THE RELATIONSHIP OF PLASMA PROTHROMBIN CONCENTRATION AND THE DEGRADATION RATE OF SERUM "PROTHROMBIN-CONVERTING FACTOR" DURING DICUMAROL THERAPY

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A previously reported study of the differential clotting action of fresh serum on fibrinogen and oxalated plasma revealed that serum obtained from recently coagulated whole blood contains a factor which causes conversion of prothrombin to thrombin. Since fibrinogen could not be converted to fibrin in any significant degree by addition of fresh serum, and since the rapidity of coagulation of oxalated plasma by the serum factor was proportional to the prothrombin concentration of the plasma, it was assumed that fresh human serum contained a "prothrombin-converting factor." In addition, it was shown that the rate of decrease of "prothrombin-converting factor" from serum on standing was relatively constant in normal individuals, whereas the rate of decrease in sera of patients treated with dicumarol was considerably slower.

A continuation of the study of the degradation rate of serum "prothrombin-converting factor" is the subject of this report. A daily analysis of plasma prothrombin by the one and two stage technics and the degradation rate of serum "prothrombin-converting factor" was made in patients who were receiving dicumarol to prevent thromboembolic complications following myocardial infarction. The daily fluctuations of each were then correlated, revealing that the degradation rate of serum "prothrombin-converting factor" usually follows closely the concentration of plasma prothrombin. As the plasma prothrombin decreased, the corresponding serum developed a slower degradation rate of the "prothrombin-converting factor." Similarly, the degradation rate of the latter rapidly increased whenever the corresponding plasma prothrombin increased.

Methods

1. Prothrombin Determination. Quick's one-stage method was utilized for determination of prothrombin. Human brain extracted in acetone and stored in vacuo served as a source of thromboplastin. All prothrombin determinations were expressed as per cent of normal by interpolation from a standardization curve, constructed from analysis of dilutions of plasma of 3 normal individuals. The two-stage determination of prothrombin was carried out by Seeger's modification of the method of Warner, Brinkhous and Smith. Collidine buffer was substituted for imidazol buffer in this method.

2. Collection of Plasma and Serum. Blood was collected by venipuncture with syringes and needles treated with silicone. Ordinary care was used to prevent contamination of the blood with tissue thromboplastin. Nine cubic centimeters of whole blood were added to graduated centrifuge tubes containing 0.0 cc. of 0.1 M potassium oxalate and thoroughly mixed by rotation. Approximately 6.0 cc. of whole blood were also placed in an ordinary glass test tube with internal dimensions of 10 by 100 mm. The exact time of blood collection was recorded. The glass test tubes containing whole blood were placed in a water bath at a temperature of 16°C. One hour later, the clotted blood was carefully rimmed with a wooden
applicator stick and the serum was separated by centrifugation and again placed in a water bath at 16 C. The oxalated plasma was separated from the formed elements by centrifugation at 2,000 RPM for fifteen minutes and used for prothrombin assay.

3. Determination of Serum "Prothrombin-Converting Factor." The clotting activity of fresh serum was determined by adding serum to oxalated fresh plasma, obtained from individuals whose plasma contained normal amounts of prothrombin. One tenth cubic centimeter aliquots of each were added to test tubes with internal dimensions of 10 by 75 mm. and the time for the first appearance of fibrin was measured with a stop watch. These determinations were made at room temperature (23 C.). Serial observations of a single serum were repeated at ten to fifteen minute intervals. These measurements were plotted on semi-logarithmic paper. When log10 of the coagulation time of plasma, after addition of serum, was plotted against incubation time of the serum (at 16 C.), the resulting points fell on a straight line. Measurement of the slopes of individual curves was used as an expression of the degradation rate of serum "prothrombin-converting factor" (hereafter called "degradation rate").

"Degradation Rate" of Serum "Prothrombin-Converting Factor" during Dicumarol Therapy. The length of time required for serum to cause clotting of oxalated plasma in each instance, is shown in figure 1. Individual sera were assayed at intervals from the time of collection of the blood and the coagulation time is represented on the ordinate. Expressed as log10, the coagulating effect is a linear function of the duration of incubation time of the serum. It will be observed, however, that the slopes of such straight lines depend upon the source of the serum. The lines of greatest slope are those obtained with sera from normal individuals; lines of lesser slope are obtained with sera from individuals receiving dicumarol. In general, the lower slopes are obtained with sera of individuals whose plasma contained the least prothrombin. Occasionally, the initial determination of the time required for coagulation of plasma after addition of serum, fell below the linear slope (fig. 1; DR = .0128). This discrepancy was shown to be the result of a combined effect of thrombin and "prothrombin-converting factor," since serum in this instance was capable of coagulating fibrinogen. Whenever the clotting time of plasma, after addition of serum, exceeded thirty seconds, however, little thrombin effect could be detected.

The equation \( \frac{\log c_1 - \log c_0}{T_1 - T_0} \) was applied to the resulting slopes to express the decrease of "prothrombin-converting factor" in terms of "degradation rate." In this equation, \( \log c_1 - \log c_0 \) represents the clotting time in seconds at two points on the slope, and \( T_1 \) and \( T_0 \) represents the elapsed time between these two observations.

In normal sera (fig. 1) the "degradation rate" was .0223 and .0263 seconds per minute for two different individuals. On the other hand (fig. 1), the "degradation rate" obtained from the sera of 3 individuals receiving dicumarol ranged between .0028–.0128 seconds per minute. Determination of "degradation rate" of 25 individuals with plasma prothrombin concentration greater than 80 per cent of normal revealed a range of .0150–.0400 seconds per minute in 23 individuals. In 96 per cent of 29 individuals whose plasma prothrombin was less than 20 per cent of normal as a result of dicumarol, the "degradation rate" ranged between .0014–.0115.

The results of a daily analysis of plasma and serum for prothrombin concentra-
tion and "degradation rate" respectively, in a patient receiving dicumarol for myocardial infarction are shown in figure 2. It will be observed that administration of dicumarol (indicated by arrows) was associated with a decrease in prothrombin and a slowing of "degradation rate" of "prothrombin-converting factor." After dicumarol administration was discontinued on the eighth day, a rise of prothrombin concentration and "degradation rate" occurred on the thirteenth day. The following day, the "degradation rate" exceeded values usually found in sera of individuals with a normal plasma prothrombin concentration despite the fact that the concentration of prothrombin in the plasma of this patient was only 60 per cent of normal. When dicumarol therapy was started again, a prompt fall in "degradation rate" resulted which preceded a fall in the plasma prothrombin. The second cessation of dicumarol brought about a delayed rise in prothrombin concentration and "degradation rate."

A second patient who received dicumarol for myocardial infarction was studied (fig. 3). An assay of plasma for prothrombin was carried out by both the one-stage
and two-stage technic to ascertain whether any discrepancy between the two determinations might be related to a change in the "degradation rate" of "prothrombin-converting factor." Attention should be directed to the initial determination of "degradation rate" in this patient, since the "degradation rate" was abnormally low at the start of therapy with dicumarol. Similar low values have been seen in other patients with myocardial infarction when blood was assayed for "degradation rate" during the acute stage of coronary occlusion.

![Graph](image)

Fig. 2.—The "degradation rate" of "prothrombin-converting factor" is plotted in relation to the concentration of plasma prothrombin. Dicumarol therapy is indicated by arrows. It will be observed that the fluctuations of prothrombin are closely paralleled by changes in "degradation rate" of "prothrombin-converting factor."

The "degradation rates" in this experiment, as in figure 2, are parallel to the rise and fall of prothrombin concentration resulting from dicumarol therapy. One cannot relate the discrepancies in prothrombin concentration determined by the two methods of assay, to any consistent change in the "degradation rate" of serum "prothrombin-converting factor."

Figure 4 is illustrative of results obtained in a study of four additional individuals. The data included in the upper, left graph were obtained from a normal, healthy adult. The other graphs depict results obtained from a study of three patients who received dicumarol following coronary occlusion. It will be observed again, that the "degradation rate" of serum "prothrombin-converting factor."
consistently reflected the effect of dicumarol on plasma prothrombin concentration.

Statistical analysis of the concentrations of plasma prothrombin and the corresponding serum "degradation rates" shows a highly significant correlation (correlation coefficient of 0.78 with 99 per cent confidence limits of 0.67 to 0.85). Daily determinations of plasma prothrombin and "degradation rate" of serum "prothrombin-converting factor," as shown in the aforementioned graphs, reveals a significant correspondence of trend. Occasionally, a decreasing "degradation rate" reflects a dicumarol effect before a decrease in plasma prothrombin can be detected. Occasionally, a rapidly increasing "degradation rate" presages an escape from dicumarol which the prothrombin determination fails to reflect. It was not feasible to rely upon "degradation rate" of "prothrombin-converting factor" as an indirect measurement of prothrombin. This was apparent particularly when plasma concentrations of prothrombin were in the midzone. In this circumstance, one encountered considerable fluctuation of "degradation rate."

It is of interest that observation of "degradation rates" in sera of patients with liver disease (cirrhosis, etc.) who had low plasma prothrombin concentrations,
were usually within the range of normal. Occasionally patients with a slightly depressed plasma prothrombin concentration had abnormally low "degradation rates" (i.e., recent myocardial infarction). The effect of dicumarol on "degradation rate" of serum "prothrombin-converting factor" is so consistent, however, that we suspect that dicumarol must produce other changes in the coagulation mechanism than mere reduction of prothrombin concentration.

**Fig. 4.—Relation of plasma prothrombin (1- and 2-stage) to "degradation rate" of serum "prothrombin-converting factor."** Four different experiments are represented. The upper left graph shows results obtained by administering dicumarol (indicated by arrows) to a healthy adult. The three remaining graphs represent results obtained in patients with coronary occlusion.

**DISCUSSION**

The remarkable slowing of the "degradation rate" of "prothrombin-converting factor" which results after administration of dicumarol, permits a conclusion that dicumarol not only affects the concentration of plasma prothrombin but also causes other changes in the coagulation mechanism which may be significant. The results of this investigation demonstrate a clear-cut relationship between the concentration of plasma prothrombin and the serum "degradation rate" which is particularly apparent when the concentration of prothrombin is depressed below 2.0 per cent of normal.

The manner in which dicumarol produces a slowing of the "degradation rate" of "prothrombin-converting factor" would appear to be an indirect influence...
comparable to reduction of prothrombin, since the effect may persist for several
days after dicumarol therapy has been discontinued. The experimental data do not
explain the mechanism of degradation of "prothrombin converting factor" nor
the action of dicumarol in causing a slowing of degradation.

Other factors in blood coagulation which have been described by Owren,7
Ware et al.,8 Alexander et al.,9 Quick10 and Milstone11 may differ from "pro-
thrombin-converting factor" merely because of a different experimental method
for their measurement. Any attempt to integrate these factors with our concept of
coaulation1 must await a suitable experimental method in order to clarify the
relationship of all these components in the coagulation reaction.

SUMMARY

A study of serum and plasma of patients receiving dicumarol reveals that a
good correlation exists between the "degradation rate" of "prothrombin-con-
verting factor" in the serum and the concentration of prothrombin in the plasma.
As prothrombin concentrations falls, the rate of degradation of "prothrombin-
converting factor" becomes slower, while a rise in prothrombin concentration is
associated with an accelerated "degradation rate."

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