EFFECT OF POSTOVULATORY OESTROGENS ON THE FINE STRUCTURE OF THE EPITHELIAL CELL IN HUMAN ENDOMETRIUM

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(Received 6th March 1973)

Summary. Treatment of secretory human endometrium with oestrogens, both *in vivo* and *in vitro*, results in a disruption of the nucleolar channel system, an organelle typically found in the epithelial cell of normal human endometrium during the postovulatory phase. The most striking effect is the loss of dense particles in immediate proximity to the channels, and the disappearance of particulate masses normally found in the nucleoplasm surrounding the channel system. Other fine structural elements, such as giant mitochondria and glycogen, are unaffected except that less glycogen migrates to the apex of the cells.

The endometrial epithelial cell of the secretory phase of the human menstrual cycle is characterized by synthesis and storage of glycogen and differentiation of the mitochondria and nucleoli. The mitochondria at the base of the cell become greatly enlarged and the nucleolar channel system develops (Dubrauszy & Pohlmann, 1960a, b; Dubrauszy & Pohlmann, 1961; Clyman, 1963a; Terzakis, 1965). This organelle has no counterpart in other mammalian endometria or in other human tissues (Terzakis, 1965). Because the principal circulating hormone following ovulation is progesterone, a combined organ culture and electron microscopic study was undertaken to see whether progesterone can differentiate proliferative or preovulatory endometrium *in vitro* (Kohorn, Rice & Gordon, 1970). The conversion of proliferative to secretory epithelium was shown to be in direct response to progesterone stimulus as monitored by glycogen production, enlargement of the mitochondria and maturation of nucleolar channel systems (Kohorn, Rice, Hemperly & Gordon, 1972). In two patients, who had normal menses but complained of infertility, nucleolar channel systems could not be detected in biopsies examined in the latter half of the cycle, although the glycogen content and mitochondria were normal. This finding suggested that nucleolar differentiation might be a prerequisite for implantation (Kohorn *et al.*, 1972).

Post-coital oestrogen administration has been shown to be effective in interrupting pregnancy (Chang & Yanagimachi, 1965; Morris & vanWagenen, 1966; Morris, vanWagenen, McCann & Jacob, 1967; Jacob & Morris, 1969)

if given 3 days after ovulation for a period of 5 days. The mechanism of action of oestrogen in preventing implantation is unknown. Since the timing of administration of oestrogen therapy coincides with the development of nucleolar channel systems, the present study was undertaken to evaluate the effects of postovulatory oestrogen on the fine structure of the endometrial epithelial cell, in particular the nucleolus. Both *in-vivo* specimens and endometrial tissue exposed to different levels of oestradiol in organ culture were examined.

Three normally menstruating volunteers provided records of basal body temperature as an index of ovulation. Endometrial biopsies were obtained at 3 days after ovulation for several cycles to establish that the volunteers produced normal secretory epithelia. In a subsequent cycle, a control biopsy was taken on the 3rd day after ovulation and 50 mg diethylstilboestrol phosphate was then administered daily for 5 consecutive days.

For electron microscopic examination, tissue was fixed in 3% glutaraldehyde in 0.05 M-phosphate buffer, pH 7.2, at room temperature for 1 hr. It was washed in the same buffer, re-fixed in osmium tetroxide, dehydrated in ethanol and embedded in Epon 812. Thin sections were stained with lead citrate and uranyl acetate and examined in a Hitachi HU-12 electron microscope.

The epithelial cells, before oestrogen administration, demonstrated well-developed nucleolar channel systems (Pl. 1, Fig. 1). The organelle consists of a labyrinth of channels, each surrounded by a dense laminar layer. Around the periphery of the lamina, electron-dense ribosomal-like particles are uniformly arranged. In addition, these nucleoli are associated with an external diffuse mass of dense particles. The core of the nucleolus contains an evenly distributed population of fine filamentous material (Terzakis, 1965; Kohorn *et al.*, 1972). Other features of the cells, glycogen and giant mitochondria, were also normal. After 5 days of diethylstilboestrol treatment, there was a marked alteration in the nucleolar channel systems (Pl. 1, Fig. 2). The most notable change was a depletion of dense particles, both those in direct proximity to the channels and the less organized peripheral mass. The number of fine filaments in the internal core was diminished, leaving a small central remnant in some cases (Pl. 1, Fig. 2). The zone of nucleoplasm around these nucleoli was relatively free of formed elements. In some channel systems, breakdown of the channels themselves was noted (Pl. 1, Fig. 3), accompanied by overall distor-

EXPLANATION OF PLATE 1

FIG. 1. The nucleolar channel system in the epithelial cell of a human endometrial biopsy taken on the 3rd day after ovulation, before administration of diethylstilboestrol. The structure consists of channels surrounded by a dense layer. Around the periphery, electron-dense particles (white pointer) are symmetrically arranged. More diffuse masses (m) of particles with less opacity are associated with the nucleolus. In the centre is a pocket of fine filaments (F). $\times 45,000$.

FIG. 2. The nucleolar channel system from a human endometrial biopsy after 5 days of diethylstilboestrol administration. The dense particles are absent. The internal core of filaments (black pointer) is disappearing. The nucleolus is surrounded by a zone of nucleoplasm (arrow) relatively free of formed elements. $\times 45,000$.

FIG. 3. The nucleolar channel system from a human endometrial biopsy after 5 days of diethylstilboestrol treatment. The organelle is distorted and shows disintegration of the channels (pointer). This nucleolus may be at a later stage of disintegration than the one in Fig. 2. $\times 45,000$.

PLATE I

(Facing p. 376)

tion of the structure. Tissue which contained these disintegrating nucleoli did not contain normal nucleolar channel systems in any of the cells examined.

After obtaining the above results, further biopsies were taken from one volunteer in another cycle on the 3rd day after ovulation, 12 hr after the first dose of oestrogen and then serially every 12 hr for a total of five samples. It was hoped that these specimens would make it possible to monitor the changes observed in the nucleoli from the onset of oestrogen therapy to the stages seen in Pl. 1, Figs 2 and 3. The control specimen contained entirely normal nucleolar channel systems. At 12 hr after oestrogen administration, however, nucleoli were present which could not be distinguished from those observed after 5 days of oestrogen therapy. It is, therefore, apparent that the profound changes observed in these nucleoli after prolonged exposure to oestrogen occur rapidly, at least as early as 12 hr, and perhaps earlier. Biopsies from endometria with established nucleolar channel systems at 3 days after ovulation were cultured (Kohorn *et al.*, 1972) in media containing 10 μ g progesterone/ml and 10, 50 or 100 μ g oestradiol/ml. In an attempt to follow the stages of disintegration of the channel systems *in vitro*, tissue was removed from culture at 3, 6, 9 and 12 hr and processed for electron microscopy. In all the cultures containing 10 or 50 μ g oestradiol/ml, normal nucleolar channel systems were seen. With 100 μ g oestradiol/ml in the medium, however, the channel systems underwent identical changes to those seen in the *in-vivo* specimens. This was observed as early as 3 hr in culture and no progression in stages of nucleolar morphological modifications could be followed. There appeared to be no differences due to time of exposure to oestradiol. Although it is not possible to evaluate the precise concentration of hormone directly affecting cells in organ culture, as in monolayer cell cultures, the results are nonetheless instructive since they demonstrate that the nucleolar channel systems can be maintained *in vitro* with steroids and that changes with high doses of oestrogen are similar to those produced *in vivo*. Further, the effect on the channel systems is immediate and, as *in vivo*, does not require prolonged exposure to oestrogens.

Shortly after ovulation, secretory endometrium exhibits large mitochondria, deposits of glycogen at the base of the cells and nucleolar channel systems (Kohorn *et al.*, 1972). As the secretory phase of the cycle progresses, the glycogen migrates to the apex of the cells. Finally, before menstruation, nucleolar channel systems are absent. They were not seen in specimens examined on Day 26 of a 28-day cycle and in some cases had disappeared as early as the 23rd day (Kohorn *et al.*, 1972). It is apparent that they have a short life-span and may disappear rapidly and simultaneously. Since stages of disintegration of nucleolar channel systems were not observed (Kohorn *et al.*, 1972), it is not possible to evaluate the nucleolar changes seen after high doses of oestrogen in terms of normal nucleolar disappearance. It seems a reasonable assumption, however, that oestrogen has a specific effect on the channel systems.

The other distinctive features of secretory epithelial cells, giant mitochondria and glycogen production, were not affected by oestrogen in either *in-vivo* or *in-vitro* tissue samples. These results confirmed previous work which demonstrated that there appeared to be no direct correlation between glycogen synthesis, giant mitochondria and differentiation of nucleoli (Kohorn *et al.*,

1970). Nucleolar channel systems were also not differentiated under the influence of the 19-nor steroids (Clyman, 1963b; Kohorn *et al.*, 1972) although the other features of secretory epithelia were normal. As assessed by light microscopic examination (Morris & vanWagenen, 1966), glycogen did not migrate from the base of the cell to the apex in secretory endometrium exposed to oestrogen. In the present study, glycogen was dispersed throughout the apex of the treated cells, although the accumulation was not as marked as in the untreated cells.

It appears that oestrogens have an immediate and striking effect on the integrity of the nucleolar channel systems. The effect does not appear to be due to alterations in circulating progesterone because plasma progesterone does not diminish until 3 days after the onset of stilboestrol administration (Gore, Caldwell & Speroff, 1973). Since high doses of oestrogen prevent implantation, these results as well as other evidence (Clyman, 1963b; Kohorn *et al.*, 1972) suggest a correlation between implantation and the nucleolar channel system.

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