of ADP and 5HT due to an intrinsic deficiency in the number of dense granules.
2. *Thrombasthenia (Glanzmann's disease):* There is failure of primary platelet aggregation.
3. *Bernard-Soulier syndrome:* Platelets are larger than normal, lack surface glycoprotein and fail to make phospholipid available or to adhere to vessel walls.
4. von Willebrand's disease: There is defective platelet adhesion as well as coagulation factor VIII deficiency.

Acquired Disorders

1. *Aspirin therapy:* It may lead to abnormal bleeding time although purpura is rare. Aspirin leads to impaired thromboxane-A_2 synthesis. So, there is failure of the release action aggregation with ADP and adrenaline.
2. *Hyperglobulinemia:* Interferes with platelet adherence, release and aggregation.
3. *Myeloproliferative disorders:* Intrinsic abnormalities of platelet function may occur in patients with essential thrombocythemia and other myeloproliferative disorders.

Bleeding Time

The duration of bleeding from a standard puncture wound of the skin is a measure of the function of platelets as well as the integrity of the vessel wall.

Duke's Method

Requirements

- Stop watch
- Lancet
- Filter paper
- Glass slide
- Alcohol sponges.

Method

1. Clean the lobe of the ear or tip of a finger with alcohol and let dry.
2. For ear—glass slide is placed behind the ear lobe and held firmly in place. This provides a firm site for incision.
3. Pierce the lobe of the ear by a firm stroke against the glass slide (or pierce the finger-tip). Discard the glass slide if ear lobe has been incised. Start the stop watch when the stab was made.
4. Bleeding of the wound should be allowed to proceed without pressure and the blood is allowed to drop on the filter paper. The paper should be moved so that each drop will fall on a fresh area. When bleeding slows, the wound is touched gently with a fresh area of the filter paper at 30 second intervals. When blood no longer stains the filter paper, the watch is stopped and the time recorded.

Normal Values

The normal range is up to 6 minutes. Between 6 and 10 minutes, the results are borderline. Over 10 minutes is definitely abnormal.

Precautions

1. In children, heel should be used.
2. In suspected cases of a bleeding disorder, the bleeding may not be controlled easily from the ear lobe hence, fingertip puncture wounds are better.
3. The area to be punctured should not be congested.
4. The size and depth of the wound may vary if one does not have a standardized technique.
5. If bleeding persists for more than 15 minutes it should be stopped by placing a dry gauge sponges over the site and applying finger pressure (the filter paper used to collect the drops of blood can be dried and saved as a record of the procedure).

Ivys's Method

(Preferred because of greater ease of standardization).

Method

1. Cleanse the inner aspect of the forearm with spirit and let dry.
2. Place a blood pressure cuff on the upper arm, inflate at 40 mm Hg, and maintain the same throughout the test.
3. Select an area on the forearm—Volar aspect which is devoid of superficial veins. Stretch the skin laterally between the thumb and forefinger and hold in a taut position.
4. Take a cork, through which a no. 11 surgical blade has been inserted with the tip extending 3 mm beyond the cork surface (both cork and blade should have been sterilized before), the blade should be withdrawn from the cork and autoclaved before being used again.
5. Hold the cork with the thumb and forefinger of the free hand, and with the heel of the hand resting on the patient's arm, quickly make two skin punctures (actually they are small incisions) in the selected area. It is important that the surface of the cork meet the skin to ensure a 3 mm deep incision. Holding the skin taut prevents the test area from being depressed when the blade enters the skin.
6. Timing is begun as soon as the incisions are made and bleeding starts.
7. Using the edge of a piece of a filter paper to collect the blood, gently touch paper to the drop of blood, which

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