(4) Tuesday 2nd Nov

PY2/13

BLOOD CORPUSCLE COUNTS USING A HAEMOCYTOMETER

Principle

A known volume of blood is diluted accurately with an appropriate solution, according to whether the count to be made is for the red or white corpuscles. There are several types of haemocytometers - you will use the improved Neubauer. Each type consists of a glass slide on which there are three platforms separated by two gutters. The counting chamber scale is engraved centrally on the central platform, which is 0.1 mm. lower than the outer platforms. When the special coverslip is pressed flat on the outer platforms, a depth of 0.1 mm. of fluid lies on the counting chamber. All counts are expressed as the number of corpuscles per cmm. of blood.

Method of use of a haemocytometer

First examine the ruling of the counting chamber under low and high power; it may be necessary to vary the aperture of the iris diaphragm and to focus the substage condenser. You are provided with a diagram of the counting chamber which gives the dimensions of the squares. The diluting pipettes, the slide and the coverslip must be scrupulously clean and dry. When necessary, clean with distilled water and dry with alcohol or acetone and lens tissue.

The blood sample is taken from the lobe of the ear or the base of the thumb nail, using a sterile "haemolet". The puncture must be such that the blood flows freely without squeezing, the first drop should be wiped away and the subsequent drop used.

Red corpuscle count

Place some of the appropriate diluent (Hayem's fluid) in a watch glass. Take the larger pipette (with the mark 101) with rubber tubing and mouthpiece attached. Make the puncture and suck blood up to just above the 0.5 mark. Quickly wipe the outside of the pipette and adjust the level of the blood to the 0.5 mark by tapping the end of the pipette against the thumb nail. Then, at once, before coagulation takes place, suck up the diluent to the 101 mark while rotating the pipette. The level may be adjusted as before. The blood is therefore diluted 200 times. Remove the rubber tube quickly and, holding the ends of the pipette between the little finger and the thumb, shake well for one minute to ensure complete mixing.

Now take the slide and with the tip of a finger slightly moisten the two outer platforms. Press the coverslip firmly down on either side so that Newton's rings are seen. Shake the pipette again and replace the rubber tube on the pipette. Blow out four drops of the liquid and, as another drop begins to form, bring the tip quickly but gently to the edge of the surface of the counting platform where it projects beyond the coverslip. The central platform must be covered by the fluid, free of bubbles, which must not overflow into the gutters. Leave 2-3 minutes, for the corpuscles to settle, before counting.

The count is made under high power. First see that the corpuscles are evenly distributed. Count the number of corpuscles in at least five groups of 16 smallest squares, that is 80 of the smallest squares (side - 0.05 mm.). It is best to count the four outer groups and the central group. Those corpuscles lying on the left hand and top rulings are included, those on the two other rulings are excluded. Add up the number of red corpuscles in these 80 squares = n.

Calculation. Each of the smallest squares encloses a volume of 1/4000 mm^3 of blood diluted 200 times.

$$\therefore \text{R.B.C. count} = \frac{n}{80} \times 4000 \times 200/mm.^3$$

White corpuscle count

The technique is similar to that described above. The blood is diluted 20 times. The diluent haemolyses the red corpuscles and shows up the white corpuscles. Using the leucocyte pipette with the same precautions as before, suck blood up to the 0.5 mark and the appropriate diluent to the mark 11. This gives a dilution of 20 times. Using all the same directions as to mixing and making the film, proceed to count the leucocytes in each of the 9 large squares of area 1 sq.mm., having a volume of 1/10 $mm.^3$ of haemolysed blood diluted 20 times. Let the total number of leucocytes be l.

$$\text{Then the leucocytes count} = \frac{l}{9} \times 10 \times 20 \text{ per } mm.^3 \text{ blood.}$$

Diluting fluids

1. For R.B.C. count - either isotonic saline or Hayem's fluid.
Hayem's fluid is: sodium chloride 0.5g., sodium sulphate 2.5g., mercuric chloride 0.05 g., distilled water 100 ml.

2. For leucocyte count - 1% acetic acid tinted with gentian violet.
This haemolyses the R.B.C. and converts the haemoglobin to acid haematin (brown), but precipitates the protein of the nucleus and stains the leucocytes.

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