Chapter 12

Coagulation Monitoring

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Summary

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The need to monitor anticoagulation during and after surgery is the reason that the cardiac surgical arena has evolved into a major site for the evaluation and use of hemostasis monitors. The rapid and accurate identification of abnormal hemostasis has been the major impetus toward the development of point-of-care tests that can be performed at the bedside or in the operating room. The detection and treatment of specific coagulation disorders in a timely and cost-efficient manner are major goals in hemostasis monitoring for the cardiac surgical patient.

MONITORING HEPARIN EFFECT

Cardiac surgery had been performed for decades using empirical heparin dosing in the form of a bolus and subsequent interval dosing. Empirical dosing continued because of the lack of an easily applicable bedside test to monitor the anticoagulant effects of heparin.

The first clotting time to be used to measure heparin's effect was the whole-blood clotting time (WBCT), or the Lee-White WBCT. This simply requires whole blood to be placed in a glass tube, maintained at 37°C, and manually tilted until blood fluidity is no longer detected. This test fell out of favor for monitoring the cardiac surgical patient because it was so labor intensive and required the

undivided attention of the person performing the test for periods up to 30 minutes. Although the glass surface of the test tube acts as an activator of factor XII, the heparin doses used for cardiac surgery prolong the WBCT to such a profound degree that the test is impractical as a monitor of the effect of heparin during cardiac surgery. To speed the clotting time so that the test was appropriate for clinical use, activators were added to the test tubes and the activated coagulation time (ACT) was introduced into practice.

Activated Coagulation Time

The ACT was first introduced by Hattersley in 1966 and is still the most widely used monitor of heparin effect during cardiac surgery. Whole blood is added to a test tube containing an activator—diatomaceous earth (celite) or kaolin. The presence of activator augments the contact activation phase of coagulation, which stimulates the intrinsic coagulation pathway. ACT can be performed manually, whereby the operator measures the time interval from when blood is injected into the test tube to when clot is seen along the sides of the tube. More commonly, the ACT is automated as it is in the Hemochron and Hemotec systems. In the automated system, the test tube is placed in a device that warms the sample to 37°C. The Hemochron device (International Technidyne Corp., Edison, NJ) rotates the test tube, which contains celite activator and a small iron cylinder, to which 2 mL of whole blood is added. Before clot forms, the cylinder rolls along the bottom of the rotating test tube. When clot forms, the cylinder is pulled away from a magnetic detector, interrupts a magnetic field, and signals the end of the clotting time. Normal ACT values range from 80 to 120 seconds. The Hemochron ACT can also be performed using kaolin as the activator in a similar manner (Fig. 12-1).

Figure 12-1 The Hemochron Response is a dual-chamber point-of-care coagulation monitor that is capable of measuring clotting times that are compatible with Hemochron technology. This system has software capability for calculation, data management, and storage of results. (Courtesy of International Technidyne Corp., Edison, NJ, http://www.itcmed.com)

The Hemotec ACT device (Medtronic Hemotec, Parker, CO) is a cartridge with two chambers that contain kaolin activator and is housed in a heat block. Blood (0.4 mL) is placed into each chamber and a daisy-shaped plunger is raised and passively falls into the chamber. The formation of clot will slow the rate of descent of the plunger, and this decrease in velocity of the plunger is detected by a photo-optical system that signals the end of the ACT test. The Hemochron and Hemotec ACTs have been compared in a number of investigations and have been found to differ significantly at low heparin concentrations.[1] However, differences in heparin concentration, activator concentration, and the measurement technique make comparison of these tests difficult and have led to the realization that the Hemochron ACT result and the Hemotec ACT result are not interchangeable. In adult patients given 300 U/kg of heparin for cardiopulmonary bypass (CPB), the Hemochron and Hemotec (Hepcon) ACTs were both therapeutic at all time points; however, at two points, the Hemochron ACT was statistically longer. This difference was even more pronounced in pediatric patients, who have higher heparin consumption rates. The apparent "overestimation" of ACT by the Hemochron device during hypothermic CPB may be due to the different volumes of blood that each assay warms to 37°C.

The ACT test can be modified by the addition of heparinase. Using this modification, the coagulation status of the patient can be monitored during CPB while the anticoagulant effects of heparin are eliminated. Because this test is a side-by-side comparison of the untreated ACT to the heparinase ACT, it also has the advantage of being a rapid test for the assessment of a circulating heparin-like substance or for residual heparinization after CPB.

With the introduction of ACT monitoring into the cardiac surgical arena, clinicians have been able to more accurately titrate heparin and protamine dosages. As a result, many investigators report reductions in blood loss and transfusion requirements, although many of these studies used retrospective analyses. The improvements in postoperative hemostasis documented with ACT monitoring are potentially attributed to better intraoperative suppression of microvascular coagulation and improved monitoring of heparin reversal with protamine.

ACT monitoring of heparinization is not without pitfalls, and its use has been criticized because of the extreme variability of the ACT and the absence of a correlation with plasma heparin levels (Fig. 12-2). Many factors have been suggested to alter the
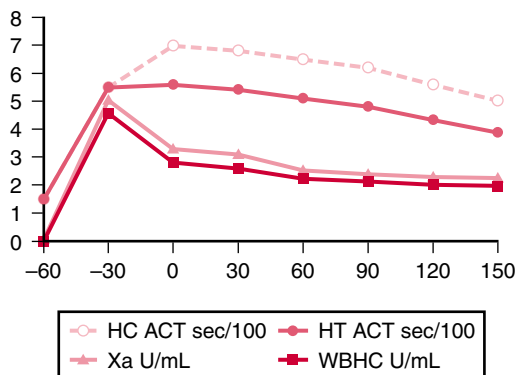

Figure 12-2 Anticoagulation measured at baseline (−60 minutes), heparinization (−30 minutes), and six time points after institution of cardiopulmonary bypass (CPB). Note the close correlation between the anti–factor Xa (Xa) activity and whole blood heparin concentration (WBHC), which does not parallel the change in Hemochron (HC ACT) or Hemotec activated coagulation time (HT ACT). (Modified from Despotis GJ, Summerfield AL, Joist JH: Comparison of activated coagulation time and whole blood heparin measurements with laboratory plasma anti-Xa heparin concentration in patients having cardiac operations. J Thorac Cardiovasc Surg 108:1076-1082, 1994.)

ACT, and these factors are prevalent during cardiac surgical procedures. When the extracorporeal circuit prime is added to the patient's blood volume, hemodilution occurs and may theoretically increase ACT. Evidence suggests that this degree of hemodilution alone is not enough to actually alter ACT. Hypothermia increases ACT in a "dose-related" fashion. It has been shown that although hemodilution and hypothermia significantly increase the ACT of a heparinized blood sample, similar increases do not occur in the absence of added heparin. The effects of platelet alterations are a bit more problematic. At mild to moderate degrees of thrombocytopenia, the baseline and heparinized ACT are not affected. It is not until platelet counts are reduced to below 30,000 to 50,000/μL that ACT may be prolonged. Patients treated with platelet inhibitors such as prostacyclin, aspirin, or platelet membrane receptor antagonists have a prolonged heparinized ACT compared with patients not treated with platelet inhibitors. This ACT prolongation is not exclusively related to decreased levels of platelet factor 4 (PF4) (PF4 is a heparin-neutralizing substance), because it also occurs when blood is anticoagulated with substances that are not neutralized by PF4. Platelet lysis, however, significantly shortens the ACT due to the release of PF4, and other platelet membrane components, which may have heparin-neutralizing activities. Anesthesia and surgery also decrease the ACT and create a hypercoagulable state, possibly by creating a thromboplastic response or through activation of platelets.

During CPB, heparin decay varies substantially and its measurement is problematic because hemodilution and hypothermia alter the metabolism of heparin. In a CPB study, the consumption of heparin varied from 0.01 to 3.86 U/kg/min and there was no correlation between the initial sensitivity to heparin and the rate of heparin decay. In the pediatric population, the consumption of heparin is increased above that of adult levels. The heparin administration protocol for pediatric patients undergoing CPB should account for a large volume of distribution, increased consumption, and a shorter elimination half-life. In monitoring the effects of heparin in pediatric patients, the minimum acceptable ACT value should be increased or an additional monitor should be used.

Heparin Resistance

Heparin resistance is documented by an inability to raise the ACT of blood to expected levels despite an adequate dose and plasma concentration of heparin. In many clinical situations, especially when heparin desensitization or a heparin inhibitor is suspected, heparin resistance can be treated by administering increased doses of heparin in a competitive fashion. If an adequately prolonged clotting time is ultimately achieved using higher-than-expected doses of heparin, a better term than *heparin resistance* would be *heparin tachyphylaxis* or "altered heparin responsiveness." During cardiac surgical procedures, the belief that a safe minimum ACT value of 300 to 400 seconds is required for CPB is based on a few clinical studies and a relative paucity of scientific data. However, inability to attain this degree of anticoagulation in the heparin-resistant patient engenders the fear among cardiac surgical providers that the patient will experience a microvascular consumptive coagulopathy or that clots will form in the extracorporeal circuit.

Many clinical conditions are associated with heparin resistance. Sepsis, liver disease, and pharmacologic agents represent just a few (Table 12-1). Many investigators have documented decreased levels of antithrombin III (ATIII) secondary to heparin pretreatment, whereas others have not found decreased ATIII levels.[2] In patients receiving preoperative heparin infusions, lower baseline ACT was the only risk factor found for predicting heparin resistance compared with patients not receiving preoperative heparin.

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Table 12-1 Disease States Associated with Heparin Resistance

Disease State	Comment
Newborn	Decreased ATIII levels until 6 months of age
Venous thromboembolism	May have increased factor VIII level
	Accelerated clearance of heparin
Pulmonary embolism	Accelerated clearance of heparin
Congenital ATIII deficiency	40% to 60% of normal ATIII concentration
Type I	Reduced synthesis of normal/abnormal ATIII
Type II	Molecular defect within the ATIII molecule
Acquired ATIII deficiency	<25% of normal ATIII concentration
Preeclampsia	Levels unchanged in normal pregnancy
Cirrhosis	Decreased protein synthesis
Nephrotic syndrome	Increased urinary excretion of ATIII
DIC	Increased consumption of ATIII
Heparin pretreatment	85% of normal ATIII concentration due to accelerated clearance
Estrogen therapy	Decreased protein synthesis
Cytotoxic drug therapy (L-asparaginase)	Decreased protein synthesis

ATIII = antithrombin III; DIC = disseminated intravascular coagulation.

Patients receiving preoperative heparin therapy traditionally require larger heparin doses to achieve a given level of anticoagulation when that anticoagulation is measured by the ACT. Presumably, this "heparin resistance" is due to deficiencies in the level or activity of ATIII. Other possible causes include enhanced factor VIII activity and platelet dysfunction causing a decrease in ACT response to heparin. In vitro addition of ATIII enhances the ACT response to heparin. ATIII concentrate is now available and represents a reasonable method of treating patients with documented ATIII deficiency.[3]

Heparin-Induced Thrombocytopenia

The syndrome known as heparin-induced thrombocytopenia (HIT) develops in anywhere from 5% to 28% of patients receiving heparin. HIT is commonly categorized into two subtypes. HIT type I is characterized by a mild decrease in platelet count and is the result of the proaggregatory effects of heparin on platelets. HIT type II is considerably more severe, most often occurs after more than 5 days of heparin administration (average onset time, 9 days), and is mediated by antibody binding to the complex formed between heparin and PF4. Associated immune-mediated endothelial injury and complement activation cause platelets to adhere, aggregate, and form platelet clots, or "white clots." Among patients developing HIT II, the incidence of thrombotic complications approximates 20%, which in turn may carry a mortality rate as high as 40%. Demonstration of heparin-induced proaggregation of platelets confirms the diagnosis of HIT type II. This can be accomplished with a heparin-induced serotonin release assay or a specific heparin-induced platelet activation assay. A highly specific enzyme-linked immunosorbent assay for the heparin/PF4 complex has been developed and has been used to delineate the course of IgG and IgM antibody responses in patients exposed to unfractionated heparin during cardiac surgery. Bedside antibody tests are being developed that may speed the diagnosis of this condition.

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The options for treating these patients are few. If the clinician has the luxury of being able to discontinue the heparin for a few weeks, often the antibody disappears and allows a brief period of heparinization for CPB without complication.[4] Changing the tissue source of heparin was an option when bovine heparin was predominantly in use. Some types of low-molecular-weight heparin (LMWH) have been administered to patients with HIT, but reactivity of the particular LMWH with the patient's platelets should be confirmed in vitro. Supplementing heparin administration with pharmacologic platelet inhibition using prostacyclin, iloprost, aspirin, or aspirin and dipyridamole has been reported, all with favorable outcomes. Tirofiban with unfractionated heparin has been used in this clinical circumstance. Plasmapheresis may be used to reduce antibody levels. The use of heparin could be avoided altogether through anticoagulation with direct thrombin inhibitors such as argatroban, hirudin, or bivalirudin. These thrombin inhibitors have become standard of care in the management of the patient with HIT II. Monitoring their effects during CPB is more complex.

Measurement of Heparin Sensitivity

Even in the absence of heparin resistance, patient response to an intravenous bolus of heparin is extremely variable. The variability stems from different concentrations of various endogenous heparin-binding proteins such as vitronectin and PF4. This variability exists whether measuring heparin concentration or the ACT; however, variability seems to be greater when measuring the ACT. Because of the large interpatient variation in heparin responsiveness and the potential for heparin resistance, it is critical that a functional monitor of heparin anticoagulation (with or without a measure of heparin concentration) be used in the cardiac surgical patient. A threefold range of ACT response to a 200-U/kg heparin dose and similar discrepancy in heparin decay rates was documented, and therefore, the use of individual patient dose-response curves is needed to determine the optimal heparin dose. This is the concept on which point-of-care individual heparin dose-response (HDR) tests are based.

An HDR curve can be generated manually using the baseline ACT and the ACT response to an in vivo or in vitro dose of heparin. Extrapolation to the desired ACT provides the additional heparin dose required for that ACT. Once the actual ACT response to the heparin dose is plotted, further dose-response calculations are made based on the average of the target ACT and the actual ACT (Fig. 12-3). This methodology was first described by Bull and associates and forms the scientific basis for the automated dose-response systems manufactured by Hemochron and Hemotec. The Hemochron RxDx system uses the heparin-response test (HRT), which is an ACT with a known quantity of in vitro heparin (3 IU/mL). Using an algorithm that incorporates the patient's baseline ACT, estimated blood volume, and HRT, a dose-response curve is generated that enables calculation of the heparin dose required to attain the target ACT. The patient's heparin sensitivity can be calculated in seconds/IU/mL by dividing the HRT by 3 IU/mL.

The RxDx system also provides an individualized protamine dose using the protamine response test (PRT). This is an ACT with one of two specific quantities of protamine, depending on the amount of circulating heparin suspected (2 IU/mL or 3 IU/mL). Using the patient's heparinized ACT, the PRT, and an estimate of the patient's blood volume, the protamine dose needed to return the ACT to baseline can be calculated based on a protamine-response curve. Jobes and coworkers reported that the heparin dose directed by the RxDx system resulted in ACT values well above the target ACT. In their patients, in vivo heparin sensitivity was higher than in vitro sensitivity. RxDx also resulted in lower protamine doses, lower

Step 1
Construct graph axes.

Step 2
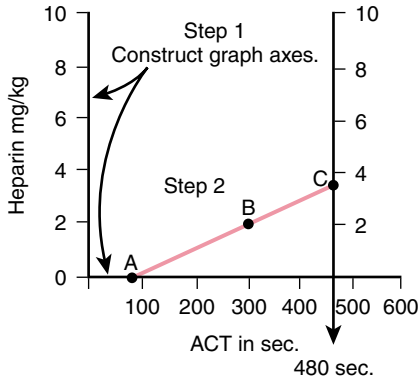
Determine initial ACT (A) and administer 2 mg/kg heparin, then measure ACT (B) and plot both values.

Extrapolate an imaginary line through "A" and "B" to intersect with 480 second line to find point "C."

Example: 3.5 mg/kg heparin is needed to produce 480 sec. ACT or 1.5 mg/kg in addition to the 2 mg/kg heparin already given.
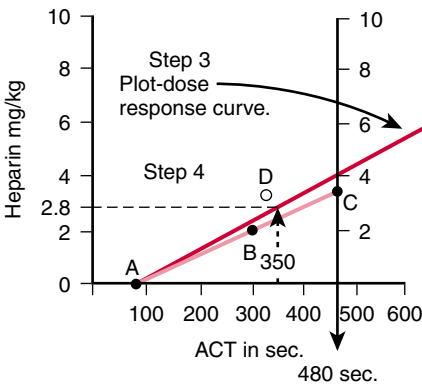
Step 3

After required heparin has been given, measure ACT. Plot point "D."

If point "D" does not superimpose on point "C," then a dose-response curve is drawn from "A" to a point midway between "C" and "D."

Step 4

After 60 minutes, measure the ACT. Determine amount of heparin in patient's circulation from the dose-response curve.

Example: Assume an ACT of 350 sec.; the heparin level would be 2.8 mg/kg. To return to 480 sec., 1.2 mg/kg of heparin is needed.

Step 5

To reverse anticoagulation, circulating heparin level is determined as in step 4. The neutralizing dose of protamine is heparin level mg/kg × 1.3.
Example: ACT of 325 seconds is measured. Heparin level is 2.6 mg/kg, and 3.4 mg/kg protamine is required.
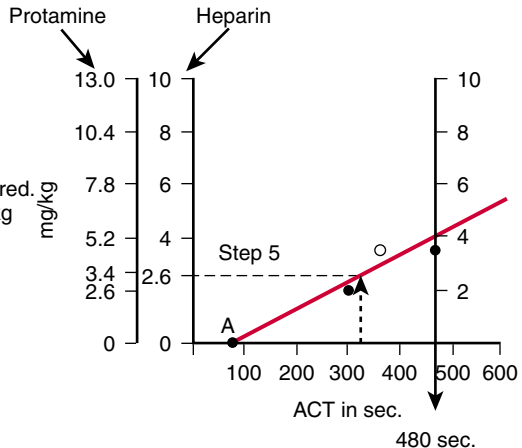

Figure 12-3 Construction of a dose-response curve for heparin. ACT = activated coagulation time. (From Bull BS, Huse WM, Brauer FS, et al: Heparin therapy during extracorporeal circulation: II. The use of a dose-response curve to individualize heparin and protamine dosage. J Thorac Cardiovasc Surg 69:685-689, 1975.)

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postoperative mediastinal tube losses, and reduced transfusion requirements compared with a ratio-based system of heparin/protamine administration.[5] In a larger study that standardized the treatment of heparin rebound, the reduced protamine dose was confirmed; however, the reductions in bleeding were not substantiated. The use of a protamine dose-response curve has been shown to successfully reduce the protamine dose in vascular surgery compared with standard weight-based protamine dosing.[6]

The Hepcon HMS system uses the HDR cartridge in the Hepcon instrument (Fig. 12-4). Each cartridge houses six chambers. Chambers 1 and 2 contain heparin at a concentration of 2.5 U/mL, chambers 3 and 4 contain heparin at a concentration of 1.5 U/mL, and chambers 5 and 6 do not contain heparin. Once information regarding patient weight, height, and CPB prime volume is entered, the information that can be obtained from this test includes the baseline ACT (chambers 5 and 6) and an HDR slope. The dose-response slope, which is the increase in ACT from 1.5 U/mL to 2.5 U/mL heparin, is extrapolated to the desired target ACT or target heparin concentration and the heparin dose is calculated.

Heparin Concentration

Proponents of ACT measurement to guide anticoagulation for CPB argue that a functional assessment of the anticoagulant effect of heparin is mandatory and that the variability in ACT represents a true variability in the coagulation status of the patient. Opponents argue that during CPB the sensitivity of the ACT to heparin is altered and ACT does not correlate with heparin concentration or with anti–factor Xa activity measurement. Heparin concentration can be measured using the Hepcon HMS system (Medtronic Hemotec), which uses an automated protamine titration technique. With a cartridge with four or six chambers containing tissue thromboplastin

Figure 12-4   The HMS heparin management system has an automated dispenser that places the appropriate volume of whole blood into each chamber of the test cartridge. A variety of assays can be performed in this instrument, depending on the cartridge used. (Courtesy of Medtronic Inc., Parker, CO, http://www.medtronic.com/cardsurgery/bloodmgmt/hmsplus.html)

and a series of known protamine concentrations, 0.2 mL of whole blood is automatically dispensed into the chambers. The first channel to clot is the channel whose protamine concentration most accurately neutralizes the heparin without a heparin or a protamine excess. Because protamine neutralizes heparin in the ratio of 1 mg of protamine per 100 U of heparin, the concentration of heparin in the blood sample can be calculated. A cartridge that monitors heparin concentration over a wide range can be used first, followed by another cartridge that can measure heparin concentrations within a narrower range. The maintenance of a stable heparin concentration rather than a specific ACT level usually results in higher doses of heparin being administered because the hemodilution and hypothermia on CPB increase the sensitivity of the ACT to heparin. The measure of heparin concentration has been shown to more closely correlate with anti–factor Xa activity measurements than the ACT during CPB, although the precision and bias of the test may not prove to be acceptable for exclusive use clinically.

In a prospective randomized trial, Despotis and colleagues demonstrated that by using a transfusion algorithm in association with Hepcon-based heparin management, chest tube drainage was minimally reduced and transfusion of non–red blood cell products could be significantly reduced relative to a group of patients who had ACT-based heparin management.[7] They attributed their results to better preservation of the coagulation system by high heparin doses because the doses of heparin administered in the Hepcon group were nearly twice the doses used in the ACT management group. The Hepcon, remains one of the more sensitive tests for detecting residual heparinization after protamine reversal because the heparin concentration can be measured by protamine titration to levels as low as 0.4 IU/mL.

High-Dose Thrombin Time

A functional test of heparin-induced anticoagulation that correlates well with heparin levels is the high-dose thrombin time (HiTT; International Technidyne Inc., Edison, NJ). The TT is a clotting time that measures the conversion of fibrinogen to fibrin by thrombin. The TT is prolonged by the presence of heparin and by hypofibrinogenemias or dysfibrinogenemias. Because the TT is sensitive to very low levels of heparin, a high dose of thrombin is necessary in the TT to accurately assay the high doses of heparin used for CPB. The HiTT is performed by adding whole blood to a prewarmed, prehydrated test tube that contains a lyophilized thrombin preparation. After the addition of 1.5 mL of blood, the tube is inserted into a Hemochron well and the time to clot formation is measured. In vitro assays indicate that HiTT is equivalent to the ACT in evaluation of the anticoagulant effects of heparin at heparin concentrations in the range of 0 to 4.8 IU/mL. Unlike ACT, HiTT is not altered by hemodilution and hypothermia and has been shown to correlate better with heparin concentration than the ACT during CPB. While on CPB, heparin concentration and HiTT decrease while the Hemochron and the Hepcon ACT increase. Another potential advantage of HiTT monitoring occurs for patients receiving aprotinin therapy. In the presence of heparin, aprotinin augments the celite ACT, possibly because its kallikrein-inhibiting capacity prolongs activation of the intrinsic coagulation pathway by XIIa. This should not be interpreted to represent enhanced anticoagulation. The kaolin ACT is less affected by aprotinin therapy than the celite ACT, perhaps because kaolin, unlike celite, *activates* the intrinsic pathway by stimulation of factor XI directly. Others have suggested that kaolin binds to aprotinin and reduces the anticoagulant effect of aprotinin in vitro. However, the heparinized kaolin ACT is still somewhat prolonged in the presence of aprotinin. HiTT is not affected by aprotinin therapy and can be used as a measure of heparinization for CPB

patients receiving aprotinin therapy. The high-dose thromboplastin time is another measure of anticoagulation that is not affected by aprotinin therapy. The high-dose thromboplastin time is a whole blood clotting time in which celite is replaced by 0.3 mL of rabbit brain thromboplastin to which 1.2 mL of blood is added. This test measures the time to coagulation via activation of the extrinsic pathway. This pathway of coagulation is also stimulated during pericardiotomy due to the rich thromboplastin environment of the pericardial cavity.

HEPARIN NEUTRALIZATION

Protamine Effects on Coagulation Monitoring

Reversal of heparin-induced anticoagulation is most frequently performed with protamine. Biologically, protamine binds to positively charged groups such as phosphate groups and may have important properties in angiogenesis and immune function. Different successful dosing plans have been proposed. The recommended dose of protamine for heparin reversal is 1 to 1.3 mg protamine per 100 U heparin; however, this dose often results in a protamine excess.

In addition to hemodynamic sequelae, protamine has adverse effects on coagulation.[8] Large doses prolong the WBCT and the ACT, possibly via thrombin inhibition. In animals and in humans, protamine has been associated with thrombocytopenia, likely due to activation of the complement cascade. The anticoagulant effect of protamine may also be due to inhibition of platelet aggregation, alteration in the platelet surface membrane, or depression of the platelet response to various agonists. These alterations in platelet function result from the presence of the heparin-protamine complex, not protamine alone. Protamine-heparin complexes activate ATIII in vitro and result in complement activation. The anticoagulant effects of free protamine occur when protamine is given in doses in excess of those used clinically; however, the risk of free protamine being the cause of a hemostatic defect is small, given the rapid clearance of protamine relative to heparin.

Monitoring for Heparin Rebound

The phenomenon referred to as heparin rebound describes the reestablishment of a heparinized state after heparin has been neutralized with protamine. Various explanations for heparin rebound have been proposed. The most commonly postulated is that rapid distribution and clearance of protamine occur shortly after protamine administration, leaving unbound heparin remaining after protamine clearance. Furthermore, endogenous heparin antagonists have an even shorter life span than protamine and are eliminated rapidly, resulting in free heparin concentrations. Also possible is the release of heparin from tissues considered heparin storage sites (endothelium, connective tissues). Endothelial cells bind and depolymerize heparin via PF4. Uptake into the cells of the reticuloendothelial system, vascular smooth muscle, and extracellular fluid may account for the storage of heparin that contributes to reactivation of heparin anticoagulation, referred to as *heparin rebound*.

Residual low levels of heparin can be detected by sensitive heparin concentration monitoring in the first hour after protamine reversal and can be present for up to 6 hours postoperatively. Increased bleeding as a result of heparin rebound may

occur, specifically when higher doses of heparin have been administered. Monitoring for heparin rebound can be accomplished using tests that are sensitive to low levels of circulating heparin. These tests are also useful monitors for confirmation of heparin neutralization at the conclusion of CPB.

HEPARIN NEUTRALIZATION MONITORS

To administer the appropriate dose of protamine at the conclusion of CPB, it would be ideal to measure the concentration of heparin present and give the dose of protamine necessary to neutralize only the circulating heparin. As a result of heparin metabolism and elimination, which vary considerably among individuals, the dose of protamine required to reverse a given dose of heparin decreases over time. Furthermore, protamine antagonizes the anti-IIa effects of heparin more effectively than the anti-Xa effects and thus varies in its potency depending on the source of heparin and its anti-IIa properties. Administration of a large fixed dose of protamine or a dose based on the total heparin dose given is no longer the standard of care and may result in an increased incidence of protamine-related adverse effects. An optimal dose of protamine is desired because unneutralized heparin results in clinical bleeding and an excess of protamine may produce an undesired coagulopathy. The use of individualized protamine dose-response curves uniformly results in a reduced protamine dose and has been shown to reduce postoperative bleeding.[9] One such dose-response test, the Hemochron PRT test, is an ACT performed on a heparinized blood sample that contains a known quantity of protamine. With knowledge of the ACT, PRT, and the estimated blood volume of the patient, the protamine dose needed to neutralize the existing heparin level can be extrapolated. The Hepcon instrument also has a protamine dose-response test, which is the protamine titration assay. The chamber that clots first contains the dose of protamine that most closely approximates the circulating dose of heparin. By measuring the circulating heparin level, the protamine dose required for its neutralization is calculated based on a specified heparin/protamine dose ratio.

At the levels of heparinization needed for cardiac surgery, tests that are sensitive to heparin become not clottable. ACT is relatively insensitive to heparin and is ideal for monitoring anticoagulation at high heparin levels but is too insensitive to accurately diagnose incomplete heparin neutralization. ACT had a high predictive value for adequate anticoagulation (confirmed by laboratory activated partial thromboplastin time [aPTT]) when greater than 225 seconds but was poorly predictive for inadequate anticoagulation when less than 225 seconds. The low levels of heparin present when heparin is incompletely neutralized are best measured by other more sensitive tests of heparin-induced anticoagulation, such as heparin concentration, aPTT, and TT. Thus, after CPB, confirmation of return to the unanticoagulated state should be performed with a sensitive test for heparin anticoagulation[10] (Box 12-1).

Thrombin Time

Thrombin time is the time it takes for the conversion of fibrinogen to fibrin clot when blood or plasma is exposed to thrombin. Fibrin strands form in seconds. Detection of fibrin formation using standard laboratory equipment involves incubation of the blood or plasma sample within the chamber in which an optical or electrical probe sits. A detector senses either movement of the probe or the creation of an electrical field (electrical detection) due to fibrin formation and hence signals the end of the test. Hemochron manufactures a point-of-care TT test that uses a lyophilized

> **BOX 12-1** *Heparin Neutralization*
>
> - The most benign form of bleeding after cardiac surgery is due to residual heparinization.
> - Treatment is with either protamine or another heparin-neutralizing product.
> - Transfusion of allogeneic blood products is rarely indicated.
> - Residual heparin can be measured by using:
>   - A protamine titration assay
>   - A heparin neutralized thrombin time assay
>   - A heparinase ACT compared with ACT or
>   - Any other heparinase test that compares itself with the test without heparinase added.

preparation of thrombin in a Hemochron test tube to which 1 mL of blood is added. Identification of fibrin formation in a Hemochron machine uses the standard Hemochron technology described previously with the ACT. The manufacturer suggests that the normal TT is 39 to 53 seconds for whole blood and 43 to 68 seconds for citrated blood. Because the TT specifically measures the activity of thrombin, it is very sensitive to heparin-induced enhancement of ATIII activity. It is a useful test in the post-CPB period for differentiating the cause of bleeding when both prothrombin time (PT) and aPTT are prolonged, because it excludes the intrinsic and extrinsic coagulation pathway limbs and evaluates the conversion of factor I to Ia. The TT is elevated in the presence of heparin, hypofibrinogenemia, dysfibrinogenemia, amyloidosis, or antibodies to thrombin. The TT is also elevated in the presence of fibrin degradation products if the systemic fibrinogen concentration is low.

The TT is an appropriate laboratory test for monitoring the degree of fibrinolytic activity in patients receiving thrombolytic therapy. Measurements of the quantity of fibrinogen, plasminogen, or plasma proteins generated during fibrinolysis are difficult to interpret and yield no prognostic information for dose adjustments. Thrombolytic agents activate the fibrinolytic system to generate plasmin, which then causes clot dissolution and decreases the quantities of fibrinogen and fibrin. This effect can be monitored using the TT. The TT should be measured at baseline (before institution of fibrinolytic therapy) and 3 to 4 hours after therapy is initiated. If it is prolonged by 1.5 to 5 times the baseline value, therapy should be considered effective. If the TT is prolonged by greater than 7 times the baseline value, an increased risk of bleeding is incurred; if the TT is not prolonged at all, therapy has failed to activate fibrinolysis.

Bedside Tests of Heparin Neutralization

Hepcon measures heparin concentration via a protamine titration assay. Cartridges with varying ranges of protamine concentration are available for use. The cartridge with the lower concentration of protamine in the titration is useful for the detection of residual circulating heparin and is sensitive to levels of heparin as low as 0.2 U/mL. Whole blood PT and aPTT assays are sensitive to deficiencies in coagulation factors and overly sensitive to low levels of heparin (aPTT); they lack specificity in assessing residual heparinization. The heparin-neutralized thrombin time (HNTT) is a TT assay with a small dose of protamine sufficient to neutralize 1.5 U/mL of heparin. Because the TT is elevated in the presence of heparin, hypofibrinogenemia, or dysfibrinogenemia, to discriminate among these three causes, HNTT and TT should be performed together. A normal HNTT in the presence of an elevated TT virtually

confirms residual heparin effect and would indicate the need for protamine administration. If HNTT is prolonged as well as the TT, the cause of bleeding may be attributed either to a fibrinogen problem or to a concentration of heparin higher than that which could be neutralized by the HNTT. In one study comparing bedside monitors of anticoagulation, the TT-HNTT difference bore a significant correlation with the aPTT elevation. Using the line of best fit, aPTT elevation of 1.5 times the control corresponded to a 31-second difference in the TT and HNTT, indicating a convenient threshold value of TT-HNTT for the administration of protamine.

Heparinase

Heparinase (Neutralase I) is an enzyme that specifically degrades heparin by catalyzing cleavage of the saccharide bonds found in the heparin molecule. As demonstrated by the ACT, heparinase in a dose of 5 µg/kg has been shown to successfully neutralize heparin effects in healthy volunteers and in patients who have undergone CPB. A dose of 7 µg/kg has been demonstrated to be even more efficacious in returning ACT to baseline values. Doses sufficient to neutralize a dose of 300 U/kg had no significant hemodynamic effects in a canine model.[11] Investigators have not found any platelet-depressive effects of heparinase in contrast to the well-documented platelet dysfunction associated with protamine therapy. Return to the unanticoagulated state after the use of heparinase has been confirmed using ACT monitoring or heparin concentration monitoring.

TESTS OF COAGULATION

Standard tests of coagulation, the PT and the aPTT, are performed on plasma to which the anticoagulant citrate has been added. Because these tests are performed on plasma, they require centrifugation of blood and are generally not feasible for use at the bedside. The aPTT tests the integrity of the intrinsic and the final coagulation pathways and is more sensitive to low levels of heparin than the ACT. Factors IX and X are most sensitive to heparin effects, and thus the aPTT will be prolonged even at very low heparin levels. The test uses a phospholipid substance to simulate the interaction of the platelet membrane in activating factor XII. (Thromboplastin is a tissue extract containing tissue factor and phospholipid. The term *partial thromboplastin* refers to the use of the phospholipid portion only.) The aPTT is prolonged in the presence of deficiencies of factors XII, XI, IX, and VIII, HMWK, and kallikrein. The aPTT reaction is considerably slower than the PT; and in order to speed activation of factor XII, an activator such as celite or kaolin is added to the assay. After incubation of citrated plasma with phospholipid and activator, calcium is added and the time to clot formation is measured. Normal aPTT is 28 to 32 seconds, which is often expressed as a ratio with a control plasma sample from the same laboratory. This is important because partial thromboplastin reagents have different sensitivities to heparin and many have nonlinear responses to heparin in various concentration ranges.

PT measures the integrity of the extrinsic and common coagulation pathways. PT will be prolonged in the presence of factor VII deficiency, warfarin sodium (Coumadin) therapy, or vitamin K deficiency. Large doses of heparin also prolong the PT because of inactivation of factor II. The addition of thromboplastin to citrated plasma results in activation of extrinsic coagulation. After a 3-minute incubation and recalcification, the time to clot formation is measured and is recorded as the

PT. Normal PT is 12 to 14 seconds; however, due to differences in the quality and lot of the thromboplastin used, absolute PT values are not standardized and are difficult to compare across different testing centers. The international normalized ratio (INR) has been adopted as the standard for coagulation monitoring. The INR is an internationally standardized laboratory value that is the ratio of the patient's PT to the result that would have been obtained if the International Reference Preparation (IRP) had been used instead of the laboratory reagents. Each laboratory uses reagents with a specific sensitivity (International Sensitivity Index [ISI]) relative to the IRP. The ISI of a particular set of reagents is provided by each manufacturer so that the INR can be reported.

Bedside Tests of Coagulation

PT and aPTT tests performed on whole blood are available for use in the operating room or at the bedside. The Hemochron PT test tube contains acetone-dried rabbit brain thromboplastin to which 2 mL of whole blood is added and the tube is inserted into a standard Hemochron machine. Normal values range from 50 to 72 seconds and are automatically converted by a computer to the plasma equivalent PT and INR. Hemochron aPTT contains kaolin activator and a platelet factor substitute and is performed similarly to the PT. The aPTT is sensitive to heparin concentrations as low as 0.2 U/mL and displays a linear relationship with heparin concentration up to 1.5 U/mL.

The Ciba Corning Biotrack 512 coagulation monitor for evaluating bedside PT and aPTT uses 0.1 mL of whole blood placed into a disposable plastic cartridge for either PT or aPTT. The sample is drawn by capillary action into a heated chamber where exposure to reagents occurs. The PT uses rabbit brain thromboplastin. The aPTT uses soybean phosphatide as the platelet substitute and bovine brain sulfatide as the activator. From the reaction chamber, blood traverses a reaction path where clot formation is detected by a laser optical system. The resulting time to clot formation is converted to a ratio of the control value by a microprocessor that has control values encoded.

Many investigators have studied the Ciba Corning Biotrack system for monitoring anticoagulation in different clinical scenarios. For patients receiving oral anticoagulant therapy, the Biotrack 512 monitor has been found to be suitable for monitoring PT and INR. The bedside Biotrack aPTT with the laboratory aPTT and heparin level in patients receiving therapeutic heparinization after interventional cardiac catheterization have been compared. The authors found a strong correlation ($r = 0.89$) between the Biotrack aPTT and the aPTT from the hospital laboratory. The correlation between Biotrack aPTT and heparin level was not strong, probably because of the many other factors such as heparin neutralization and clearance that affect the heparin concentration in vivo. Another study in patients receiving heparin compared the Ciba Corning Biotrack aPTT assay with standard laboratory aPTT and documented that Biotrack was less sensitive to heparin than the laboratory aPTT; however, the correlation coefficient of these two tests was $r = 0.82$. In patients on warfarin therapy, the Biotrack aPTT was more sensitive than the laboratory aPTT and yielded consistently higher results for the aPTT value. In another study in patients being anticoagulated for nonsurgical applications, the bedside aPTT was similar to the standard aPTT in its prediction of treatment in simple therapeutic algorithms. However, in more complex clinical situations, there was less agreement between the bedside aPTT and laboratory aPTT.[12]

In a comparison of bedside coagulation monitors after cardiac surgical procedures, acceptable accuracy and precision levels for Hemochron and Ciba Corning Biotrack

PT in comparison with standard laboratory plasma PT, were documented, making them potentially valuable for use in the perioperative period. Neither Hemochron nor Ciba Corning aPTT reached this level of clinical competence compared with standard laboratory tests. Others have documented that this monitor seems to be more precise for PT than for aPTT. Because of rapid turn-around times, these point-of-care co-agulation monitors may be useful in predicting patients who will bleed after cardiac surgery and have also been successfully used in transfusion algorithms to decrease the number of allogeneic blood products given to cardiac surgical patients.[13]

Fibrinogen Level

Fibrinogen concentration is traditionally measured using either clottable protein methods, endpoint detection techniques, or immunochemical tests. Of the former, the most commonly used fibrinogen assay relies on the method of Clauss. This method involves a 10-fold dilution of plasma, which ensures that fibrinogen is the rate-limiting step in clot formation. Subsequently, an excess of thrombin is added to the sample and the time to clot formation is measured. The clotting time is inversely related to the fibrinogen concentration. Because this assay relies on detection of actual clot, it can be affected by fibrin degradation products, polymerization inhibitors, or other inhibitors of fibrin formation. Because of the thrombin excess, small clinical concentrations of heparin do not affect fibrinogen determination according to the Clauss technique.

A whole blood point-of-care fibrinogen assay is available using the Hemochron system. The specific test tube contains a lyophilized preparation of human thrombin, snake venom extract, protamine, buffers, and calcium stabilizers. The test tube is incubated with 1.5 mL of distilled water and heated in the Hemochron instrument for 3 minutes. Whole blood is placed into a diluent vial, where it is 50% diluted, and from this vial, 0.5 mL of diluted whole blood is placed into the specific fibrinogen test tube. The clotting time is measured using standard Hemochron technology as described previously. The fibrinogen concentration is determined by comparison with a standard curve for this test. Normal fibrinogen concentration of 180 to 220 mg/dL correlates with a clotting time of 54 ± 2.5 seconds. Fibrinogen deficiency of 50 to 75 mg/dL correlates with a clotting time of 150 ± 9.0 seconds.

Unlike the method of Clauss, the endpoint detection assays rely on the detection of changes in turbidity of plasma when clot is formed. This technique does not require the maintenance of a stable cross-linked fibrin product and therefore does not report underestimated fibrinogen measurements due to the presence of inhibitors. Immunochemical measures of fibrinogen concentration are a direct and accurate measurement technique; however, they are expensive and time consuming and require specialized laboratory facilities.

MONITORING FIBRINOLYSIS

Fibrinolysis, the dissolution of fibrin, is the normal modifier of hemostasis that ensures that coagulation does not proceed unchecked. It occurs in the vicinity of a clot and dissolves clot when local endothelial healing occurs. Fibrinolysis is mediated by the serine protease plasmin, which is the product of the cleavage of plasminogen by tissue plasminogen activator. Fibrinolysis is a normal phenomenon in response to clot formation; when it occurs systemically, it represents a pathologic condition.

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Fibrinolysis can be primary or secondary. Primary fibrinolysis occurs when fibrinolytic activators are released or produced in excess and do not represent a response to the coagulation process. Examples of primary fibrinolysis include the release of plasminogen activators during liver transplantation surgery and the exogenous administration of fibrinolytic agents such as streptokinase. During primary fibrinolysis, plasmin cleaves fibrinogen, yielding fibrinogen degradation products. These end products can be measured using immunologic techniques.

When fibrinolysis is a result of enhanced activation of the coagulation system, secondary fibrinolysis ensues. A well-known extreme form of secondary fibrinolysis is seen during disseminated intravascular coagulation, when both systemic coagulation and fibrinolysis are occurring in excess. During CPB, fibrinolysis is most likely secondary to the microvascular coagulation that is occurring despite attempts at suppression using high doses of heparin.

The identification of fibrinolysis can be accomplished through either direct measurement of the clot lysis time (manual or viscoelastic tests) or measurement of the end products of fibrin degradation. The manual clot lysis time simply involves the placement of whole blood into a test tube. This blood clots in a matter of minutes. Visual inspection determines the endpoint for observation of clot lysis, and this time period is the clot lysis time. This technique is considerably time consuming and requires constant observation by the person performing the test.

Viscoelastic Tests

Viscoelastic tests measure the unique properties of the clot as it is forming, organizing, strengthening, and lysing. As a result, fibrinolysis determination by this methodology requires that time elapse during which clot formation is occurring. It is subsequent to clot formation and platelet-fibrin linkages that clot lysis parameters can be measured. For this reason, viscoelastic tests often require longer than 1 hour to detect the initiation of fibrinolysis; however, if fibrinolysis is enhanced, results can often be obtained in 30 minutes.

End Products of Fibrin Degradation

Other methods for quantifying fibrinolysis include measurement of the end products of fibrin degradation. Fibrin degradation products are the result of the cleavage of fibrin monomers and polymers and can be measured using a latex agglutination assay. When plasmin cleaves cross-linked fibrin, dimeric units are formed that comprise one D-domain from each of two adjacent fibrin units. These "D-dimers" are frequently measured by researchers in clinical and laboratory investigations. They are measured by either enzyme-linked immunosorbent assays or latex agglutination techniques and thus are not available for on-site use. Controversy still exists regarding whether D-dimer level or fibrin degradation products are the most sensitive test for detecting fibrinolysis, but most agree that the presence of D-dimers is the most specific for cross-linked fibrin degradation.[14]

Monitoring the Thrombin Inhibitors

A new class of drugs, the selective thrombin inhibitors, is a viable alternative to heparin anticoagulation for CPB. These agents include hirudin, argatroban, and other experimental agents. A major advantage of these agents over heparin is that they are able to effectively inhibit clot-bound thrombin in an ATIII-independent fashion. The platelet thrombin receptor is believed to be the focus of thrombin's procoagulant effects in states of thrombosis such as after coronary artery angioplasty. Because

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surface-bound thrombin is more effectively suppressed, thrombin generation can be reduced at lower levels of systemic anticoagulation than are achieved during anti-coagulation by the heparin-ATIII complex. This translates into less bleeding despite the lack of a clinically useful antidote for the thrombin antagonists.[15] Thrombin antagonists are also not susceptible to neutralization by PF4 and thus are not neu-tralized at endothelial sites where activated platelets reside. They are also useful in patients with HIT in whom the administration of heparin and subsequent antibody-induced platelet aggregation would be dangerous.

Hirudin

Hirudin, a coagulation inhibitor isolated from the salivary glands of the medicinal leech (*Hirudo medicinalis*), is a potent inhibitor of thrombin that, unlike heparin, acts independently of ATIII and inhibits clot-bound thrombin as well as fluid-phase thrombin. Hirudin does not require a cofactor and is not susceptible to neutraliza-tion by PF4. This would seem to be beneficial in patients in whom platelet activa-tion and thrombosis are potential problems. Recombinant hirudin (r-hirudin) was administered as a 0.25-mg/kg bolus and an infusion to maintain the hirudin concen-tration at 2.5 μg/mL as determined by the ecarin clotting time in studies by Koster and coworkers.[16] The ecarin clotting time, modified for use in the TAS analyzer, has been used in large series of patients with HIT. Compared with standard treatment with heparin or LMWHs, r-hirudin–treated patients maintained platelet counts and hemoglobin levels and had few bleeding complications if renal function was normal. Hirudin is a small molecule (molecular weight 7 kDa) that is eliminated by the kid-ney and is easily hemofiltered at the end of CPB.

Bivalirudin

Bivalirudin is a small 20–amino acid molecule with a plasma half-life of 24 minutes. It is a synthetic derivative of hirudin and thus acts as a direct thrombin inhibitor. Bivalirudin binds to both the catalytic binding site and the anion-binding exosite on fluid phase and clot-bound thrombin. The part of the molecule that binds to throm-bin is actually cleaved by thrombin itself, so the elimination of bivalirudin activity is independent of specific organ metabolism. Bivalirudin has been used successfully as an anticoagulant in interventional cardiology procedures as a replacement for heparin therapy. In fact, in interventional cardiology, bivalirudin has been associated with less bleeding and equivalent ischemic outcomes compared with heparin plus a platelet inhibitor. This may be the result of bivalirudin being both an antithrombin anticoagulant and an antithrombin at the level of the platelet. Merry and colleagues showed equivalence with regard to bleeding outcomes and an improvement in graft flow after off-pump cardiac surgery when bivalirudin was used (0.75-mg/kg bolus, 1.75-mg/kg/hr infusion).[17] Case reports confirm the safety of bivalirudin use dur-ing CPB, although current trials are under way. Monitoring of anticoagulant activity is performed using the ecarin clotting time with similar prolongation to that seen with hirudin anticoagulation.[18] The ecarin clotting time has a closer correlation with anti-IIa activity and plasma drug levels than does the ACT. For this reason, standard ACT monitoring during antithrombin therapy is not preferred if ecarin clotting time can be measured.

The anticoagulant effects of the thrombin antagonists can be monitored using the ACT, aPTT, or the TT. The bleeding time may also be prolonged.

Bivalirudin has been favorably compared with heparin in patients undergo-ing coronary angioplasty for unstable angina. The half-life of aPTT prolongation is

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> **BOX 12-2** *Thrombin Inhibitors*
>
> - These anticoagulant drugs are superior to heparin.
> - They inhibit both clot-bound and soluble thrombin.
> - They do not require a cofactor, activate platelets, or cause immunogenicity.
> - Heparin remains an attractive drug owing to its long history of safe use and the fact that it has a specific drug antidote, protamine.
> - These drugs include hirudin, argatroban, and bivalirudin.

approximately 40 minutes, and reductions in formation of fibrinopeptide A are evidence of thrombin inhibition and fibrinogen preservation. Careful monitoring should be used because there may be a rebound prothrombotic state after cessation of therapy, which could lead to recurrence of anginal symptoms (Box 12-2).

MONITORING PLATELET FUNCTION

Circulating platelets adhere to the endothelium via platelet surface receptors that bind exposed collagen and become activated. This initiates platelet activation, because collagen is a potent platelet activator. The unstimulated platelet, which is discoid in shape, undergoes a conformational change when activated. The activated platelet is spherical, extrudes pseudopodia, and expresses an increased number of activated surface receptors that can be measured to quantify the degree of platelet reactivity. The intensity of this platelet activation occurs in proportion to the quantity and nature of the platelet stimulus and increases in a graded fashion with increasing concentrations of agonists. The glycoprotein (GP) IIb/IIIa receptor is the primary receptor responsible for fibrinogen binding and the formation of the platelet plug.

Platelet Count

Numerous events occur during cardiac surgical procedures that predispose patients to platelet-related hemostasis defects. The two major categories are thrombocytopenia and qualitative platelet defects. Thrombocytopenia commonly occurs during cardiac surgery as a result of hemodilution, sequestration, and destruction by nonendothelial surfaces. Platelet counts commonly fall to 100,000/μL or slightly below; however, the final platelet count is greatly dependent on the starting value and the duration of platelet destructive interventions (i.e., CPB). Between 10,000/μL and 100,000/μL, bleeding time decreases directly; however, at platelet counts greater than 50,000/μL, neither the bleeding time nor platelet count has any correlation with postoperative bleeding in cardiac surgical patients. On the other hand, platelet size or mean platelet volume does have some correlation with hemostatic function. Larger, younger platelets are more hemostatically active than smaller ones. Mean platelet volume multiplied by the platelet count gives an estimation of overall platelet mass and is referred to as the *plateletcrit*. It is important to appreciate the inverse relationship between platelet volume and platelet count when using a measure such as the plateletcrit to assess the viability of the existing platelet population. Because the mean platelet volume is dependent on the method of specimen collection, the anticoagulant used, and temperature of the storage conditions, its reproducibility is dependent on standardized laboratory procedures.

Qualitative platelet defects occur more commonly than thrombocytopenia during CPB procedures. The range of possible causes of platelet dysfunction includes

traumatic extracorporeal techniques, pharmacologic therapy, hypothermia, and fibrinolysis; the hemostatic insult increases with the duration of time spent on CPB. The use of bubble oxygenators, noncoated extracorporeal circulation, and cardiotomy suctioning may cause platelets to become activated, initiate the release reaction, and partly deplete platelets of the contents of their alpha granules. Many of these changes are only transiently associated with CPB. The hematologic changes associated with CPB have been characterized. While the platelet count falls and reaches a plateau at 2 hours after CPB, mean platelet volume reaches its nadir at 2 hours after CPB and then begins to rise during the ensuing 72 hours. The relative thrombocytopenia seen up to 72 hours after cardiac surgery is not consistently associated with a bleeding diathesis. Similarly, the clotting proteins fibrinogen, factor VIII/von Willebrand factor (vWF), and factor VIII-C also rise to levels above baseline in the 2 to 72 hours after CPB.

Large doses of heparin have been shown to reduce the ability of the platelets to aggregate and to reduce clot strength. This effect is not reversed when protamine is administered; however, it may be mitigated by the prophylactic administration of aprotinin. The adverse effects of heparin on platelet function may be due to its ability to inhibit the formation of thrombin, the most potent invivo platelet activator. However, heparin also activates the fibrinolytic system, a system that, through plasmin and other activators, has the ability to depress platelet function through other mechanisms. Additionally, various degrees of fibrinolysis occur after CPB. Circulating plasmin causes dissolution of the GPIb platelet receptor and decreases the adhesiveness of platelets. Because fibrinolysis is partly responsible for the platelet dysfunction seen after heparin administration and CPB, the efficacy of antifibrinolytic agents as hemostatic drugs can be better appreciated. In addition to reducing platelet adhesiveness to vWF, the fibrin degradation products formed depress platelet responsiveness to agonists.

Protamine-heparin complexes and protamine alone also contribute to platelet depression after CPB. Mild to moderate degrees of hypothermia are associated with reversible degrees of platelet activation and platelet dysfunction, which may be partly mitigated by the use of aprotinin therapy. Overall, the potential coagulation benefits of normothermic CPB compared with hypothermic CPB require further study in well-conducted randomized trials (Box 12-3).

Bleeding Time

The bleeding time is performed by creating a skin incision and measuring the time to clot formation via the platelet plug. The Ivy bleeding time is performed on the volar surface of the forearm above which a cuff is inflated to 40 mm Hg (above venous pressure). Using a template, two parallel incisions are made and the incisions blotted with filter paper every 30 seconds until no further bleeding occurs. The time from incision to cessation of blood seepage is the template bleeding time. The Duke bleeding time is performed on the earlobe and has advantages for cardiac surgery because the earlobe is more accessible and less likely to be subjected to the peripheral vasoconstriction seen after hypothermia. However, because neither the width/depth of the incision nor the venous pressure can be controlled in the Duke bleeding time, the Ivy bleeding time is considered the superior test. Normal bleeding time is 4 to 10 minutes.

Numerous prospective blinded investigations have confirmed that bleeding time has little or no value in predicting excessive hemorrhage after cardiac surgery. Even in patients receiving therapeutic doses of aspirin, an increase in bleeding time does not necessarily translate into an increase in mediastinal tube drainage or transfusions

> ## BOX 12-3 *Platelet Function*
>
> - The measure of platelet count does not correlate with bleeding after cardiac surgery.
> - Patients frequently have extreme degrees of thrombocytopenia but do not bleed because they have adequate platelet function.
> - It is the measure of platelet function that correlates temporally with the bleeding course seen after cardiac surgery.
> - The thromboelastogram maximal amplitude, mean platelet volume, and other functional platelet tests are very useful in transfusion algorithms.

if reinfusion and blood conservation techniques are used aggressively. There is substantial evidence that platelet-directed therapy in the form of platelet transfusions or desmopressin acetate shortens a prolonged bleeding time in patients with clinical hemorrhage. Because the bleeding time does not follow the temporal course of postoperative coagulopathy, the bleeding time may be a nonspecific and impractical test for detecting an existing platelet defect but may be suitable for following patient response to platelet-directed therapies.

Aggregometry

Activated platelets undergo aggregation, which is initially a reversible process. Activation also induces the release of substances from alpha and dense platelet granules and platelet lysosomes. Because platelet granules contain many platelet agonists, the release of granular contents further stimulates platelet activation and is responsible for the secondary phase of platelet aggregation. This secondary phase of platelet aggregation is dependent on the release of thromboxane and other substances from the platelet granules, is an energy-consuming process, and is irreversible.

Aggregometry is a useful research tool for measuring platelet responsiveness to a variety of different agonists. The end result, platelet aggregation, is an objective measure of platelet activation. Platelet aggregometry uses a photo-optical instrument to measure light transmittance through a sample of whole blood or platelet-rich plasma. Platelet-rich plasma undergoes a decrease in light transmittance on the early phase of platelet activation due to the change in platelet shape from discoid to spherical. When exposed to a platelet agonist such as thrombin, adenosine diphosphate (ADP), epinephrine, collagen, or ristocetin, the initial reversible aggregation phase results in increased light transmittance due to the platelet aggregates that decrease the turbidity of the sample. The larger the platelet aggregates, the greater is the transmittance of light. In the absence of further activation, disaggregation occurs and the plasma sample becomes turbid. However, when the platelet release reaction occurs, thromboxane and other activators are released from the platelet alpha granules and the phase of secondary, or irreversible, aggregation occurs. This results in a further increase in light transmittance.

Platelet-Mediated Force Transduction

An instrument that measures the force developed by platelets during clot retraction has been shown to be directly related to platelet concentration and function[19] (Hemodyne). The apparatus consists of a cup and a parallel upper plate. The cup is filled with blood or the platelet-containing solution, and the upper plate is lowered onto the clotting solution. Clot forms and adheres to the outer edges of the cup and

to the plate above. A thin layer of oil is deposited onto the surfaces that are exposed to air. The upper plate is coupled to a displacement transducer that translates displacement due to platelet retraction into a force. Normal values for platelet force development have been suggested by the investigators. The antiplatelet effects of heparin have been evaluated using this force retractometer. Using this instrument, investigators have shown that high heparin concentrations completely abolish platelet force generation. Furthermore, the concentration of protamine required to reverse the anticoagulant effects of heparin is not sufficient to reverse these antiplatelet effects. The antiplatelet effects of protamine alone have also been evaluated using this monitor.

BEDSIDE PLATELET FUNCTION TESTING

Thromboelastography

The coaguloviscometers that were developed in the 1920s formed the basis of viscoelastic coagulation testing that is now known as thromboelastography. Thromboelastography in its current form was developed by Hartert in 1948 and has been used in many different clinical scenarios to diagnose coagulation abnormalities.[20] Although not yet truly portable, the thromboelastograph (TEG; Haemoscope, Skokie, IL) can be performed "on site" either in the operating room or in a laboratory and provides a rapid whole blood analysis that yields information about clot formation and clot dissolution. Within minutes, information is obtained regarding the integrity of the coagulation cascade, platelet function, platelet-fibrin interactions, and fibrinolysis. The principle is as follows: whole blood (0.36 mL) is placed into a plastic cuvette into which a plastic pin is suspended; this plastic pin is attached to a torsion wire that is coupled to an amplifier and recorded; a thin layer of oil is added to the surface of the blood to prevent drying of the specimen; and the cuvette oscillates through an arc of 4 degrees, 45 minutes at 37°C. When the blood is liquid, movement of the cuvette does not affect the pin. However, as clot begins to form, the pin becomes coupled to the motion of the cuvette and the torsion wire generates a signal that is recorded. The recorded tracing can be stored by computer, and the parameters of interest are calculated using a simple software package. Alternatively, the tracing can be generated on line with a recording speed of 2 mm/min. The tracing generated has a characteristic conformation that is the signature of the TEG (Fig. 12-5).

The specific parameters measured by the TEG include the reaction time (R value), coagulation time (K value), a angle, maximal amplitude (MA), amplitude 60 minutes after the MA (A60), and clot lysis indices at 30 and 60 minutes after MA (LY30 and LY60, respectively). The reaction time, R, represents the time for initial fibrin formation and is a measure of the intrinsic coagulation pathway, the extrinsic coagulation pathway, and the final common pathway. R is measured from the start of the bioassay until fibrin begins to form and the amplitude of the tracing is 2 mm. Normal values vary depending on the types of activator used and range from 7 to 14 minutes using celite activator and are as short as 1 to 3 minutes using tissue factor activator. The K value is a measure of the speed of clot formation and is measured from the end of the R time to the time that the amplitude reaches 20 mm. Normal values (3 to 6 minutes) also vary with the type of activators used. The a angle, another index of speed of clot formation, is the angle formed between the horizontal axis of the tracing and the tangent to the tracing at 20-mm amplitude. Alpha values normally range from 45 to 55 degrees. Because both the K value and the a angle are measures of the speed of clot strengthening, each is improved by high levels of functional fibrinogen. MA (normal is 50 to 60 mm) is an index of clot strength as determined
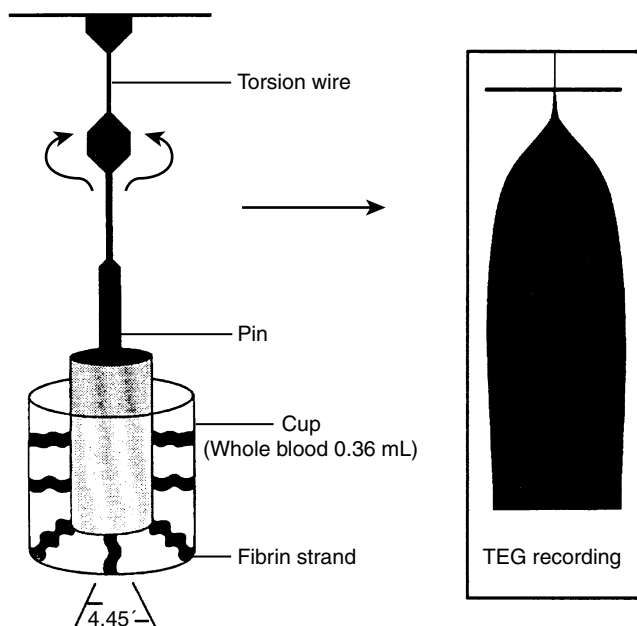
Figure 12-5 Schematic diagram of the thromboelastograph instrumentation (*left*) and a sample tracing (*right*). A whole blood sample is placed into the cup into which a plastic pin is suspended. This plastic pin is attached to a torsion wire that is coupled to an amplifier and recorder. See text for details. (From Mallett SV, Cox DJA: Thromboelastography. Br J Anaesth 69:307-313, 1992.)

by platelet function, the cross-linkage of fibrin, and the interactions of platelets with polymerizing fibrin. The peak strength of the clot, or the shear elastic modulus "G," has a curvilinear relation with MA and is defined as $G = (5000 \cdot MA)/(96 - MA)$. The percent reduction in MA after 30 minutes reflects the fibrinolytic activity present and is normally not more than 7.5%.

Characteristic TEG tracings can be recognized to be indicative of particular coagulation defects. A prolonged R value indicates a deficiency in coagulation factor activity or level and is seen typically in patients with liver disease and in patients on anticoagulants such as warfarin or heparin. MA and a angle are reduced in states associated with platelet dysfunction or thrombocytopenia and are even further reduced in the presence of a fibrinogen defect. LY 30, or the lysis index at 30 minutes after MA, is increased in conjunction with fibrinolysis. These particular signature tracings are depicted in Figure 12-6.

TEG is a useful tool for diagnosing and treating perioperative coagulopathy in patients undergoing cardiac surgical procedures due to a variety of potential coagulation defects that may exist. Within 15 to 30 minutes, on-site information is available regarding the integrity of the coagulation system, the platelet function, fibrinogen function, and fibrinolysis. With the addition of heparinase, TEG can be performed during CPB and can provide valuable and timely information regarding coagulation status.[21] Because TEG is a viscoelastic test and evaluates whole blood hemostasis interactions, it is suggested that TEG is a more accurate predictor of postoperative hemorrhage than routine coagulation tests that analyze individual components of the hemostasis system. A number of clinical trials have confirmed that in cardiac surgical patients TEG has a greater predictive value and greater specificity than routine coagulation tests for diagnosing patients known as "bleeders." Tuman
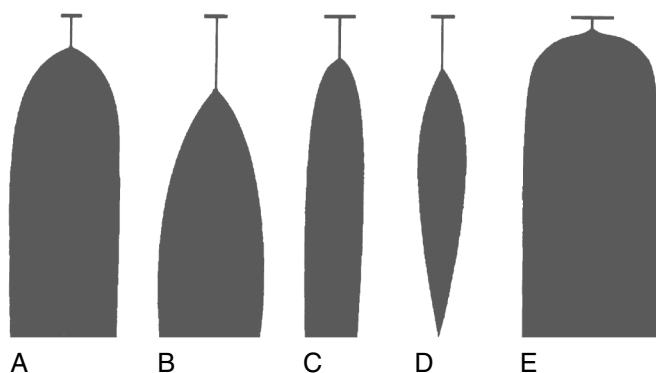
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Figure 12-6   Signature thromboelastograph tracings. Tracing identification from left to right. A = normal; B = coagulation factor deficiency; C = platelet dysfunction or deficiency; D = fibrinolysis; E = hypercoagulability. (From Mallett SV, Cox DJA: Thromboelastography. Br J Anaesth 69:307-313, 1992.)

and associates studied 42 patients, of whom 9 were classified as bleeders.[21] A routine coagulation screen consisting of ACT, PT, aPTT, and platelet count had only a 33% accuracy for predicting bleeding, whereas TEG and Sonoclot (Sienco Inc., Morrison, CO) (another viscoelastic test) had 88% and 74% accuracy, respectively. Other investigators have also found that TEG abnormalities predict postoperative bleeding and, using TEG parameters, they were also able to identify a population of patients who respond to therapy with desmopressin acetate.

In a large retrospective evaluation in more than 1000 patients, Spiess and associates found that the institution of a transfusion algorithm using TEG resulted in a significant reduction in the incidence of mediastinal exploration and in the rate of transfusion of allogeneic blood products.[22] Because of its ease of use and application at the bedside, TEG has been used in many research settings to assess drug effects on platelet function and clot strength.

Thromboelastography Modifications

Thromboelastography was originally performed using recalcified citrated whole blood or celite activator. The addition of recombinant human tissue factor as an activator can accelerate the rate of thrombin formation and thus the formation of fibrin. This serves to shorten the time required for development of the MA. Because the MA is primarily reflective of clot strength and platelet function, this information can be obtained more quickly with tissue factor enhancement. The recombinant tissue factor is a thromboplastin agent and is available from a number of manufacturers.[23]

An application of TEG in the clinical arena is its use in monitoring GPIIb/IIIa receptor blockade and ADP receptor blockade in patients treated with specific antiplatelet agents. TEG with tissue factor acceleration speeds the appearance of MA and is accurate for monitoring the platelet inhibition by large concentrations of GPIIb/IIIa receptor blockers. Using this technique with platelet-rich plasma, the reduction of the MA has been used as an index of platelet inhibition by GPIIb/IIIa receptor blockers in the catheterization laboratory. Comparison with the baseline MA yields a relative measure of the degree of platelet inhibition.

Because the MA is a function of the platelet-fibrinogen interaction, a reduction in the MA can be accomplished by the addition of potent GPIIb/IIIa receptor blockade to the assay. The resultant MA, in the presence of excessively high GPIIb/IIIa

receptor blockade, is primarily due to the fibrinogen concentration and the strength of fibrin alone. This value (called MA_f) correlates strongly with plasma fibrinogen concentration.

The thienopyridine ADP-receptor blockers clopidogrel and ticlopidine are widely used in cardiovascular medicine. The ability to measure the platelet defect induced by these drugs is very difficult unless sophisticated laboratory techniques such as ADP-aggregometry are used. Aggregometry yields accurate results; however, it is not readily available in the perioperative period as a point-of-care test. Native TEG analysis does not measure the thienopyridine-induced platelet defect because the formation of thrombin in the assay has an overwhelming effect on the development of the TEG MA. A modification of the TEG removes thrombin from the assay and studies a nonthrombin clot, strengthened by the addition of ADP. Figure 12-7 depicts the different signature TEG tracings that are used to calculate the platelet contribution to MA when a platelet inhibitor is present. This assay was specifically created to measure the platelet inhibition by ADP antagonists such as clopidogrel and is referred to as the platelet mapping assay. The MA_{kh} is the maximal activation of platelets and fibrin and is the largest amplitude that can be achieved. The MA_f is the MA that is obtained when a thrombin-depleted fibrin clot is formed without a platelet contribution. The MA_{pi} is the MA_f contribution plus the platelet contribution. MA_{pi} is created by adding an activator such as ADP to the MA_f assay (for clopidogrel testing). Only platelets that can be activated by ADP contribute to the MA_{pi}. The following formula calculates the percent reduction in platelet activity using this assay.

$$100 - \left[\left(MA_{pi} - MA_f \right) / \left(MA_{kh} - MA_f \right) \right] \times 100$$

Clopidogrel, ticlopidine, and even aspirin inhibition can now be studied at the point-of-care using this modification.[24]

Tests of Platelet Response to Agonist

HemoSTATUS

Despite the introduction of numerous point-of-care coagulation analyzers that allow for rapid determination of a patient's coagulation status, the qualitative measure of platelet function, at the bedside, remains an elusive challenge. HemoSTATUS (Medtronic Inc., Parker, CO) is a point-of-care platelet function assay that uses the Hepcon monitoring system to measure platelet reactivity. A six-channel cartridge measures the heparinized kaolin-activated ACT without platelet activator (channels 1 and 2) and with incrementally increasing doses of platelet-activating factor [PAF] (channels 3 to 6). The ACT of the PAF-activated channels will be shortened due to the ability of activated platelets to speed coagulation.

The potential ability to measure the qualitative function of platelets using a point-of-care assay provides innumerable advantages for clinicians caring for cardiac surgical patients. Platelet dysfunction is one of the more common hemostasis defects incurred during CPB, yet it is difficult to specifically measure platelet function rapidly and at the bedside. Viscoelastic tests conveniently measure platelet function, but their use in transfusion algorithms is limited by a lack of specificity to the measure of platelet dysfunction. Transfusion algorithms have been suggested to result in reduced transfusions in cardiac surgical patients and have incorporated only the measure of platelet number, because the on-site ability to measure platelet function has been so elusive. Inclusion of a measure of platelet function into a transfusion algorithm would potentially reduce allogeneic transfusions even further.

An initial investigation of HemoSTATUS in cardiac surgical patients was performed by Despotis and colleagues.[25] The authors studied 150 patients and
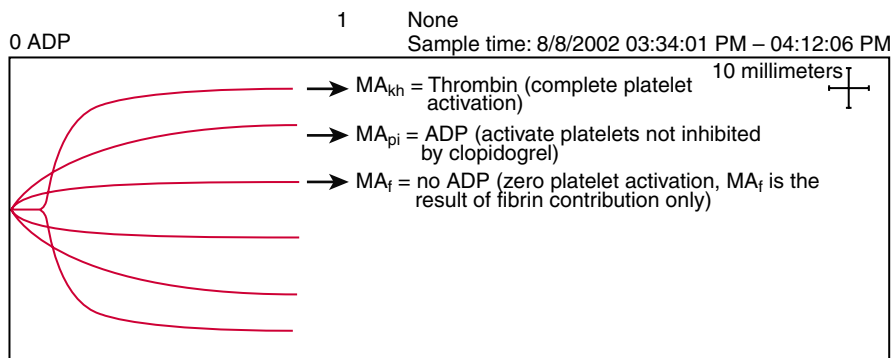
Figure 12-7 Thromboelastograph tracings using the modification to measure platelet inhibition by the thienopyridine drugs clopidogrel or ticlopidine. The measurements made are the maximal amplitude allowing thrombin activation of platelets (MA_{kh}). This is the standard kaolin-activated MA. Also measured is the maximal amplitude measuring only the fibrinogen component of MA (MA_f) using a fibrinogen activator, and the maximal amplitude measuring the platelet contribution to MA by platelets able to be activated by ADP (MA_{pi}). This is using a fibrinogen activator plus ADP. Only platelets that are responsive to ADP will contribute to the MA_{pi}.

conducted multivariate analyses to evaluate the relationship between postoperative blood loss and multiple demographic, operative, and hemostatic measurements. They demonstrated a significant correlation between HemoSTATUS measurements on arrival in the intensive care unit (ICU) and 4-hour postoperative mediastinal tube drainage ($r = -0.85$, channel 5; $r = -0.82$, channel 6). Using receiver operating characteristic (ROC) curves for the detection of excessive mediastinal tube drainage, the accuracy of a number of hemostasis assays was measured. The highest predictability for bleeding was found in both the channel 5 clot ratio and the bleeding time. The PT, aPTT, and platelet count had much lower predictive value. HemoSTATUS-derived clot ratios also had the capability to detect enhanced platelet function after the administration of pharmacologic platelet therapy (desmopressin acetate) and after the transfusion of platelet concentrates. Subsequent investigations in cardiac surgical patients have confirmed a significant yet weak correlation of HemoSTATUS with postoperative bleeding but have not found this test to be superior to TEG or routine coagulation tests in its predictive value.

Other Platelet Function Tests

Ultegra (Accumetrics, San Diego, CA), or "rapid platelet function assay," is a point-of-care monitor designed specifically to measure the platelet response to a thrombin-receptor-agonist peptide (TRAP). This technology was approved by the U.S. Food and Drug administration for use as a platelet function assay.

The Platelet Function Analyzer (PFA-100) (Dade Behring, Miami, FL) is a monitor of platelet adhesive capacity that is valuable in its diagnostic abilities to identify drug-induced platelet abnormalities, platelet dysfunction of von Willebrand's disease, and other acquired and congenital platelet defects.

"Platelet Works" Ichor (Array Medical, Somerville, NJ) is a test that uses the principle of the platelet count ratio to assess platelet reactivity. The instrument is a Coulter counter that measures the platelet count in a standard EDTA-containing tube. Platelet count is also measured in tubes containing the platelet agonist risto-cetin, ADP, epinephrine, collagen, or thrombin. Addition of blood to these agonist tubes causes platelets to activate, adhere to the tube, and be effectively eliminated from the platelet count. The ratio of the activated platelet count to the nonactivated

platelet count is a function of the reactivity of the platelets. Early investigation in cardiac surgical patients indicates that this assay is useful in providing a platelet count and that it is capable of measuring the platelet dysfunction that accompanies CPB.

SUMMARY

- Monitoring the effect of heparin is done using an activated coagulation time (ACT), a functional test of heparin anticoagulation. The ACT is susceptible to elevation due to hypothermia, hemodilution, and the use of aprotinin (celite ACT).
- Heparin resistance can be congenital or acquired. Pretreatment heparin exposure predisposes a patient to altered heparin responsiveness due to either antithrombin III (ATIII) depletion, platelet activation, or activation of extrinsic coagulation.
- Heparin-induced thrombocytopenia type I is benign and is a normal aggregation response of platelets to heparin. Heparin-induced thrombocytopenia type II is an abnormal immunologic response to the heparin/platelet factor 4 complex and is sometimes associated with overt thrombosis.
- Protamine neutralization of heparin can be associated with "protamine reactions," which include vasodilatory hypotension, anaphylactoid reactions, and pulmonary hypertensive crises (types 1, 2, and 3).
- Before considering a transfusion of plasma, it is important to document that the effect of heparin has been neutralized. This can be done using a heparinase-neutralized test or a protamine-neutralized test.
- Point-of-care tests are available for use in transfusion algorithms that can measure coagulation factor activity (international normalized ratio, activated partial thromboplastin time) and platelet function.
- Fibrinolysis is common after cardiopulmonary bypass when antifibrinolytic therapy is not used.
- New thrombin inhibitor drugs are available for anticoagulation in patients who cannot receive heparin. These can be monitored using the ecarin clotting time or a modified ACT. Bivalirudin and hirudin are the two new drugs that have been used most often in cardiac surgery.
- Platelet dysfunction is the most common reason for bleeding after cardiopulmonary bypass. There are point-of-care tests that can be used to measure specific aspects of platelet function.
- The degree of platelet inhibition as measured by standard or point-of-care instruments has been shown to correlate with decreased ischemic outcomes after coronary intervention. However, cardiac surgical patients who are receiving antiplatelet medication are at increased risk for postoperative bleeding.

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