BC AL/336/3/9

(6) Tuesday 16th Nov

HISTOLOGY OF BLOOD

1. Rouleaux Formation. Place a small drop of freshly drawn blood on a slide and observe after two to five minutes the granulated appearance. Cover with a slip and examine under the microscope. Many of the red cells will have adhered to one another by their broad surfaces and have assembled into long columns resembling piles of coins - these columns are often curved and branched.

2. Permanent Preparation of Human Blood. The leucocytes or white blood corpuscles are best studied in thin even films of blood, fixed and stained appropriately to show the characteristics of their nuclei and cytoplasm.

To obtain a thin and even film of blood, the slides and coverslips must be absolutely grease-free; they are therefore cleaned in acid dichromate mixture, rinsed thoroughly in distilled water and dried from alcohol.

All the stains used below are made up in methyl alcohol so that it is very important that the films should be covered while staining. Methyl alcohol fixes the blood corpuscles, but only when water-free. The stains are cosinates of methylene blue, they therefore contain both an acid and a basic staining component.

Preparation of Blood Films.

(a) Coverslip Technique. Place a small drop of blood in the centre of a coverslip and immediately lower another crosswise on it. The blood spreads quickly between the two coverslips. Draw apart by slipping one over the other. Dry by waving in the air.

(b) Slide Technique. Place a small drop of blood near one end of a slide. Take a second slide (this should be a good thick slide with a smooth edge), hold at an angle of 45° to the first and slide it up to the drop. As soon as the blood spreads right across, push the second slide firmly and rapidly along the surface of the first slide making a thin uniform film. Dry by waving in the air.

Staining Methods.

(N.B. Films must be absolutely dry before staining.)

(a) Jenner's Stain. Place slide or cover slip in suitable dish. Cover film with Jenner's stain and leave covered for 3-5 minutes. Wash in neutral distilled water until pink. Blot dry and examine under L.P. - R.B.C.'s are coppery red, the nuclei of white corpuscles are blue. Mount in Canada Balsam and examine under H.P.
(Note. This stain demonstrates the granules in the cytoplasm of the leucocytes well: the nuclei are bright blue.)

(b) Leishmann's Stain. Place 7-8 drops of stain on the slide - this will spread in a thin layer over the film. Leave covered for 2 minutes, dilute with 15 drops of neutral distilled water and leave for another 10 minutes. Wash in neutral distilled water until pink, and blot dry. Examine under L.P. - R.B.C.'s are again coppery red and the nuclei of white corpuscles purple. Mount in C.B. and examine under H.P.
(Note. The granules in the cytoplasm of the leucocytes are not so clear as in films stained by Jenner's method. The nuclei are very clearly stained purple; this film is therefore suitable for a differential white cell count.)

3. Demonstration of Amoeboid Movement and Phagocytosis. In these experiments living cells from warm blooded animals are studied so the slides are examined in a hot box at 37°C and the coverslip is ringed round with vaseline to prevent evaporation.

(a) Amoeboid movement and Phagocytosis by Leucocytes. A small drop of freshly drawn human blood and a drop of carmine suspension in isotonic saline are placed side by side on a slide, covered with a slip and examined at 37°C. All leucocytes are capable of amoeboid movement, though the neutrophils exhibit the greatest motility. The neutrophils only will be seen ingesting the carmine particles.

(b) Supravital Staining of Leucocytes. An alcoholic solution of Janus Green and Neutral Red is poured on to a slide, drained off by placing the slide vertically and dried so that a thin film of the stains remains. A small drop of blood is placed on the slide, covered with a slip and examined at 37°C.

Note. Granules in the neutrophils are a brownish-red. Rod-shaped mitochondria, blue-green in colour, are well seen in lymphocytes and monocytes.

4. Staining of Marrow Smear. Cut a long bone longitudinally or split open a rib bone and take a small piece of red marrow from as near the epiphysis as possible. Place between two coverslips and rub together and slip apart. When smear is dry, stain with Jenner's stain (keeping covered) for 7-10 minutes. Drain off stain, cover with dilute Giemsa and leave for 10-15 mins. Wash in neutral buffer, blot dry and examine. Mount in C.B. after 5-10 mins. Jenner's stain demonstrates the granules in the developing cells; Giemsa shows up the nuclei.

Make a blood film from the same animal and stain with Jenner. Correlate staining reaction of leucocytes in marrow with those in the blood.

5. Staining of Reticulocytes. To prepare slide for supravital staining: pour a drop of 1% Brilliant Cresyl Blue in absolute alcohol on to a clean slide and drain it off; leave the slide in a vertical position to dry. Apply a drop of blood to the slide, cover with a coverslip and leave for 2 minutes. Remove coverslip and allow the film to dry. Stain with Jenner, as for blood. This fixes the corpuscles as well as staining the leucocytes.

September 1963. G.D.