determine the fibrinogen value in mg per dl for the Ci-trol Normal. This result should fall within the ranges given for that lot number of Ci-trol Normal.

2. Centrifuge blood at 2500 RPM for 10 minutes to obtain platelet-poor plasma.
3. Reconstitute Thrombin Reagent with 1.0 ml of distilled water. Mix carefully.
4. Reconstitute Ci-trol Normal (or SNP) with 1.0 ml of distilled water. Allow to sit for 10 to 15 minutes. Mix gently.
5. Make a 1:10 dilution of both the plasma and Ci-trol Normal: place 0.9 ml of Owren's Veronal Buffer into appropriately labeled 12 × 75-mm test tubes (one tube for the Ci-trol Normal and one tube for each specimen to be tested). Add 0.1 ml of Ci-trol Normal to the control tube and 0.1 ml of patient plasma to each of the appropriately labeled patient tubes. Mix carefully.
6. Pipet 0.2 ml of the diluted Ci-trol Normal into each of two 12 × 75-mm test tubes.
7. Incubate foregoing tubes at 37°C for at least 2 minutes, but no longer than 5 minutes.
8. Pipet 0.1 ml of Thrombin Reagent (kept at room temperature) into the first tube and immediately start the stopwatch.
9. While keeping the tube in the water bath, immediately begin running the wire loop through the mixture. At the first sign of fibrin formation, stop the stopwatch and record the result.
10. Repeat steps 7 through 9 for the duplicate specimen. Average the two results. Both clotting times should agree with each other within 1.5 seconds. If they do not, repeat steps 7 through 9.
11. Repeat steps 7 through 10 for each patient specimen.
12. Determine the fibrinogen concentration for the Ci-trol Normal and each patient specimen by referring to the previously prepared fibrinogen curve. The Ci-trol Normal result should agree within the ranges given for that lot number of Ci-trol Normal. If it does not, the cause must be found, and the entire test repeated on all of the specimens.

DISCUSSION

1. The preceding procedure may be performed on both the Fibrometer and Mechrolab Clot-Timer with excellent results.
2. When the fibrinogen value is below 50 mg per dl, the plasma should be diluted 1:5 (0.2 ml of plasma added to 0.8 ml of Owren's Veronal Buffer), or 1:2 (0.4 ml of plasma added to 0.4 ml of Owren's Veronal Buffer). Perform the fibrinogen in duplicate as just outlined, average the results, and determine the fibrinogen value from the curve. Divide these results by 2 (for the 1:5 dilution) or 5 (for the 1:2 dilution). If there is no clot formed with the 1:2 dilution, report a result of less than 15 mg per dl.
3. When the fibrinogen value is above 800 mg per dl, the plasma should be diluted 1:20 (0.1 ml of plasma added to 1.9 ml of Owren's Veronal Buffer). Perform the fibrinogen in duplicate as just outlined, average the results, and determine the fibrinogen value from the curve. Multiply these results by 2.
4. In the presence of significant levels of fibrin degradation products (above 100 μg/ml) or heparin (above 0.6 USP units/ml), the test results will be invalidly low.

PROTHROMBIN CONSUMPTION TEST

The prothrombin consumption test is merely a prothrombin time carried out on serum. It tests mainly for the coagulation

factors present in stage 1 of the intrinsic system, namely, factors VIII, IX, and platelet factor 3, which are necessary for plasma thromboplastin formation. The normal prothrombin consumption time is over 30 seconds. Under 20 seconds is abnormal, and times of 20 to 30 seconds are considered borderline. An abnormal prothrombin consumption is found in factors VIII, IX, and platelet factor 3 deficiencies. Deficiencies in factor XI or XII, although of less importance, will be detected since they are also necessary components of stage 1 and needed for plasma thromboplastin generation. If, however, the blood is allowed to clot before proceeding with the test, factor XI and XII deficiencies will not be detected, other than by a prolonged clotting time.

REFERENCE

Cartwright, G.E.: *Diagnostic Laboratory Hematology*, Grune & Stratton, Inc., New York, 1963.

REAGENTS AND EQUIPMENT

1. Water bath, 37°C.
2. Thromboplastin-calcium chloride mixture.
3. Fibrinogen reagent. (Available from General Diagnostics Division, Warner-Chilcott Laboratories, Morris Plains, N.J.) Care should be taken not to shake this vial after it has been reconstituted. Vigorous mixing may cause denaturation of the fibrinogen.
4. Test tubes, 13 × 100 mm.
5. Stopwatch.

SPECIMEN

Whole blood, 1 to 3 ml, placed in a plain test tube, 13 × 100 mm in size. A normal control blood should be obtained at the same time the patient's blood is collected.

PRINCIPLE

When the formation of plasma thromboplastin is normal, all but trace amounts of prothrombin are converted to thrombin. If a prothrombin time is then performed on the serum, with the addition of fibrinogen (and thromboplastin-calcium reagent), the resulting prothrombin time should be prolonged due to decreased amounts of prothrombin. When plasma thromboplastin formation is defective, however, prothrombin conversion to thrombin is decreased, and there is an excess of prothrombin present in the serum. When fibrinogen and thromboplastin-calcium reagent are added to this serum, a shortened clotting time therefore results.

PROCEDURE

1. As soon as the patient and control bloods are drawn, place them in a 37°C water bath and observe for clotting.
2. Incubate the blood at 37°C for 50 minutes, after the blood has clotted.
3. Centrifuge the blood at 2500 RPM for 5 minutes.
4. Remove the serum from both tubes and incubate at 37°C, until exactly 60 minutes have elapsed since the blood clotted.
5. Mix 2.0 ml of thromboplastin-calcium reagent with 1.0 ml of fibrinogen.
6. Pipet 0.2 ml of thromboplastin-fibrinogen mixture into each of four, 13 × 100-mm test tubes, and place in the 37°C water bath for 2 minutes.
7. Pipet 0.1 ml of control, or patient's serum, into the preceding tube, simultaneously starting the stopwatch.
8. With a nichrome wire loop, sweep through the mixture at a rate of two sweeps per second. As soon as a fine web or clot forms, stop the watch and record the results.
9. Repeat steps 7 and 8, performing duplicate clotting times on the patient and control serums. Results on the normal control serum must be above 30 seconds in order for the test to be considered valid.

DISCUSSION

1. In drawing blood, contamination with tissue thromboplastin must be avoided. If this occurs, or serum is hemolyzed, the specimen must be redrawn.
2. There is a modification of the prothrombin consumption test using adsorbed plasma (containing factors I, V, VIII, XI, and XII) and thromboplastin-calcium reagent. This method is able to detect a deficiency in factor V in addition to those previously listed. The procedure is the same as previously described, using 0.1 ml of barium sulfate-adsorbed plasma, 0.2 ml of thromboplastin-calcium reagent, and 0.1 ml of patient's serum. A prothrombin consumption time of 25 seconds or above is considered normal.
3. A severe deficiency in prothrombin yields a normal prothrombin consumption time. Also, a normal result is obtained when there are deficiencies present in more than one factor, such as in Owren's disease (factor V and VIII deficiencies), when one of the decreased factors is present in stage 2 of the coagulation process.
4. If the prothrombin time is abnormal, a prothrombin consumption should not be performed.
5. In the presence of thrombocytopenia (decreased platelet count) or abnormally functioning platelets, the prothrombin consumption test need not be performed because platelets are necessary for plasma thromboplastin formation.

FIBRIN STABILIZING FACTOR

Factor XIII, known as the fibrin stabilizing factor, is responsible for converting the fibrin clot to a more stable form. It is thought to exist in the plasma in an inactive form, and is activated by thrombin during the fibrinogen-to-fibrin conversion. When factor XIII is present, the fibrin clot formed is insoluble in 5 M urea and should not dissolve in the urea when left standing for 24 hours. A deficiency in this factor is very rare.

REFERENCES

Harker, L.A.: *Hemostasis Manual*, 2nd Edition, F. A. Davis, Philadelphia, 1974.

Dacie, J.V., and Lewis, S.M.: *Practical Haematology*, 5th Edition, Churchill Livingstone, New York, 1975.

REAGENTS AND EQUIPMENT

1. Urea, 5.0 M.
   Urea (desiccator-dried, reagent grade) 300.30 g
   Distilled water 900 ml
   Dissolve urea, and dilute to 1000 ml with distilled water. Stable at room temperature for several months.
2. Calcium chloride, 0.05 M.
   Anhydrous calcium chloride 5.55 g
   Dilute to 1000 ml with distilled water.
3. Normal control plasma.
4. Test tubes, 13 × 100 mm.
5. Water bath, 37°C.

SPECIMEN

Citrated plasma: one part 0.11 M sodium citrate to nine parts whole blood, or oxalated plasma: one part 0.1 M sodium oxalate to nine parts whole blood.

PRINCIPLE

The patient's plasma is clotted by the addition of calcium chloride. Urea (5 M) is added to the clot. If factor XIII is not present in the patient's plasma, the clot is dissolved in less than 24 hours by the urea.

PROCEDURE

1. Pipet 0.5 ml of patient's plasma into each of two test tubes. Repeat, pipetting 0.5 ml of normal control plasma into each of two additional tubes.