2. Add 0.5 ml of 0.05 M calcium chloride to the four tubes.
3. Incubate the resulting fibrin clots at 37°C for 30 minutes.
4. Loosen the clots from the sides of the test tubes by gently tapping the sides of the tube.
5. Transfer one of the patient's clots and one of the normal control clots to respective tubes containing 5 ml of 5 M urea. Transfer both the remaining patient clot and the normal control clot to a third tube containing 5 ml of 5 M urea.
6. Allow the mixtures to stand at room temperature.
7. Check the clots at the end of 1 hour, 2 hours, 3 hours, and 24 hours, and note if the clots have dissolved.
8. Report the length of time it took for the patient's clot to dissolve after urea was added. If the clot is still present at the end of 24 hours, report that the clot was insoluble after 24 hours.

DISCUSSION

1. In the absence of factor XIII, the clot usually dissolves within 2 to 3 hours. The tube containing the normal plasma clot should remain intact for 24 hours, as should the mixed clot of the patient and normal control. If the mixed clot is dissolved, it suggests that it may be due to some other mechanism, such as fibrinolysis, rather than a deficiency in factor XIII.
2. Monochloroacetic acid (1%, w/v) may be used in place of the 5.0 M urea for performance of this test.
3. A positive control (a clot that will dissolve in 5.0 M urea) may also be performed along with the patient test using thrombin and EDTA plasma. Add 10 NIH units of thrombin (0.5 ml of 20 NIH units/ml of thrombin) to 0.5 ml of EDTA plasma. Place the resultant clot in 5.0 ml of 5 M urea. This clot should be dissolved in 24 hours due to the lack of calcium that is necessary for the action of factor XIII.

HICKS-PITNEY TEST

The Hicks-Pitney test is a modification of the thromboplastin generation test. It is a rapid screening test for disorders of thromboplastin generation and detects deficiencies in factors V, VIII, IX, X, XI, and XII. The test does not, however, distinguish between these defects. If the patient's prothrombin time is normal, a deficiency in factor V or X may be ruled out.

Hicks and Pitney Method

REFERENCE

Hicks, N.D., and Pitney, W.R.: A rapid screening test for disorders of thromboplastin generation, Br. J. Haemat., 3, 227, 1957.

REAGENTS AND EQUIPMENT

1. Water bath, 37°C.
2. Calcium chloride, 0.025 M.

Anhydrous calcium chloride	1.38 g
Distilled water	500 ml

3. Normal control plasma (plasma substrate).
4. Partial thromboplastin (platelet substitute). (Commercially available.)
5. Sodium chloride, 0.85% (w/v).
6. Test tubes, 13 × 100 mm.
7. Stopwatch.

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. Collect one tube each from patient and normal control.

PRINCIPLE

The patient's diluted plasma is mixed with a platelet substitute and calcium chloride. The ability of the patient's plasma to form thromboplastin is measured by adding this generation mixture to normal plasma and determining the clot-

ting time. Normally, sufficient thromboplastin is generated in 3 to 5 minutes to clot a normal plasma in 7 to 12 seconds.

PROCEDURE

1. Centrifuge patient and control bloods at 2500 RPM for 10 minutes immediately after collection.
2. Remove plasma and place in respective tubes in crushed ice.
3. Dilute control plasma and patient's plasma 1:10 with 0.85% sodium chloride (0.1 ml plasma in 0.9 ml of 0.85% sodium chloride). Place both diluted plasmas on ice.
4. Add 3 ml of 0.025 M calcium chloride to a 13 × 100-mm test tube and incubate at 37°C.
5. Place five 13 × 100-mm test tubes in the 37°C water bath and pipet exactly 0.1 ml of undiluted normal control plasma (plasma substrate) into each tube.
6. Prepare the control generation mixture in a 13 × 100-mm test tube, in the 37°C water bath, by the following procedure:
   A. Pipet 0.5 ml of diluted normal control plasma into the test tube.
   B. Add 0.5 ml of partial thromboplastin.
   C. Incubate for 2 minutes.
   D. Add 0.5 ml of 0.025 M calcium chloride and simultaneously start a stopwatch. If a clot forms in this generation mixture at any time, it should be removed so that it will not interfere with pipetting.
7. When 55 seconds have elapsed on the stopwatch, pipet 0.1 ml of the generation mixture. With the other hand, pipet 0.1 ml of the 0.025 M calcium chloride.
8. When 1 minute has elapsed on the stopwatch, blow the 0.1 ml of generation mixture into one of the tubes containing 0.1 ml of plasma substrate. Immediately blow the 0.1 ml of calcium chloride into the tube and simultaneously start a second stopwatch.
9. Determine the clotting time of this mixture by the tilt tube method. If clotting has not occurred within 40 to 45 seconds, have a second person continue tilting the tube until clotting occurs. Record results. If the clotting time is greater than 60 seconds, record as over 60 seconds.
10. Repeat steps 7, 8, and 9 at 1-minute intervals for all five tubes containing the 0.1 ml of plasma substrate. (Start the second clotting time when 1 minute 55 seconds have elapsed on the first stopwatch.) Do not stop the first stopwatch at any time during this procedure.
11. If any of the five tubes has a clotting time of 7 to 12 seconds within the 5-minute incubation of the generation mixture, the test is considered normal. If the control test run on the plasma substrate is abnormal, the entire test must be repeated.
12. Place five 13 × 100-mm test tubes in the 37°C water bath. Pipet exactly 0.1 ml of undiluted normal control plasma (plasma substrate) into each tube.
13. Prepare the patient's generation mixture in a 13 × 100-mm test tube in the 37°C water bath by the following procedure:
   A. Pipet 0.5 ml of diluted patient's plasma into the test tube.
   B. Add 0.5 ml of platelet substitute to the tube.
   C. Incubate for 2 minutes.
   D. Add 0.5 ml of 0.025 M calcium chloride and simultaneously start a stopwatch. If a clot forms in this generation mixture at any time, it should be removed so that it will not interfere with pipetting.
14. Repeat steps 7 through 10 as described for the control. Failure of any of the five clotting times to clot in less than 12 seconds, using the patient's

generation mixture, indicates a deficiency in factor VIII, IX, XI, or XII, if the prothrombin time is normal. It may also indicate the presence of an anticoagulant.

THROMBOPLASTIN GENERATION TEST

The thromboplastin generation test measures the efficiency with which plasma thromboplastin is formed. It detects factor VIII and factor IX deficiencies and is able to distinguish between the two. Factor XI and XII deficiencies may be detected but cannot be differentiated from each other. If the patient's platelets are used in the test, a platelet abnormality may also be detected.

REFERENCES

Biggs, R., and Douglas, A.S.: The thromboplastin generation test, J. Clin. Path., 6, 23, 1953.

General Diagnostics: *Manual for Teaching Blood Coagulation Techniques in the Routine Laboratory*, Warner-Chilcott Laboratories, Morris Plains, New Jersey, 1964.

REAGENTS AND EQUIPMENT

1. Barium sulfate, powdered.
2. Water bath, 37°C.
3. Calcium chloride, 0.025 M.

Anhydrous calcium chloride	1.38 g
Distilled water	500 ml

4. Thromboplastin-calcium chloride mixture.
5. Sodium chloride, 0.85% (w/v).
6. Partial thromboplastin (platelet substitute). (Available commercially.)
7. Ice bath (crushed ice).
8. Test tubes, 13 × 100 mm.
9. Stopwatch.

SPECIMEN

One tube of clotted blood and one tube of oxalated blood (one part 0.1 M sodium oxalate to nine parts whole blood), from both a normal patient (to be used as control), and from the patient to be tested.

PRINCIPLE

The patient's diluted serum and adsorbed plasma are mixed with calcium chloride and a substitute platelet factor. This constitutes the patient's thromboplastin generation mixture. The amount of plasma thromboplastin formed by the patient's coagulation factors is measured by the ability of this mixture to clot a normal plasma (which supplies, mainly, prothrombin and fibrinogen). The patient's generation mixture is incubated for a total of 6 minutes. At 1-minute intervals during this time, samples of the generation mixture are added to a normal plasma, and the clotting time determined. Normally, there will be enough thromboplastin generated during this 6 minutes to clot normal plasma in 12 seconds or less. When an abnormal result is obtained, normal adsorbed plasma (source of factors I, V, VIII, XI, XII) and normal serum (source of factors VII, IX, X, XI, XII) are substituted, one at a time, in order to determine which corrects the patient's defect. If the prothrombin time is normal, factors I, II, V, VII, and X are assumed to be normal. Therefore, deficiencies in factors VIII, IX, XI, or XII may be detected.

PROCEDURE

1. Preparation of patient and normal control plasma. (Source of factor VIII.)
   A. Centrifuge the normal control and patient's anticoagulated bloods at 2500 RPM for 10 minutes within 15 minutes of collection.
   B. Remove the plasma, and pipet 1.0 ml of each plasma into a separate 13 × 100 mm test tube containing 100 mg barium sulfate. Place the remaining patient's plasma in the refrigerator in case it is needed for future tests. Refrigerate the re-