THE PROTHROMBIN CONSUMPTION TEST: ITS CLINICAL AND THEORETIC IMPLICATIONS

By Armand J. Quick, M.D., and Jean E. Favre-Gilly, M.D.

In 1947, a simple procedure was described for estimating the available thromboplastin of the blood, which was named the prothrombin consumption test. It is based on the principle that by determining the prothrombin before and after coagulation is complete, a measure of the plasma thromboplastin that reacts with prothrombin is obtained. By means of this test it was established that little prothrombin is consumed in the clotting of either hemophilic blood or platelet-depleted plasma.

In the original test, blood was allowed to remain one hour at 37°C. after coagulation before the prothrombin of the serum was determined. While satisfactory results were obtained with hemophilic and thrombocytopenic bloods, occasional inconsistencies were encountered that could not be explained. In searching for the cause of these aberrant results, the important finding was made that when normal blood clots in a test tube, all of the fibrinogen is converted to fibrin before a detectable diminution of prothrombin occurs. This leads to the logical conclusion that the fibrin clot, being uniformly dispersed through the mass of blood, presents an enormous adsorbing surface which quickly and effectively removes the nascent thrombin, thereby preventing sufficient accumulation to initiate the chain reaction that is mediated through the labilizing action of thrombin on the platelets. Almost all of the consumption of prothrombin therefore occurs only after the serum has been separated from the clot either mechanically by centrifugation, or spontaneously through clot retraction.

As a result of the observation that prothrombin consumption is markedly influenced by the separation of serum from the clot, the original test was modified in order to control the adsorption factor of fibrin. Instead of waiting one hour after coagulation before determining the prothrombin of the serum, several test tubes, each containing the same volume of blood, were allowed to coagulate. Every fifteen minutes a tube was centrifuged and the prothrombin of the serum determined at once and after fifteen minute intervals. Since the conversion of prothrom-
PROTHROMBIN CONSUMPTION TEST

Bin is very rapid immediately following the break in the intimate contact of the serum with the fibrin reticulum; thrombin will form and accumulate during centrifugation; therefore the prothrombin time done directly will be abnormally short, since it measures thrombin already present plus the amount formed during the test. By adding sodium citrate to the clotted blood just prior to centrifugation, thrombin formation is stopped, and a true prothrombin value in serum is obtained.

METHODS

The prothrombin consumption test requires the same reagents as the original one-stage method for determining prothrombin. The thromboplastin is prepared from acetone-dehydrated rabbit brain which consistently yields a prothrombin time of 11 to 12½ seconds for normal human plasma.

Tricalcium phosphate-treated plasma (calcium phosphate plasma). Tricalcium phosphate quantitatively removes component A from oxalated plasma. It does not remove the labile factor nor fibrinogen; therefore calcium phosphate plasma serves as a ready and convenient source of fibrinogen when determining the prothrombin of serum.

Calcium phosphate plasma is prepared as follows: A measured volume of a 0.005 M suspension of tricalcium phosphate (1 cc. for every cc. of oxalated plasma to be treated) is transferred to a test tube. By centrifuging, the gelatinous calcium phosphate is packed and the surplus water poured off. The required volume of fresh oxalated human plasma is added, mixed with the absorbant and repeatedly stirred with a small glass rod for five minutes at room temperature. The calcium phosphate is removed by centrifugation, and the clear adsorbed plasma poured into a clean test tube.

Prothrombin time of serum. The calcium phosphate plasma (0.1 cc.) is mixed with 0.1 cc. thromboplastin and 0.1 cc. of 0.02 M calcium chloride. Into this mixture 0.1 cc. of the serum is blown by means of a serologic pipet and the time required for a clot to form accurately determined.

Prothrombin consumption test. Blood obtained by venepuncture is distributed in 2 cc. portions to 8 test tubes (100 x 13 mm.). These are placed in a water bath at 37 C. The time required to form a solid clot is noted and fifteen minutes later 0.1 cc. of 0.4 M sodium citrate is added to one tube. This and a second tube are put in an International Clinical centrifuge and spun at full speed for one minute. After an additional half minute required to stop the centrifuge, the prothrombin time is immediately determined in the noncitrated serum and then in the citrated serum. For the latter, 0.04 M calcium chloride is used. The prothrombin time of the two sera is determined for three consecutive fifteen minute periods. Thirty minutes after coagulation, 0.1 cc. of 0.02 M sodium citrate is added to tube 3, which with tube 4 is centrifuged and the prothrombin time of the serum determined immediately and for two additional fifteen minute periods. At the end of forty-five minutes following coagulation, tubes 5 and 6 are taken out of the water bath, to one sodium citrate is added and both centrifuged and the prothrombin determined. At sixty minutes, tubes 7 and 8 are similarly treated.

For ordinary clinical studies it may not be necessary to follow the prothrombin consumption test in 8 test tubes. Three tubes will suffice. The first is centrifuged fifteen minutes after coagulation, the second after thirty and the third after sixty minutes. The prothrombin time is determined in each tube immediately after centrifugation and every fifteen minutes within the first hour after coagulation.

RESULTS AND DISCUSSION

Prothrombin consumption in the clotting of normal blood. In table 1 the prothrombin consumption observed in two typically normal healthy subjects is presented. It will be observed that in subject 1, the consumption of prothrombin is considerably slower and less complete than in subject 2. Thus, in the first individual only 50 per cent of the prothrombin was consumed in thirty minutes, whereas 70 was converted in the second during that period. At the end of one hour, the maximum quantity of prothrombin converted in the serum of subject 1 was approximately 85 per cent, whereas 95 per cent was consumed in the serum of subject 2.
Such marked variations in the activation are interesting, because the concentrations of the factors that constitute the prothrombin complex are remarkably constant in normal healthy individuals. It will be important to study these differences in the availability of thromboplastin as determined by the prothrombin consumption in relation to thrombotic tendencies. While it has been postulated since the time of Virchow that hypercoagulability of the blood is one of the triads that causes thrombosis, no reliable evidence can be found for its support. Hypercoagulability as determined by the coagulation time is not only meaningless, but occasionally definitely erroneous, as will be brought out in the discussion of thrombocytopenia. Since the prothrombin consumption test offers a new and promising means to determine the thromboplastin factors quantitatively, its value in postoperative or other conditions in which intravascular clotting commonly occurs will be investigated.

**Table 1.** The Prothrombin Consumption During and after the Coagulation of Normal Blood

<table>
<thead>
<tr>
<th>Tube</th>
<th>Time after formation of a solid clot</th>
<th>15</th>
<th>30</th>
<th>45</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Prothrombin time of serum in seconds</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subject 1</td>
<td></td>
<td>6*</td>
<td>16</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12</td>
<td></td>
<td></td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6*</td>
<td>28</td>
<td>29</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15</td>
<td>16</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10*</td>
<td>32</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14*</td>
<td>14</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10*</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>15*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subject 2</td>
<td></td>
<td>6*</td>
<td>41</td>
<td>43</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11*</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11*</td>
<td>53</td>
<td>53</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td></td>
<td>19*</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>17*</td>
<td>18</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td></td>
<td>29*</td>
<td>32</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>173*</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>18*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Prothrombin determined immediately after centrifugation.
† Sodium citrate added to unretracted clot immediately before centrifugation.

The prothrombin consumption in hemophilic blood. Evidence has accumulated since the time of Alexander Schmidt that the defect in hemophilia is a lack of thromboplastin. But Addis believed that the coagulation defect in hemophilia was due to a qualitative change in prothrombin which was responsible for its slow conversion to thrombin. Eagle presented evidence showing that the platelets were functionally normal as well as the prothrombin, but that its activation was delayed, for which he could offer no adequate explanation. Brinkhous was the first to study quantitatively the rate of prothrombin conversion in hemophilia and he...
concluded that reaction was very slow. This he attributed to the sluggishness
with which the formed elements of the blood liberate thromboplastin. More
recently, he has postulated that hemophilic blood lacks a factor which is required
for the lysis of platelets. In contrast to this explanation, Quick has postulated
that the agent responsible for platelet lysis is thrombin itself and that the basic
defect in hemophilia is the lack of thromboplastinogen. The platelets have been
found entirely normal. Their seeming stability is due to the lack of thrombin
formation caused by the deficiency of thromboplastinogen. The new concept in-
troduced by Quick is that platelets do not furnish thromboplastin, but the enzyme
which activates plasma thromboplastinogen.

A study of the prothrombin consumption of two hemophils is given in table 2.
The data are typical. Similar results have been obtained on 20 other hemophilic
patients. Oddly, the prothrombin consumption time immediately after the clotted
blood is centrifuged becomes fixed and often does not change in twenty-four hours
or longer. Frequently the serum prothrombin time is shorter than that of the
oxalated plasma and the usual range is nine to twelve seconds. This shortening of
the prothrombin time does not occur until thrombin has accumulated, for on
adding sodium citrate prior to centrifuging, to the clotted blood, a normal
prothrombin of eleven to twelve seconds is obtained.

The diagnostic value of the prothrombin consumption test in hemophilia is
obvious. It is particularly helpful in the diagnosis of the disease in very young
children who present difficulties in the collection of blood completely free of tissue
juice contamination. Often the diagnosis is delayed for months and even years
because of failure to obtain a prolonged coagulation. This actually happened in
the case of the second patient. The correct diagnosis was not made until he was
nearly seven years old. In one hospital his condition had been diagnosed as purpura and a splenectomy advised.

The prothrombin consumption in thrombocytopenic purpura. Since it was found that the removal of platelets from plasma markedly inhibited the conversion of prothrombin, it could be anticipated that a faulty or delayed prothrombin consumption occurs in thrombocytopenic purpura. This was verified with clinical cases by Soulier and by Quick, Shanberge and Stefanini. The latter studied one case in which the prothrombin consumption time improved as the platelets increased and clinical recovery occurred and another case in which splenectomy caused a rapid recovery as indicated by the platelet count and the prothrombin consumption test. In those studies the one hour old serum was employed.

In the present investigation several cases were studied by the new technic. The results obtained on 2 of these cases are presented in table 3. It is clear that when the platelet count is low, little prothrombin is converted, but the prothrombin consumption time is not as fixed and constant as in hemophilia. It tends to increase as the serum stands. There is apparently a slow conversion of prothrombin which is to be expected if the hypothesis is correct that the platelets liberate the activating enzyme of thromboplastinogen.

Since a close relationship exists between the platelet count and the prothrombin consumption, the latter test complements the platelet count and can probably be substituted for it when the latter is not available. Since the recognition of thrombocytopenic purpura is relatively simple, the test very likely will contribute little diagnostically. It may, however, become helpful in the condition in which a qualitative change in the platelets exist. Such a condition has been postulated but never satisfactorily demonstrated by concrete tests and experiments.

The most important contribution that the prothrombin consumption test has made to the problem of thrombocytopenic purpura is the establishment that a
demonstrable defect in coagulation occurs despite the normal coagulation time. There is good evidence clinically that neither the low platelet count nor the defective prothrombin consumption is responsible for the petechiae, the ecchymosis or the mucous membrane oozing. These are due to damage or dysfunction of the capillaries caused perhaps by a specific agent. It is logical to suppose, however, that the coagulation defect arising from the thrombocytopenia superimposed on the capillary hyperpermeability accentuates the hemorrhagic state.

The prothrombin consumption in hypoprothrombinemia. Since the discovery in 1943 that prothrombin activity is not confined to one discrete compound, but to several factors which have been designated as components of the prothrombin complex, the problem has become rather complicated. There is a growing agreement that one of these factors diminishes fairly readily on storage, and is not adsorbed by tricalcium phosphate. This agent has been named the labile factor by Quick. The second factor, component A, is adsorbed by tricalcium phosphate, disappears from the blood in vitamin K deficiency, is probably inactivated by sodium citrate, and is diminished in one type of congenital hypoprothrombinemia.11, 12 The third factor, component B, is least clearly defined.* Its existence is postulated to explain the type of hypoprothrombinemia that is both congenital and hereditary and in which no deficiency in the labile factor nor component A occurs.11, 12 Most characteristic in this type of hypoprothrombinemia is the fixed prothrombin level. In one family the prothrombin time is consistently sixteen seconds in the mother and in a daughter and in one son. Recently a second family has been studied in which the prothrombin time is fixed at fourteen seconds and has appeared in three generations.

In table 4 are recorded the prothrombin consumption tests observed in the blood of a patient treated with dicumarol and in a boy suffering from a congenital deficiency of component A. The prothrombin consumption in the latter is strikingly complete. Fifteen minutes after the clotted blood was centrifuged the prothrombin time of the serum increased to 155 seconds which represents 2 per cent of prothrombin activity. In marked contrast, the blood from the patient on dicumarol therapy which had a prothrombin time of twenty five seconds showed a relatively poor conversion of prothrombin during coagulation. These results cannot be satisfactorily explained until more is known concerning the interaction of the various components of the prothrombin complex. Until such information becomes available the results of a prothrombin consumption test in hypoprothrombinemia will be difficult to interpret and the test will be of limited value clinically.

The relation of prothrombin consumption to hemostasis. The most remarkable finding that has accrued from this investigation is that in the test tube only a minute amount of prothrombin is converted to thrombin in the coagulation of all the
fibrinogen of the blood. Even after the clotted blood has remained in the water bath for fifteen minutes or longer, so little prothrombin is utilized that the amount cannot be estimated. It is only after separation of the serum from the clot takes place that prothrombin begins to decrease rapidly. Obviously fibrin, itself, is the most important physiological anti-thrombin. Potentially, 1 cc. of blood can yield enough thrombin to coagulate all the blood of the body. Heretofore, it was difficult to explain how this powerful latent clotting capacity of the blood was held in check. It is now clear that the strong adsorptive property of fibrin not only guards against the accumulation of thrombin, but also prevents the autocatalytic reaction involving the labilization of platelets by thrombin from being set in motion.

<table>
<thead>
<tr>
<th>Table 4.—The Prothrombin Consumption Time in Dicumarol and Congenital (Deficiency of Component A) Hypoprothrombinemia</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plasma Prothrombin Time</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Subject (Dicumarol) 1</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>5</td>
</tr>
<tr>
<td>6</td>
</tr>
<tr>
<td>Subject (Congenital) 2</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
</tbody>
</table>

* Prothrombin determined immediately after centrifugation.
† Sodium citrate added to unretracted clot immediately before centrifugation.

In the test tube left undisturbed no significant change in the prothrombin concentration occurs until clot retraction takes place. But this phenomenon as observed in a test tube is purely artificial since the walls are rigid and unlike the more elastic walls of a vein. Clot retraction as seen in a test tube has not been demonstrated in vivo and, furthermore, its physiological significance is not known. To be sure, Quick in his monograph presented a pen drawing to show how clot retraction might draw in the torn edges of an injured vessel and thus anchor the fibrin clot. Seegers and Sharp apparently were sufficiently impressed with this concept to reproduce it as a color plate. Unfortunately the internal force that contracts the clot is weak, and it is difficult to see how this mechanism could function in arteriolar bleeding since such a vessel has not enough flaccidity to permit a weak force to narrow the lumen. It seems fairly certain that normally little separation of serum occurs in intravascular clotting, and that therefore no rapid conversion of prothrombin to thrombin takes place in vivo.
It would be idle even in the light of these new observations to speculate how hemostasis is achieved, but a few possible suggestions can be offered. Platelet accumulation and agglutination at the site of injury is undoubtedly the early response as Zucker convincingly has shown. As soon as platelets disintegrate thrombin is produced and some fibrin must form. As platelets are lysed, a vasoconstrictor is liberated which contracts all the vessels in the affected area, thus sharply localizing the process. A clot enmeshing the formed elements of the blood, including platelets, will form in the traumatized area. Due to the antithrombic action of fibrin, little thrombin becomes available to labilize platelets; therefore the disintegration of these cells is slow, and the liberation of the vasoconstrictor principle minimal but sustained. The possibility that the fibrin clot is more than a mechanical plug cannot be ignored. It is likely that the fibrin reticulum is the means whereby the coagulation reaction is held in check and that by this means the conditions for sustained hemostasis are maintained.

**SUMMARY**

The prothrombin consumption test, which originally was carried out on serum one hour after coagulation, is modified. Blood is distributed to several test tubes, and after fixed time intervals, the tubes are centrifuged. The prothrombin of the serum of each tube is determined immediately and every fifteen minutes within the limits of one hour from the time the blood is taken.

The prothrombin consumption shows considerable variations in normal individuals. In hemophilia and in thrombocytopenia it is very incomplete. In hypoprothrombinemia the prothrombin may be very complete as in congenital hypoprothrombinemia of the Component A deficiency type, or surprisingly incomplete as in dicumarol hypoprothrombinemia. The possible significance of prothrombin consumption in relation to hemostasis is discussed.

**REFERENCES**

THE PROTHROMBIN CONSUMPTION TEST: ITS CLINICAL AND THEORETIC IMPLICATIONS

ARMAND J. QUICK and JEAN E. FAVRE-GILLY