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.

DISCUSSION

1. An increased pH above 7.70 causes a delay in precipitate formation. It is therefore important to use buffered sodium citrate as the anticoagulant. Sodium oxalate produces too alkaline a pH.
2. The presence of heparin, or contamination with a few red cells, does not alter the results of this test.

FIBRIN-SPLIT PRODUCTS

Fibrin- (fibrinogen) split products may be demonstrated in the blood of patients with primary fibrinolysis and during the process of disseminated intravascular coagulation with secondary fibrinolysis.

The Thrombo-Wellcotest procedure described here is a rapid, sensitive test for fibrin- (fibrinogen) split products present in the blood. The normal level of serum fibrin-split products in the adult is 2.1 to 7.7 μg per ml.

Thrombo-Wellcotest

REFERENCE

Wellcome Reagents Limited: *Thrombo-Wellcotest. Rapid Latex Test for Detection of Fibrinogen Degradation Products*, Wellcome Research Laboratories, Beckenham, Kent, England, 1977.

REAGENTS AND EQUIPMENT

1. Sample collection tubes (contain thrombin to cause rapid and complete clotting, and soya bean enzyme inhibitors to prevent the breakdown of fibrin).
2. Glycine buffer.
3. Latex suspension. (The latex particles have been sensitized with an anti-fibrin-split product globulin.)
4. Positive control serum.
5. Negative control serum.
6. Glass test slide.
7. Disposable pipet droppers.
8. Disposable mixing rods.
   (Note: All preceding reagents are available from Wellcome Reagents Division, Burroughs Wellcome Co., Research Triangle Park, N.C. 27709.)
9. Test tubes, 10 × 75 mm.

SPECIMEN

Obtain 3 ml of whole blood. Immediately transfer from the syringe 2 ml to the sample collection tube. These tubes may also be used with a Vacutainer system and will draw 2 ml of blood. As soon as the blood is in the tube, mix well by inverting several times.

PRINCIPLE

Whole blood is added to thrombin (to ensure complete clotting) and soya bean enzyme inhibitors (to prevent any breakdown of fibrin). After incubation, the patient's serum is diluted and mixed with anti-fibrin-split products latex particles. If fibrin- (fibrinogen) split products are present, the latex particles will agglutinate.

PROCEDURE

1. Obtain the blood specimen and place 2 ml into the sample collection tube. Immediately mix the tube by inverting several times.
2. The blood in the sample tube should clot quickly, within one-half minute. As soon as clotting has occurred, ring the clot with an applicator stick to allow good clot retraction.
3. Allow the blood sample to stand at room temperature, or 37°C, for 30 minutes to allow for clot retraction.
4. As soon as the serum has separated from the clot, remove the serum from the sample collection tube with a disposable dropper. (If red cells are present in the serum, centrifuge serum and separate from the cells. The serum may be obtained more quickly by centrifuging the specimen after clotting is complete.)
5. Label two 10 × 75-mm test tubes for each patient sample being tested, 1:5 and 1:20.