A Single Differential Staining Method for Vaginal Smears

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(Plate 1)

Since the observation of STOCKARD and PAPANIKOLAOU (1917) on the cyclic cytological changes of vaginal smears in guineapigs in accordance with their estrous cycle, many morphological studies have been made on vaginal smears of different mammals by many investigators. Recently, the examination of vaginal smears has come to be significant not only in the determination of each stage of the estrous cycle and in sexual hormone tests, but also in the clinical use. As for the staining of vaginal smears, there are many methods using such a single stain as methylene blue and Giemsa stain or such a compound stain as Masson's trichrome and Heidenhain's azan stain. Especially, SHORR (1940a, 1940b, 1941), PAPANIKOLAOU (1942), and CUYLER (1932) have reported a single differential staining method which is generally used for vaginal smears. The principle of this method of staining consits in the demonstration of changes in stainability of epithelial cells by using a staining solution composed of some acidic dyes. For instance, orange G, scarlet red, and fast green are used in Shorr's method, by which cornified cells are stained blilliant orange-red and non-cornified cells blue. By Papanikolaou's method, in which light green, Bismarck brown, and eosin Y are used, acidophilic cells are stained red to orange and basophilic cells green or greenish blue.

The method presented in this paper is essentially the same as the methods of the above-mentioned authors. In this method are used three kinds of acidic dyes which differ in dispersity and color-tone: picric acid, aniline blue, and ponceau 2R. This method is based especially upon the theory of MÖLLENDORFF (1924) and SEKI (1936, 1954) on "the ultrastructural density of tissues and its stainability". It is very simple and brings about a rapid differentiation for vaginal smears. So it will be useful for rapid inspection of vaginal smears.

(I) STAINING METHOD

1. Vaginal smear is sucked up by a small pipette, with a rubber cap, containing a small amount of physiological salt solution which is used for washing the surface of the vaginal mucous membrane. It is placed on a slide glass and spread out thinly with tip of the pipette.

2. The slide, when almost dried, is fixed with 10 per cent formalin solution

for about 10 to 15 minutes.

3. It is rinsed with running tap water for about 10 minutes.

4. The slide glass is pressed lightly with filter paper to remove the excessive water on it and stained with the staining solution mentioned below for about 10 minutes on the thermostat at about 37°C, being wavered occasionally.

The staining solution is prepared as follows: 0.5 per cent aqueous solution of aniline blue, picric acid, and ponceau 2R are mixed at the rate of 1: 3: 3, respectively. Then glacial acetic acid is added to the whole amount of the mixture at the rate of 1.5 per cent. For aniline blue Merck's product is used and for ponceau 2R and picric acid Katayama's products are employed.

It is preferable to use the staining solution a few days after preparation than immediately after preparation. The solution can be preserved for several months when well stoppered.

5. The slide may be rinsed for a very short time. However, rinsing may be skipped. Prolonged washing is undesirable, since a yellow and blue tone of the preparation is removed.

6. It is carried directly into 90 per cent alcohol, dehydrated through absolute alcohol, and made transparent in xylol. Ten dippings may be enough in each of the alcohol.

In preparing a smear slide, it is most important not to allow the smear to dry excessively before fixation, since drying alters the staining properties of the cells contained in the smear. In any case, moreover, drying is undesirable during the whole process of staining. The thickness of the smear has influence also on the stainability of the cells. Therefore, care must be taken of the secretion of the estrous stage which is rich in cell elements and liable to flock into masses. An excellent picture stained is shown in an area which is poor in cell elements in the center of a smeared specimen. When many smears are examined simultaneously on the same slide glass, it is convenient to place one or two drops of fixative, with a pipette, on each of the smears as they are collected to prevent them from excessive drying. Then the slide with smears on it is finally put in the fixing bottle. The specimen must be examined immediately, as it is decolorized gradually.

(II) RESULTS OF STAINING

This study was performed on the vaginal smears of the mouse. When the slide stained by this method is placed on a piece of white paper, it is possible to determine macroscopically from the specific tint of its staining color to what stage of the estrous cycle it belongs. Precise microscopic observation reveals a sharp difference in color tone between cornified and non-cornified cells. In the so-called cornified cells, which are large in size, containig no visible nuclei, the cell body is stained in a yellow tint homogeneously. In the non-cornified cells, which have nuclei, the cytoplasm takes various color tones, such as reddish-yellow, red, reddish-purple, purple, blue and the nuclei are stained yellowish-red slightly, as shown

in the figures attached. Among the other constituents of the smears, the leucocytes are stained blue or, sometimes, reddish-blue and mucus blue.

Fig. 1 shows the vaginal smears of a mouse in the diestrous stage. A large number of leucocytes stained in a blue color and a small number of younger nucleated cells are presented. Either cornified or pre-cornified cells in the process of cornification are hardly found.

Fig. 2 shows a picture of the proestrous stage. Leucocytes are decreased in number and large-sized, nucleated cells stained in various color tones increased in number. A few cornified cells are seen.

Fig. 3 shows a picture of the estrous stage. Only cornified cells stained in a yellow color are presented.

Fig. 4 shows a picture of the metaestrous stage. Leucocytes and nucleated cells in various color tones are seen. A few cornified cells still remain.

In these figures, it should be noted that the cytoplasm is stained yellow in the cornified cell and in various color tones in the non-cornified cell. Those findings indicate that the degree of cornification is different between the two kinds of cells. Such difference in staining color is explained as follows: according to SEKI, picric acid producing a yellow tint is of the highest dispersity, aniline blue producing a blue tint is of the lowest, and ponceau 2R producing a red tint is of moderate dispersity. In accordance with the advance in the process of cornification of cells, the ultrastructure of cell elements becomes dense gradually. Consequently, the denser the ultrastructure of a cell, the more stainable the cell may be with such small-moleculed dye as picric acid. That is, the more cornified cells are present, the more remarkable the yellowish tone is. It has been already confirmed by HINO (1948) in guineapigs with the azan staining method that the ultrastructure of epithelial cells in vaginal smears becomes dense with the progress of cornification.

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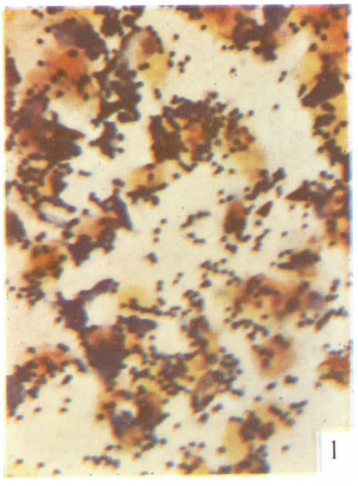
EXPLANATION OF PLATE

Fig. 1. The vaginal smears of a mouse in the diestrous stage. ×150.
Fig. 2. The vaginal smears in the proestrous stage. ×150.
Fig. 3. The vaginal smears in the estrous stage. ×150.
Fig. 4. The vaginal smears in the metaestrous stage. ×150.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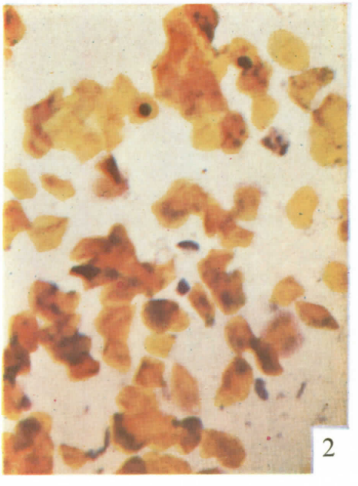
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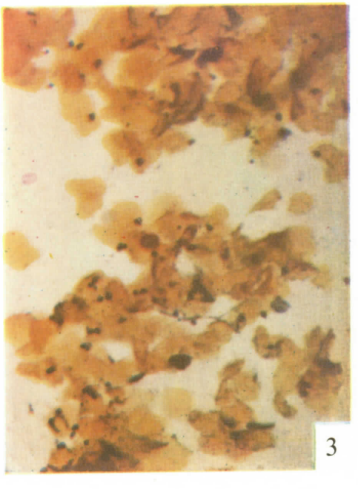
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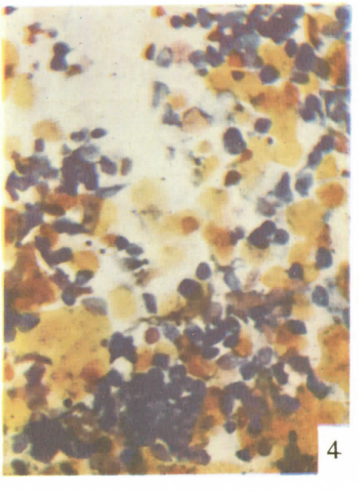
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