Use of Different Tissue Thromboplastins in the Control of Anticoagulant Therapy

By Marc Verstraete, M.D., Patricia A. Clark, and Irving S. Wright, M.D.

As anticoagulant therapy has become more widespread and a greater knowledge has been accumulated regarding the mode of action of coumarin derivatives, problems arising from the use of different thromboplastin preparations have been subjected to greater scrutiny. This paper presents an evaluation of the dependability and significance of different types of tissue thromboplastin in the determination of effects of coumarin derivatives. The main difference between thromboplastin extracts seems to be that brain preparations have a factor VII-like activity. As the exact evaluation of factor VII activity appears to be of primary importance in patients treated with coumarin derivatives, a thromboplastin preparation that does not contain factor VII activity is recommended.

As the indications for anticoagulant therapy become more extended, some problems in the management of anticoagulant therapy with coumarin derivatives and their laboratory control become increasingly important.

Until recently very little was known about the mode of action of coumarin derivatives. It has been shown that the activity of factor VII and subsequently of prothrombin decreases during coumarin therapy. This diminution is usually directly related to the antithrombotic activity of coumarin derivatives. Douglas has recently shown that the prothrombin level of the blood does not fall below 50 per cent of normal during adequate therapy with 3,3'-carboxymethylenebis (4-hydroxycoumarin) ethyl ester (Tromexan). The prolongation of the prothrombin time by the 1-stage method is partly due to the deficiency of factor VII and the moderate depression of prothrombin is insufficient to decrease blood coagulability. Recent studies have also demonstrated that the activity of other clotting factors, such as plasma thromboplastin component (PTC, factor IX, Christmas factor) and factor X decreases during coumarin therapy. The influence of coumarin derivatives on the latter 2 factors is not detected by the 1-stage prothrombin determination because potent tissue thromboplastin by-passes the first phase of the clotting mechanism in which both components participate. If the diminished activity of PTC and factor X is partly responsible for the desired hypocoagulability of the blood, the 1-stage prothrombin time method gives an incomplete account of the coumarin-induced changes. The heparin tolerance test, as a complementary test to prothrombin determinations, has given important information for the evaluation of the action of anticoagulants. This technic, however, is too cumbersome to become a routine procedure.

Other problems are the presumably changing sensitivity of a given patient to a coumarin derivative and the varying dosage requirements by different individuals. These changes are evaluated on the basis of prothrombin assays carried out in the same laboratory with identical reagents.

A further shortcoming in the control of anticoagulant therapy is the lack of uniformity of prothrombin times in different laboratories using various technics and possibly different thromboplastins. Even the prothrombin time values obtained in one laboratory using the same technic but different thromboplastin preparations show significant differences.

This communication is a report of a comparative study of the mode of action and properties of different thromboplastins used in the 1-stage prothrombin time test.

Methods

Collection of Plasma. Blood was drawn into a syringe and added to tubes containing 0.1 M sodium...
oxalate in the proportion of 9 parts blood to 1 part sodium oxalate. Tubes that appeared to be under-filled or overfilled were rejected. The oxalated blood was centrifuged at 1,700 r.p.m. for 10 minutes and the plasma was removed.

Collection of Serum. Five to 10 ml of venous blood were placed in a centrifuge tube. The clotted blood was stored in a water bath at 37 C. for at least 4 hours before the serum was removed. The presence of free thrombin in the serum was evaluated by adding 0.1 ml of serum to 0.1 ml of fibrinogen at 37 C. (fibrinogen Warner-Chilcott 300 mg. per cent). The prothrombin content was assessed in the mixture of 0.1 ml of serum and 0.1 ml of barium sulfate-treated plasma by adding 0.2 ml of thromboplastin-calcium chloride suspension and observing the clotting time. For these experiments the rabbit lung extract, Difico-Aplastin, was used. Fifty milligrams of dried lung extract were suspended in 2.5 ml of 0.85 per cent sodium chloride and mixed with 2.5 ml of 0.02 M calcium chloride.

Preparation of Thromboplastins. Eight different commercial thromboplastin extracts were selected for this study. They were prepared in accordance with the methods described by the manufacturers. There were 4 rabbit lung preparations (Difico-Aplastin, Geigy Thromboplatin, Roche Thromboplatin, Warner-Chilcott Thromboplatin), 2 rabbit brain preparations (Alban Permaplastin, Difico Baeto-thromboplatin), 1 mixture of horse brain and lung extracts (Schiefelin Soluplastin), and 1 mixture of equal parts of rabbit lung and brain extracts (Warner-Chilcott Simplastin).*

Barium Sulfate Adsorption. One milliliter of oxalated plasma or serum was incubated at 37 C. for 10 minutes and shaken with 50 mg. of barium sulfate (Mallineckrodt, analytic reagent) and incubated at 37 C. for 10 minutes. The barium sulfate was separated by centrifuging at 1,700 r.p.m. for 10 minutes and the supernatant plasma was removed with a pipette.

Imidazole Buffer. Buffer solution, pH 7.4, ionic strength 0.018, was prepared by mixing 2.5 parts of imidazole solution (13.6 Gm. per 100 ml. of distilled water), 1.86 parts of 0.1 N hydrochloric acid, and 5.64 parts of distilled water.

Oxalated Buffer consisted of 9 parts of buffer solution and 1 part of 0.1 M sodium oxalate. Ionic strength of this solution was 0.638.

One-stage Prothrombin Time (Link-Shapiro). For the prothrombin time of whole plasma, approximately 0.5 ml of plasma was transferred into a 75-

by 100-mm. test tube and placed in a constant temperature bath at 37 C.

From the thromboplastin-calcium chloride suspension, 0.2 ml was transferred into 100 by 12-mm. test tubes with a 0.2-ml. pipette (micro blood sugar). This suspension was blown into the test tubes with care to empty the pipette completely after each transfer. These tubes were placed in the rack beside the plasma samples in the constant temperature bath. As soon as the contents of the tubes reached the bath temperature, the prothrombin time of the plasma was determined by transferring 0.1 ml of the warmed plasma to a tube containing 0.2 ml of the thromboplastin-calcium suspension. The plasma was blown quickly from the pipette and at the same time the stop watch was started. The tube was tapped sharply to mix the solution. A small stirrer made of no. 22 nichrome wire with a small loop on the end was then introduced. If any small droplets were present on the sides of the tube, they were removed by passing the stirrer over them, thus making certain that all of the constituents were at the bottom of the tube. Only 2 or 3 seconds elapsed from the time the plasma was added to the thromboplastin-calcium chloride suspension. The mixture was stirred so that the stirrer loop swept across the test tube from one side to the other twice per second.

Results

Different commercial thromboplastin preparations, tested on the same day by the same technician using the same method, gave values ranging from 12 to 20 seconds for normal undiluted plasma. These differences in activity were greater when plasma with a prolonged 1-stage prothrombin time was tested. In order to determine if a conversion factor could be used to compare the results with different thromboplastins, dilution studies were performed. A hyperbolic relationship was found between the clotting time and the reciprocal of dilutions of normal plasma with barium sulfate-treated plasma. However, when prothrombin times versus reciprocal of plasma concentration were plotted, different curves were obtained for the various thromboplastins, although the technic and diluent were the same (fig. 1A). Figure 1B illustrates the results obtained with different vials of the same thromboplastin preparation, D-A; almost parallel straight lines resulted. In view of the differences in the curves of figure 1A, the 1-stage prothrombin times obtained with different thromboplastins cannot be compared directly and a conversion factor can-
not be used to correlate them. However, it is possible to make a calibration curve of one specific thromboplastin preparation.

Choice of the Diluent. When Quick introduced his valuable method of prothrombin determination in 1935, 14 0.85 per cent sodium chloride was recommended as diluent. With plasma dilutions with 0.85 per cent sodium chloride, activity curves were plotted for the different thromboplastin preparations tested. When these curves were compared, they were found to differ considerably from each other (curves D in fig. 2A–D). There are, however, several objections to the use of 0.85 per cent sodium chloride, \( \mu = 0.145 \), as diluent. The determination of the clotting time is less accurate in the higher plasma dilutions because the fibrinogen concentration is low. Apart from the dilution of prothrombin, the concentration of 2 important accelerators (factor V and VII) of the thromboplastin-prothrombin-thrombin reaction is also decreased. One of them (factor V), is normally not affected by coumarin derivatives and is not supposed to be present in tissue thromboplastins. A decrease of the factor V content in the system leads, therefore, to a prolongation of the 1-stage prothrombin time that is not related to coumarin therapy. The final oxalate concentration also changes in the plasma diluted with 0.85 per cent sodium chloride. With imidazole buffer at pH 7.4, \( \mu = 0.618 \), (curve A, fig. 2A–D) the prothrombin times of serially diluted normal plasma were considerably shorter than in the previous experiments with 0.85 per cent sodium chloride. The presence of 0.01 M sodium oxalate in the imidazole buffer (\( \mu = 0.658 \)) brought the dilution curves closer to those obtained with
CONTROL OF ANTICOAGULANT THERAPY

0.85 per cent sodium chloride as diluent (curves B, fig. 2A–D). From a consideration of the above-mentioned objections, a definite improvement in the technic was the use of barium sulfate-treated plasma as diluent (curves C of fig. 2A–D).\(^1\) Adsorbed plasma provided factor V and fibrinogen but was devoid of factor VII, prothrombin, plasma thromboplastin component (PTC), partially free of plasma thromboplastin antecedent (PTA) and did not change the pH of the final mixture. The progressive decrease of PTC and PTA in the normal plasma dilutions did not influence the 1-stage prothrombin time because tissue thromboplastin by-passes their activity in the clotting mechanism.

In conclusion, for each thromboplastin tested, different dilution curves were obtained for the various diluents.

In an analysis of the data obtained with 4 different thromboplastins, a definite difference was noted between the plasma dilution curves for thromboplastin extracts derived from rabbit brain (A-P, D-T) and rabbit lung respectively (D-A, W.C.-T.). With the brain preparations there was only a moderate difference between the 1-stage prothrombin times in corresponding plasma concentration, with oxalated imidazole buffer or barium sulfate-treated normal plasma as diluent. Neither of the corresponding curves had a diverging slope in the lower plasma concentrations (curves B and C of fig. 2A and 2B). However, when the same experiments were repeated with thromboplastin prepared from rabbit lung, a marked difference between the results with oxalated imidazole buffer or barium sulfate-treated plasma was noted (curves B and C of fig. 2C and 2D). The di-

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*Fig. 2. Dilution curves of normal plasma with various diluents: (A) imidazole buffer pH 7.4, (B) imidazole buffer 7.4 containing 0.01 M sodium oxalate, (C) barium sulfate-treated normal plasma, (D) 0.85 per cent sodium chloride. The 1-stage prothrombin times were performed (A) with brain thromboplastin (A-P), (B) with brain thromboplastin (B-T), (C) with lung thromboplastin (D-A), (D) with lung thromboplastin (W.C.-T).*
VERSTRAETE, CLARK, AND WRIGHT

Do plasma dilutions with imidazole buffer pH 7.4 with oxalated imidazole buffer with BaSO4 treated plasma with 0.85% NaCl.

Lung thromboplastin: D-A

Lung thromboplastin: W.C.-T

Fig. 2. Continued.

Convergence between both curves became greater in the lower plasma concentrations.

The ideal diluent of the reference curve constructed for the guidance of coumarin therapy should be human plasma devoid of all the clotting factors that are influenced by the action of coumarin derivatives. It is difficult to define the content of this ideal diluent, since the exact influence of coumarin derivatives on blood clotting factors is not sufficiently understood. Therefore, the most suitable diluting agent is the plasma of a patient with a very prolonged 1-stage prothrombin time, induced by the action of a coumarin derivative (more than 3 minutes for undiluted plasma). Such plasma from human subjects is only rarely available. For this reason, animal blood was used for the following experiments. Daily doses of 10 mg. of 3 - (1’ - phenyl - propyl) - 4 - hydroxy coumarin (Marcumar) per Kg. body weight were administered orally to fasting rabbits, and blood was taken by cardiac puncture when the hypocoagulability was so marked that external bleeding occurred. The 1-stage prothrombin time of the pooled rabbit plasma was more than 3 minutes. The prothrombin time of normal rabbit plasma, tested with the same thromboplastin, ranged between 7 and 9 seconds (rabbit lung preparation D-A). Normal fresh rabbit plasma was diluted with the coumarin rabbit plasma and the 1-stage prothrombin time was determined with 2 brain thromboplastins (A-P and D-T), 2 lung thromboplastins (G-T and R-T), and 2 thromboplastins that are a mixture of lung and brain extracts (S-S and W.C.-S.). The same experiments were repeated on normal rabbit plasma diluted with barium sulfate-treated rabbit plasma. The results of these tests are shown in figure 3. From a consideration of a given
thromboplastin, it is clear that the dilution curves with barium sulfate-treated rabbit plasma or coumarin rabbit plasma are practically superimposable. If human blood would behave in this respect as rabbit blood, it would indicate that for clinical purposes, barium sulfate-treated normal plasma may replace the “ideal” plasma with a very long 1-stage prothrombin time. This statement applies only to 1-stage prothrombin times performed in controlling therapy with coumarin derivatives.

Properties of Brain and Lung Thromboplastins. When the thromboplastins were diluted with calcium chloride and aliquots used with a fixed quantity of normal plasma in the 1-stage prothrombin time test, almost parallel curves were obtained in plotting clotting times against thromboplastin concentration (fig. 4). This result indicates that the basic difference between both groups of preparations is not directly related to the optimal concentration of materials that induce blood coagulation nor to the proportional concentration of substances that enhance or inhibit clotting.

The activity of both types of thromboplastin were further investigated in systems containing decreasing concentrations of prothrombin or factor VII. The clottable substrate consisted of 0.1 ml. of normal plasma diluted to various concentrations with the same plasma previously treated with barium sulfate. Added to this mixture were 0.1-ml. samples of either factor VII-free plasma containing prothrombin (citrated human plasma filtered through a 20 per cent asbestos filter pad) or prothrombin-free serum, containing factor VII. The factor VII-free plasma or prothrombin-free serum was diluted with oxalated imidazole buffer to complement the dilutions of the normal plasma (i.e., 0.1 ml. of 75 per cent normal plasma and 0.1 ml. of 25 per cent serum or factor VII-free plasma).

Filtration through 20 per cent asbestos pads renders human citrated plasma free of factor VII and removes only 10 to 20 per cent of the
original prothrombin concentration of the plasma. It is, however, important not to use the first 10-ml. filtrate, which is poor in prothrombin as well. Once the filter pad is saturated with prothrombin, only factor VII is adsorbed. Care should be taken not to overuse the filter beyond its adsorption capacity for factor VII. The presence of prothrombin in the filtrate is tested by adding in a constant volume increasing amounts of serum (source of factor VII) in order to obtain a 1-stage prothrombin time as close as possible to this of the unfiltered plasma. Usually 0.1 ml. of human serum, diluted 1 in 2 with imidazole buffer, will almost normalize the 1-stage prothrombin time of the filtered plasma (0.1 ml.) (1-stage prothrombin time before filtration, 14 seconds; of the filtrate, more than 3 minutes; of the filtrate-serum mixture, 16 seconds). If a normal prothrombin time can be obtained on the mixture of filtrated plasma and diluted serum, it can be concluded that the filtration did not remove substantial amounts of prothrombin.

The serum samples were free of active thrombin and the traces of remaining prothrombin were too small to interfere significantly in the experiments. The influence of salts, the pH, and the protein concentration on the 1-stage prothrombin time test and fibrinogen conversion has been stressed recently. The specimens were, therefore, diluted with oxalated buffer in order to keep the oxalate content and pH in the final mixture constant.

The thromboplastins were diluted with 0.25 M calcium chloride to the same extent as the normal plasma diluted with barium sulfate-treated plasma. The results of these experiments are shown in figures 5A and 5B. The points plotted represent the mean of 3 experiments performed in duplicate on 3 different days (tables 1 and 2).

When the factor VII content in the system was constant and the prothrombin level the only variable (fig. 5A), all the thromboplastin preparations tested appeared to have a similar activity. However, the results of the 1-stage

![Diagram](http://circ.ahajournals.org/)
prothrombin time test were different when the clotting system had a fixed activity of prothrombin, but a varying content of factor VII (fig. 5B). The curves obtained with the various thromboplastins were now distributed over a large area and a significant difference between brain and lung preparations was apparent. The curves of prothrombin times measured with the brain extracts A-P and D-B were considerably flatter than those of lung preparations D-A, R-T, G-T, and W.C.-T. The prothrombin time curves for the thromboplastins consisting of mixture of both animal lung and brain extracts (S-S, W.C.-S) had an intermediate position.

In subsequent experiments, a fixed amount (0.1 ml.) of different buffer dilutions of normal
### Table 1. One-Stage Prothrombin Times in Seconds (Link-Shapiro Modification) C.T. Clotting Times of Three Experiments (Data for Fig. 5A)

<table>
<thead>
<tr>
<th>Concentration of prothrombin</th>
<th>75% C.T.</th>
<th>50% C.T.</th>
<th>25% C.T.</th>
<th>12.5% C.T.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thromboplastin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Geigy (G-T)</td>
<td>19.0</td>
<td>31.5</td>
<td>51.5</td>
<td>74.5</td>
</tr>
<tr>
<td></td>
<td>17.5</td>
<td>19.2</td>
<td>24.6</td>
<td>38.2</td>
</tr>
<tr>
<td>Bacto brain (D-T)</td>
<td>14.4</td>
<td>17.5</td>
<td>30.1</td>
<td>22.3</td>
</tr>
<tr>
<td>Permaplastin (A-P)</td>
<td>18.3</td>
<td>22.2</td>
<td>29.6</td>
<td>23.9</td>
</tr>
<tr>
<td></td>
<td>18.1</td>
<td>20.0</td>
<td>23.9</td>
<td>29.6</td>
</tr>
<tr>
<td>Simplantin (W.C.-G.)</td>
<td>18.3</td>
<td>22.2</td>
<td>29.6</td>
<td>23.9</td>
</tr>
<tr>
<td></td>
<td>18.1</td>
<td>20.0</td>
<td>23.9</td>
<td>29.6</td>
</tr>
<tr>
<td>Aplastin (D-A)</td>
<td>18.2</td>
<td>23.5</td>
<td>34.7</td>
<td>43.0</td>
</tr>
<tr>
<td></td>
<td>18.1</td>
<td>20.0</td>
<td>23.9</td>
<td>29.6</td>
</tr>
<tr>
<td>Warner-Chilcott (W.C.-T.)</td>
<td>18.5</td>
<td>23.5</td>
<td>34.7</td>
<td>43.0</td>
</tr>
</tbody>
</table>

The thromboplastin suspensions were diluted with CaCl₂ M/40 to the same extent as the prothrombin concentration.

The factor VII content of the mixture is kept constant in these experiments.

### Table 2.—One-Stage Prothrombin Times in Seconds (Link-Shapiro Modification) C.T. Clotting Times of Three Experiments (Data for Fig. 5B)

<table>
<thead>
<tr>
<th>Concentration of factor VII</th>
<th>75% C.T.</th>
<th>50% C.T.</th>
<th>25% C.T.</th>
<th>12.5% C.T.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thromboplastin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Geigy (G-T)</td>
<td>17.5</td>
<td>18.9</td>
<td>33.2</td>
<td>55.8</td>
</tr>
<tr>
<td></td>
<td>18.6</td>
<td>24.8</td>
<td>46.7</td>
<td>109.0</td>
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<tr>
<td>Bacto brain (D-T)</td>
<td>12.8</td>
<td>13.5</td>
<td>17.0</td>
<td>28.0</td>
</tr>
<tr>
<td>Permaplastin (A-P)</td>
<td>15.6</td>
<td>16.3</td>
<td>21.1</td>
<td>31.0</td>
</tr>
<tr>
<td>Aplastin (D-A)</td>
<td>18.7</td>
<td>20.9</td>
<td>26.3</td>
<td>32.7</td>
</tr>
<tr>
<td>Warner-Chilcott (W.C.-T.)</td>
<td>24.2</td>
<td>23.8</td>
<td>37.8</td>
<td>122.8</td>
</tr>
<tr>
<td>Simplantin W.C.-G.</td>
<td>15.0</td>
<td>16.6</td>
<td>27.1</td>
<td>48.0</td>
</tr>
<tr>
<td>Roche R-T</td>
<td>16.1</td>
<td>16.4</td>
<td>22.6</td>
<td>47.7</td>
</tr>
<tr>
<td></td>
<td>27.3</td>
<td>40.6</td>
<td>93.3</td>
<td>155.5</td>
</tr>
<tr>
<td></td>
<td>22.9</td>
<td>33.4</td>
<td>68.0</td>
<td>131.3</td>
</tr>
</tbody>
</table>

The thromboplastin suspensions were diluted with one-fortieth molar CaCl₂ to the same extent as the factor VII.

The prothrombin content of the mixture is kept constant in these experiments.
TABLE 3.—One-Stage Prothrombin Times Performed on Mixtures of Equal Parts of Dicumarol Plasma and (A) Imidazole Buffer at pH 7.4, (B) Barium Sulfate-Treated Serum, (C) Normal Serum—The Serum was Diluted with Imidazole Buffer

<table>
<thead>
<tr>
<th>One ml. Dicumarol plasma plus</th>
<th>Thromboplastin used in testing pooled dicumarol plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. 0.1 ml. buffer</td>
<td>28.8</td>
</tr>
<tr>
<td>B. 0.1 ml. BaSO₄, serum 1:5</td>
<td>31.8</td>
</tr>
<tr>
<td>C. 0.1 ml. serum diluted</td>
<td></td>
</tr>
<tr>
<td>1:40</td>
<td>25.2</td>
</tr>
<tr>
<td>1:20</td>
<td>23.8</td>
</tr>
<tr>
<td>1:5</td>
<td>15.0</td>
</tr>
<tr>
<td>A. 0.1 ml. buffer</td>
<td>26.8</td>
</tr>
<tr>
<td>C. 0.1 ml. serum diluted</td>
<td></td>
</tr>
<tr>
<td>1:50</td>
<td>18.8</td>
</tr>
<tr>
<td>1:40</td>
<td>17.9</td>
</tr>
<tr>
<td>1:20</td>
<td>17.2</td>
</tr>
<tr>
<td>1:10</td>
<td>17.4</td>
</tr>
<tr>
<td>1:5</td>
<td>19.1</td>
</tr>
<tr>
<td>1:2</td>
<td>22.6</td>
</tr>
</tbody>
</table>

TABLE 4.—Activity of Lung Thromboplastin D-A and Brain Thromboplastin D-T on Normal Plasma and Dicumarol Plasma before and after Treatment with Different Concentrations of Barium Sulfate (2.5 mg., 5 mg., 10 mg., 25 mg., and 50 mg. per ml. Thromboplastin-Calcium Chloride Mixture), Incubation Time, 15 Minutes at 37°C.

<table>
<thead>
<tr>
<th>Thromboplastin</th>
<th>Not Adsorbed with BaSO₄</th>
<th>Adsorbed with BaSO₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal plasma</td>
<td>19</td>
<td>21.2 24.2 23.8 30.6</td>
</tr>
<tr>
<td>Dicumarol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1:25</td>
<td>27.6</td>
<td>33.2 34.2 35.0 45.8</td>
</tr>
<tr>
<td>2:25</td>
<td>28.2</td>
<td>41.2 39.4 41.0 62.2</td>
</tr>
<tr>
<td>3:25</td>
<td>38.6</td>
<td>41.4 47.6 48.0 58.0</td>
</tr>
<tr>
<td>D-T</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal plasma</td>
<td>16.0</td>
<td>16.4 16.2 16.4 17.2</td>
</tr>
<tr>
<td>Dicumarol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1:25</td>
<td>22.0</td>
<td>21.0 21.2 22.0 19.6</td>
</tr>
<tr>
<td>2:25</td>
<td>25.4</td>
<td>25.2 24.0 25.8 25.0</td>
</tr>
<tr>
<td>3:25</td>
<td>26.2</td>
<td>25.0 25.8 26.8 25.0</td>
</tr>
</tbody>
</table>

The addition of 1 ml. of normal serum to 5 ml. of thromboplastin-calcium chloride mixture included that the difference in activity between both systems may be masked by addition of normal serum to coumarin plasma, but not by addition of barium sulfate-treated serum.

The opposite experiment was to treat the different thromboplastin-calcium chloride mixtures with barium sulfate (table 4). Even in a concentration of 50 mg. of barium sulfate per ml of mixture, significant changes in the activity of brain thromboplastin were not noted. A lung preparation had a pronounced decrease in activity after similar treatment, even when very low concentrations of barium sulfate were used (2.5 mg./ml.).

The addition of 1 ml. of normal serum to 5 ml. of thromboplastin-calcium chloride mixture...
enhanced the activity of brain and lung preparations. A moderately prolonged coumarin plasma reached even shorter values than the normal control time (table 5). If the serum (2 ml.) was shaken with ether (2 ml.) for 2 hours at room temperature before mixing with thromboplastin-calcium chloride, the 1-stage prothrombin time of the same coumarin plasma became equal to the control time (table 5B). However, if the serum was treated with barium sulfate before mixing with the thromboplastin-calcium chloride, the 1-stage prothrombin times became longer as compared with times obtained with regular thromboplastin (table 5C).

A further differentiation between lung and brain preparations could not be obtained by heating of the thromboplastin-calcium chloride mixtures to 60 C. or 90 C. for 10 minutes, because the activity decreased rapidly for both preparations.

Discussion

According to our clinical experience, the 1-stage plasma prothrombin time test is still the preferred method for the measurement of the action of coumarin derivatives. Although the exact influences of these compounds on the blood clotting factors are still obscure, the decrease of prothrombin and factor VII activity should be the guide for the administration of anticoagulants. Because coumarin derivatives produce a factor VII deficiency that may lead to bleeding without a striking depression of prothrombin, it is essential to evaluate factor VII in the plasma.

In order to make the 1-stage prothrombin time test reflect the factor VII and prothrombin content of the plasma, the thromboplastin used should be devoid of these factors. For example, Russell’s viper venom combined with lipids had to be discarded because it possessed “convertin” activity.15 When the action of rabbit brain and lung tissue thromboplastins was compared on plasmas of patients treated with coumarin derivatives, shorter times were obtained with brain preparations. In comparing the 2 groups of thromboplastins, it was found that both have a similar activity in a system with a fixed content of prothrombin but a decreased level of factor VII.

One possible interpretation of the observed difference between rabbit lung and brain thromboplastins is that brain preparations have a factor VII-like activity that does not seem to be present in lung preparations. Therefore, rabbit brain thromboplastins are more active than lung preparations in blood samples in which the concentration of factor VII is depressed. However, they have the same activity as lung preparations when the clotting system has a normal factor VII activity but low prothrombin activity.

The mode of action of rabbit brain thromboplastin is, therefore, similar to Russell’s viper venom combined with lipids, which is considered to have a “convertin” activity (equivalent to the combination of antihemophilic globulin, PTC., factor VII and platelet lipid factor).18 19 The activity of the Russell’s viper venom-lipid suspension is, however, considerably higher than that of brain extracts. The action of brain thromboplastin in the plasma prothrombin time is, therefore, partly independent of the factor VII content of the plasma tested. The practical clinical consequence is that when laboratories use brain thromboplastin, the clinicians have to give higher doses of coumarin derivatives in order to obtain the commonly accepted therapeutic prothrombin time than when the slower acting lung thromboplastin is used. A striking illustration of this is a large hospital where 2 laboratories are doing prothrombin times, using 2 different types of thromboplastin. When the blood is tested in one laboratory (using a brain thromboplastin), an average maintenance dose of 100 to 125 mg. of Dicumarol is required to maintain a prothrombin time of 25 to 35 seconds. However, when the tests are performed in the other laboratory, a daily dose of 25 to 50 mg. of Dicumarol is sufficient to maintain the prothrombin time in the same therapeutic range. The concept that a range expressed in absolute seconds can be accepted for all laboratories leads to absurd confusion. For example, a patient who appears
to be safely controlled on anticoagulant therapy according to the findings of one laboratory, might seem to be dangerously overdosed according to the results of another laboratory.

Most authors agree that the therapeutic range of the 1-stage prothrombin time is between 25 and 35 seconds or $1\frac{1}{2}$ to $2\frac{1}{2}$ the control time. Although most thromboplastins have a rather identical prothrombin time on fresh plasma, normal plasma has to be diluted much more to reach this therapeutic range, if a rabbit brain preparation rather than a lung preparation is used. To obtain a 1-stage prothrombin time of 30 seconds, normal plasma has to be diluted with barium sulfate-treated plasma to 34 per cent and 30 per cent if the prothrombin tests are performed with brain preparations (A-P and D-T respectively) and to 60 per cent and 45 per cent if a lung preparation is used (D-A and W.C.-T. respectively).

These observations indicate the hazard of referring to a prothrombin time without mentioning the control time, the type of diluent, and the thromboplastin used. In view of the marked difference in activity, it is almost impossible to determine a general therapeutic range for all thromboplastins commercially available. Previous attempts were based on the erroneous assumption that the thromboplastins are of fairly uniform activity.

Dilution curves of normal plasma with different diluents (0.85 per cent sodium chloride, imidazole buffer pH 7.4, oxalated buffer and barium sulfate-treated normal plasma) are quite different for each thromboplastin tested, although the different slopes are more similar for brain preparations than for lung preparations. When prothrombin times are reported in terms of concentrations of prothrombin, the diluent chosen to make the dilution graph should, therefore, be mentioned. As can be seen in figure 2D, curves D and C, a 20 per cent concentration (0.85 per cent sodium chloride dilution curve) corresponds to 35 per cent concentration (barium sulfate dilution curve) when W.C.-T. thromboplastin is used.

**Summary**

An analysis of the results of prothrombin time tests with different types of thromboplastin sheds some light on the problem why the administration of coumarin is difficult to standardize in different centers. Our present ideas on the subject, based on experimental data, may be summarized as follows.

Several factors of the clotting mechanism are influenced by coumarin derivatives. The action of some of these factors is by-passed in the 1-stage prothrombin time test. The decrease of the prothrombin and factor VII levels may be evaluated in the 1-stage prothrombin time determination (Quick test). The prolongation of the prothrombin times are, however, predominantly due to the decrease of factor VII activity, the prothrombin content remaining around 50 per cent of normal during an adequate anticoagulant therapy. It is unlikely that this degree of depression of prothrombin is of major significance in interfering with the coagulation mechanism in the protection against thromboembolism. It may, however, play a minor role, which has yet to be evaluated quantitatively. An exact evaluation of factor VII is, therefore, important for the guidance of anticoagulant therapy and the method of choice is the one that is most sensitive to changes in factor VII concentration. The 1-stage prothrombin time test with a rabbit lung thromboplastin seems the most suitable method because rabbit brain preparations exhibit a factor VII-like activity that is not present in rabbit lung preparations.

The 1-stage prothrombin time assay of normal plasmaserially diluted with barium sulfate-treated plasma gives dilution curves that have a different slope for each thromboplastin tested, but are similar for different vials of one thromboplastin preparation.

When 0.85 per cent sodium chloride, imidazole buffer at pH 7.4, oxalated imidazole buffer, at pH 7.4, and barium sulfate-adsorbed normal plasma are used as diluents of normal plasma, different curves are obtained for each thromboplastin tested. With brain thromboplastin preparations, there is only a moderate difference between imidazole buffer curves and barium sulfate-treated plasma as diluent. However, when the same experiments were repeated with thromboplastin prepared from rabbit lung tissue, a marked difference between
the results obtained with the 2 diluents was observed.

The prothrombin times of clottable mixtures with a constant factor VII content and decreasing prothrombin activity obtained with lung and brain thromboplastins are spread over a small area. The same thromboplastins tested in a system with constant prothrombin activity but decreasing factor VII content give widespread prothrombin times. Brain preparations give short times; lung preparations give long times, and mixtures of lung and brain preparations take an intermediate position. The main difference between both thromboplastin extracts seems to be that brain preparations have a factor VII-like activity.

As the exact evaluation of the factor VII activity seems to be of primary importance in patients treated with coumarin derivatives, the use of a thromboplastin preparation devoid of factor VII activity is recommended.

**SUMMARIO IN INTERLINGUA**

Un analyse del resultatos de tests del tempore prothrombinic con differente typos de thromboplastina elucida a un certe grado le question proque le administration de coumarina es difficile a standardisar a differente centros. Nostre presente ideas in re iste tema—basate super datos experimental—pote esser summarisate sequentemente:

Plure factores del mechanismo coagulatori es influentiate per derivatos de coumarina. Le effetto de plures de iste factores es evitate in le uniphasic test del tempore prothrombinic. Le reduction de prothrombina e de factor VII pote esser evaluatate in le uniphasic determination del tempore prothrombinic (test de Quick). Tamen, le prolongation del tempores prothrombinic es predominantemente le resultato de un reduction del activitate de factor VII, durante que le contento de prothrombina remane in le vicinitate de 50 pro cento del contento normal durante un adequate theralgia anticoagulante. Il non es probable que iste grado de depression de prothrombina es de signification major como obstruction del mechanismo coagulatori in le protection contra thromboembolism. Del altere latere, il es possibile que illo ha un rolo de signification minor, que ha non ancora essite evolutate quantitativemente. Per consequente,

un exacte evaluation de factor VII es importante como guida in le theraipa anticoagulante, e le metodo de selection es le metodo que es le plus sensibile a alterationes in le concentration de factor VII. Le uniphasic test del tempore prothrombinic con un thromboplastina de pulmon de conilio pare esser le methodo le plus appropriate, proque preparatos ab cerebro de conilio exhibi un activitate del typo factor VII que non es presente in preparatos ab pulmon de conilio.

Le essayo uniphasic del tempore de prothrombina in plasma normal con dilution serial per plasma tractate con sulfato de barium resulta in curvas de dilution que exhibi diferente inclinos con omne le thromboplastinas testate sed que es simile pro differente tubos in que le mesme preparato thromboplastinic es usate.

Quando plasma normal es diluite con 0,85 pro cento de chloruro de natrium, tampon imidazol a pH 7,4, tampon imidazol oxalate a pH 7,4, o plasma normal adsorbite a sulfato de barium, differente curvas es obtenite pro omne le thromboplastinas testate. In le caso de preparatos de thromboplastina cerebral, il ha solmente moderate differentias inter le curvas correspondente al uso de tampon imidazol e de plasma tractate con sulfato de barium como diluentes. Tamen, quando le mesme experimentos eseva repetite con thromboplastina prepare ab tessuto pulmonar de conilios, un marcate differentia eseva observate inter le resultatos obtenite con le 2 diluente:

Le tempores prothrombinic de mixturas coagulabile con constant contento de factor VII e decrescente activitate prothrombinic obtenite con thromboplastinas pulmonar e cerebral es pauc variaete. Le mesme thromboplastinas testate in un sistema con constant activitate prothrombinic sed con un decrescente contento de factor VII resulta in multo divergente tempores prothrombinic. Preparatos cerebral produce breve tempores. Preparatos pulmonar produce longe tempores. Mixturas de preparatos pulmonar e cerebral produce tempores intermediari. Le principal differentia inter le duo extractos thromboplastinic pare esser que pre-
paratos cerebral ha un activitate resimilante factor VII.

Proque le evaluatation exacte del activitate de factor VII pare esser de importantia primari in patientes tractate con derivatos de coumarina, le uso de preparatos thromboplastinie sin activitate de factor VII es recommendate.

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Let the medicine therefore be given in the doses, and at the intervals mentioned above:—let it be continued until it either acts on the kidneys, the stomach, the pulse, or the bowels; let it be stopped upon the first appearance of any one of these effects, and I will maintain that the patient will not suffer from its exhibition, nor the practitioner be disappointed in any reasonable expectation.—William Withering. An Account of the Foxglove, and Some of Its Medical Uses. Birmingham, 1785.
Use of Different Tissue Thromboplastins in the Control of Anticoagulant Therapy
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