefficients of Correlation. An example of the marked difference between the correlation coefficients for the platelet clumping time and prothrombin time for 2 individuals is shown in figure 1. In table 4 the distribution of the coefficients of correlation is shown. The clotting time gives a good correlation with the platelet clumping time (0.500 or better) in 18 of 21 subjects, and the plasma thromboplastin time and prothrombin time give as good a correlation in 14 and 11 subjects respectively.

Value of Clotting Tests as an Index of Platelet Clumping Time. From the clinical standpoint, it is important to determine which of the 3 clotting tests under discussion is most useful in estimating the platelet clumping time, since this latter test is, at present, too difficult for routine use. In the assessment of
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Table 2
Correlation Between Platelet Clumping Time, Prothrombin Time, Clotting Time and Plasma Thromboplastin Time

<table>
<thead>
<tr>
<th></th>
<th>Coefficient of correlation</th>
<th>Untrimmed values</th>
<th>Trimmed values</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Platelet clumping time and prothrombin time</td>
<td>+0.321</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>Platelet clumping time and plasma thromboplastin time</td>
<td>-0.523</td>
<td>-0.514</td>
</tr>
<tr>
<td>C</td>
<td>Platelet clumping time and clotting time</td>
<td>+0.729</td>
<td>+0.710</td>
</tr>
</tbody>
</table>

Significance of absolute differences between coefficients of correlation:
- A & B: <.005**
- A & C: <.001***
- B & C: <.001***

1See table 1 footnote.

Table 3
Homogeneity of the Coefficients of Correlation

<table>
<thead>
<tr>
<th>Correlation</th>
<th>Significance of heterogeneity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clotting time and platelet clumping time</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>Plasma thromboplastin time and platelet clumping time</td>
<td>&lt;.1</td>
</tr>
<tr>
<td>Prothrombin time and platelet clumping time</td>
<td>&lt;0.001***</td>
</tr>
</tbody>
</table>

1See table 1 footnote.

The efficiency with which these individual tests estimate platelet clumping time, 3 questions arise about the variation between subjects:

1. Are the regression slopes similar, i.e., does a given change in the predicting test indicate the same change in platelet clumping time in all subjects or is there a wide variation from subject to subject? (2) Are the intercepts similar, i.e., does a given value in the predicting test indicate the same platelet clumping time in all subjects or is there a wide variation from subject to subject? (3) Is the scatter around the regression line similar, i.e., is the predicting test as reliable a measure of the platelet clumping time in all subjects or does it lead on the average to larger errors of estimate in one subject than in another?

The answering of these questions is handicapped in this study by heterogeneity of variance so that ordinary analysis of covariance cannot be done. Therefore, other statistical methods were applied in an attempt to solve the problem: (1) An analysis of the regression coefficients of the trimmed values for each of the 21 subjects is shown in table 5. Clearly, the clotting time is the most reliable of the 3 variables and gives rise to the least error if the individual regression line is replaced by a "universal" regression slope. (2) If the intercepts were the same and sampling error made unimportant by large numbers of readings on each patient, the points located by the means of these values (the mean of one test as the ordinate, the mean of the other as the abscissa) would lie in a straight line. This is examined in figure 2 which shows also the regression lines and the correlation coefficients between these means. Although none of the absolute differences between the correlation coefficients is quite significant, in this sample clotting time has again proved to be the best of the 3 tests. (3) Scatter can be conveniently studied by comparing correlation coefficients between subjects. We have already shown that the best homogeneity of the correlation is exhibited by clotting time, while correlations between the prothrombin time and platelet clumping time are frankly heterogeneous.

It may, therefore, be noted that although prothrombin time as a predictor of platelet clumping time is seen to best advantage (since only the trimmed values have been used throughout) in all 3 criteria of usefulness, clotting time has proved clearly the most reliable. When we add to this the fact that the
Table 4

Distribution of Coefficients of Correlation for Twenty-One Subjects

<table>
<thead>
<tr>
<th>Range of coefficients of correlation</th>
<th>Platelet clumping time and clotting time</th>
<th>Platelet clumping time and plasma thromboplastin time</th>
<th>Platelet clumping time and prothrombin time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of subjects Untrimmed</td>
<td>Trimated</td>
<td>No. of subjects Untrimmed</td>
</tr>
<tr>
<td>1.0000 to 0.7500</td>
<td>11</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>0.7500 to 0.5000</td>
<td>7</td>
<td>8</td>
<td>11</td>
</tr>
<tr>
<td>0.5000 to 0.2500</td>
<td>3</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>0.2500 or worse</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Total subjects</td>
<td>21</td>
<td>21</td>
<td>21</td>
</tr>
</tbody>
</table>

†The correlations between plasma thromboplastin times and the platelet clumping times are negative.

The relationship between the prothrombin time and platelet clumping time for 2 extreme cases: in Subject I, the correlation (trimmed) is good \( r = +0.810 \); in Subject II, very poor \( r = -0.010 \). The correlation approaches unity (the ideal) as the acute angle between the regression lines approaches zero. Platelet clumping time is plotted on a logarithmic scale.

Clotting time also gives much the best correlation with the platelet clumping time, its superiority as a measure of platelet clumping time during Dicumarol therapy is obvious.

Correlation Between Platelet Adhesive Index and Prothrombin Time, Clotting Time and Plasma Thromboplastin Time

The clotting time is the best and the prothrombin time the poorest index of platelet adhesiveness (table 6). Clotting time is significantly more effective than prothrombin time \( p < 0.025 \) untrimmed or trimmed and almost significantly better than the plasma thromboplastin time.

Significance of the Difference Between Subjects. The results for the \( \chi^2 \) test (table 7) indicate that the differences in the coefficients of correlation between the clotting time and platelet adhesive index differ from subject to subject by amounts attributable to sampling error. This was also true of correlation coefficients between the plasma thromboplastin time and platelet adhesive index. However, in the case of the prothrombin time and platelet adhesive index, the odds are 100 to 1 (untrimmed and trimmed figures) against the differences being due to sampling error. This suggests different individuals may have different coefficients of correlation between the prothrombin time and the platelet adhesive index, whereas the clotting time appears to have a consistent degree of correlation from subject to subject.

Distribution of Coefficients of Correlation. This is shown in table 8. While the prothrombin time was a good index of the platelet adhesive index in 4 of 14 subjects, the clotting time was as good an index of change in platelet adhesiveness in 11 of 14 subjects.

Value of Clotting Test in Determining Platelet Adhesive Index

Evaluation of the data with regard to this point by the same methods used for platelet clumping time showed that the clotting time gave a better index of the platelet adhesive value from subject to subject than either the prothrombin time or plasma thromboplastin time.
Table 5

<table>
<thead>
<tr>
<th>Regression</th>
<th>Mean of regression coefficients (m)</th>
<th>Range of coefficients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lower</td>
<td>Upper</td>
</tr>
<tr>
<td>PCT on WBCT</td>
<td>+1.200</td>
<td>+2.458</td>
</tr>
<tr>
<td>PCT on PTT</td>
<td>-0.08015</td>
<td>+0.0074</td>
</tr>
<tr>
<td>PCT on PT</td>
<td>+0.01288</td>
<td>+0.03370</td>
</tr>
</tbody>
</table>

The efficiency with which adhesive index may be estimated from the other tests was examined by the same technics as for the platelet clumping time. The range of the regression slopes between subjects is summarized in table 9. In this instance, the most consistent predictor test is the plasma thromboplastin time but the superiority of the clotting time is obscured by one freak reading.

The correlation coefficients between the several trimmed means are as follows:

Means of A.I. and means of W.B.C.T.  \( r = -0.756 \)
Means of A.I. and means of P.T.T.  \( r = 0.548 \)
Means of A.I. and means of P.T.  \( r = -0.465 \)

None of the absolute differences is significant but again the clotting times give the best relationship which compares with \( r = -0.738 \) for the correlation between platelet clumping time and adhesive index.

Finally, the consistency of the correlation coefficients has been examined in table 7 from which it appears that clotting time is the best test. It is clear then that clotting time is the best predictor test of adhesive index as well as platelet clumping time.

Part II

Differences in Prothrombin Time Technics

While different tissue thromboplastins may give similar prothrombin time values when tested against normal plasma, they may show marked discrepancies when used against plasma from patients taking Dicumarol. In figure 3, the differences between prothrombin times done in the routine hospital laboratory and the research laboratory are shown. The hospital laboratory uses a commercial tissue thromboplastin (Simplastin* and the re-

*Warner-Chilcott Laboratories, Toronto, Canada.

Relationships between the several variables plotted on appropriate scales (see text). Each circle indicates the trimmed means for 1 subject. Note the excellent agreement between plasma thromboplastin time and prothrombin time.
Table 6

Correlation Between Platelet Adhesive Index, Prothrombin Time, Plasma Thrombo-
plastin Time and Clotting Time

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Platelet adhesive index and prothrombin time</td>
<td>Platelet adhesive index and plasma thromboplastin time</td>
<td>Platelet adhesive index and clotting time</td>
</tr>
<tr>
<td>Coefficients</td>
<td>Untrimmed -0.374</td>
<td>+0.452</td>
<td>-0.590</td>
</tr>
<tr>
<td></td>
<td>Trimmed -0.358</td>
<td>+0.436</td>
<td>-0.579</td>
</tr>
<tr>
<td>Significance of</td>
<td>Untrimmed values</td>
<td>Trimmed values</td>
<td></td>
</tr>
<tr>
<td>absolute differences</td>
<td>&lt;0.5</td>
<td>&lt;0.025*</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>between coefficients</td>
<td>A &amp; B</td>
<td>A &amp; C</td>
<td>B &amp; C</td>
</tr>
<tr>
<td>of correlation</td>
<td>&lt;0.025*</td>
<td>&lt;0.025*</td>
<td>&lt;0.1</td>
</tr>
</tbody>
</table>

*See table 1 footnote.

Figure 3

The prothrombin time values as determined in the hospital laboratory (●—●) and research laboratory (●—●) for a subject receiving Dicumarol therapy. The dotted line represents twice the subject's control value.

Hemorrhage and Platelet Clumping Time

Seven episodes of hemorrhage occurred during this study (table 10). Two were episodes of gastrointestinal bleeding associated with proven duodenal ulcers. In both instances, the platelet clumping time, clotting time and prothrombin time values were not as prolonged as in the remaining cases of bleeding where no organic lesion was recognized. In all the spontaneous episodes of bleeding, consisting of excessive bruising, epistaxis or marked hematuria, the clotting time and platelet clumping time were considerably prolonged. Although the prothrombin time was prolonged in most instances, it was within therapeutic limits (1 1/2 to 2 1/2 times normal) in one case (subject 4).

Discussion

The first morphologically recognizable changes in endogenous thrombosis are the clumping and fusion of platelets. The fibrin coagulum occurs at a later stage. Moreover, the clumping and fusion of platelets are important mechanisms in hemostasis. Changes in the clotting factors involved in the early stages of the intrinsic (intravascular) coagulation mechanism precede the onset of the platelet reaction. Therefore, in view of the importance of platelets, it is vital to decide 2 questions: what the effect of Dicumarol on the platelet is, and what relationship exists between measures of the extrinsic clotting system and of the intrinsic clotting system and platelet function. These 2 questions will be discussed separately.

Dicumarol Therapy and Platelet Function

The results show that Dicumarol in sufficient doses delays the onset of platelet clumping and makes platelets less adhesive. These findings agree with those of some investiga-
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tors,6 but conflict with those of others.8,9 In Sharp's8 observations made on 4 patients, the anticoagulant therapy was regulated by the prothrombin time. It is, therefore, possible that the subjects had not received anticoagulant adequate to affect platelet clumping. Moreover, in view of the differences between our "normal" range and Sharp's values, it would appear that differences in technic may also account in part for the discrepancies. Using Sharp's test, Zucker and Borelli22 reported a "normal" range similar to ours. Fulton9 and associates found that administration of Dicumarol and related drugs to hamsters enhanced platelet adhesiveness. It is evident, however, that their dosage was insufficient to produce much effect on the prothrombin time and L. Horlick (personal communication) has found that platelets may become more adhesive in human subjects receiving small doses of Dicumarol. It is possible, therefore, that increased platelet adhesiveness may occur during Dicumarol therapy particularly if the dose is not large enough.

Platelet Function and Other Clotting Tests

These results show that measures of the intrinsic clotting mechanism (clotting time, plasma thromboplastin time) show a better correlation with changes in the platelet clumping than does the prothrombin time which measures mainly the extrinsic system. Furthermore, while the clotting time appears to be a consistent index of platelet clumping in all subjects, the prothrombin time as an index, varies from subject to subject. In some cases, it is as reliable as the clotting time, while in others it appears to be of no value. Although there are many factors which may account for this variation in the prothrombin time, one which may be of some importance is the sensitivity of this test to changes in Factor VII. Although it was originally recognized that the main effect of Dicumarol was on Factor VII,13 it is now known that several factors are affected including those involved in the early stages of the intrinsic mechanism.23-25 Since the intrinsic coagulation mechanism is independent of Factor VII activity,13 it is not surprising that the prothrombin time is sometimes a poor index of changes in platelets.

That the plasma thromboplastin time does not give as good an index of changes in platelet clumping as the clotting time is to some extent unexpected, particularly since Factor IX (Autoprothrombin I, Christmas Factor, P.T.C.) is reduced to levels near zero during adequate Dicumarol26 therapy and is thought to be necessary for the onset of platelet clumping and viscous metamorphosis.20 However, it may be that Factor IX is not as important for the onset of platelet clumping as we at present think.8,21,26 Furthermore, it appears that this test may measure Hageman and Stuart factor changes as well, which could also influence the final correlation.

It is conceivable that the formation of the fibrin clot could have been depressed by Dicumarol without obvious change in platelet function. In view of the fact that, as has already been stated, platelet clumping occurs before fibrin formation, this would seem to be unlikely. Evaluation of the data in this study shows that this has not in fact occurred. It is of considerable interest that changes in the prothrombin time and in the plasma thromboplastin time show an excellent correlation. There is evidence that prothrombin, Factor VII and Factor IX are closely related. Seegers and associates have found that Factor VII (Autoprothrombin I) and Factor IX (Autoprothrombin II) can be derived from purified prothrombin.27-29 The good correlation between the degree of change in the pro-

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Table 7

<table>
<thead>
<tr>
<th>Correlation coefficient</th>
<th>Probability that differences are due to sampling error</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Untrimmed values</td>
</tr>
<tr>
<td>Platelet adhesive index and clotting time</td>
<td>&lt;0.70</td>
</tr>
<tr>
<td>Platelet adhesive index and plasma thromboplastin time</td>
<td>&lt;0.2</td>
</tr>
<tr>
<td>Plasma adhesive index and prothrombin time</td>
<td>&lt;0.01**</td>
</tr>
</tbody>
</table>

†See table 1 footnote.
Table 8

<table>
<thead>
<tr>
<th>Range of coefficients of correlation</th>
<th>Platelet adhesive index and clotting time</th>
<th>Platelet adhesive index and plasma thromboplastin time</th>
<th>Platelet adhesive index and prothrombin time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of subjects</td>
<td>Untrimmed</td>
<td>Trimmer</td>
</tr>
<tr>
<td>1.000 to 0.7500</td>
<td>5</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>0.7500 to 0.5000</td>
<td>6</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>0.5000 to 0.2500</td>
<td>2</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>0.2500 or worse</td>
<td>1</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Total subjects</td>
<td>14</td>
<td>14</td>
<td>14</td>
</tr>
</tbody>
</table>

Table 9

<table>
<thead>
<tr>
<th>Regression</th>
<th>Mean of regression coefficients</th>
<th>Range of regression coefficients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lower</td>
<td>Upper</td>
</tr>
<tr>
<td>A.I. on W.B.C.T.</td>
<td>-1.5548</td>
<td>-0.5040</td>
</tr>
<tr>
<td>A.I. on P.T.T.</td>
<td>0.0702</td>
<td>0.15230</td>
</tr>
<tr>
<td>A.I. on P.T.</td>
<td>-0.01160</td>
<td>0.03581</td>
</tr>
</tbody>
</table>

The findings reported in this paper and those in others raise an important question in regard to clinical trials of Dicumarol. It is possible that the investigators who have not
found Dicumarol therapy to reduce the incidence of thrombotic episodes have simply failed to achieve a sufficient degree of change in the clotting mechanism. In view of the large variation between techniques for doing prothrombin times and the shortcomings of this test as an index of platelet and clotting changes in some cases, this seems to be a not unreasonable point of view. Also in studies where a beneficial therapeutic effect has been demonstrated, there have still been thrombotic episodes and hemorrhagic complications. It would seem likely that some of these failures occur in those for whom the prothrombin time is a poor index of the over-all clotting change. Pending clinical trials satisfactory both in method of patient selection and method of control, we will not know the full extent to which therapy with Dicumarol and related drugs influence the course of thrombotic vascular disease.

**Summary**

One of the problems of Dicumarol administration has been determination of the adequacy of therapy. A fundamental stage in thrombus formation in the circulation is platelet clumping. Since platelets are involved in both hemostasis and thrombosis, a study of changes in platelet function may prove useful. The effects of Dicumarol on platelet clumping and adhesiveness have, therefore, been studied and related to changes in other clotting mechanisms.

Comparison of platelet function in control periods and during Dicumarol administration shows that this drug lowers the adhesive index and prolongs the platelet clumping time to a highly significant degree. The accuracy of estimates of these 2 platelet functions given by 3 other tests, prothrombin time, whole blood clotting time and plasma activity in the thromboplastin generation tests have been compared. By any criterion, degree of correlation, consistency of regression slopes, intercepts or scatter about regression lines, clotting time was the most efficient of the 3 tests for evaluating platelet function. The prothrombin time was the least efficient. Furthermore, hemorrhage from excessive dosage was closely related to the platelet clumping time and clotting time. It should be pointed out, however, that in about one-third of the patients the prothrombin time was as good an index of platelet clumping time as clotting time. While prothrombin times done with 2 different sources of thromboplastin may give congruous results in untreated patients, considerable discrepancies may appear during Dicumarol therapy.

If prothrombin time is to be used as a guide to Dicumarol therapy, it is important to establish in each patient separately its relia-
bility as a measure of the behavior of the clotting mechanism as a whole. For those in whom it seems reliable, an individual "therapeutic range" should be determined. For other cases, some other suitable test should be employed. It is emphasized that regulation of Dicumarol dosage is an individual problem and that only those tests shown to be reliable indices of safe effective modifications of clotting mechanism in the individual patient should be used.

Summario in Interlingua

Un del problemas in la administration de Dicumarol es la determination del adequa utia del therapia. Un studio fundamental in le formation del thrombo in le circulation es le aggregation de plachettas. Viste que plachettas participa in le processos hemo-static si ben que thrombotie, un studio del alterationes in le function del plachettas es possibilmente de valor. Essa studiata, per consequente, le effectos de Dicumarol super la aggregation e le adhesivitate del plachettas e le relation inter iste effectos e alterationes in cetero mechanismos de coagulation.

La comparation del function plachettal durante periodos de controlo e durante le administration de Dicumarol demonstra que lo droga reduce le indice de adhesivitate e prolonga le tempore de aggregation del plachettas a grados que es altemente significativo. Le accuratia del estimationes de iste duo functiones plachettal esseva comparata secundo tres altere tests, i.e. le test del tempore de prothrombina, le test del tempore de coagulation de sanguine total, e le activitate de plasma in le tests de generation de thromboplastina. Secundo omne le criterios—grado de correlation, uniformitate del regressive coefficiences de direction, interceptos, dispersion circum lineas de regression—le tempore del conglutitation esseva le plus efficace del tres tests pro le evalutation del function plachettal. Le tempore de prothrombina esseva le minus efficace. In plus, hemorrhagias ab exessos de dosage esseva intimamente correlationate con le tempore de aggregation plachettal e le tempore de coagulation. Tamen, il debe esser signalate que in circa un tertio del patientes le tempore de prothrombina equivalence le tempore de coagulation como indicio del aggregation de plachettas. Durante que le tempore de prothrombina determinata per medio de thromboplastina ab duo differente fontes es ben capace a produc congrue resultatos in non-tractate patientes, grados considerable de discrepania pote occurrere durante le therapia a dicumarfol.

References


Dicumarol Therapy: Some Effects on Platelets and Their Relationship to Clotting Tests
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