Venous blood is collected in a plasticized tube containing sodium citrate (approximately 1 part citrate/9 parts plasma). Platelet poor plasma is obtained by centrifugation at 4°C. The plasma is diluted with distilled H₂O (1:10) and acidified by the addition of acetic acid or CO₂ to a pH of 5.9. This step causes precipitation of the euglobulins: plasminogen activators, plasminogen, plasmin, fibrinogen, factor V, and factor VIII. The inhibitors of fibrinolysis (anti-activators and anti-plasmins) remain in the supernatant and are discarded. The euglobulin pellet is resuspended in buffer solution. Thrombin or calcium is added to the euglobulin suspension to form a clot. The specimen is incubated in a 37°C water bath and observed every fifteen minutes until clot lysis is complete. Normal values are between 3 and 20 hrs. Lysis times less than 1 hour are indicative of severe fibrinolysis. The significance of an elevated lysis time has not been fully evaluated. The spontaneous fibrinolytic activity of plasma may be mathematically expressed as 1000/ECL time (min).

The significance of an elevated lysis time has not been fully evaluated.

FIG. 1. Activation and inhibition of the fibrinolytic system.
INTRODUCTION

The fibrinolytic system is a critical regulator of fibrin deposition and thrombosis. The components of the system are diagrammed above. Plasminogen, probably synthesized in the liver, is a circulating inactive proenzyme which is found in concentrations of 10-15 mg/dl. Plasminogen is converted into the active serine protease plasmin by a variety of plasminogen activators. These activators are widely distributed in a variety of tissues (tissue activator), plasma (activated Hageman factor/Kallikrein system) and urine (Urokinase). In addition to endogenous activators, a variety of bacterial products and pyrogens interact with the fibrinolytic system. The plasminogen activator of greatest physiologic importance is the tissue activator synthesized in venous endothelium and the vasa vasorum of muscular arteries. The release mechanism of the vascular-derived activator is poorly understood. To counterbalance the powerful proteolytic effects of plasmin, there are numerous fibrinolytic inhibitors which are normally present in approximately 30-fold excess concentration. These circulating anti-plasmins and anti-activators include alpha-1-antitrypsin, alpha-2-macroglobulin, antithrombin III, C1-inhibitor, and alpha-2-anti-plasmin.

The spontaneous fibrinolytic activity of blood may be measured by simply observing the time for clot lysis of whole blood collected in a glass tube (Whole Blood Clot Lysis Time). Due to the presence of natural inhibitors of fibrinolysis, clot lysis will occur only in cases of profoundly elevated fibrinolysis. In the Dilute Plasma Clot Lysis Test, dilution of the plasma results in differential depression of the inhibitors and allows lysis to occur in normal subjects or cases of slightly elevated fibrinolysis. The lysis time will generally be 18-24 hours. The ECL test is based on the concentration and precipitation of the fibrinolytic factors in the "euglobulin" fraction with removal of the fibrinolytic inhibitors in the supernatant. Thus with most inhibitors removed, lysis time will be shortened. The ECL test is felt to reflect the spontaneous fibrinolytic activity of plasma and most closely corresponds to the level of plasminogen activators.

TECHNICAL PROBLEMS

Fibrinolytic activity is increased when plasma comes in contact with glass, probably due to the activation of the Hageman factor/Kallikrein system which is capable of generating plasmin. Thus, glass contact must be minimized. Venous occlusion is known to cause release of plasminogen activators from venous endothelium and particular attention to blood drawing technique is necessary. The method of precipitation of the euglobulin fraction is critical and the ECL times will change significantly with slight variations in pH and plasma dilution. Most (62%) of the fibrinogen is precipitated with the euglobulins, but this will vary if pH is not controlled. Some anti-plasmin activity, probably C1-inactivator, will co-precipitate with the euglobulins even if pH and ionic strength of the plasma dilution are carefully standardized. A recent modification of the ECL test utilizes the addition of sodium flufenamate to the euglobulin fraction to neutralize any remaining C1-inactivator (not done in the UCSF lab). Precipitation of euglobulins in the presence of dextran sulfate polymer will markedly increase the recovery of plasminogen activator and pro-plasminogen activator. Heparin and EACA (epsilon-amino-caproic acid) remain in the supernatant. Most authors strongly advise against storage of the
plasma prior to test performance. Storage at 20°C or 36°C will markedly decrease fibrinolytic activity. However, storage at -20°C may not affect outcome. For reproducible results, the test should probably be run within two hours of blood collection. The type of anticoagulant used will affect results. Citrate is recommended. EDTA will increase fibrinolysis whereas heparin will reduce it. Variations in the ECL time will occur with alterations in the fibrinogen level since the patient's fibrin clot is the substrate in the assay. Care should be taken to avoid undue handling of the tubes during performance of the ECL test. The endpoint (complete clot dissolution) is usually easy to read, although difficulties may occur in lipemic sera.

**CLINICAL APPLICATIONS**

The ECL test and related assays have been used to investigate a variety of factors which influence spontaneous fibrinolysis. Epinephrine, stress, and exercise will markedly increase fibrinolytic activity either by neuronally mediated release of plasminogen activator or through their effects on vascular permeability. ACTH and corticosteroids potentiate the effects of epinephrine in stimulating activator release. Numerous drugs, including procainamide, nicotinic acid, sulfonylureas, phenformin, reserpine, pitressin, androsterone and DDAVP will increase fibrinolytic activity. Some of these may find therapeutic application as antithrombotic agents. Various surgical procedures produce a biphasic response in fibrinolysis. Many neurologic procedures, including ventriculography and pneumoencephalography, have been shown to markedly increase spontaneous fibrinolysis and on occasion provoke severe bleeding. Post-operatively, fibrinolysis usually decreases, reaching a nadir on postoperative days 1 and 2. Studies have indicated that large rapid falls in fibrinolytic activity as measured by the ECL test are associated with increased incidence of post-operative deep venous thrombosis. In approximately 95% of normal subjects, venous occlusion stimulates fibrinolysis in blood collected from the occluded vein. This potentiation of fibrinolysis with occlusion is more marked in arm than in leg veins. Subjects with idiopathic deep venous thrombosis frequently show no such enhancement of fibrinolysis upon venous occlusion. Release of plasminogen activators from venous endothelium of occluded vessels may be physiologically important in controlling thrombosis in situations of stasis. Fibrinolysis has also been reported to be reduced in individuals with multiple risk factors for myocardial infarction and in patients with peripheral atherosclerotic disease, vasculitis, and Raynaud's phenomenon.

Thrombolytic agents such as Urokinase and Streptokinase markedly accelerate fibrinolysis through direct and indirect mechanisms (see chart). The ECL test was formerly used to monitor patients receiving thrombolytic therapy for acute myocardial infarction. Currently, however, only PT, PTT, TT, platelets and fibrinogen are commonly monitored during intracoronary streptokinase infusion. The ECL test has been used to monitor patients receiving urokinase therapy for the treatment of DVT.

The ECL test has been used in the investigation of primary fibrinogenolysis (hyperplasminemia). Various tissues, including prostate, uterus, and colon appear to be exceptionally rich in plasminogen activator substances. Surgical manipulation of these organs may give rise to a consumptive coagulopathy based on widespread
intravascular activation of fibrinolysis. Plasmin has a wide range of substrates, including factors V and VIII, and will interfere with coagulation by multiple mechanisms. Production of abnormal quantities of plasminogen activator has also been described in certain tumors and acute leukemias. In primary fibrinogenolysis, there will be a marked increase in the spontaneous fibrinolytic activity of plasma. Increased fibrinolytic activity may also be seen, although usually to a lesser extent, in DIC.

There are no studies providing detailed comparisons of coagulation and fibrinolytic parameters in primary fibrinogenolysis versus DIC. The utility of the ECL test in making this distinction is uncertain. In clinical practice, DIC should be ruled out on clinical and laboratory grounds before primary defects in fibrinogenolysis are suspected. There is little agreement on the specific clinical indications for employing the ECL test in general patient management. At present, the test appears useful mainly as an investigative and research tool. Additionally, the variety of factors sited above which affect fibrinolysis detract from the specificity and diagnostic utility of the ECL test.

FURTHER DEVELOPMENTS

In the ECL test, the patient's own fibrin clot serves as the substrate for fibrinolysis. Different substrates may also be used, including casein (caseinolysis) and synthesis esters (esterolysis). The most important modification of the ECL time is the Fibrin Plate assay in which a sample of plasma or euglobulin fraction is placed directly on the surface of a petrie dish containing human or bovine fibrin. At approximately 20 hours, the zone size of fibrinolysis is measured. There is a relatively good correlation between assays of fibrinolysis as measured in the fibrin plate and the ECL test. The fibrin plate assay has a more precise and easily quantifiable endpoint and demands less technician intervention. However, it is not suitable for stat determinations. A recent modification of the ECL test used the addition of radio-labelled fibrinogen to the euglobulin fraction. The rate of radioactivity release is proportional to the fibrinolytic activity of the test plasma. This technique is very precise and has been used to investigate fibrinolytic activity in families with mild bleeding tendencies. For many research and clinical purposes, the ECL test has been supplanted by recently developed assays based on a chromogenic substrate (S-2251) which is a specific substrate for plasmin and plasminogen activated streptokinase. Using the chromogenic substrate technique, plasminogen activator, plasminogen, plasmin, and anti-plasmins may be easily and readily quantified.

REFERENCES


