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Synthesis of a mucous glycoprotein in the human uterus

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Scrapings of endometrium and uterine contents of 10 women were analysed. In the uterine lumina of two women that used synthetic progestagens, a considerable amount of mucus was present. We fractionated the mucus by CsCl density equilibrium centrifugation into glycoprotein and protein fractions. With sugar and amino acid analysis the glycoprotein could be classified as a typical epithelial glycoprotein, resembling the cervical glycoprotein. It contains neuraminic acid (6.2%) and sulfate (8.4%). From the uteri of the other 8 women, who did not use hormones, a small amount of a similar glycoprotein could be isolated.

mucus; endometrium

Introduction

Many histological and histochemical studies have been done on human endometrium (reviewed by Gordon, 1974), but biochemical information about specific secretory proteins and/or glycoproteins is scarce. Uterine secretions are important because of their role in the capacitation of spermatozoa and the nidation of the blastocyst, and by their possible contribution to the cervical mucus. It is supposed that synthesis and secretion of uterine products begin during the estrogen priming of the uterus and culminate around ovulation in the production of a serous fluid, the uterine milk (Datnow, 1973). Sengel and Stoebner (1970) showed that periodic acid schiff (PAS) stainable material is present in the glandular lumina during the luteal phase; the secretory products seem to form a gel-like mass that occludes the endometrial glands. The quantitative contribution of these uterine products to the cervical mucus is unknown. Datnow (1973) supposed that the cyclic variations in cervical mucus are largely due to variations in this contribution.

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Biochemical data on the secretory products of the uterus are hardly available. Moricard (1966) supposed sulfated mucopolysaccharides, while according to Lambaradios et al. (1976) glycoproteins containing glucosamine are secreted. Modulation of a number of proteins by estrogen has been reported by Skipper et al. (1980). The nature of the glycoproteins and mucopolysaccharides in the uterus is unknown. The results of Moricard and Lambaradios et al. can be explained by the production in the uterus of an epithelial mucous glycoprotein. This type of glycoprotein contains glucosamine and often sulfate (see the review by Clamp et al., 1978). In this article we demonstrate the presence of such a glycoprotein in the uterus, under various hormonal conditions.

Material and methods

Uterine contents and endometrium

Uterine contents and parts of endometrium were obtained from 10 women who underwent total hysterectomy without any sign of pathology of the uterine mucosa. Only uteri from women in the reproductive age were considered. Eight of them did not use contraceptive agents. In one case the uterus was dominated by medroxyprogesterone acetate (Depo-provera) (patient I), and in one other case an oral contraceptive was used (patient II). Routinely, the uterus was opened longitudinally and the mucosa was scraped off with a curette. The obtained material contained uterine fluid, endometrial tissue and blood. From patient I, however, only the uterine content was obtained, after removal of the cervix uteri, by careful aspiration with a tuberculine syringe. No blood contamination was visible. In 2 cases the cervical mucosa was also scraped off. The phase of the cycle was not determined precisely, although the preovulatory and postovulatory phase could be discerned by the appearance of the cervical mucus and the time of the patients' latest menses.

Biochemical techniques

The material was homogenized with an Elvejhem potter and sonicated in a Branson Sonifier for 1 min. The homogenate was then centrifuged for 10 min at $2000 \times g$, to remove connective tissue. Subsequently, we fractionated the supernatant of the homogenate by CsCl density equilibrium centrifugation according to Creeth and Denborough (1970) at $150{,}000 \times g$ for at least 48 h in a Beckman L5-65 centrifuge after adding CsCl to 40% w/w final concentration. After the run the gradient was divided into approximately 18 fractions. Macromolecular material was precipitated by adding to each fraction volume 4 vols. of water, 0.5 vol. of 2 M potassium acetate solution (pH 5.0), and 3 vols. of cold ethanol. After incubation at $-20°C$ overnight, the precipitates were spun down at $2000 \times g$, and washed one time with 96% ethanol. After drying the precipitates were dissolved in water. In each fraction hexose was measured with the orcinol reagent, according to Francois et al. (1962), DNA with the method of Burton (1956), and protein with the method of Lowrey et al. (1951). Sulfate was measured by the method of Dodgson (1962). To determine the sugar composition gas liquid chromatography was performed according to the procedure of Kamerlingh et al. (1975). Amino acid composition was analysed with a Liquimat II (Kontron).

Protein patterns were obtained by electrophoresis on 7–15% polyacrylamide gel, in 1% sodium dodecyl sulfate after reduction of the samples with β-mercaptoethanol. The gels were stained with Coomassie Brilliant Blue R 250 or with the PAS reagent.

Results

Uterine contents after administration of progestagens

Patient I. After removal of the cervix about 2 g wet weight of translucent mucus was aspirated from the uterine lumen. By CsCl density equilibrium centrifugation we obtained fractions rich in mucous glycoprotein and fractions rich in protein. In Fig. 1B hexose, DNA and protein patterns are depicted. Hexose is a measure for the mucous glycoprotein content. The highest amount was found in the fraction with a buoyant density of about 1.5. Gas liquid chromatography of this and nearest fractions shows a sugar composition, characteristic for mucous glycoproteins of epithelial origin. No glycogen is present in the fractions heavier than 1.5 g/ml.

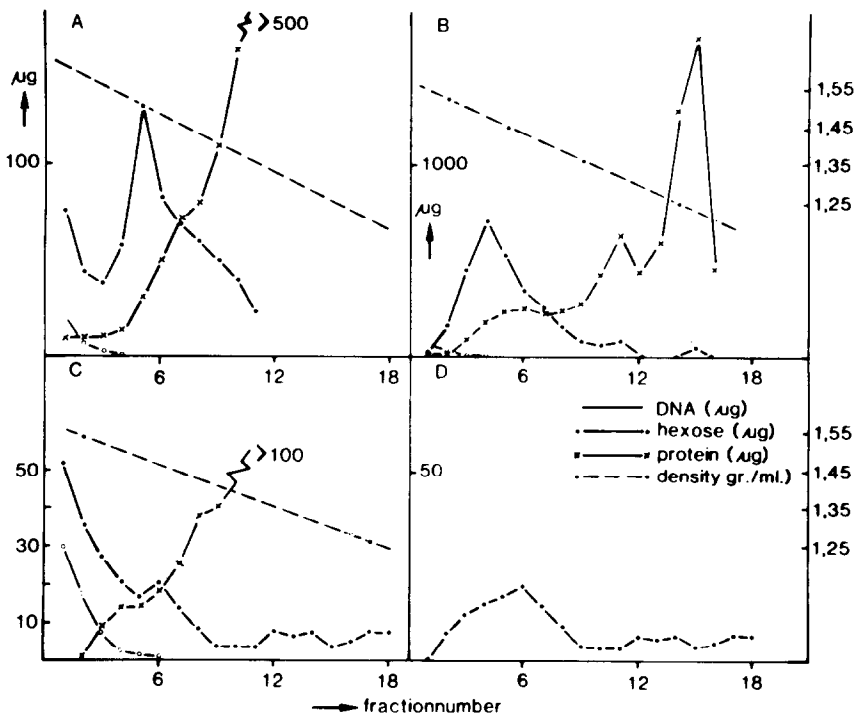

Fig. 1. (A) The results of density gradient centrifugation of a scraping of cervical mucosa. The mucous glycoprotein, characterized by the high hexose content, accumulates at about 1.5 g/ml density. (B) The same for the luminal mucus from the uterus under influence of medroxyprogesterone acetate, without contamination by endometrial tissue. At density 1.5 g/ml a hexose peak is present. Only small amounts of DNA are present. Protein concentrates at lower density. (C) The results for scrapings of a normal uterus (postovulatory phase) with much glycogen (represented by the high hexose values in the highest density fractions) and a small glycoprotein peak at density 1.5, which becomes more apparent after correction for DNA and glycogen content (D).

Electrophoresis of the glycoprotein containing fractions reveals a slowly migrating component which stains with PAS and slightly with Coomassie Blue. It behaves on the acrylamide gel in the same way as the cervical glycoprotein (compare the second with the third lane in Fig. 2). Apart from mucous glycoprotein the mucus contains a large amount of proteins, accumulating mainly in the fraction(s) with density 1.2–1.3. Electrophoresis of this fraction shows that serum proteins dominate (Fig. 3), although other proteins are present.

Patient II. The scraping contained, apart from endometrial tissue and blood, a large amount of luminal mucous material. On CsCl gradients the same distribution of hexose rich and protein rich fractions was obtained. Because of the blood

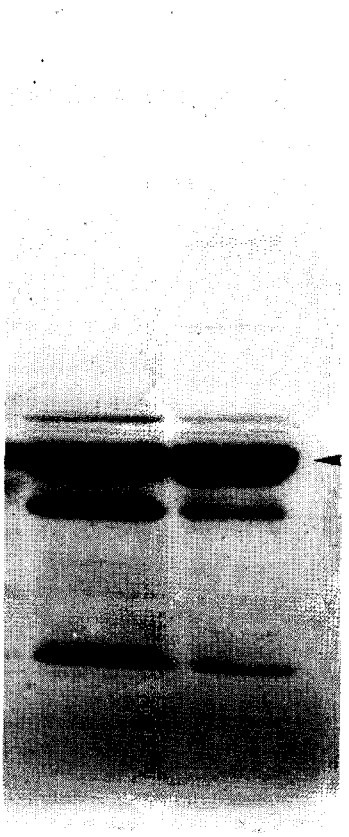

Fig. 2. Electrophoresis of the isolated uterine mucous glycoprotein from a normal (preovulatory) uterus (left lane), from a progestagen treated uterus (middle lane), and from the preovulatory cervical glycoprotein (right lane). In the latter two the isolated glycoprotein fraction is still contaminated by proteins. The mucous glycoprotein (arrow) hardly penetrates the gel and is stainable with PAS (not shown) and slightly with Coomassie Blue (this gel).

Fig. 3. Electrophoresis of the protein rich fraction from (left lane) the luminal mucus of the uterus under influence of medroxyprogesterone acetate and (right lane) blood plasma. The arrow designates albumin (molecular weight, 67,000). Staining: Coomassie Blue. The patterns are not completely similar.

TABLE I

Composition of uterine glycoprotein (patient II) (weight percentages related to dry weight)

Hexose	25.1	
fucose		11.8
galactose		13.3
Amino sugar	25.6	
glcNAc		17.4
galNAc		8.2
Sialic acid	6.2	
Protein	24.1	
serine		2.4
threonine		4.0
proline		3.2
Sulfate	8.4	

contamination much higher protein concentrations were measured; the protein pattern after electrophoresis resembled that of blood. The analysis of the sugar and amino acid moiety of the glycoprotein fraction is given in Table I. Both show a composition typical for mucous glycoprotein, the protein backbone being characterized by a high serine, threonine and proline content. No xylose, indicating contamination with proteoglycans could be detected. Electrophoretically, the glycoprotein fractions behaved identically to those from patient I.

Uterine content in normal women

Three specimens were classified as preovulatory and five as postovulatory. The CsCl gradient centrifugation resulted in small amounts of hexose positive material in the high density fractions (Fig. 1C). As the orcinol reagent also reacts with DNA, the amount of the latter was determined separately with the method of Burton, that does not cross-react with mucous glycoprotein. From control studies we know that glycogen concentrates in the first high density fraction and decreases rapidly in the following fractions. Therefore, we could correct the hexose values in the high density fractions for DNA and glycogen contribution. After these corrections the hexose values of the gradient fractions (Fig. 1D) showed a peak at about density 1.5. In one case we treated the hexose rich fractions with α-amylase to digest glycogen. At density 1.5 hexose positive material remained, whereas in the highest density fractions no high molecular weight material was precipitable, except DNA. As only small amounts of material were available, we could not perform a sugar and amino acid analysis, but analysed the fractions by electrophoresis only. A small amount of PAS and Coomassie Blue positive material remained at the origin of the polyacrylamide gel in the same way as the luminal glycoprotein described before (compare the first lane with the second lane of Fig. 2).

The fractionation patterns did not show important differences between pre- and postovulatory uterine material. The preovulatory specimens originated from the late proliferative phase, as indicated by the presence of clear and watery cervical mucus in the cervical canal and contained glycogen and mucous glycoprotein in about the

same ratio as the postovulatory specimens. Postovulatory material contained more DNA and a higher glycoprotein to DNA ratio although that difference was not statistically significant.

In Fig. 1A the fractionation of cervical scrapings (preovulatory) is depicted. As described earlier by us (Van Kooij et al., 1980) hexose accumulates around density 1.5, thus around the same density as the uterine glycoprotein. No xylose could be detected with sugar analysis, indicating the absence of dermatan and chondroitin in the supernatant of the centrifuged homogenate. In the scraped uterine mucosa from patient II, treated in the same way as the cervical scrapings, no xylose could be detected either. This lessens the probability that significant amounts of connective tissue proteoglycans are present throughout our analysis.

Discussion

From our results it appears that the uterus is able to produce mucus. This is quite clear in the two patients where the uterus was dominated by progestagens like medroxyprogesterone acetate. It is not likely that the material obtained from patient I and II originates from the cervical mucosa, because only a very small amount of mucus was present there. The main components of the uterine mucus of these two patients are serum derived proteins and a glycoprotein which resembles epithelial mucous glycoprotein by sugar and amino acid compositon (Clamp et al., 1978). It has a rather high sulfate content (8.4%). This glycoprotein can well be responsible for the intense PAS staining, observed by Flowers et al. (1974) in the gland cells and lumina of progestagen treated uteri. These authors suggest that the secretion mechanism is disturbed by progestagens and that the greater part of the endometrial products enter the uterine lumen via cell desquamation. However, the large amount of uterine mucus of patient I contained relatively small amounts of DNA, pointing to less desquamation than Flowers et al. suggested.

The discovery of this uterine mucous glycoprotein after progestagen administration makes it probable that under normal conditions too, such a glycoprotein should be secreted. Indeed, the 8 uterine scrapings of untreated women contain hexose positive material that accumulates at a density of about 1.5 in CsCl gradients. Because we recovered only very small amounts of material sugar analysis and amino acid analysis were not possible.

Such a mucous glycoprotein might well be involved in the formation of PAS-stainable gel-like material observed by Sengel and Stoebner (1970) in the uterine glands. Histochemically, Flowers et al. (1974) discovered the appearance of PAS positive, amylase resistant material in the uterine gland cells. This PAS staining was most prominent in the luteal phase of the cycle. This might explain the tendency to a higher glycoprotein to DNA ratio in the 5 postovulatory relative to the 3 preovulatory scrapings. In line with this is the large amount of mucinous material in the two patients using progestational contraceptives. This is in agreement with the observation of Flowers et al., 1974, who saw a substantial increase in PAS positive, amylase resistant material in the endometrial cells under the influence of quingestanol acetate, a similar progestagen to medroxyprogesterone acetate. Electronmicroscopically, they observed more secretory vesicles than in untreated control uteri.

The high protein content and the physical appearance of the uterine mucus, present under influence of progestational contraceptives, suggest similarity with postovulatory cervical mucus. Therefore, this mucus could act as a barrier to sperms, producing an additional contraceptive effect.

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