3. Borate solution, pH 9.0.
   Sodium chloride 9.0 g
   Sodium borate 1.0 g
   Dilute to 100 ml with distilled water.
4. Water bath, 37°C.
5. Test tube, 15 × 125 mm.

SPECIMEN

Oxalated plasma: one part 0.1 M sodium oxalate to nine parts whole blood. Citrated plasma should not be used, since the presence of citrate tends to increase fibrinolytic activity.

PRINCIPLE

The plasma euglobulins are precipitated with 1% acetic acid and resuspended in a borate solution. The euglobulins are then clotted by the addition of calcium chloride. The clot is incubated, and time of lysis reported.

PROCEDURE

1. Collect blood with a plastic or siliconized syringe. Mix with the appropriate anticoagulant and immediately place on ice.
2. Centrifuge the blood specimen at 2500 RPM for 10 minutes, and immediately proceed with the test. The procedure must be carried out within 20 minutes of blood collection.
3. Into a 15 × 125-mm test tube, pipet 9.0 ml distilled water, 0.5 ml patient's plasma, and 0.1 ml 1% acetic acid.
4. Refrigerate the preceding mixture for 30 minutes at 4°C to allow for euglobulin precipitation.
5. Centrifuge at 2500 RPM for 5 minutes.
6. Pour off the supernatant and invert the tube on filter paper to drain.
7. Add 0.5 ml of the borate solution and place in a 37°C water bath.
8. Stir the mixture gently with a glass rod.
9. Add 0.5 ml 0.025 M calcium chloride to the mixture.
10. Record the time of clot formation.
11. Incubate the tube in the 37°C water bath, and periodically check for clot lysis. When clot lysis begins, check the tube every 5 minutes until the lysis is complete.
12. Report results as the length of time from clot formation to complete clot lysis.

PROTAMINE SULFATE

The protamine sulfate procedure tests primarily for the presence of fibrin monomers. During stage 3 of the coagulation process when thrombin acts on fibrinogen, fibrin monomers are formed that then polymerize to form a fibrin clot. Also detected in this procedure are early fibrin-(fibrinogen) split products (fragments X and Y). During the process of fibrinolysis, plasmin breaks down fibrin and fibrinogen to fragments X, Y, D, and E. Under certain pathologic conditions, intravascular coagulation may be stimulated. In these instances there is widespread appearance of fibrin clots in the blood vessels of the microcirculation. Fibrin monomers are therefore present in the plasma. Due to the presence of coagulation, there is stimulation of the fibrinolytic system and the formation of fibrin- (fibrinogen) split products. Rapid detection of the presence of fibrin monomers is an important aid in the diagnosis of disseminated intravascular coagulation. Normally, there should be no fibrin monomers present in the plasma.

REFERENCES

Dade Division, American Hospital Supply Corporation: *Protamine Sulfate* Set, Dade Division, American Hospital Supply Corporation, Miami, Florida, 1975.

Williams, W.J., Beutler, E., Erslev, A.J., and Rundles, R.W.: *Hematology*, 2nd Edition, McGraw-Hill Book Company, New York, 1977.

REAGENTS AND EQUIPMENT

1. Protamine sulfate reagent, 0.2% (w/v). (Obtainable from Dade Division, American Hospital Supply Corporation, Miami, Florida.) Reconstitute with 3.0 ml distilled water. This reagent, when reconstituted, is good for 8 hours when stored at 4°C.
2. Fibrin monomer control. (Obtainable from Dade Division, American Hospital Supply Corporation, Miami, Florida.) To reconstitute this positive control, add 1.5 ml distilled water while continually agitating the vial. If the control is not reconstituted correctly, some of the fibrin monomers present may polymerize into small fibrin threads. If only a few form, these may be removed with wooden applicator sticks without affecting the quality of the control.
3. Test tubes, 13 × 100 mm and 10 × 75 mm.
4. Sodium chloride, 0.85% (w/v).
5. Pipets, 1.0 ml and 0.2 ml.

SPECIMEN

Citrated plasma: one part 3.8% sodium citrate to nine parts whole blood. To prepare 3.8% sodium citrate, dissolve 3.8 g sodium citrate in distilled water and dilute to 100 ml.

PRINCIPLE

Fibrin monomers and early fibrin- (fibrinogen) split products present in the plasma precipitate in the presence of a weak solution of protamine sulfate.

PROCEDURE

1. Centrifuge blood immediately after collection at 2500 RPM for 15 minutes.
2. Label five 13 × 75-mm test tubes as indicated: 1:5, 1:10, 1:20, 1:40, and 1:80.
3. Pipet 1.0 ml of 0.85% sodium chloride to each of the preceding tubes except for the tube labeled 1:5.
4. Place the 3.0 ml of reconstituted protamine sulfate reagent into the 1:5 labeled tube.
5. Pipet 1.0 ml of the protamine sulfate reagent from the 1:5 tube into the test tube labeled 1:10. (Use a separate pipet for each dilution outlined in steps 6 through 9.)
6. Mix the 1:10 tube well and transfer 1.0 ml of this dilution into the 1:20 test tube.
7. Mix the 1:20 tube well and transfer 1.0 ml into the 1:40 test tube.
8. Mix the 1:40 tube and transfer 1.0 ml of this dilution into the test tube labeled 1:80.
9. Using 10 × 75-mm test tubes, label five tubes for each patient specimen and control: 1:5, 1:10, 1:20, 1:40, and 1:80.
10. Pipet 0.2 ml of patient plasma into each of the five preceding tubes. Pipet 0.2 ml of fibrin monomer control to each of the five labeled control tubes.
11. Using a 0.2 ml pipet, add 0.2 ml of the corresponding protamine sulfate reagent to the appropriate control and patient tubes. To ensure immediate mixing, blow the reagent directly into the bottom of each test tube.
12. Allow the tubes to sit at room temperature undisturbed for 30 minutes.
13. At the end of a half hour gently tilt each tube and observe for clot formation. If no clot is seen, carefully shake the tube and examine for fibrin strands. Occasionally there may be slight precipitation of the fibrinogen. When the tube is mixed, this precipitate should clear. Do not interpret this as a clot or fibrin formation.
14. Report the results as positive if there is clot or fibrin formation in any of the five tubes. Negative results will show

no clot or fibrin formation in any of the five tubes.

DISCUSSION

1. Due to the fact that the preceding procedure is extremely sensitive, a positive result must be interpreted in conjunction with clinical findings and additional laboratory data.
2. This procedure may be performed on patients receiving heparin since this will not interfere with the test results. However, heparin should not be used as the anticoagulant in place of sodium citrate.
3. A more simplified procedure than the preceding one may be performed by adding 0.1 ml protamine sulfate to 1.0 ml citrated plasma, allowed to incubate at 37°C for 15 minutes. The presence of fibrin strands or a granular precipitate indicates a positive test.

ETHANOL GELATION TEST

The ethanol gelation test is designed to detect the presence of fibrin monomers present in the plasma. It is a screening procedure to be utilized as an aid in the diagnosis of disseminated intravascular coagulation and in distinguishing this condition from primary fibrinolysis.

Breen and Tullis Method

(Modified by H. Glueck)

REFERENCES

Breen, F.A., Jr., and Tullis, J.L.: Ethanol gelation: A rapid screening test for intravascular coagulation, Ann. Intern. Med., 69, 1197, 1968.

Breen, F.A., Jr., and Tullis, J.L.: Ethanol gelation test improved, Ann. Intern. Med., 71, 433, 1969.

REAGENTS AND EQUIPMENT

1. Sodium hydroxide, 0.1 N.
2. Ethyl alcohol, 50% (v/v).
3. Buffered citrated anticoagulant.
   Sodium citrate, 0.11 M 3 parts
   Citric acid, 0.1 M 2 parts
4. Test tubes, 12 × 75 mm.

SPECIMEN

Collect blood using a plastic syringe, and mix nine parts whole blood to one part buffered citrate anticoagulant. Collect normal control blood at the same time patient's blood is obtained.

PRINCIPLE

During the process of disseminated intravascular coagulation, the level of fibrin monomer (intermediate product of fibrinogen breakdown to fibrin) in the blood increases. The fibrin monomer is precipitated from the plasma by ethyl alcohol and forms a gel or precipitate.

PROCEDURE

1. Centrifuge buffered citrated blood at 2500 RPM for 20 minutes to obtain platelet-poor plasma.
2. Into two appropriately labeled 12 × 75-mm test tubes, place nine drops of patient's plasma and control plasma.
3. Add one drop of 0.1 N sodium hydroxide to each tube.
4. Mix well.
5. Carefully layer 0.15 ml 50% ethyl alcohol over the mixture in each tube.
6. Allow tubes to sit undisturbed for 1 minute.
7. Inspect the interface (line between the plasma and ethyl alcohol) for a line of precipitation.
8. Precipitation, or gel formation, constitutes a positive test.
9. If the test is negative after 1 minute, allow the tubes to sit for 9 additional minutes. At the end of this time, if a precipitate or gel forms, add one more drop of 0.1 N sodium hydroxide and gently shake the tube. If the precipitate formed is nonspecific, it will disappear. Persistence of the precipitate constitutes a positive test.