Comparison of the Thrombotest with the Modified Quick Test

Drug-Induced Hypoprothrombinemia Countered with Phytonadione

By Milton Shoshkes, and Melvin Odze, M.D.

With the technical assistance of Paul J. Lasser

C OUMARIN and indanedione derivatives are widely used anticoagulants. These compounds induce hypoprothrombinemia by depressing the synthesis of factor II (prothrombin), factor VII (proconvertin), factor IX (plasma thromboplastin component, Christmas factor), and factor X (Stuart-Prower factor), and this hypoprothrombinemia is neutralized most effectively by phytonadione (vitamin K1). The laboratory control of their dosage has depended primarily upon the Quick one-stage prothrombin time or some modification of this test. This simple test has survived competitive trials with other similar laboratory tests because it is accurate and dependable in the hands of technicians of average laboratory skill. Rodman and his associates emphasized that the most serious faults found with the Quick test can be ascribed to the fluctuations that follow the use of the several varieties of dilution curves and types of thromboplastin preparations available, and that these criticisms could be eliminated easily if there were a general adherence to a consistent standard of technic and materials.

The thrombotest of Owren, another type of one-stage test, has been reported to mirror the drug-induced depressions of the prothrombin complex with greater accuracy and sensitivity. It has been said, as well, to be sensitive to factor IX depressions, which cannot be detected by the Quick test. A further advantage cited for this new test was its greater laboratory flexibility, namely, that capillary blood as well as venous blood could be used with equal accuracy, and that citrated venous blood, when collected in siliconized or plastic (Lusteroid) tubes, could be stored up to 48 hours without a significant impairment of accuracy of the determination.

The thrombotest has become available commercially and clinical experiences with its use in this country have been accumulating rapidly. Initially, these reports were enthusiastic, but subsequent publications have been more guarded. Quick and Hussey found that the thrombotest was insensitive to pure factor IX deficiency, and, in similar vein, Lempert and Poller failed to show a relationship between the thromboplastin generation test and the thrombotest. Moreover, Rappaport and Ames demonstrated that there was little fear that there might be a significant danger of bleeding from excessive and unmeasured depressions in factor IX in patients who had been maintained on anticoagulants within therapeutic ranges.

Another significant clinical reservation was founded on the fact that thrombotest values consistently and predictably range lower than comparable Quick test values. This increased sensitivity could handicap anticoagulant therapy, for a “safe” level of 10 to 30 per cent when measured by the thrombotest is 24 to 50 per cent by the comparable Quick test. Since this range of anticoagulation is higher than has been generally recommended, one naturally would expect the lesser number of hemorrhagic complications claimed when the thrombotest has been used for drug dosage...
control. It would appear doubtful, however, that the thromboembolic disease would be as well treated with an adequate hypoprothrombinemia judged according to present-day standards.

This laboratory has been interested in the rate and effectiveness of the antagonism of coumarin derivatives by phytonadione. In the course of these studies, we have recently compared the use of the Quick test to two specific tests for factors VII and IX combined and found that the one-stage technic was equal in sensitivity. The present report is of a similar nature in comparing the thrombotest to the Quick test as to which is the more sensitive indicator of phytonadione antagonism.

Methods and Materials

Forty-five patients were studied. All received bis-hydroxycoumarin (Dicumarol) or warfarin (Coumadin) daily for the usual indications for anticoagulant therapy. They had been on these drugs for at least 1 week and most of these subjects had been under therapy for 3 to 4 weeks. A control period was established by monitoring the prothrombin times on the first day of the experiment at 9:00 a.m. (0 hours), 1:00 p.m. (4 hours), and 9:00 a.m. the following morning (24 hours). A single 10-mg. dose of colloidally dispersed phytonadione* then was given intravenously and the prothrombin times were determined at 1:00 p.m. (28 hours) and 9:00 a.m. (48 hours).

The thrombotest procedure was performed according to the method of Owren, with use of venous blood collected with glass syringes and disposable needles, and expressed into Lusteroid tubes containing the sodium citrate solution. The thrombotest reagent was obtained from Nyegaard and Co., Oslo, Norway, and precalibrated dilution curves were included by this company for converting clotting times into per cent. The quick test was determined by the Link-Shapiro modification, with use of Soluplastin. A pool of four normal sera, diluted with saline, was used to construct a curve for converting clotting times into per cent. All comparative tests were done within 10 minutes of one another. In an added observation, 45 specimens collected in Lusteroid tubes with citrate solution for the thrombotest were stored for 24 hours further in a blood-bank refrigerator, and the thrombotest was repeated.

Results

Table 1 summarizes the comparable one-stage prothrombin-time technics. The Quick-test control mean values ranged from 47 to 52 per cent with standard deviations from the mean fixed at 19 and standard errors of 2.8 to 3.0. This hypoprothrombinemia is generally higher than recommended for therapeutic levels, but it was the level adhered to by the medical residents at this calendar period and not of our own choice. At the 28-hour observation (or 4 hours after the phytonadione injection) the mean prothrombin time had risen to 65 per cent, remaining at this general level over the following 24 hours. The reproducibility of these determinations is reaffirmed by the standard deviations of only 11 to 15 and the standard errors of only 1.6 to 2.2. In contrast, the thrombotest times began

Table 1
Comparison between the Responses of the Prothrombin Times as Measured by the Modified Quick Test and Thrombotest to the Intravenous Administration of 10 Mg. of Phytonadione

<table>
<thead>
<tr>
<th>Time in hours</th>
<th>Quick-test prothrombin time (in per cent)</th>
<th>Thrombotest prothrombin time (in per cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>0</td>
<td>47</td>
<td>19</td>
</tr>
<tr>
<td>4</td>
<td>53</td>
<td>19</td>
</tr>
<tr>
<td>24</td>
<td>49</td>
<td>19</td>
</tr>
</tbody>
</table>

*AquAMEPHYTON, kindly supplied by Richard T. Smith, M.D., Director, Medical Services; Merck, Sharp & Dohme Research Laboratories, West Point, Pennsylvania.
A comparison between the modified Quick test and thrombotest plasma activities in per cent of normal.

at a lower level of 39 to 40 per cent with a slightly greater variation as reflected by the standard deviations, which ranged from 19 to 23, and standard errors of between 2.8 and 3.5. Following the phytonadione injection, the prothrombin times rose to 54 per cent at both 28 and 48 hours. The relatively higher standard error of 3.2 for each of these two observation periods reflects the wider fluctuation recorded by the thrombotest prothrombin times. These fluctuations were quite unpredictable and occasionally ranged disconcertingly far from the companion Quick-test times. The increased sensitivity of the thrombotest (ranging between 0.5 and 0.67 of the Quick test) is best appreciated by referring to figure 1, which is a correlation curve between the Quick test and the thrombotest. The recommended therapeutic ranges of 10 to 30 per cent for each test are drawn upon the graph for better visualization. This illustration demonstrates quite pointedly that the majority of the thrombotest values were within the therapeutic ranges recommended, whereas the concurrently determined Quick-test values were most often higher than recommended.

Figure 2 is a separate correlation curve between the immediately determined thrombotest samples and those stored for 24 hours in the blood-bank refrigerator. This curve shows unacceptable variations in these prothrombin times after storage. These deviated in both directions and occurred despite our adherence to strict precautions in drawing the blood samples.

Discussion

Our observations show that, in general, there is a good correlation between these two tests. The return of the prothrombin times toward normal were of the same general order of magnitude, occurred simultaneously, and showed no added vitamin effect after 4 hours. However, the greater lability of the thrombotest was at times disconcerting. We were especially disturbed by the occurrence of the unexpectedly large fluctuations in some of the individual determinations. For example, on three occasions, the thrombotest times were over 100 per cent, while the corresponding Quick test times ranged between 35 to 60 per cent. Reference to figure 1 will graphically emphasize these examples and others less extreme. Of lesser importance was the need for special needles, a special citrate solution, and special Lusteroid tubes to perform this test. These precautions had a positive benefit by drawing closer attention to the technic of
obtaining the blood samples. Yet the special handling necessary could interfere with a technician's effort when a large number and variety of blood chemistry samples must be drawn in a short time, as is the usual hospital practice.

The failure to confirm the stability of stored samples for 24 hours was unexpected. Owren's laboratory determines the thrombotest times of venous blood samples that have been mailed by physicians cooperating with their own investigations. The wide range of changes found after only 24 hours makes us hesitate to recommend this technic. The hazards inherent in mailing samples would include, not only the unavoidable time lag required for results to go back and forth, but also this added potential error of storage. If a good laboratory is not on hand, this potentially dangerous type of treatment should not be used.

This study fails to find any real advantage for substituting the thrombotest for the modified Quick test. Of itself, the thrombotest is an acceptable and generally accurate one-stage test for measuring prothrombin time, but its greater sensitivity creates serious qualifications for general use. Since our own clinical experience in anticoagulation, as well as that of the great majority of other clinicians of this country, has been based upon its laboratory control with the Quick test, there would be a great loss of future opportunity for retrospective comparative analyses of data if a new level of anticoagulation were substituted now. For example, this disadvantage might become enormously important when attempting to compare some future population treated with hypoprothrombinemic anticoagulant therapy according to thrombotest prothrombin times of 10 to 30 per cent (actually 24 to 50 per cent by Quick test), and trying to relate results to a similar group who had been treated by a Quick test control of 10 to 30 per cent. This increased sensitivity of the thrombotest offers no special inducement to switch technics, since the speed and range of fluctuations are similar. There have been two recent reviews, one by Miale and the second by Rodman and Pastor, that together exhaustively examine the pros and cons of this problem, and both maintain that there are strong reasons for maintaining the Quick test as the basic guide for anticoagulant therapy.

Summary

Forty-five patients, given anticoagulant therapy with prothrombinopenic drugs, were given 10 mg. of phytonadione intravenously. Thrombotest prothrombin times and modified Quick-test prothrombin times were compared as indices for measuring the resulting increase in prothrombin times. These increases were similar generally for both tests. The thrombotest times were lower, approximately 0.5 to 0.67 of the comparable Quick-test times, and on several occasions, grossly inaccurate results were found with the thrombotest. Blood samples stored for 24 hours in Lusteroid tubes lost a significant reproducibility of thrombotest time. Therapeutic thrombotest-time levels are inadequate for acceptable hypoprothrombinemia as judged by the Quick-test times.

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Book Collecting

There are many methods of acquiring rare books, but the most interesting is the search 
for "nuggets" in the smaller secondhand shops. If time is of value, this method is not to 
be recommended, but if the pleasure of pursuit is an object, it certainly has its fasci-
nation... .

There is a little shop in the south of London, more or less famous for its "curiosa" and 
oddments of the printed page... This shop has a sixpenny bin out in front; within are 
many shelves filled with the nondescript in literature, but in a room upstairs—never pene-
trated by customers—may be treasures... .

On my last visit the negotiations proceeded in this order, now retarded, now advanced, 
by a book scout who was present, and the climax, somewhat delayed, was this remark: 
"I did pick up some manuscripts in Wales last week which may be medical." Returning 
from another trip upstairs, he laid before me three volumes of manuscript. A casual 
inspection, then a start; one volume had written on the title page, "An Introduction to 
the Study of Physic," and below a holograph preface was the signature, "William 
Heberden."...

The next morning I took the manuscripts to the Royal Society of Medicine, knowing 
that I could rely upon Mr. Powell, the librarian, to direct me in my quest. A search of 
the catalogs, indices and bibliographies showed no similar title, and in a study of the 
available biographies no mention of any such essay was found; but the facsimile signa-
ture of Heberden under the portrait in "Pettigrew's Medical Portrait Gallery," seemed 
identical with that of the manuscript... On another day I sought further confirmation 
at the Royal College of Physicians... .

These volumes are fresh and immaculate and written throughout in the beautiful script 
of William Heberden, which was so characteristic that Mr. Barlow identified the writing 
in my volumes at a single glance. Equally striking was the fact that the make-up of these 
volumes—the paper, the quire arrangement, the format and the limp vellum binding— 
was identical with the three volumes I had found.—Prefatory Essay, by LEROY 
CRUMMER.

WILLIAM HEBERDEN. An Introduction to the Study of Physic. New York, Paul B. Hoeber, 

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