present. If the patient is bleeding, a specific inhibitor such as anti-factor VIII is suspected, and a factor VIII activity assay is performed. Although anti-factor IX and other inhibitors have been documented, anti-factor VIII is the most common. The Bethesda titer procedure, discussed later in this chapter, is used to confirm the presence of specific anti-coagulation factor antibodies.

If the PTT of the initial or incubated mixture fails to correct and the patient is not bleeding, the laboratory practitioner suspects LA and automatically orders an LA profile, as described in Chapter 39. LA profiles are available from tertiary care facilities and specialty reference laboratories.

Thrombin Clotting Time

Thrombin Clotting Time Reagent and Principle

Commercially prepared bovine thrombin reagent at 5 National Institutes of Health (NIH) units/mL cleaves fibrinopeptides A and B from plasma fibrinogen to form a detectable fibrin polymer (Figure 42-10).

Thrombin Clotting Time Procedure

Reagent thrombin is warmed to 37° C for a minimum of 3 and a maximum of 10 minutes. Thrombin deteriorates during incubation and must be used within 10 minutes of the time incubation is begun. An aliquot of PPP, usually 100 µL, is also incubated at 37° C for a minimum of 3 and a maximum of 10 minutes. The operator pipettes 200 µL of thrombin into the PPP aliquot, starts a timer, and records the interval to clot formation. TCT tests may be performed in duplicate and the results averaged.

Thrombin Clotting Time Quality Control

The medical laboratory practitioner tests a normal control sample and an abnormal control sample with each batch of TCT assays and records the results. The normal control results should fall within the laboratory's reference interval. The abnormal control results should be prolonged to the range reached by the TCT in moderate hypofibrinogenemia. If the results fall outside the laboratory protocol's control limits, the reagents, control, and equipment are checked; the problem is corrected; and the control is retested. The actions taken to correct out-of-limit tests are recorded. Control results are analyzed at regular intervals (weekly is typical) to determine the longitudinal validity of the procedure.

Specimen errors that affect the PT likewise affect the TCT (Table 42-7).

Reporting of Thrombin Clotting Time Results and Clinical Utility

A typical TCT reference interval is 15 to 20 seconds, although the reference interval should be established locally. The TCT is prolonged when the fibrinogen level is less than 100 mg/dL (hypofibrinogenemia) or in the presence of antithrombotic materials such as FDPs, paraproteins, or heparin. Afibrinogenemia (absence of fibrinogen) and dysfibrinogenemia (presence of fibrinogen that is biochemically abnormal and nonfunctional) also cause a prolonged TCT. Before a prolonged TCT may be considered as evidence of diminished or abnormal fibrinogen, the presence of antithrombotic substances, such as heparin, FDPs, or paraproteins, must be ruled out. The TCT is part of the PTT mixing study protocol and is used to determine whether heparin is present whenever the PTT is prolonged.[76]

The TCT may also assess the presence of the oral direct thrombin inhibitor dabigatran. The TCT provides binary (qualitative) evidence for dabigatran; if drug is present, the TCT is markedly prolonged. A normal TCT rules out dabigatran.

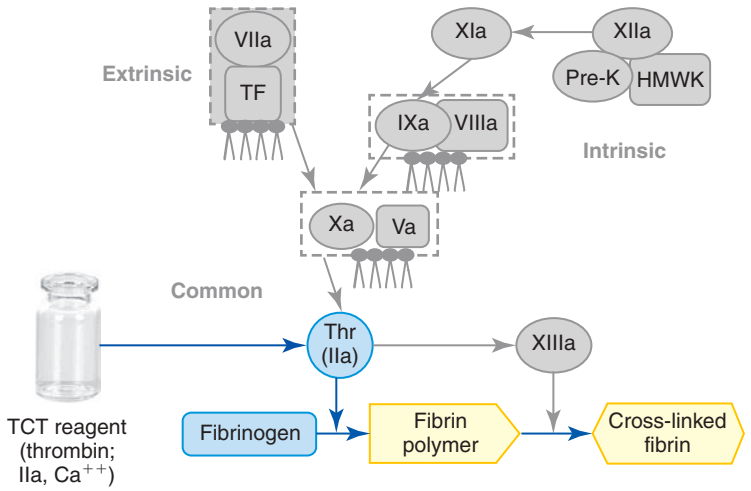

Figure 42-10 Thrombin clotting time (TCT, also reptilase time) coagulation pathway. The reagent activates the coagulation pathway at the level of thrombin and tests for the polymerization of fibrinogen (see colored area in figure). The TCT is prolonged by unfractionated heparin; direct thrombin inhibitors; fibrin degradation products; M-proteins; and dysfibrinogenemia, hypofibrinogenemia, and afibrinogenemia. The reptilase time is unaffected by heparin but is prolonged by dysfibrinogenemia, hypofibrinogenemia, and afibrinogenemia. Neither the TCT nor reptilase time detects factor XIII deficiency. *HMWK,* High-molecular-weight kininogen (Fitzgerald factor); *NCS,* negatively charged surface; *Pre-K,* prekallikrein (Fletcher factor); *PL,* phospholipid; *TF,* tissue factor; *Thr,* thrombin (activated factor II, or IIa; serine protease); *Va, VIIIa,* activated factors V and VIII (serine protease cofactors); *VIIa, IXa, Xa, XIa,* activated factors VII, IX, X, XI (serine proteases); *XIIa,* activated factor XII (serine protease, but not part of in vivo coagulation); *XIIIa,* activated factor XIII (transglutaminase).

A TCT modification, the plasma-diluted TCT, provides a quantitative measure of dabigatran when used with calibrators of specific drug concentrations.[77]

The fibrinogen assay described in a subsequent section is a simple modification of the TCT. In the fibrinogen assay, the concentration of reagent thrombin is 50 NIH units/mL, or about 10 times that used in the TCT, and the patient specimen is diluted 1:10. This dilution minimizes the effects of heparin or antithrombotic proteins. The reptilase time procedure described below is identical to the TCT procedure, except that the reptilase reagent is insensitive to the effects of heparin.

Reptilase Time

Reptilase Time Reagent and Principle

Reptilase is a thrombin-like enzyme isolated from the venom of *Bothrops atrox* that catalyzes the conversion of fibrinogen to fibrin (Pefakit Reptilase Time; Pentapharm, Inc., Basel, Switzerland). In contrast to thrombin, this enzyme cleaves only fibrinopeptide A from the fibrinogen molecule, whereas thrombin cleaves both fibrinopeptides A and B.[78] The specimen requirements, procedure, and quality assurance protocol for the reptilase time test are the same as those for the TCT. The reagent is reconstituted with distilled water and is stable for 1 month when stored at 1° C to 6° C. Reptilase time reagent is a poison that may be fatal if it directly enters the bloodstream.

Reptilase Time Clinical Utility

Reptilase is insensitive to heparin but is sensitive to dysfibrinogenemia, which profoundly prolongs the assay time. The reptilase time test is also useful for detecting hypofibrinogenemia or dysfibrinogenemia in patients receiving heparin therapy. The reptilase time is prolonged in the presence of FDPs and paraproteins.

Russell Viper Venom

Russell viper venom (RVV) from the *Daboia russelii* viper, which triggers coagulation at the level of factor X, was once used as an alternative to the prothrombin time. The assay was named the Stypven time, but is now obsolete. Russell viper venom is used in a dilute form to detect and confirm lupus anticoagulant, an assay called the dilute Russell viper venom time described in Chapter 39.

COAGULATION FACTOR ASSAYS

Fibrinogen Assay

Fibrinogen Assay Principle

The clot-based method of Clauss, a modification of the TCT, is the recommended procedure for estimating the functional fibrinogen level.[79,80] The operator adds reagent bovine thrombin to dilute PPP, catalyzing the conversion of fibrinogen to fibrin polymer. In the fibrinogen assay, the thrombin reagent concentration is 50 NIH units/mL. The PPP to be tested is diluted 1:10 with Owren buffer. There is an inverse relationship between the interval to clot formation and the concentration of functional fibrinogen. Because the thrombin reagent is concentrated and the PPP is diluted, the relationship is linear when the fibrinogen concentration is 100 to 400 mg/dL. Diluting the PPP also minimizes the antithrombotic effects of heparin, FDPs, and paraproteins; heparin levels less than 0.6 units/mL and FDP levels less than 100 μg/dL do not affect the results of the fibrinogen assay provided the fibrinogen concentration is 150 mg/dL or greater.

The interval to clot formation is compared with the results for fibrinogen calibrators. A calibration curve is prepared in each laboratory and updated regularly.

Fibrinogen Assay Procedure

Fibrinogen Assay Thrombin Reagent. Most laboratory managers prefer commercially manufactured diagnostic lyophilized bovine thrombin reagent for fibrinogen assays. Pharmaceutical topical thrombin also may be used. The reagent is reconstituted according to manufacturer instructions and used immediately or aliquotted and frozen. If thrombin is to be frozen, it should be prepared in a stock solution of 1000 NIH units/mL and frozen at −70° C until it is ready for use. When thawed, the thrombin is diluted 1:2 with buffer, is stable for only a few hours, and cannot be refrozen.

Fibrinogen Assay Calibration Curve. The laboratory practitioner prepares a calibration curve every 6 months at a minimum and with each change of reagent lot numbers, with a shift in QC, and after major maintenance. The curve is prepared by reconstituting commercially available lyophilized fibrinogen calibration plasma. Using Owren buffer, five dilutions of the calibration plasma are prepared: 1:5, 1:10, 1:15, 1:20, and 1:40. An aliquot of each dilution, usually 200 μL, is transferred to each of three reaction tubes or cups, warmed to 37° C, and tested by adding 100 μL of working thrombin reagent at 50 NIH units/mL. Time from addition of thrombin to clot formation is recorded, results of duplicate tests are averaged, and the values in seconds are graphed against fibrinogen concentration (Figure 42-11). Because patient PPPs are diluted 1:10 before testing, the 1:10 calibration plasma dilution is assigned the same fibrinogen concentration value as that of the undiluted reconstituted calibration plasma.

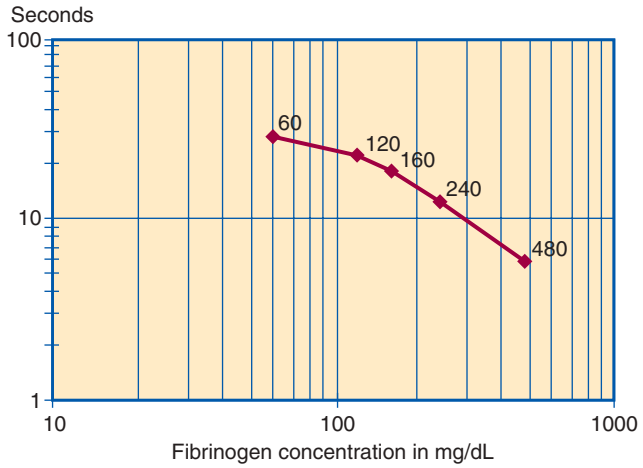

Figure 42-11 Fibrinogen calibrator curve plotted on log-log axes.