The Effect of Dicumarol on Co-Thromboplastin, a Factor in Blood Concerned with the Conversion of Prothrombin to Thrombin

By Frank D. Mann, M.D., Nelson W. Barker, M.D. and Margaret Hurn, M.S.

It is generally accepted that dicumarol decreases the amount of prothrombin in the blood, but this is neither its sole nor necessarily its principal effect. In 1947, we reported that in addition to this decrease, dicumarol causes a deficiency in the conversion of prothrombin to thrombin. This conclusion was reached by comparison of results obtained from one-stage and two-stage tests for prothrombin. The addition of another procedure, the co-thromboplastin assay, permits us to inquire more closely into the nature of this deficiency in conversion produced by dicumarol.

Methods

One-stage Test for Prothrombin (Quick)

Results of this test show a summation of significant factors in regard to the coagulation of blood. The test was performed as described by Hurn and associates. The normal prothrombin time ranges from seventeen to nineteen seconds. By comparison with characteristic dilution curves of normal plasma it is possible to express the results of the one-stage test as “per cent of normal.” This sometimes affords a convenient use of data, especially in plotting graphs, since the relationship between clotting time and “per cent of normal” always deviates greatly from linearity. No more absolute or specific significance, however, should be attached to the percentage than to the actual clotting time since a given abnormal percentage may be caused by a number of different types of alterations which are not at all comparable. Abnormalities detected by the one-stage method may be analyzed further by other more specific procedures.

Two-stage Determination of Prothrombin (Warner, Brinkhous and Smith)

In this test the total content of prothrombin of plasma is measured, provided conditions are so arranged that the prothrombin is quantitatively converted to thrombin. The test was performed as described by Hurn and Mann except that when testing plasma of patients receiving dicumarol, 0.1 cc. of essentially prothrombin-free serum was added to 2 cc. of prothrombin conversion mixture. This did not appreciably alter the yield of thrombin from normal plasma but materially increased the yield from certain specimens of plasma from patients receiving dicumarol. In our experience the range of concentration of prothrombin of normal persons was appreciable, that is, from 244 to 452 units per cc. We consider this to represent true variation of the material rather than of the method since it is possible to demonstrate differences of this order of magnitude between normal persons tested at the same time with the same reagents, while consistent results can be obtained for the same person. An average value of 320 units per cc. was used in computing “per cent of average normal.”

Co-thromboplastin Assay

This test measures the activity of plasma or serum with respect to a reaction with tissue thromboplastin which appears to occur before prothrombin itself enters into the reaction.
Co-thromboplastin appears unaffected by certain procedures, such as aging in vitro, which markedly affect the conversion of prothrombin to thrombin. Thus co-thromboplastin differs from the Ac-globulin of Seegers and the labile factor of Quick. The test was performed as previously described except that we prepared our own acetone-dehydrated rabbit brain. All batches of thromboplastin did not prove to be suitable for the test; only those giving results quantitatively and qualitatively similar to those previously reported were used. The control plasma used in all tests for which data are reported in this communication, except that for Patients 10, 11 and 12, was from the same normal person. Values are expressed in terms of percentage of the activity of this control. All daily tests for the 6 patients were made with the same batch of acetone-dehydrated rabbit brain.

![Diagram](image.png)

**Fig. 1.**—A brief response to dicumarol.

**Observations**

Six patients were observed both preceding the administration of dicumarol and daily for four to six days during treatment with dicumarol (figs. 1, 2 and 3). Data obtained were supplemented by tests of one to two days' duration performed on patients at various stages of anticoagulant therapy. The control values for the one-stage and co-thromboplastin tests tended to be somewhat low; this seems to be common for hospitalized patients. As in our previous study, dicumarol initially affected the results of the one-stage test much more than that of the two-stage test. The decrease in the concentration of prothrombin indicated by the two-stage test did not occur as soon after administration
of dicumarol was begun as did the decrease indicated by the one-stage test, and it was also of smaller magnitude. Co-thromboplastin activity showed a sharp initial decrease of about the same magnitude as the decrease of prothrombin indicated by the one-stage test. While the patient was recovering from the effects of dicumarol, the concentration of prothrombin, as shown by the one-stage test, and co-thromboplastin activity increased sooner and to a greater extent than the increase in prothrombin shown by the two-stage test. Characteristically, as the effects of dicumarol increased, the activity of prothrombin, shown by the one-stage test, and the activity of co-thromboplastin in terms of percentage of average normal were materially less than the level of prothrombin indicated by the two-stage test. As the effects of dicumarol decreased, the differences were decreased or even reversed. After several weeks of treatment, when it was not obvious whether the effects of dicumarol were increasing or decreasing, the decrease in co-thromboplastin activity tended to be moderately lower than the decrease in prothrombin indicated by the two-stage test (table 1). Although many of the changes in prothrombin demonstrated by the one-stage test paralleled changes in co-thromboplastin activity, there were definite exceptions to this trend (fig. 3) in which both two-stage and co-

![Diagram](attachment:image.png)

**Fig. 2.—Effect of dicumarol in the case of a relatively sensitive patient.**
thromboplastin assays changed in the opposite direction to the change shown by the one-stage test. At present we cannot quantitatively explain all changes associated with the one-stage test even with both the two-stage and co-thromboplastin assays.

Of all phases of this subject, that pertaining to the effect of vitamin K is most difficult to discuss, because in any case it cannot be said exactly what would...
have occurred without it. Persons who showed a striking initial response to dicumarol usually showed a rapid increase in prothrombin indicated by the one-stage test and in co-thromboplastin activity after the intravenous administration of vitamin K (72 mg. menadione bisulfite, table 2), while the concentration of prothrombin indicated by the two-stage test remained relatively unaffected. Since a decrease in co-thromboplastin activity is the principal change and since this increase normally precedes the increase in prothrombin indicated

**Table 2. Effect of Dicumarol Followed by Vitamin K on Sensitive Patients**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Treatment with dicumarol</th>
<th>Prothrombin time (Quick)</th>
<th>Two-stage test for prothrombin</th>
<th>Co-thromboplastin, per cent of activity of control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Seconds</td>
<td>Per cent of normal</td>
<td>Units per cc.</td>
</tr>
<tr>
<td>5</td>
<td>Two days</td>
<td>72</td>
<td>9</td>
<td>215</td>
</tr>
<tr>
<td></td>
<td>One day later after vitamin K</td>
<td>27</td>
<td>30</td>
<td>215</td>
</tr>
<tr>
<td>6</td>
<td>Two days</td>
<td>116</td>
<td>5</td>
<td>232</td>
</tr>
<tr>
<td></td>
<td>One day later after vitamin K</td>
<td>56</td>
<td>10</td>
<td>215</td>
</tr>
<tr>
<td>7</td>
<td>Four days</td>
<td>105</td>
<td>6</td>
<td>119</td>
</tr>
<tr>
<td></td>
<td>One day later after vitamin K</td>
<td>58</td>
<td>10</td>
<td>132</td>
</tr>
<tr>
<td>8</td>
<td>Five days</td>
<td>126</td>
<td>&lt;5</td>
<td>150</td>
</tr>
<tr>
<td></td>
<td>One day later after vitamin K</td>
<td>53</td>
<td>11</td>
<td>150</td>
</tr>
<tr>
<td>9</td>
<td>Five days</td>
<td>114</td>
<td>5</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>One day later after vitamin K</td>
<td>41</td>
<td>15</td>
<td>89</td>
</tr>
</tbody>
</table>

**Table 3. Effect of Poorly Controlled Treatment with Dicumarol on Coagulation Factors of Blood**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Approximate time on dicumarol</th>
<th>Prothrombin time (Quick)</th>
<th>Two-stage test for prothrombin</th>
<th>Co-thromboplastin, per cent of activity of control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Seconds</td>
<td>Per cent of normal</td>
<td>Units per cc.</td>
</tr>
<tr>
<td>10</td>
<td>Unknown</td>
<td>202</td>
<td>&lt;5</td>
<td>62</td>
</tr>
<tr>
<td>11</td>
<td>One month</td>
<td>203</td>
<td>&lt;5</td>
<td>38</td>
</tr>
<tr>
<td>12</td>
<td>Two weeks</td>
<td>240</td>
<td>&lt;5</td>
<td>14</td>
</tr>
</tbody>
</table>

by the two-stage test, recovery after the administration of vitamin K seems to resemble recovery without the use of the vitamin, except that it is accelerated to a somewhat variable degree.

Data are included on 3 patients in whom bleeding was definitely attributable to poorly controlled treatment with dicumarol administered elsewhere (table 3). Two of these patients, Cases 10 and 11, (table 3) had appreciable although greatly subnormal amounts of prothrombin; the amount of co-thromboplastin was decreased even more. Although a difference could not be discerned between the amount of co-thromboplastin of these patients and that of some who did
not bleed, the latter patients exhibited extremely low co-thromboplastin for brief intervals only. Furthermore the values observed were at the lower limit of detectability by the assay method. The co-thromboplastin of the third patient (Case 12) was not measured and the concentration of prothrombin indicated by the two-stage test was the lowest we have observed for a human being after the administration of dicumarol. The outcome in this case was fatal; the other 2 patients recovered. The one-stage test on all 3 patients showed greatly reduced concentrations. It should be mentioned that at least 1 of these patients (Case 11) had received massive doses of vitamin K at least twenty-four hours previous to the performance of the tests.

**COMMENT**

In communications concerning primarily with the methods used (including citation of pertinent literature) reasons are set forth for the belief that the co-thromboplastin test measures a reaction which involves thromboplastin but at a time preceding the conversion of prothrombin to thrombin. Whether this reaction depends on a specific factor, co-thromboplastin, is left unsettled for the present; it does not appear to depend on what is known as “labile factor,” “factor V” or “Ac-globulin.” A relatively small decrease in Ac-globulin has been reported to follow treatment with dicumarol. This change has not been demonstrable with one-stage methods and hence presumably is not responsible for the striking conversion defect of dicumarol plasma which was demonstrated by one-stage methods.

The co-thromboplastin assay seems to provide a method of study of this conversion defect which is more specific than the one-stage test for prothrombin, but, it must be added immediately, which is not applicable to routine clinical use. Thus, further understanding of the mechanism of the co-thromboplastin reaction would appear to be a plausible avenue to understanding the conversion defect of dicumarol plasma. The anomalous increase in prothrombin activity revealed by the one-stage test and coincident with decreases in both co-thromboplastin activity and two-stage determinations for prothrombin indicates that we still do not have the whole story of the effect of dicumarol. For that matter, there are still other indications that this effect is indeed complex.

The question of the physiologic and practical significance of the foregoing methods remains to be considered. It is our practice, consistent with much experience from which we see no reason to depart, to regard the Quick prothrombin time as a measure of the effect of dicumarol. As measured by the tests in terms of deviation from normal, deficiency of co-thromboplastin often seems to be a larger factor in prolonging the Quick prothrombin time than does actual concentration of prothrombin. From the mechanism of the co-thromboplastin reaction one would expect marked differences in the extent to which a given co-thromboplastin deficiency would affect the one-stage test performed with different thromboplastins. Thus we have found that certain tissue thromboplastins are very insensitive to the conversion deficiency of dicumarol plasma, resembling Russell viper venom in this respect. It should be emphasized that such insensitivity is not detected by testing with normal control plasma.
It has been reported that bleeding definitely attributable to dicumarol may occur when the Quick prothrombin time is in the usually accepted therapeutic range. Such a statement would appear justified only if accompanied by positive evidence that the one-stage method used was at least approximately comparable to that with which the therapeutic range was established. On the contrary, the statement has been made without any information whatever as to the method used to determine the concentration of prothrombin and data reported suggest that the method used was highly variable. In 1945, Hurn, Barker and Magath presented evidence for the need of special standardization of thromboplastin extracts used in control of dicumarol therapy; this evidence is in good agreement with our findings in this study.

It is by no means claimed that even the more specific tests used are absolute quantitations of the physiologic state of the coagulation factors of the blood. The tests merely measure deviation from normal, the significance of which must be learned from experience. It is hoped, however, that in the future as in the past such measurements may aid in the acquiring of worthwhile experience.

SUMMARY

The deficiency in the conversion of prothrombin to thrombin which develops after administration of dicumarol appears to be due largely to decreased co-thromboplastin activity. Both the development of this deficiency and the recovery from it tend to precede the accompanying changes in prothrombin itself, as measured by the two-stage method. In terms of deviation from normal, the deficiency in conversion often exceeds in magnitude the deficiency of prothrombin itself.

REFERENCES

8 ALEXANDER, BENJAMIN, DE VRIES, ANDRE AND GOLDSTEIN, ROBERT: A factor in serum which accelerates the conversion of prothrombin to thrombin. II. Its evolution with special reference to the influence of conditions which affect blood coagulation. Blood 4: 739-746, 1949.
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