EFFECT OF ESTROGEN LEVEL IN THE BODY ON STAINING PROPERTIES OF NUCLEIC ACIDS OF THE UTERINE EPITHELIUM

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By the use of the basic dyes methyl green and pyronine by Kurnik's method, changes can be observed in the staining properties of the nuclei in the uterine epithelium of normal sexually mature mice in the course of the sex cycle, and also in the epithelium of ovariectomized mice and of mice injected with dihydrostilbestrol.

It was shown that DNA in the epithelium lining the uterine cavity and uterine glands is labile, its affinity for methyl green and pyronine depends on the level of estrogens circulating in the body, and a definite optimum for the concentration and duration of action of estrogens exists. Deviation from this optimum reduces affinity for methyl green and produces pyroninophilia of the nuclei.

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In sexually mature female rodents the epithelium lining the uterine cavity undergoes changes throughout the estrous cycle: its height, release, and mitotic activity are modified. The latter is characterized by a bimodal curve [1-3, 8]: the first rise coincides with proestrus, the second with the beginning of diestrus. Periods of increase in number of dividing cells are preceded by periods of increase in the number of cells synthesizing DNA [12, 14, 21, 24], and by its conversion into a high-polymer state [11, 12, 15].

In 1947, Kurnik [16] suggested a method of detecting nucleic acids in high- and low-polymer states by means of basic dyes, methyl green and pyronine.

It has been shown experimentally [4-6, 9, 16-20] that affinity for methyl green is possessed only by high-polymer DNA, while low-polymer DNA is stained by pyronine. A decrease in adsorption of methyl green and manifestation of pyroninophilia in the cell nucleus are associated with changes in the state of the DNA, namely with its depolymerization and weakening of the bond with protein in nucleoproteins [4].

By using Kurnik's method in the investigation described below, we attempted to discover whether changes take place in the degree of polymerization of DNA in nuclei of the uterine epithelium during DNA synthesis as the cells prepared to divide in various stages of the estrous cycle, and also after ovariectomy and administration of various doses of dihydrostilbestrol.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred female albino mice weighing 20-25 g. Altogether 119 animals were used in the experimental series (17 groups each containing 6-10 animals) and 40 females were used in five control groups. The control animals were sacrified in the period of diestrus, on the 1st, 2nd, and 3rd days of estrus and the experimental animals on the 10th-20th day and 3.5-6 months after castration and also after 1, 2, 3, 5, 6, and 7 injections of dihydrostilbestrol (given once daily starting from the 15th, 17th, or 20th day after castration, in doses of 0.8 mg as a 2% oily solution). The animals were always sacrificed at 11-12 A.M. The uterus was quickly removed and fixed with Carnoy's fluid without subsequent treatment in 70% and 96% alcohol. The material was embedded in paraffin wax. Nucleic acids were detected by the methods of Feulgen, Unna-Pappenheim, Brachet, and Kurnik. Sections from control and experimental blocks were mounted on the same slide, so that they could be stained under identical conditions.

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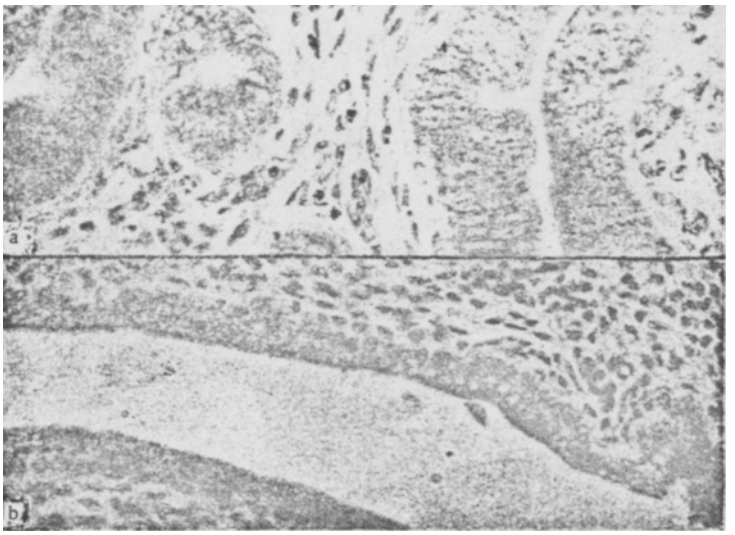

Fig. 1. Changes in height and relief of uterine epithelium in mice. a) Uterine epithelium in proestrus; b) uterine epithelium on 10th day after castration. Fixation by Carnoy's method, staining by Brachet's method, 10 × 40.

EXPERIMENTAL RESULTS

Histochemical study of the uterine sections showed that throughout the estrous cycle, starting from proestrus, the height of the uterine epithelium gradually diminished and affinity for basic dyes, methyl green and pyronine, of the nuclei in the epithelium lining the uterine cavity and the uterine glands was modified. This was probably due, on the one hand, to disturbance of the water and salt balance of the cell [23], and on the other hand to a decrease in synthesis of biopolymers [13, 14]. The nuclei of the epithelial cells became denser and changed in color: from green or bluish green in proestrus to azure (during the 1st and 2nd days of estrus), to azure-violet (the 3rd day of estrus) to violet (in the uterine epithelium of ovariectomized mice). The relief of the epithelium was smoothed out, the number of uterine glands was reduced, the height of the epithelium decreased by two-thirds, and the nuclei showed affinity for pyronine at all periods after castration (Fig. 1).

After castration the epithelium lining the uterine cavity lost most of its RNA but much of it remained in the epithelium of the uterine glands. A control with ribonuclease showed that the violet color of the nuclei was not due to an increase in content of nuclear RNA, because after prolonged treatment with the enzyme the initial color of the nuclei was not restored. This was also observed after control treatment of preparations obtained from castrated females. It is difficult to imagine that pyroninophilia of the nuclei was the result of staining newly formed protein of the chromosomes [12, 15], because staining of the nuclei with pyronine was observed in those stages of the cycle when synthetic processes in the uterine epithelial cells are sharply inhibited (3rd day of estrus, diestrus). Synthetic processes were depressed to an even greater degree in the castrated animals, yet at the same time the pyroninophilia of the nuclei of their uterine epithelium was not marked. The likeliest explanation is that pyroninophilia in the presence of hyposecretion and castration indicates changes in the staining properties of the nuclei or of their DNA.

Administration of dihydrostilbestrol to castrated females evidently restored normal cell metabolism (soon after the 1st injection). The nuclei increased in size and resumed their elongated shape characteristic of nuclei of the epithelium lining the uterine cavity, while the mitotic activity of the epithelium increased sharply [3, 7, 8].

Redistribution of RNA was observed in the epithelial cells: in control and ovariectomized females RNA was always found in the apical portion of the cell as a bright red border, while in ovariectomized females receiving one or two injections of dihydrostilbestrol, RNA was distributed uniformly throughout the cytoplasm as bright red filaments displacing the nucleus toward the center of the cell (Fig. 2A). Inhibition of cell division developing after the 3rd injection of dihydrostilbestrol was accompanied by a de-

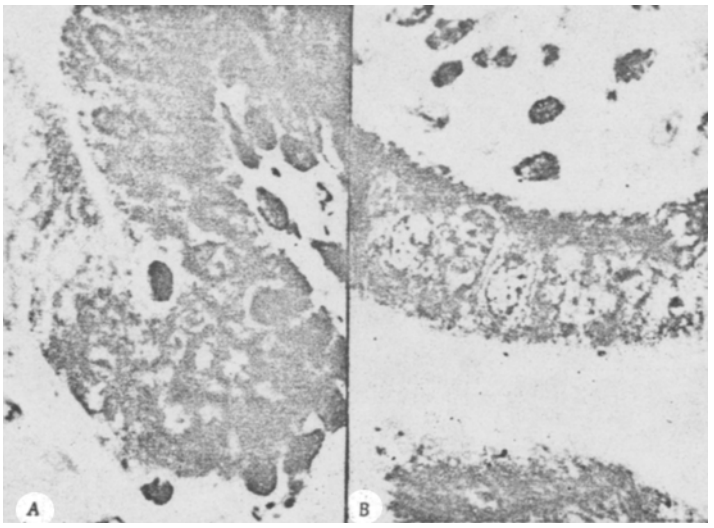

Fig. 2. Distribution of RNA in uterine epithelium of mice. A) Uterine epithelium of mouse receiving one injection of dihydrostilbestrol on 15th day after castration; RNA distributed uniformly throughout cytoplasm; B) uterine epithelium of mouse receiving three injections of dihydrostilbestrol; RNA displaced into basal part of cells, nuclei displaced toward center. Fixation by Carnoy's method, staining by Brachet's method, 10 × 100.

crease in the intensity of staining of the nuclei both by Feulgen's method and with methyl green. RNA was displaced into the basal portion of the cell (Fig. 2B).

The changes in staining properties of the nuclei described above were found in all cells of the epithelium and not merely in some of them.

Periodic changes in the epithelium of sexually mature mice, characterized by changes in mitotic activity, are therefore accompanied by changes in the affinity of nuclear DNA for methyl green: in proestrus the DNA has an affinity for methyl green, while in estrus and diestrus the DNA is pyroninophilic.

In castrated females a decrease in mitotic index is accompanied by an increase in affinity for pyronine. Consequently, affinity of DNA in the uterine epithelium for methyl green depends on the body level of estrogens, a definite optimum being found in the concentration and duration of action of estrogens. A deviation toward either side from this optimum lowers the affinity of the nucleus for methyl green and causes it to exhibit pyroninophilia.

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