THE PRACTICAL IDENTIFICATION OF ENDOTHELIAL LEUKOCYTES IN DIFFERENTIAL BLOOD COUNTING

FOURTH REPORT OF STUDIES ON THE MONONUCLEAR CELLS OF
THE BLOOD *

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A method for the identification of endothelial leukocytes in the peripheral blood has been reported by one of us.¹ This method, as was pointed out in its description, is not well adapted to routine work on account of the impossibility of bringing all cells into contact with the carbon particles of the suspension employed to determine the phagocytic properties of the leukocytes. The purpose of this paper is to record the application to blood films of an indophenol staining reaction which brings out characteristic cytoplasmic granules in the endothelial leukocytes.

Leukocytes Granule Stain.—To apply the stain, a blood film made in the usual way on a 22-mm. square coverglass is covered for one half minute with 5 drops of an alphanaphthol-methyl-violet solution in order to fix the preparation; the alcoholic stain is then diluted with an equal amount of distilled water, and the dilute stain allowed to act for five minutes. The preparation is washed with water, dried with filter paper, counterstained for two or three minutes with 0.01 per cent. basic fuchsin (Grübler), washed, dried in the air, and mounted in balsam.

To prepare the alphanaphthol-methyl-violet solution, add 0.2 gm. alphanaphthol (Merck reagent), 0.015 gm. methyl violet 5 B. (Grübler) and 0.2 c.c. hydrogen peroxid to 100 c.c. of warm 80 per cent. alcohol (made from absolute alcohol). The hydrogen peroxid used should contain approximately 3 per cent by weight of the gas as determined by titration with decinormal potassium permanganate.

Reaction of the Blood Cells to the Stain.—Nuclei and cytoplasm are colored red, except the cytoplasmic granules in neutrophils, eosinophils and endothelial leukocytes, which are blue. The granules of basophils are of a distinctive red color. The granules of the eosinophils are large and the central portion appears unstained so that these granules have a very characteristic ringlike appearance not seen in the granules of other leukocytes. The platelets take the red stain faintly; the erythrocytes are pink.

Since the red cytoplasm of lymphocytes is entirely free from bluish granules, the only differentiation requiring discussion is that between

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1. McJunkin, F. A.: THE ARCHIVES INT. MED., 1918, **21**, 57.

the neutrophils and endothelial leukocytes. That these two cells have an entirely different origin has been shown elsewhere by one of us.²

The character of the granules of the endothelial leukocytes and the relationship between granules and nucleus distinguish them from polymorphonuclear neutrophils. Of these two points, the first is of greater importance. The granules of the endothelial leukocytes are discrete and the cytoplasm is distinctly seen between them, while the neutrophilic granules are so thickly placed that little of the cytoplasm can be seen. The neutrophilic granules are larger and more regular in shape than those of the endothelial leukocytes (Plate, A, B, C). In pathologic blood in which mononuclear myeloblastic cells (neutrophilic myelocytes) are present the character of the granules assumes a greater significance. However, since the granules of neutrophilic myelocytes and of the so-called metamyelocytes are even more prominent than those in the polymorphonuclear neutrophils, there is no chance of confusing myelocytes and endothelial leukocytes, although both are mononuclear. The differential character of the endothelial leukocyte is the presence of blue granules in a mononuclear cell. Although the nucleus of this leukocyte frequently has a broken outline, it does not consist of pyknotic nuclear masses connected by filaments. The reason that endothelial leukocytes cannot be identified in films stained with a polychrome blood stain is that some of these cells, such as the one shown in B in the plate, are entirely devoid of granulation and cannot, therefore, be distinguished from lymphocytes when they approach these cells in size.

Endothelial leukocytes after incubation with carbon suspensions according to the method previously referred to¹ contain carbon. That the carbon-containing mononuclear leukocytes (Plate, D, E, F) react typically to the stain is proof that the cells under consideration in this and previous reports are one and the same.

Since some of the endothelial leukocytes contain no granules when stained with polychrome blood stains, and since they approach lymphocytes in size and shape, reported increases in the nonlymphocytic mononuclear leukocytes must be accepted with qualifications. Increases in the so-called "transitional" leukocytes have been reported in typhoid fever, Hodgkin's disease, malaria, and other conditions by various observers. F. A. Evans³ found 40 per cent. of this variety of leukocyte in a case of secondary syphilis under treatment with salvarsan.

The authors have examined the blood in four cases of syphilis under treatment with preparations of arsenphenamin (arsenobenzol brand) with the results recorded in the following paragraphs.

² McJunkin, F. A.: *Am. Jour. Anat.*, 1918. In press.

³ Evans, F. A.: *THE ARCHIVES INT. MED.*, 1916, **17**, 1.

REPORT OF CASES

CASE 1.—H. S., man, aged 21; hereditary syphilis; Wassermann + + +. Feb. 6 to March 22, 1918, five doses arsphenamin and five doses salicylate of mercury; last dose of arsphenamin seven days before examination of the blood. The blood examination showed 6,950 leukocytes per cubic millimeter, with 7.2 per cent. endothelial leukocytes, 60.6 neutrophils, 28.7 lymphocytes, 2.6 eosinophils, and 0.9 basophils.

CASE 2.—E. S., man, aged 25; secondary syphilis; Wassermann + + + +. Two doses of arsphenamin; last dose twelve days before examination of the blood. Blood examination showed 9,650 leukocytes with 6.1 per cent. endothelial leukocytes, 56 neutrophils, 34.8 lymphocytes, 2.3 eosinophils, and 0.8 basophils.

CASE 3.—J. A., man, aged 40; lesion on the tongue; Wassermann + + + +. Feb. 8 to April 5, 1918, received four doses of arsphenamin; last dose ten days before examination of the blood. The blood examination shows 6,700 leukocytes with 8.35 per cent. endothelial leukocytes, 51 neutrophils, 37.24 lymphocytes, 2.02 eosinophils, and 1.39 basophils.

CASE 4.—E. W., man, aged 29; syphilis ten years previously; at the time of examination there were difficulties in the movements of the left leg and arm; Wassermann "doubtful." April 12, 19 and 26, and May 3 and 10, he received intravenous injections of arsphenamin. The following percentages of endothelial leukocytes were present: April 10, 5.65; April 11, 3.35; April 13, 3.24; April 15, 4.83; April 17, 3.3; April 20, 5.05; April 23, 4.9; April 25, 4.6; April 27, 5.3; April 30, 7; May 4, 6.38; May 7, 6.03; May 11, 4.23; May 15, 4.5. The number of leukocytes varied between 6,200 and 10,600.

In thirty-four differential counts made on the blood of normal adults the average percentage of endothelial leukocytes was found to be 5 per cent.; an average of these leukocytes on the seventeen counts made on the four cases of syphilis receiving arsphenamin is 5.3 per cent. The percentages are based on counts of from 1,000 to 2,000 leukocytes. It would seem, therefore, that increases in this variety of cell are not produced by the intravenous injection of arsphenamin.

CONCLUSIONS

The endothelial leukocytes of the peripheral blood can be stained differentially. That the stain is specific is shown by staining blood in which this variety of leukocyte is characterized by ingested carbon.

EXPLANATION OF PLATE

Drawn with camera lucida and oil-immersion objective.

A, B, and C are cell groups from normal blood stained with leukocytic granule stain: A, endothelial leukocyte, neutrophil and erythrocyte; B, endothelial leukocyte, neutrophil and lymphocyte. If it were not for the distinct blue granules in the endothelial leukocyte, it would be difficult or impossible to differentiate it from some of the lymphocytes; C, endothelial leukocyte, erythrocyte, and eosinophil. Note the ringlike appearance of the eosinophilic granules.

D, E, and F are cell groups from normal blood incubated at 37.5 C. with a carbon suspension; films stained with leukocytic granule stain: D, lymphocyte, erythrocyte and endothelial leukocyte containing carbon; E, erythrocyte, neutrophil, and endothelial leukocyte containing particles of carbon; F, erythrocyte, lymphocyte, and endothelial leukocyte containing numerous carbon particles that have been ingested by phagocytosis.