mm deep skin puncture, about 5–6 cm below the cubital fossa. Note the time.

4. Remove the blood every 30 seconds by absorbing it along the edges of a clean filter paper by gently touching the wound with it, till the bleeding stops. This is the end-point.

Note
Instead of one prick, two lancet stabs may be given, 5 cm apart, one after the other, and the BT noted in them separately.

Normal bleeding time with this method is upto 9 minutes.

[IV] Simplate method. Though the "Duke" and the "Ivy" bleeding time methods are fairly reliable, it is not possible to control the depth of the wound made by a lancet or a blade. However, by careful standardization, it has become possible to do so. The most widely used technique uses a 'template' or an automated scalpel to control the depth and length of the wound—usually 1 mm deep and 9 mm long—and a blood pressure cuff inflated to 40 mm Hg to distend the capillary bed of the forearm.

Normal bleeding time = < 7 minutes.

Note
Although a BT of over 10 minutes has a slightly increased risk of bleeding, the risk becomes great when the BT exceeds 15 or 20 minutes.

[V] Capillary fragility test of Hess (also called "Tourniquet" test). This is an important test to assess the mechanical fragility of the capillaries (and formation of a platelet plug) by raising the pressure within them. It may reveal latent purpura.

1. Mark a 1 inch diameter circle on the front of the forearm, and using blue ink, mark any pink, purple, or yellow spots within the circle.
2. Apply a blood pressure cuff on the upper arm and note the systolic and diastolic pressures. Then, after a pause of about 2 minutes or so, raise the pressure to midway between systolic and diastolic levels and maintain it there for 15 minutes. Appearance of more than 10 new petechiae (pink or red spots in the skin) is a positive test, which may be seen in various types of purpura and vessel wall abnormalities.

Comments
The BT test is an in vivo test of platelet function, and "Ivy" method is probably the most reliable. However, a peripheral blood film is always examined for the number of platelets and their morphology. The students should note that platelets are involved both in BT and CT tests and one is normally affected without the other. If the BT is prolonged due to low platelet count (thrombocytopenic purpura), the platelets that are available are sufficient to give a normal clotting time.

PLATELET COUNT

Despite their small size (2–4 μm) and being non-nucleated fragments of cytoplasm, the platelets contain a wide variety of chemical substances that play an important role in vasoconstriction, hemostatic plug formation, activation of factor X, conversion of prothrombin to thrombin, and in clot retraction that results in permanent sealing of a ruptured vessel. Thus they take part in almost all stages of hemostasis.

Platelet counting. There are two methods for this count: *direct method* and the *indirect method.* Automated counters are also available.

A. DIRECT METHODS

You will require: • Microscope • RBC pipette • Counting chamber with cover slip • Equipment for fingerpick • Rees-Ecker diluting fluid—OR—Freshly prepared 1.0% ammonium oxalate solution.

PROCEDURES

I. Ammonium Oxalate Method. This fluid destroys red cells but preserves platelets; it also acts as an anticoagulant.

1. Get a finger- prick and draw blood up to the mark 1.0. Suck the diluting fluid to the mark 101.
2. Mix the contents thoroughly and wait for 20 minutes. The red cells will be hemolyzed, leaving only the platelets. Mix the contents once again and charge the chamber on both sides.

Place the charged chamber on wet filter paper and cover it with a petri dish to avoid evaporation.

3. Focus the RBC square under HP; adjust the diaphragm and position of condenser till you see the platelets – which appear as small, round or oval structures lying separately, highly refractile bodies with a silvery appearance. Rack the microscope continuously and count the platelets in 5 groups of 16 squares each, as was done for red cell count.

Knowing the dilution (1 in 100) employed and the dimensions of the squares, calculate the number of platelets in 1 mm^3 of undiluted blood.

II. Rees-Ecker Method. The Rees-Ecker fluid contains the following:

• Brilliant cresyl blue = 0.1 g • Sodium citrate = 3.8 g • Formalin 40% formaldehyde) = 0.2ml • Distilled water = 100 ml	(The dye stains platelets formalin prevents fungal growth and lyses red cells, citrate prevents clotting and makes the fluid isotonic

1. Draw freshly filtered diluent to the mark 0.5 in the RBC pipette. Get a finger-prick and draw blood in the pipette so that the diluent reaches the mark 1.0. Wipe the tip and fill the pipette with diluent once again to the mark 101. This gives a dilution of 1 in 200.
2. Roll the pipette gently between your palms for 3–4 minutes. (Taking the diluent first in the pipette prevents clumping and disintegration of platelets which occurs if blood is taken directly into the pipette).
3. Discard the first two drops and charge both sides of the chamber in the usual manner. Place it on a wet filter paper and cover with a petri dish, and wait for 10 minutes to allow the platelets to settle.
4. Count the platelets (which appear as bluish, round or oval bodies, highly refractile on racking the microscope) in 5 groups of 16 squares each, as was done for red cells. Calculate their number in 1 mm^3 of undiluted blood.

Note
The chamber and the pipette must be cleaned with absolute alcohol to remove any dust particles, etc. to which platelets could adhere. Use a lint-free piece of cloth for final cleaning.

B. INDIRECT METHOD

1. Place a drop of 14% magnesium sulfate solution on your finger tip, and get a prick through this drop. Blood oozes directly into the solution which prevents clumping, and disintegration of platelets.
2. Spread a blood film with the diluted blood, dry it, and stain it with Leishman's stain.
3. Examine the stained film under oil immersion lens. Count the platelets and red cells in every 5th field until 1000 red cells have been counted. Determine the "platelet ratio", i.e. the ratio of platelets to red cells (usually, there is 1 platelet to 16–18 red cells).
4. Do the RBC count from a fresh finger-prick in a counting chamber, and calculate the count in 1 mm^3 of undiluted blood.

Calculation of platelet count. With the knowledge of platelet count, and the RBC count, the actual number of platelets per mm^3 blood can now be calculated.

(While doing DLC in a stained blood film, the platelets appear in groups of 3–15, and most of them show different degrees of disintegration. In the present case, however, the platelets lie separately from each other and their morphology can also be studied).

Normal platelet count = 250,000 - 500,000/mm^3.

C. AUTOMATED METHOD

It is a very accurate method. It is carried out on an electronic cell counter (See page 1). The red cells and platelets in the diluted blood sample pass through an aperture. The particles between 2 and 10 μm^3 (fl; femtoliters) are counted as platelets., the measuring range being 0-99.9 x 10^3 /fl, and the coefficient of variation being within 1.5%. A platelet distribution graph can also be plotted.