DNA CONTENT IN GLANDULAR EPITHELIAL CELLS OF THE ENDOMETRIUM DURING MENSTRUATION

S. Kh. Khakimova, R. N. Stepanova, and L. S. Strochkova

UDC 612.662-08:[612.627.014.3:612.6+612.627.015.3:547.963.32

The DNA content in the cell nuclei of the endometrial glands was measured cytospectrophotometrically in nine women of reproductive age during menstruation. No change was found in the DNA content on different days of menstruation. Both during desquamation (days 1-2 of the cycle) and during regeneration (days 3-4) it corresponded to the standard diploid level. Mitotic activity was determined in 36 samples of endometrium. It was completely absent in the glandular epithelium during menstruation.

KEY WORDS: menstruation; cytospectrophotometry; desquamation and regeneration of the endometrium; DNA content; mitotic activity.

The origin of the epithelial cells lining the uterine cavity after desquamation, despite many years of study, is still far from solution. In the opinion of many investigators during menstruation the functional layer of the endometrium undergoes necrobiosis and desquamation. In the phase of regeneration the cells of the residual glands of the basal layer divide mitotically very quickly and re-epithelize the denuded surface of the mucous membrane [4, 6]. Some workers [7] postulate metaplasia of the stroma into epithelial cells or amitotic division of the glandular cells under these circumstances [11].

It must be pointed out that in attempts to resolve this problem the routine methods of histology have been used, although because of its static nature the morphological picture is inadequate and can give only indirect information on the nature of the processes taking place in the phase of menstruation. The contradictory views which have been expressed on the source of the cells for re-epithelization of the endometrium calls for different methodological approaches in order to find a solution.

EXPERIMENTAL METHOD

Samples of mucous membrane obtained from the uterus of 36 clinically healthy women of reproductive age with a biphasic menstrual cycle were examined. Biopsy was carried out between the 1st and 4th days of the cycle. Strips of endometrium were fixed in Carnoy's fluid, dehydrated, and embedded in paraffin wax. Sections 5μ thick were stained with hematoxylin and light green for investigation of their morphology and to count mitoses. Sections were cut to a thickness of 3μ from nine samples of endometrium on the 1st-3rd days of the cycle and stained by Feulgen's method. Photometry of the cell nuclei of the glandular epithelium was carried out with a single-beam probe cytophotometer at a wavelength of 500 nm. In each section the optical density of at least 50 gland cells and 30 small lympocytes was determined. The area of the same nuclei of the epithelium and lymphocytes was measured with a planimeter. The DNA content was determined as the product of the optical density and the area of cross-section of the cell nucleus and expressed in conventional units. The mean DNA content in the nucleus of a small lymphocyte was taken as the standard DNA content corresponding to a diploid set of chromosomes. The distribution of the cells in the population by DNA content is shown as histograms in Fig. 1. The results were subjected to statistical analysis. The DNA content in cells of the endometrial glands on the 4th day of the menstrual cycle was determined previously [5].

Department of Obstetrics and Gynecology, Faculty of Internal Medicine, Tadjik Medical Institute, Dushanbe. Laboratory of Cytology, Institute of Human Morphology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 86, No. 7, pp. 81-84, July, 1978. Original article submitted January 10, 1978.

 0007-4888/78/8607-0928 $07.50

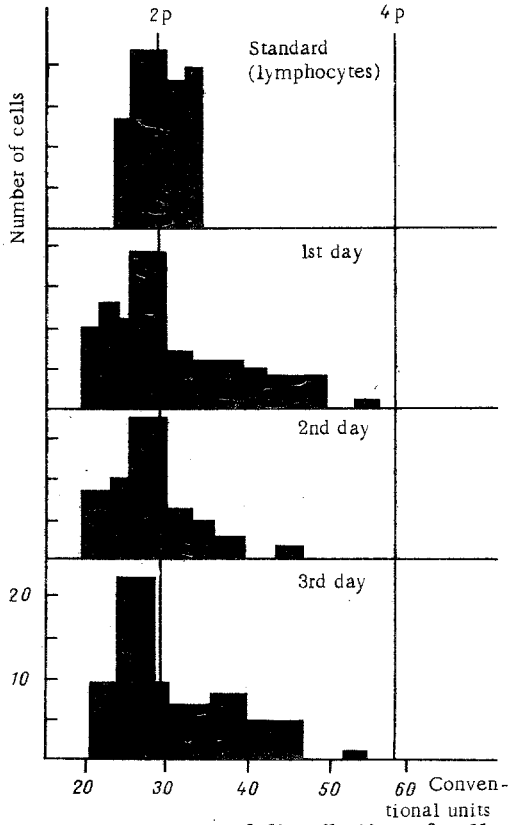

Fig. 1. Histogram of distribution of cell nuclei of endometrial glandular epithelium by DNA content during phase of menstruation.

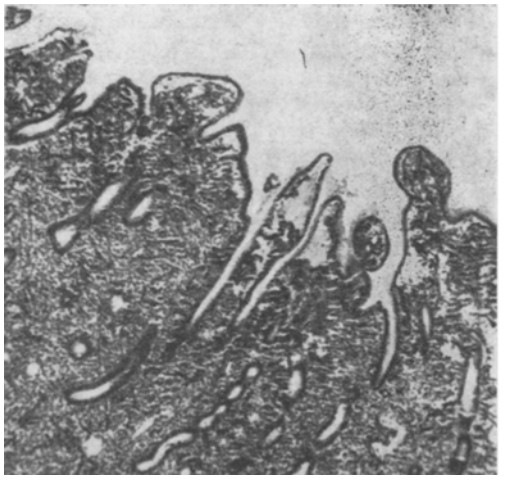

Fig. 2

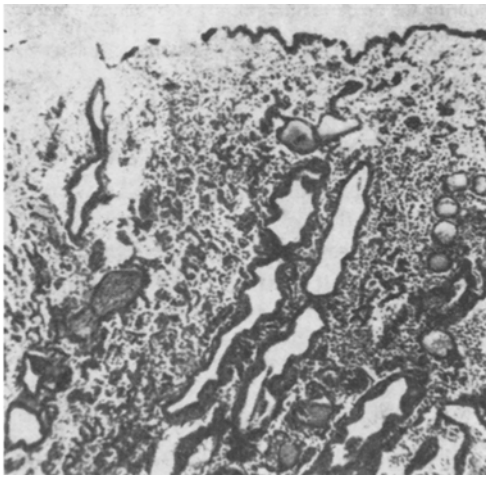

Fig. 3

Fig. 2. Second day of menstruation. Area of endometrium with persistent epithelial cover. Vessels dilated and congested with blood. Glands collapsed. Hematoxylin and light green, 56×.

Fig. 3. Fourth day of menstruation. Surface of endometrium epithelized. Outlines of glands rounded. Stroma compact. Hematoxylin and light green, 56×.

EXPERIMENTAL RESULTS

In some samples of endometrium the loss of tissue after the beginning of menstruation was small, whereas in others destruction and desquamation of parts of the functional layer were more marked. The glands were collapsed. Subepithelial hemorrhages were present and the tissues were infiltrated by polymorphs. On subsequent days the disintegration process was intensified in certain parts of the functional layer. Meanwhile areas of mucous membrane with its structure preserved, and lined with intact epithelium, were constantly found (Fig. 2). No mitoses were seen in the glandular epithelium throughout the phase of menstruation. At the end of regeneration (the 4th day of the cycle) the surface of the mucous membrane was wave-like. Except in small areas it was covered with a layer of epithelium, the cells of which appeared to be "climbing" up the prominences formed by disorganized cellular material and blood at the sites of desquamation (Fig. 3).

During menstruation desquamation of the endometrium is thus focal in character, and even in these areas the desquamation processes do not take place so intensively as is usually considered in accordance with the traditional view. Fragments of the functional layer, lined with a persistent layer of epithelium, always remain intact.

The mean DNA content in the lymphocyte nucleus was found to be 29.26 ± 0.76 conventional unit (c.u.). After the beginning of menstruation the mean DNA content in the nuclei of the glandular cells was 30.46 ± 1.91 c.u. The differences between these values are not statistically significant ($P > 0.05$). The modal class of the distribution was formed by diploid cells (52.34%). Of the total population, 17.76% of cells had an intermediate DNA level and only 7.47% were tetraploid. Despite the presence of cells with premitotic nuclei, cell division was absent in the epithelium. The mean DNA content in the cell nuclei of the glands on the 2nd and 3rd days of menstruation was 27.88 ± 0.56 and 29.42 ± 1.94 conventional units respectively; in every case the difference is not significant ($P > 0.2$). Just as on the 1st day of menstruation, the distribution was dominated by the class of diploid cells (55.88 and 66.67% respectively on the 2nd and 3rd days of the cycle), there were 16.18 and 19.23% of intermediate cell nuclei, and only single cells with premitotic levels of DNA (Fig. 1). At the end of menstruation (the 4th day of the cycle) the DNA content in the nuclei of the glandular cells also corresponded to the diploid standard. The distribution was dominated by diploid cells (57.78%), there were 22.22% of cells with an intermediate DNA level, and only single tetraploid cells.

Both in the phase of desquamation (the 1st and 2nd days of menstruation) and during regeneration (the 3rd and 4th days) from 11.54 to 26.47% of hypodiploid cells (cells with a DNA content below 2p) were found; this result was evidently explained by imperfections of the method.

In the phase of menstruation, cells of the glandular epithelium of the endometrium are thus distributed by DNA content between 2p and 4p, and invariably subpopulations of cells with intermediate and tetraploid nuclei are present; most cells accumulate in the region of the diploid mode, and cells with a premitotic DNA content account for only 1.67-7.47% of the total population studied.

Before starting mitosis the cell must have at least a tetraploid DNA content. The results show that in the phases of desquamation and regeneration the dominant class of cells in the glandular epithelium of the endometrium consists of diploid cells; cells with a premitotic DNA content are exceptional. Furthermore, both during desquamation and during regeneration cell division is completely inhibited in the glands of the mucous membrane. Other workers [10, 15] also found that diploid cells are predominant in the glandular epithelium of the endometrium during menstruation and that tetraploid cells are almost completely absent. Because of this, during regeneration intensive mitotic division of the glandular cells does not take place. Neither of these facts – the rapid epithelization and total absence of mitosis at that time – can be easily explained in terms of the generally accepted view.

Attempts have been made to surmount this difficulty by postulating the presence of amitotic division of the glandular cells during regeneration [11]. However, the only possible fully adequate form of cell division in mammals is recognized to be division with a typical mitotic cycle [2, 3]. The possibility of metaplastic transformation of stromal cells into epithelial cells has also been suggested on the basis of the common mesodermal origin of both types [7, 12]. It is difficult to agree with this if one takes into account determination and the differentiated state of the tissues in the adult.

The opinion is held [8, 14] that after desquamation fully competent epithelial cells persist and, during regeneration, they creep over the defects in the epithelial lining and cover them. Persistence of the epithelial cover during desquamation of the endometrium in women has been described by several authors [1, 9, 13].

The present investigation showed that during menstruation the destruction and desquamation of the endometrium are focal in character, and under these circumstances fragments of the functional layer lined by preserved epithelium always remain. Most cells of the residual glands are diploid and, for that reason, cannot divide by mitosis; consequently, they cannot take part in the re-epithelization of the raw surface of the uterine mucous membrane during menstruation.

LITERATURE CITED

1. E. Bumm, Textbook of Obstetrics [in Russian], St. Petersburg and Kiev (1910).
2. A. A. Zavarzin, DNA Synthesis and Kinetics of Cell Populations in Mammalian Ontogeny [in Russian], Leningrad (1967).
3. H. Oberling and W. Bernhard, in: Functional Morphology of the Cell [Russian translation], Moscow (1963), p. 316.
4. E. N. Petrova, Histological Diagnosis of Diseases of the Uterus [in Russian], Moscow (1964).
5. R. N. Stepanova, "The DNA content and mitotic activity of endometrial cells of women in the menstrual cycle," Candidate's Dissertation, Dushanbe (1975).
6. R. Schróder, The Normal Menstrual Cycle of the Uterine Mucous Membrane [Russian translation], Leningrad (1938).
7. M. C. Baggish, S. I. Pauerstein, and J. D. Woodruff, Am. J. Obstet. Gynec., 99, 459 (1967).
8. A. David, A. Kaplun, and D. Serr, Am. J. Obstet. Gynec., 117, 473 (1973).
9. A. Ferenczy, Am. J. Obstet. Gynec., 124, 64 (1976).
10. E. Johannisson and K. Hagenfeldt, Acta Endocrinol. (Copenhagen), 67, Suppl. 153, 81 (1971).
11. E. Novac and E. R. Novac, Gynecologic and Obstetric Pathology, Philadelphia (1958).
12. C. N. Papanicolaou, Am. J. Obstet. Gynec., 25, 30 (1933).
13. J. Rock, C. Garcia, and M. Menkin, Ann. New York Acad. Sci., 75, 831 (1959).
14. J. G. Schenker, M. Saks, and W. Polishuk, Am. J. Obstet. Gynec., 11, 970 (1971).
15. D. Wagner, R. Richart, and J. Terner, Am. J. Obstet. Gynec., 100, 90 (1968).

ACTION OF ALLOGENEIC SERUM FROM A PREGNANT PNEUMONECTOMIZED RAT ON EMBRYONIC LUNG TISSUE

I. I. Orlova, N. G. Lisatova, and S. D. Mikhal'chenko

UDC 591.3.591.39

Allogeneic blood serum from a rat undergoing unilateral pneumonectomy during pregnancy was shown to stimulate growth of a primary monolayer culture of embryonic Wistar rat lung, directed chiefly toward fibroblasts. This effect was manifested most clearly on the 3rd day of culture.

KEY WORDS: allogeneic serum; primary monolayer culture; pneumonectomy.

Much clinical and experimental evidence has now been obtained to show that injury to an organ of a pregnant animal affects the development of the homonymous organ of its fetus [1, 3, 5-9, 11, 13]. Murashova [10] showed that unilateral pneumonectomy on a pregnant rat leads to the more rapid development of the lungs in its fetuses. Under these circumstances a specific growth-stimulating factor appears in the blood and reaches the homonymous fetal organ by the humoral route. During postnatal development the progeny of such an animal develops a state of inborn predisposition to diseases of the corresponding organs and systems and they constitute a group of increased risk [4, 14].

Laboratory of Immunoembryology, Institute of Human Morphology, Moscow. Department of Biology, Moscow Medical Stomatologic Institute.

(Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 86, No. 7, pp. 84-87, July, 1978. Original article submitted December 29, 1977.