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A new method for counting the reticulocyte number

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A new method for counting the reticulocyte number*

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Abstract

The counting of reticulocyte number by the routine method on the dye fillms often leads to a poor result. This can be avoided by counting them on the collodion dye film on which the almost equal distribution of reticulocytes can be attained.

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A NEW METHOD FOR COUNTING THE RETICULOCYTE NUMBER

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In the clinical examination of anemic diseases it is important to count the reticulocyte number in the circulating blood, by which the hematopoietic activity of the bone marrow can be detected in many cases, though when one wants to know the exact hematopoietic activity of the bone marrow, it is necessary to calculate the reticulocyte index proposed by SENO[1]. In general, the counting of reticulocytes is done on the cells stained supravitally on a dye film prepared by smearing the alcohol dye solution. But this method often leads to an error by giving a smaller or a larger number than the actual one. The authors tried to establish a method by which the actual number can be known exactly. In the following a simple and superior method will be described.

METHOD

To begin with 0.1 gram of Nile blue or brilliant cresyl blue is dissolved in 50 cc. of pure ethanol and then an equal volume of amyl-acetate containing 0.1 per cent collodion is added. Using this solution, a dye film is prepared on an object glass by the routine method, smearing and drying. A small droplet of blood placed on a cover slide is sealed on the film. The observation is carried out after five minutes in wet.

Comparison of the reticulocyte number found on the collodion dye film and thal on the general dye film: As the cover slides those of 1.8 cm. in size were used. A small amount of blood taken from the ear vein of an anemic rabbit was put on the center of the cover slide or of the object glass and then sealed on the dye film extending the blood in the whole area of the cover glass giving a slight pressure by the finger tip. The samples in which the blood did not spread to the whole area of the cover slide or the exess of blood was protruding were excluded. Through the centre of the coverslide the counting was made from one side to the other side in straightline in the nine fields selected at a nearly equal distance

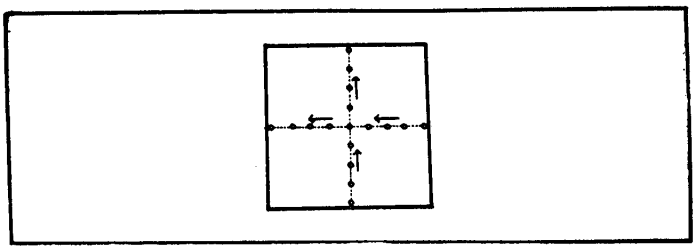

Fig. 1

(Fig. 1). Thus on one slide the counting was made in the fields lying on two straight lines crossing, and these were repeated on the three different slides, six countings in the same blood sample.

As indicated in Figs. 2 a and b, a markedly irregular distribution of reticulocytes is seen by staining on a Nile blue film prepared by smearing alcoholic dye solution, showing a greater number of reticulocytes in the peripheral parts and a less number in the central part. This tendency becomes marked when the blood droplet is put directly on the object glass having dye film. On the other hand, almost an equal number of reticulocytes can be found in each field when reticulocytes are stained on a collodion dye film (Fig. 2 c). Comparing these findings, the true number of

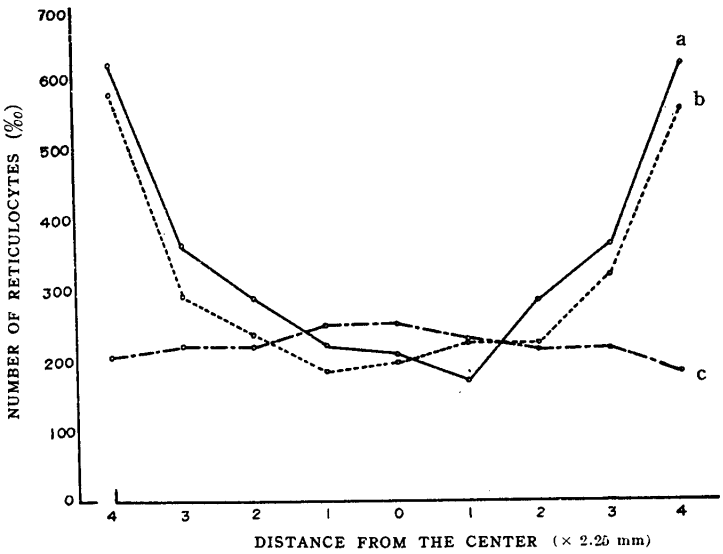

Fig. 2.

reticulocytes will be obtained near the central part when the cells are stained by routine method. The increase in number in the peripheral

part will be due to the reticulocyte-like deformation of the matured cells by an extremely high concentration of dye, where dye accumulates by being pushed aside from the central part when the blood droplet is spread. A relatively lower number in the central part will be due to the low concentration of dye caused by the same mechanism as just described.

In the case of supravital staining on the collodion dye films dye does not move from central part to the periphery and then reticulocytes equally distributed in the whole area. The slightly high distribution in the central part will be due to the higher adhesiveness of the reticulocytes than the matured cells, by which they may not move as rapidly as the matured cells when the blood droplet spreads from the central part to the periphery.

SUMMARY

The counting of reticulocyte number by the routine method on the dye fillms often leads to a poor result. This can be avoided by counting them on the collodion dye film on which the almost equal distribution of reticulocytes can be attained.

REFERENCE

1. SENO, S., KAWAI, K. et al. : Maturation of reticulocytes and related phenomena, III mie med. J. 4. suppl. 1. 19. 1953