DNA CONTENT IN EPITHELIAL NUCLEI OF ENDOMETRIAL GLANDS IN MENOPAUSAL DYSHORMONAL HYPERPLASIAS

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The content of nuclear DNA in the epithelium of the endometrial glands of six healthy women of childbearing age and 10 women with menopausal dyshormonal glandular-cystic hyperplasia was investigated microspectrophotometrically. The DNA content in the normal uterine mucosa in the various phases of the menstrual cycle was unchanged, but in dyshormonal hyperplasia of the endometrium the DNA content was almost twice the normal value; a moderate increase in polyploidy also was observed.
KEY WORDS: menstrual cycle; endometrial hyperplasia; microspectrophotometry; DNA content.

An important aspect of the study of the pathogenesis of dysfunctional uterine bleeding due to dyshormonal endometrial hyperplasia is the assessment of the state of metabolism by histochemical methods. Determination of the dynamics of the DNA content in the cell nuclei of the uterine mucosa is interesting for two reasons. First, two fundamental processes requiring intensive protein synthesis take place in the endometrium, representing alternating phases of the menstrual cycle: proliferation and secretion. Second, the endometrium is a unique target tissue in which processes of cell growth and differentiation are controlled by sex hormones.

Histochemical investigation of the DNA content in the cell nuclei of the endometrial glands of women with a normal menstrual cycle and in certain pathological states has been carried out by a method of visual assessment of the intensity of the Feulgen reaction [4-6, 10] and also by the use of quantitative techniques [3, 7, 9]. However, the results of these investigations are heterogeneous and contradictory.

The object of this investigation was a microspectrophotometric study of the content of nuclear DNA in the glandular epithelium of the endometrium in individual phases of the normal menstrual cycle and in menopausal dyshormonal hyperplasias.

EXPERIMENTAL METHOD

The endometrium obtained by diagnostic or therapeutic curettage of the uterine cavity of 10 women aged from 45 to 52 years, suffering from dysfunctional uterine bleeding, was used (glandular-cystic hyperplasia of the endometrium was found histologically). The control group consisted of six women of reproductive age with a normal menstrual cycle (in three women a phase of proliferation and in the other three a phase of secretion was determined histologically).

The specific optical density of the nuclei was determined by means of an integrating scanning microspectrophotometer [1] at a wavelength of 575 nm (area of beam applied 0.25 μ^2) in sections 5 μ thick stained in batches by the Feulgen method. In each section the concentration of nuclear DNA was determined in 30 cell nuclei from similar glands. The area of 30 nuclei of the glandular epithelium was determined in the same regions by means of a micrometer. The product of the specific optical density and the area of the section was an index of the mean DNA content per nucleus. The DNA content in 30 nuclei of small lymphocytes was deter-

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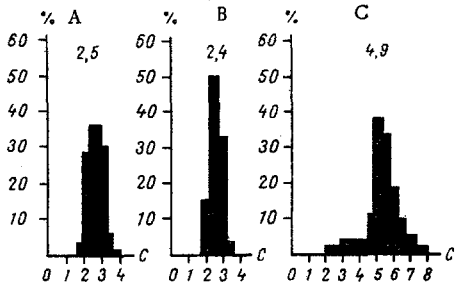

Fig. 1. Histograms of DNA content (in ploidy units) in epithelial nuclei of endometrial glands. Ordinate, number of cells (in %); abscissa, ploidy value (c). a) Phase of proliferation; b) phase of secretion; c) dyshormonal endometrial hyperplasias. Numbers above columns indicate DNA accumulation index.

mined in the same sections and the mean DNA content per nucleus was taken as the standard for its content in the nucleus of a diploid cell (2c). This was used for calculating the DNA content in the cell nuclei of the endometrial glands in ploidy units. To estimate the total DNA content, a generalized index of the kinetics of the DNA content was used, namely the DNA accumulation index (in ploidy units) [2], which is a weighted arithmetic mean. The experimental results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

Microspectrophotometric examination showed that the mean DNA content per nucleus of a small lymphocyte is 6.10 ± 0.25.

The mean DNA concentration in the nuclei of the glandular epithelium in the proliferative phase was 0.314 ± 0.006; the mean area of the nuclei for this group was 24.0 ± 0.33 μ^2, and the DNA content per nucleus was 7.1 ± 0.15.

The mean DNA concentration in the nucleus of an epithelial cell in the secreting endometrium was 0.212 ± 0.006, i.e., significantly less than in the phase of proliferation ($t > 3$). The mean area of cross section of the nuclei for the secreting endometrium was 32.0 ± 0.29 μ^2 (1.3 times greater than for the proliferating endometrium) and the mean DNA content per nucleus was 6.98 ± 0.20.

Both in the proliferating and in the secreting endometrium the modal class of the distribution was formed by paradiploid cells (85%), and the number of paratetraploid cells was small (Fig. 1). The DNA accumulation index for the cell nuclei of the endometrial glands in the phase of proliferation was 2.5 and in the phase of secretion 2.4.

For the normal menstrual cycle the mean DNA content per nucleus of the glandular epithelium is thus maximal in the phase of proliferation and much lower (one third lower) in the phase of secretion. However, the mean DNA content per nucleus does not change significantly during the menstrual cycle, on account of the increase in volume of the nuclei of the glandular epithelium during secretory transformation of the uterine mucosa. The increase in volume of the nuclei while the DNA content remains constant is probably linked with the accumulation of other karyoplasmic proteins in the nuclei in connection with increased secretion. These results are in agreement with Khesin's view [8] that the increase in volume of the nuclei is connected with their functional swelling and is a reliable criterion of increased function of the cells, i.e., activation of their specific activity.

In dyshormonal glandular-cystic hyperplasias of the endometrium the mean DNA concentration in the nuclei of the glandular epithelium was comparatively high (0.614 ± 0.018); considerable variability of this index was observed in individual observations (from 0.300 ± 0.02 to 0.903 ± 0.016). Endometrial hyperplasia also was characterized by a high degree of variation of the index of the area of cross section of the nuclei (from 27.7 ± 0.32 to 46.6 ± 0.41 μ^2). However, the mean group index for the area of the nuclei in endometrial hyperplasia did not differ significantly from that in the secreting endometrium (32.8 ± 0.44 and 32.0 ± 0.29 μ^2, respectively).

The DNA content also varied considerably in individual observations (from 11.5 ± 0.25 to 30.7 ± 0.20), and for the group as a whole it was 20.1 ± 0.30, i.e., almost three times greater than the corresponding index for the endometrium in the phase of proliferation and secretion during the normal menstrual cycle.

The distribution of the cells by DNA content (in ploidy units) showed predominance of paratetraploid cells (70%), and the number of cells with a paradiploid DNA content was much smaller than normally (10%); a considerable number of paraoctaploid cells also appeared (20%). The DNA accumulation index in the nuclei of the hyperplastic endometrium was increased to 4.9.

In dyshormonal glandular-cystic hyperplasia of the endometrium a high DNA concentration and an increase in volume of the nuclei of the glandular epithelium were discovered. The area of cross section of the nuclei was greater than in the endometrium in the phase of proliferation but it was not greater than that in the secreting mucosa. The nuclear DNA content was considerably higher than in the normal endometrium, as a result of an increase both in the DNA concentration and in the nuclear volume. Endometrial hyperplasia also was characterized by an increase in heterogeneity of the epithelium of the glands, evidence of which was given by the predominance of paratetraploid cells, the appearance of paraoctaploid cells, and the increase in the DNA accumulation index. The results point to activation of the genetic material in the cells and to a moderate increase in polyploidy, evidence of an increase in clonal instability in the glandular tissue. The doubling of the DNA accumulation index in dyshormonal hyperplasias in some cases may be the basis for further transformation of the endometrium and its malignant change [2].

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