Clot formation and dissolution in vivo can be considered as a highly integrated cellular/humoral process that acts through 3 interrelated pathways: primary hemostasis, coagulation, and fibrinolysis. Primary hemostasis is defined as the platelet–vessel wall interaction at the site of vascular injury that initiates when flowing platelets recognize (through their receptors) and bind (through the adhesive protein von Willebrand factor) subendothelial collagen. Any perturbation of this interaction may result in hypo- or hyperactivity, which increase the risk of hemorrhage or thrombosis, respectively.

Coagulation acts through 2 opposing drivers. The first, represented by the procoagulant factors, is triggered by tissue factor (TF), a cellular receptor in damaged tissues that forms a complex with activated factor VII (FVIIa) at the site of injury (Fig. 1A). TF-FVIIa starts a series of reactions mediated by negatively charged phospholipids on the membranes of activated platelets that lead to thrombin generation and fibrinogen-to-fibrin conversion (Fig. 1A). The anticoagulant driver originates from thrombin that, once complexed with its endothelial receptor thrombomodulin (TM), activates protein C (PC) bound to the endothelial PC receptor (EPCR) (Fig. 1B). Activated PC (APC) is a potent anticoagulant that, in combination with its cofactor protein S (PS), downregulates thrombin generation through the inhibition of the activated forms of FVIII and FV (Fig. 1, B and C). The anticoagulant driver is also potentiated by antithrombin (AT) that, in complex with endothelial heparin-like substances, inhibits thrombin directly and indirectly through the inactivation of the activated forms of the coagulation FVII, FIX, and FX (Fig. 1C). Thrombin generation is also downregulated by the tissue factor pathway inhibitor (TFPI), which inhibits TF-FVIIa and FXa (Fig. 1C). The balance between the pro- and anticoagulant drivers is essential to prevent unwanted thrombin formation in physiologic conditions. Again, any perturbation of this balance may result in hypo- or hypercoagulation, which increase the risk of hemorrhage or thrombosis, respectively.

Fibrinolysis is a highly regulated mechanism that, upon deposition of fibrin within the vasculature, converts plasminogen to plasmin, which in turn degrades fibrin (Fig. 1D). Under physiologic conditions, the conversion of plasminogen to plasmin is regulated by activators such as tissue plasminogen activator (tPA), urokinase plasminogen activator (uPA), and FXIIa. These activators (profibrinolytic drivers) are opposed by antiactivators such as tPA inhibitors [mainly plasminogen activator inhibitor (PAI)], plasmin inhibitor (PI), and thrombin-activatable fibrinolysis inhibitor (TAFI), which act as antifibrinolytic drivers. Here also, any perturbation of this balance may result in hyperfibrinolysis (increased risk of hemorrhage) or hypofibrinolysis (increased risk of thrombosis).

Over the years, many in vitro tests have been developed to investigate hemostasis, but none of them has proven suitable to meet the call for a global robust test able to truly mimic what occurs in vivo.

The skin bleeding time (or alternative tests) and platelet aggregometry can be used to assess primary hemostasis, but their results are not invariably correlated with clinical outcome in natural models of impaired primary hemostasis. These tests are also difficult to standardize.

The time-honored prothrombin and activated partial thromboplastin times (PT and APTT) were designed many years ago to investigate coagulation in patients with jaundice (PT) or as a guide to assess purification of coagulation factors from plasma (APTT). PT and APTT have been later used as laboratory tools to detect congenital deficiencies of coagulation factors (1). PT, APTT, and their modifications are still the simplest laboratory tools to diagnose the most frequent congenital hemorrhagic coagulopathies such as the hemophilias and allied disorders. Their use is based on the rationale that congenital deficiencies in any of the procoagulant factors translate into defective thrombin generation and, hence, into defective fibrinogen-to-fibrin conversion and prolonged PT or APTT. How-
Fig. 1. Simplified scheme of thrombin generation and fibrin formation (A); protein C activation (B); naturally occurring anticoagulants (C); and fibrin dissolution (D).

T, thrombin; HRGP, histidine-rich glycoprotein.

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ever, as knowledge of the mechanisms of regulation of coagulation improved, it became clear that in physiologic conditions procoagulants are counteracted by their anticoagulant counterparts, thus leading to a balanced mechanism. As a consequence, those conditions characterized by congenital deficiency of anticoagulant factors (in the face of normal procoagulants) should result in heightened thrombin generation and, therefore, in shortened PT/APTT, but congenital deficiencies of AT, PC, or PS present with normal PT or APTT. The above observations provide evidence that PT and APTT are sensitive to the effect induced by the procoagulants, but much less so to the effect induced by the anticoagulants.

HOW CAN THIS DIFFERENT BEHAVIOR BE EXPLAINED?
Because of their design, PT and APTT cannot account for the anticoagulant effect of APC, simply because TM (the main activator of PC in vivo), being located on endothelial cells, is not available in plasma, and thus PC cannot be fully activated by thrombin and cannot express its full anticoagulant activity. As a matter of fact, liver cirrhosis, a natural model of acquired coagulopathy in which both pro- and anticoagulants are concomitantly decreased, presents with prolonged PT and APTT but normal thrombin generation, when the latter is assessed by including exogenous TM in the assay system (2). AT also needs to be activated in vivo by the heparin-like substances that are located on endothelial cells, but not in plasma. Therefore, the anticoagulant effect of AT is unlikely to be fully represented by PT and APTT. Finally, another reason that makes PT and APTT unsuitable to represent what truly occurs in vivo is that they are performed in platelet-poor plasma and thus cannot account for the important role in supporting thrombin generation that is mediated by the presence of platelets (3).

Attempts to resolve the unsuitability of PT and APTT in investigating coagulation have led to the development of global assays such as thromboelastometry/thromboelastography (ROTEM/TEG) and thrombin generation tests in platelet-poor plasma, platelet-rich plasma, or whole blood. Other global assays currently under investigation include clot waveform analysis (4) and simultaneous thrombin and plasmin generation in platelet-poor plasma (5).

ROTEM/TEG helps assess the viscoelastic properties of clotting blood (including plasma, platelets, and blood cells) and should theoretically mimic what occurs in vivo much more than PT or APTT. The basic concept of ROTEM/TEG was described as early as 1948 (6) but was not widely used until recently because of the difficult interpretation of the viscoelastic tracings and the lack of standardization. More recently, the combination of the technique with computer software made ROTEM/TEG a bedside test suitable to help guide the management of bleeding, critically ill patients in whom PT/APTT are much less efficient.

Thrombin generation tests described in the early 1950s by Macfarlane and Biggs (7) were later developed by Hemker et al. (8) into laboratory tools useful for investigating the mechanisms that regulate thrombogenesis. The possibility to run these tests not only in platelet-rich plasma but also in whole blood (9, 10) opens new opportunities in the investigation of coagulation that were not possible until recently.

Fibrinolysis is investigated either by the measurement of its individual components (pro- and antifibrinolytic drivers) or through global tests designed to assess the time for clot dissolution upon addition of optimal amounts of tPA. The first approach does not account for the balance of the 2 drivers that operate in vivo, and the second is neither standardized nor automated.

In this issue of Clinical Chemistry, Skewis et al. (11) have tried to circumvent the above limitations by describing a unique technology that exploits completely new avenues to investigate global hemostasis in whole blood. The method, based on the measurement of the transverse relaxation time of the nuclear magnetic resonance of water molecules in clotting blood, referred to as T2 magnetic resonance (T2MR), uses tiny amounts of blood and produces results in a few minutes. The method is highly innovative and combines the relative simplicity of sample handling with good reproducibility and the use of whole blood. These features make it a promising candidate to investigate global hemostasis in a milieu similar (although not identical) to that operating in vivo. Future improvement should be aimed at mimicking the flow conditions operating in vivo and the presence of such endothelial receptors as TM and the heparin-like substances that are needed to fully activate the naturally occurring anticoagulants PC and AT. So far, the technique has been assessed to look at the contribution that individual components such as hematocrit, PT, and clot structure may have on the T2MR signal. The results of the new test show good correlation with some of the traditional parameters of hemostasis and also with the clot structure as assessed by electronic microscopy. Future studies are needed to validate T2MR in the investigation of well-defined natural models of hemostasis rearrangements that are characterized by bleeding or thrombosis risk.

Skewis et al. (11), by using sophisticated methods not even dreamt of at the time PT and APTT were developed, provide evidence that it is possible to explore new avenues suitable to fill the gap between in vivo and ex vivo hemostasis that still prevents accurate
laboratory investigation of patients at risk of hemorrhage or thrombosis.

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