forms over the wound every 30 seconds. Do not rub or remove the clot. Do not touch the skin. Any disruption of formed fibrin or clot will prolong the bleeding time.

8. The bleeding time is reported when no blood stain is seen on the filter paper after a gentle touch. It is reported in intervals of 30 seconds. One can measure both wounds and average them, or take the reading of the last one to stop bleeding.

**Normal Values**

Normal values are 1 to 6 minutes. More than 6 minutes should be taken as abnormal.

**Interpretation**

1. Results of duplicate tests performed on the same individual should agree within 2 to 3 minutes at most.
2. Bleeding time is prolonged:
   - When platelet count < 100,000/mm$^3$
   - In patients on aspirin therapy.
   - In acquired fibrinogen disorders.

(If the platelets are young even in a thrombocytopenia patient, the bleeding time may not be raised as young platelets have enhanced hemostatic capabilities).

When platelet counts are low, one can calculate the expected bleeding time with the following formula:

$$\text{Bleeding time} = 30.5 \times \frac{\text{Platelet count/cu mm}}{3850}$$

A bleeding time longer than that calculated from platelet numbers alone, suggests defective platelet function in addition to reduced number. It is also possible to detect above-normal hemostatic capacity in cases in which active young platelets comprise the entire population of circulating platelets.

### *Clinical Implications*

1. Bleeding time is prolonged when the level of platelets is decreased or when the platelets are qualitatively abnormal, as in
   a. Thrombocytopenia
   b. Platelet dysfunction syndromes
   c. Decrease or abnormality in plasma factors such as von Willebrand's factor and fibrinogen
   d. Abnormalities in walls of the small blood vessels—vascular defects
   e. Severe liver disease
   f. Leukemia
   g. Aplastic anemia
   h. DIC disease.
2. Bleeding time can be either normal or prolonged in von Willebrand's disease. It will definitely be prolonged if aspirin is administered prior to testing.
3. A single prolonged bleeding time does not prove the existence of hemorrhagic disease because a larger vessel may have been punctured. The puncture should be done twice (on the contralateral side) and the average of the bleeding times can be taken.

### *Interfering Factors*

1. The normal range may vary when the puncture is not of standard depth and width.
2. Touching the incision during the test will break off any fibrin particles and prolong the bleeding time.
3. Heavy alcohol consumption (as in alcoholics) may cause bleeding time to be increased.
4. Prolonged bleeding time will result from the ingestion of 10 g of aspirin up to 5 days before the test.
5. Other drugs that may cause the bleeding time to be increased include:
   - Dextran
   - Streptokinase—streptodornase
   - Mithramycin
   - Pantothenyl alcohol.

### *Patient Preparation*

1. Explain the purpose and procedure of the test to patient.
2. Warn patient not to consume aspirin for 5 days prior to test.
3. Advise patient not to consume alcohol in any form.

## Coagulation Time

### *Capillary Tube Method of Wright*

Blood is collected in about a dozen capillary tubes from a finger prick made after aseptic precautions. The tubes are sealed with plasticine and immersed in water bath at 37°C. After 4 minutes, remove the first tube from the bath and expel the blood in it with one end immersed in a dish containing water. Repeat this every 30 seconds with the other tubes till the blood is expelled in a worm clot and note the time.

An alternative way of determining the end point is to break the capillary tubes every 30 seconds until a clot is seen between the two broken ends. By these methods, the normal clotting time is 5 to 10 minutes at 37°C and longer if performed at room temperature. This test should be avoided as tissue thromboplastin contaminates the oozing blood and hence, false reports may be obtained.

### *Lee and White's Method*

*Principle:* Whole blood, when removed from the vascular system and exposed to a foreign surface, will form a solid

clot. Within limits, the time required for the formation of the solid clot is a measure of the coagulation system.

**Requirements**

1. Stop watch
2. Equipment for collection of blood
3. Clean, dry glass test tubes (10 × 75 mm)
4. Water bath (37°C).

**Method**

1. Make a clean venipuncture with as little trauma to (or time spent passing through) the connective tissue between skin and vein as possible. One may routinely or in selected cases use the two-syringe technique, whereby one rinses the needle of all interstitial tissue fluid by drawing back 1 cc. of blood after entering the vein. Then remove the first syringe from the needle and quickly place on a second clean and dry syringe and draw back blood for the test.
2. Timing is begun when the blood first enters the syringe. The second syringe in the 'Two-syringe' technique.
3. Draw 3–5 mL of blood and withdraw the syringe and needle. Disconnect the needle. Place approximately 1 mL of blood in each of three (10 × 75 mm) test tubes.
4. Place the tubes in a stand so that they remain upright and undisturbed, at room temperature for 10 minutes. If a 37°C water bath is available one may do the entire test at 37°C, and shorter clotting times will be found (if the test has been done at 37°C, do not wait for more than 5 minutes).
5. After 10 minutes (or 5 minutes) take the first of the tubes and gently tip it every 30 seconds to test for clotting. Do not tip it further than necessary to get the information.
6. When the first tube is clotted (can be inverted without blood running down the edge of the tube), record the time and start the tipping of the second tube every 30 seconds until it is also found to be clotted. Then do the same with the third tube (tipping is intended to allow one to ascertain when blood is clotted—not as a means of hastening clotting or of assuring mixing of the blood).
7. The time recorded for the clotting of the third tube is taken as the clotting time (the purpose of the first two tubes is to tell one when to start looking in the third tube, since the agitation of tipping does hasten the clotting).

Some choose to tip the tubes in rotation (at 37°C) every 15 seconds, or tip all tubes at once, and average the results of the three tubes.

## *Normal Values*

Normal times depend on method used. Normal range at 37°C is usually 5 to 10 minutes. Normal times at room temperature will vary with the degree of temperature present and the method used. If one uses the method which waits 10 minutes before starting to tip, then normal values may go as high as 22 to 25 minutes, especially in the cool season. Values shorter than 10 minutes should be suspected and the test repeated using the two-syringe technique to rule out contamination by tissue fluid (in the heat of April, May or June warm tropical climate blood will clot before 10 minutes without having been contaminated by tissue fluids). If one uses the method which waits 5 minutes before tipping begins, normal results are between 8 to 18 minutes. Longer than 20 minutes is abnormal. If clotting occurs in less than 7 minutes, the test should be repeated using two-syringe technique.

## *Precautions and Errors*

1. The venipuncture must be without trauma to avoid contamination with tissue thromboplastin.
2. If all three tubes are clotted at 10 minutes (or 5 minutes) when one starts to tip the first tube, the test is unsatisfactory and should be repeated. If blood was drawn by single syringe technique, the most likely explanation is contamination, of the blood by tissue thromboplastin. If a two-syringe technique is used, it suggests that the patient's blood is hypercoagulable.
3. Vigorous agitation of the tubes will significantly shorten the coagulation time. So tipping should really be very gentle just to see if the blood has clotted.

## *Clinical Implications*

1. Severe deficiencies of any of the coagulation factors must be present before the coagulation time will be prolonged. Fibrinogen for example, needs to be decreased to 50 mg/100 mL or less before the coagulation time is affected, the normal range of fibrinogen is 200 to 400 mg/100 mL.
2. When prothrombin is diminished to a level of 30% of normal, there will be a small change in coagulation time.
3. Prolonged coagulation time will be noted in afibrinogenemia and marked hyperheparinemia.

## *Interfering Factors*

1. *Quality of venipuncture:* The venipuncture must be carefully done because either tissue thromboplastin obtained as a contaminant when the venipuncture is done, or hemolyzed red blood cells suctioned when the blood is drawn, can cause a marked shortening of the coagulation time. The time required for a severe hemophiliac's blood to clot can be shortened from 1 hour to a normal value when a poor venipuncture is done.

2. *Type of test tube:* The coagulation time will be lengthened to 20 to 40 minutes if plastic or silicone coated test tubes are used.
3. *Drugs:* Increased coagulation time may be seen with:
   - Mithramycin
   - Tetracyclines
   - Anticoagulants
   - Azathioprine
   - Carbenicillin.

   Decreased coagulation time may be seen with:
   - Corticosteroids
   - Epinephrine.

## Clot Retraction

### *Principle*

When whole blood is allowed to clot spontaneously, the initial coagulum is composed of all elements of the blood. With time the coagulum reduces in mass and fluid serum is expressed from the clot. This is due to an action of platelets on the fibrin network.

### *Requirements*

- Equipment for collecting blood
- Clean, dry plain glass graduated centrifuge tube
- Timer
- Water bath 37°C.

### *Method*

1. 5 mL blood is obtained with a standard two-syringe technique and transferred to the centrifuge tube.
2. Incubate it at 37°C in vertical position.
3. Record degree of retraction after 1, 2, and 4 hours. It may be necessary to loosen the clot gently from the wall of the test tube if contraction is not apparent at the end of 1 hour. The degree and rate of retraction should be noted. Note also any digestion of clot or discoloration of serum.

Clot retraction is directly related to platelet count, hence, it is impaired in thrombocytopenia, but is normal in hemophilia. In the method just described, one can remove the clot by using a hooked long needle and the volume of serum left behind can be measured. The percentage of clot can be calculated from the initial 5 mL of blood taken. In normal individuals, the clot percentage is about 50% at the end of one hour of the original blood volume taken.

### *Interpretation*

1. Patients with qualitative or quantitative platelet disorders have samples with scant serum and a soft, plump, poorly demarcated clot.
2. The clot is small and serum voluminous if the patient has a low hematocrit.
3. Patients with polycythemia have poor clot retraction because the large numbers of captured red cells separate fibrin stands and interfere with platelet contraction.
4. If fibrinogen levels are low, the initial clot is so fragile that the delicate strands rupture and red cells spill out into the serum when retraction begins.
5. Serum contamination by red cells is especially striking if fibrinolysis is abnormally brisk, as often happens with reduced fibrinogen levels. Sometimes in these cases, the incubated tube contains only cells and plasma with no fibrin clot at all.

### *Errors*

1. When fibrinogen is reduced in amount, the clot may be very small and retraction may be interpreted as normal even though it is inadequate.
2. In the presence of active fibrinolytic activity, the clot may dissolve.
3. In normal blood the exuded serum will be clear and free of RBC's. The presence of significant number of RBC's in the serum suggests fibrinolytic activity.
4. With a low hematocrit value, the mass of the clot will be proportionately small and may give enormously high values.

## Heparin Therapy

### *Protocols and Blood Coagulation Tests*

1. Heparin combines in the blood with an alpha globulin (heparin cofactor) for a potent antithrombin.
2. The intravenous injection of heparin will give an immediate anticoagulant effect, so it is used when rapid effects are desired.
3. Because of heparin not remaining in the blood very long, the clotting time is measured before each injection.
4. The coagulation time is ordinarily maintained at two to two and one half times the normal limit.
5. To evaluate the effect of heparin, the blood is tested for coagulation time:
   - Before therapy is started for baseline
   - One hour before the next dose is administered
   - Dependent upon the status of patient during heparin therapy (signs of bleeding).
6. Protamine sulfate is the antidote for heparin overdose and hemorrhage.