to 120 seconds. However, plasmas containing a standard number of platelets are difficult to obtain.

3. A modification of the plasma clotting time is called the activated recalcification time, and employs the use of 0.1 ml of platelet-rich plasma, 0.1 ml of 0.03 M calcium chloride, and 0.1 ml of 1% celite as an activator. The normal clotting time in this procedure is less than 50 seconds.

THROMBIN TIME

The thrombin time tests the third stage of coagulation, the conversion of fibrinogen to fibrin. It measures the availability of functional fibrinogen. The normal thrombin time for this procedure is 15 to 20 seconds. Prolonged times are found when the fibrinogen level is below 100 mg per dl, when the function of fibrinogen is impaired, and in the presence of thrombin-inhibitors, such as heparin or fibrin-split products. The thrombin time is a most sensitive test in detecting heparin inhibition. The thrombin time may be normally prolonged in the newborn and in multiple myeloma (the abnormal globulin interferes with the polymerization of fibrin).

REFERENCES

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REAGENTS AND EQUIPMENT

1. Stock thrombin (100 units per 1 ml). Reconstitute one vial of Bovine Thrombin, Topical, 5000 NIH units (Parke, Davis & Company, Detroit, Mich.) with 5 ml saline diluent. Add 100 mg barium sulfate, and incubate at 37°C for 20 minutes. Centrifuge at 2500 RPM for 5 minutes. Carefully remove the supernatant and add it to 20 ml 0.85% sodium chloride and 25 ml glycerin. This mixture, stored at 0°C, is stable for several months.
2. Tris buffer, pH 7.35.

Sigma 121 Primary Standard Biochemical Buffer	6 g
Sodium chloride	6.6 g
Hydrochloric acid, 0.1 N	440 ml

   Dilute to 1000 ml with distilled water. Store at 4°C.
3. Normal control plasma.
4. Water bath, 37°C.
5. Nichrome wire loop.
6. Stopwatch.
7. Test tubes, 13 × 100 mm.

SPECIMEN

Plasma obtained from whole blood collected in sodium oxalate, sodium citrate, or EDTA.

PRINCIPLE

A measured amount of thrombin is added to plasma. The length of time for a fibrin clot to form is recorded as the thrombin time.

PROCEDURE

1. Centrifuge blood at 2500 RPM for 10 minutes to obtain platelet-poor plasma.
2. Immediately before use, prepare working thrombin solution by diluting 0.1 ml of stock thrombin with 0.9 ml Tris buffer. Incubate at 37°C. (This solution is stable for 20 minutes at 37°C.)
3. Incubate a sufficient amount of Tris buffer at 37°C.
4. Place 0.2 ml of patient's plasma, or normal control, into a 13 × 100-mm test tube.
5. Add 0.2 ml Tris buffer to the tube, mix, and allow to incubate for 1 minute.
6. At the end of 1 minute, pipet 0.2 ml of working thrombin solution into the tube, simultaneously starting the stopwatch.
7. With a nichrome wire loop, sweep through the mixture, two times per

second, until a clot is formed. Stop the watch and record the thrombin time.

8. Run a normal control with each series of thrombin times. Each specimen must be tested in duplicate.

DISCUSSION

1. Duplicate tests performed on the same plasma sample should check within ±1.5 seconds of each other.
2. Whenever thrombin is used, plastic or siliconized pipets should be employed to pipet the thrombin.
3. The concentration of thrombin in the working thrombin solution should be at a concentration that gives a clotting time of 15 to 20 seconds on normal plasma. When the stock thrombin solution is first prepared, it may be necessary to use a 1:12 or greater dilution when preparing the working thrombin mixture. As the stock solution ages, the reverse is true, and a dilution of 1:8 or less with Tris buffer may be required.
4. If the thrombin time is greater than 25 seconds, repeat the procedure, using a 1:1 mixture of the patient's plasma and normal control to test for inhibitors. If inhibitors are present, the thrombin time will not be shortened.

FIBRINOGEN TITER

A deficiency in fibrinogen is a rare occurrence. However, when it does occur, it may produce severe hemorrhage, and little time should be lost in diagnosing the problem. A lack of fibrinogen may be caused by a congenital defect, and it may also be found in certain obstetric and surgical cases. A chronic deficiency of fibrinogen may occur in such cases as liver disease, where production may be defective. Elevated fibrinogen levels are normally found in pregnancy, near term or after delivery. The fibrinogen titer is useful in detecting a deficiency in fibrinogen and in detecting an alteration in the conversion of fibrinogen to fibrin. The normal fibrinogen titer is 1:128 to 1:256. A titer below 1:64 is abnormal.

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REAGENTS AND EQUIPMENT

1. Water bath, 37°C.
2. Glass test tubes, 13 × 100 mm.
3. Sodium chloride, 0.85% (w/v).
4. Pipets, 1 ml, plastic.
5. Thrombin (100 units per 1 ml). Reconstitute one vial of Bovine Thrombin, Topical, 5000 NIH units (Parke, Davis & Company, Detroit, Mich.) with 5 ml of saline diluent. Add 100 mg of barium sulfate and incubate at 37°C for 20 minutes. Centrifuge at 2500 RPM for 5 minutes. Carefully remove the supernatant and add it to 20 ml 0.85% sodium chloride and 25 ml glycerin. This mixture, stored at 0°C, will be stable for several months. Alternatively 50 units of human thrombin (Fibrindex, Ortho Diagnostics, Raritan, N.J.) may be dissolved in 0.5 ml 0.85% sodium chloride (use this mixture only as a fresh preparation).
6. Control plasma from a normal individual.

SPECIMEN

Citrated plasma: one part 0.11 M sodium citrate to nine parts whole blood, or oxa-