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.

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-