time. Less than 1 ml of blood in the tube yields a shortened clotting time.
2. Poor venipuncture technique, causing hemolysis or causing tissue thromboplastin to mix with the blood, shortens the clotting time.
3. Incubation at 37°C is important if the normal values for the test have been determined using this technique. Temperatures lower than 37°C retard the clotting time.
4. Bubbles entering the syringe when the blood sample is being obtained increase the rate of coagulation.
5. Unnecessary agitation of the blood shortens the coagulation time.
6. At the completion of the Lee and White clotting time, it is suggested that one tube remain in the 37°C water bath to be checked after 2 and 4 hours for clot retraction. Also, the tube may be allowed to remain in the water bath overnight, and checked the next day for abnormal clot lysis.
7. The Lee and White clotting time may also be performed using siliconized glass test tubes in place of the plain glass test tubes used in the previously described method. Using the same procedure, the normal clotting time (using siliconized tubes and tilting the tubes every 5 minutes) is 20 to 60 minutes. This method is more sensitive to coagulation deficiencies than the unsiliconized test tube procedure. However, because of the time involved, it is not a practical method for the routine hematology laboratory.

CLOT RETRACTION

When blood coagulation is complete, the clot normally undergoes contraction, where serum is expressed from the clot, and the clot becomes denser. Thrombosthenin, released by the platelets,is responsible for clot retraction. In addition, the number of platelets present also affects the clot retraction time. If the platelet count is below 50,000 per cu mm, poor clot retraction may occur. In rare instances where the platelet count is normal, there may be poor clot retraction due to an abnormality present in the platelets. Normally, clot retraction begins within 30 seconds after the blood has clotted. At the end of one hour, there should be appreciable clot retraction, and almost complete retraction by the end of 4 hours. Clot retraction should be complete within 24 hours. An abnormal clot retraction time is found in Glanzmann's thrombasthenia.

REFERENCE

Cartwright, G.E.: *Diagnostic Laboratory Hematology*, Grune & Stratton, Inc., New York, 1963.

REAGENTS AND EQUIPMENT

1. Water bath, 37°C.
2. Glass test tubes, 13 × 100 mm.

SPECIMEN

One of the tubes containing 1 ml of whole blood, used in the Lee and White clotting time, or 3 ml of whole fresh blood, placed in a 13 × 100-mm glass test tube.

PRINCIPLE

Whole fresh blood is placed in a 37°C water bath, and inspected at 1, 2, 4, and 24 hours for the presence of a retracted clot.

PROCEDURE

1. If a Lee and White clotting time was not performed, obtain 3 ml of blood and dispense carefully into a 13 × 100-mm glass test tube.
2. Place tube of blood in the 37°C water bath and allow the blood to clot.
3. As soon as the blood has clotted, inspect the clot at 1, 2, 4, and 24 hours for the formation of a retracted clot. The clot should be firm and retracted from the sides of the tube. It generally

occupies a little more than half of the original volume.

4. If a Lee and White clotting time was performed, use one of the three tubes of blood and check for clot retraction at 1, 2, 4, and 24 hours after the blood clotted.
5. Results are reported as the length of time it took for the clotted blood to retract. As an alternative method, the results may be reported as: normal, if clot retraction has occurred at 2 to 4 hours; poor, if retraction occurs after 4 hours and within 24 hours; nil, if no retraction occurs after 24 hours.

DISCUSSION

1. Clot retraction should be almost complete within 4 hours. In abnormal states, there may be variable degrees of retraction or no retraction at all.
2. Shaking or jarring of the tube of blood should be avoided. This may lead to a shortened clot retraction time.
3. Clot retraction varies inversely with the plasma fibrinogen concentration. That is, if the plasma fibrinogen level is elevated, clot retraction may be poor.
4. Clot retraction may be affected by the red cell mass. In blood containing a large mass of red cells, the degree of retraction is limited because of the large volume of red cells within the clot. In anemic states, the reverse occurs, and the degree of clot retraction is increased.
5. Generally there is a small amount of what is termed red cell fallout during clot retraction. This is seen as a few red cells at the bottom of the tube that have fallen from the clot. The significance of an increased amount of red cell fallout is not known. However, when the fibrinogen level is slightly decreased, there will be an increased number of free red cells at the bottom of the tube. Whenever red cell fallout is increased, a notation on the patient's report should be made.

CLOT LYSIS

The clot used in the clot retraction procedure should be kept at 37°C and examined at the end of 8, 24, 48, and 72 hours for clot lysis. Normally, there is no clot lysis before 72 hours. If the clot that was initially formed becomes fluid in less than 72 hours, abnormal clot lysis is present. The time at which lysis was observed is reported as the clot lysis time. If no lysis occurred, the results are reported as, "no clot lysis after 48 and 72 hours."

PROTHROMBIN TIME

The prothrombin time is a useful screening procedure for deficiencies in factors II, V, VII, and X. Deficiencies in factor I, although rare, may also be detected. This test may be used to follow the course of anticoagulant therapy in patients receiving coumarin drugs. Factors II, VII, IX, and X are inhibited by the coumarin drugs, with factor VII showing decreased activity first. Common causes of a prolonged prothrombin time are vitamin K deficiency, certain liver diseases, specific coagulation deficiencies, and coumarin drug therapy. The normal prothrombin time is generally 11 to 13 seconds. However, these values differ according to the method and reagents used in the performance of the test. Therefore, each laboratory should determine its own set of normal values.

Quick's One-Stage Prothrombin Time Method

REFERENCE

Quick, A.J.: *Bleeding Problems in Clinical Medicine*, W. B. Saunders Company, Philadelphia, 1970.

REAGENTS AND EQUIPMENT

1. Water bath, 37°C.
2. Thromboplastin-calcium chloride mixture.