of the cell surface have been reported[10]. Previous ultrastructural studies on synchronized plasmacytoma cells demonstrated that cells in the $G_1$ phase have on their surface tiny slender cytoplasmic projections (seen also in the phase contrast microscope, Figures 2a and 4c); they disappear in the S phase[7]. This might be due to reduced stability of surface structures during $G_1$ phase. But it is as yet uncertain whether this phenomenon is related to the heparin sensitivity.

Zeiosis was also observed when monolayer cultures of fibroblasts were exposed to heparin (Figure 5a and b). After trypsinization, similar concentrations of heparin produced large non-reversible blebs in all cells except those in mitosis (Figure 5c). Fibroblasts tend to produce small reversible blebs after trypsinization; however, heparin seemed to accentuate the effect considerably. Preceding trypsinization made the cells fragile, and blebs detached easily.

Although heparin is known to produce morphological changes in cells[3], the mechanism behind such manifestations has not been explained. Fibroblasts and ascites cells incubated with heparin have been shown to adsorb this polyanion to the cell surface both reversibly and irreversibly[11]. The present observations suggest that the primary target for heparin is to be sought among components of the cellular periphery. The plasma membrane and its adjoining glycocalyx or 'cell coat', rich in heterosaccharide materials, are included within the concept of a larger functional complex[12]. As the necessary structural and functional information is lacking, the biology of the cell surface has been the subject for much speculation. The compounds of the cell surface are believed to play fundamental roles in cell-to-cell interactions in development and differentiation, cell transformation, and malignancy. It should be emphasized that heparin is structurally close to compounds of the cell surface. As trypsinized cells, presumed to have lost most of their glycocalyx[13] including their heparan sulphate[10], respond to heparin more vigorously than non-trypsinized cells, perhaps the target for heparin is to be found in the plasma membrane proper and not in the stabilizing glycocalyx.

*Summary.* Plasmacytoma cells exposed to heparin exhibited zeiotic blebs in the $G_1$ phase, S phase, and early $G_2$ phase. Zeiosis was not seen in mitotic cells. This heparin effect was reversible. Also fibroblasts were sensitive to heparin. After trypsinization of fibroblasts, heparin produced large non-reversible zeiotic blebs in the cells, except in those in mitosis. The primary target for heparin is apparently to be sought among components of the cellular periphery.

A. ÅBRO and K. A. ABRAHAM

*Departments of Anatomy and Biochemistry, University of Bergen, Årstadveien 19, N–5000 Bergen (Norway), 3 June 1975.*

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## Histochemistry of the Lumenal Cell Surfaces of the Mucosa of the Oviducts and the Uterus of the Rat. Changes in Prepuberty, Estrous Cycle, Castration, Hormone Replacement and Pseudopregnancy

It was reported that the apical surface of the epithelial cells of the isthmus of the rat oviduct was more intensely PAS-positive than the ampulla and fimbriated end, but no changes were recorded during the estrous cycle[1]. Also, PAS-positive material was noted at the cell surfaces as well as in the apical cytoplasm of the epithelial cells of the guinea-pig uterus, varying during the cycle[2,3]. Similar observations were made on the mouse uterus[4], and on the prepuberal rat oviduct[5]. The purpose of the present study was to characterize further by histochemical techniques the surface coat of the rat oviducts and uterus at prepuberty, during the estrous cycle, after castration with and without hormone replacement and in pseudopregnancy.

*Materials and methods.* Albino female rats, kept with a 12 h schedule of light and darkness, were fed a balanced diet and water ad libitum.

1. *Prepuberal rats.* 16 normal rats were sacrified from the 10th up to the 30th day of age. Pseudopregnancy was induced in another group of 25-day-old rats. 26 rats were injected s.c. with 75 IU of PMSG (Eleagol, Elea) in 0.5 ml of 0.9% sodium chloride followed 60 h later with 25 IU of HCG (Endocorion, Elea) in 0.5 ml of 0.9% sodium chloride, s.c.[6]. 8 control rats received 0.5 ml of 0.9% sodium chloride. Animals were sacrificed between the 6th and the 21 th day after the last injection.

2. *Adult rats.* 21 rats, 2 to 4 months old, which showed a regular 4-day cycle controlled by exfoliative cytology, were sacrificed.

3. *Castrated rats.* 17 adult rats were bilaterally castrated. 15 rats were subjected to a sham operation. Groups of experimental and control animals were sacrificed 15 days after castration. 3 castrated rats received s.c. for 15 days 30 µg estradiol benzoate (Progynon B-Schering) daily. 3 castrated rats were injected daily with 2 mg progesterone (Prolution-Schering) for 15 days.

*Light microscopy.* Segments of the oviducts and uterus at the level of the uterine horns, were fixed in 10% neutral buffered formaldehyde pH 7.0 for 24 h at 4 °C. The following histochemical techniques for carbohydrates were applied to tissue sections: periodic acid-Schiff and diastase digestion[7]; colloidal iron[8]; alcian blue (pH 1.0 and 2.5)[8]; alcian blue (pH 1.0 and 2.5)-PAS sequence[8];

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Table I. Histochemical reactions in the lumenal surface coats of the rat oviducts and uterus

| Organ | Method | Prepuberty (days) | | | Estrous cycle | | | | Bilateral castration | Sham operation | Bilateral castration | | 8th-day Pseudo-pregnancy |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 10 | 14 | 20 | Diestrus | Procstrus | Estrus | Metestrus | | | Estradiol | Proges-terone | |
| Oviducts (fimbriated-end) | PAS | R1 | R1 | R1 | R1 | R1 | R2 | R2 | R1 | R2 | R2 | R2 | R2 |
| | AB | B1 | B1 | B1 | B1 | B2 | B2 | B2 | B1 | B2 | B2 | B2 | B2 |
| | AB-PAS | B1 | B1 | B1 | B1 | B1 | B2 | B2 | B1 | B2 | B2 | B2 | B2 |
| Oviducts (ampulla and isthmus) | PAS | R1 | R1 | R2 | R2 | R3 | R3 | R4 | R1 | R3 | R3 | R3-4 | R4 |
| | AB | B2 | B2 | B2 | B2 | B2 | B3 | B4 | B2 | B3 | B3 | B4 | B4 |
| | AB-PAS | B to PV2 | B to PV2 | B to PV2 | PV to PR2 | PV to PR3 | PV to PR4 | PV to PR4 | B to PV1 | PV to PR4 | PV to PR3 | PV to PR4 | PV to PR4 |
| Uterus | PAS | 0 | 0 | R1 | R1 | R1 | R2 | R2 | R1 | R2 | R2 | R2 | R2 |
| | AB | B2 | B2 | B2 | B2 | B2 | B3 | B3 | B2 | B3 | B3 | B3 | B3 |
| | AB-PAS | B to PV1 | B to PV1 | B to PV1 | B to PV2 | B to PV2 | B to PV3 | B to PV3 | B to PV1 | B to PV3 | B to PV3 | B to PV3 | B to PV3 |

R, red; B, blue; PV, purple violet; PR, purple red.

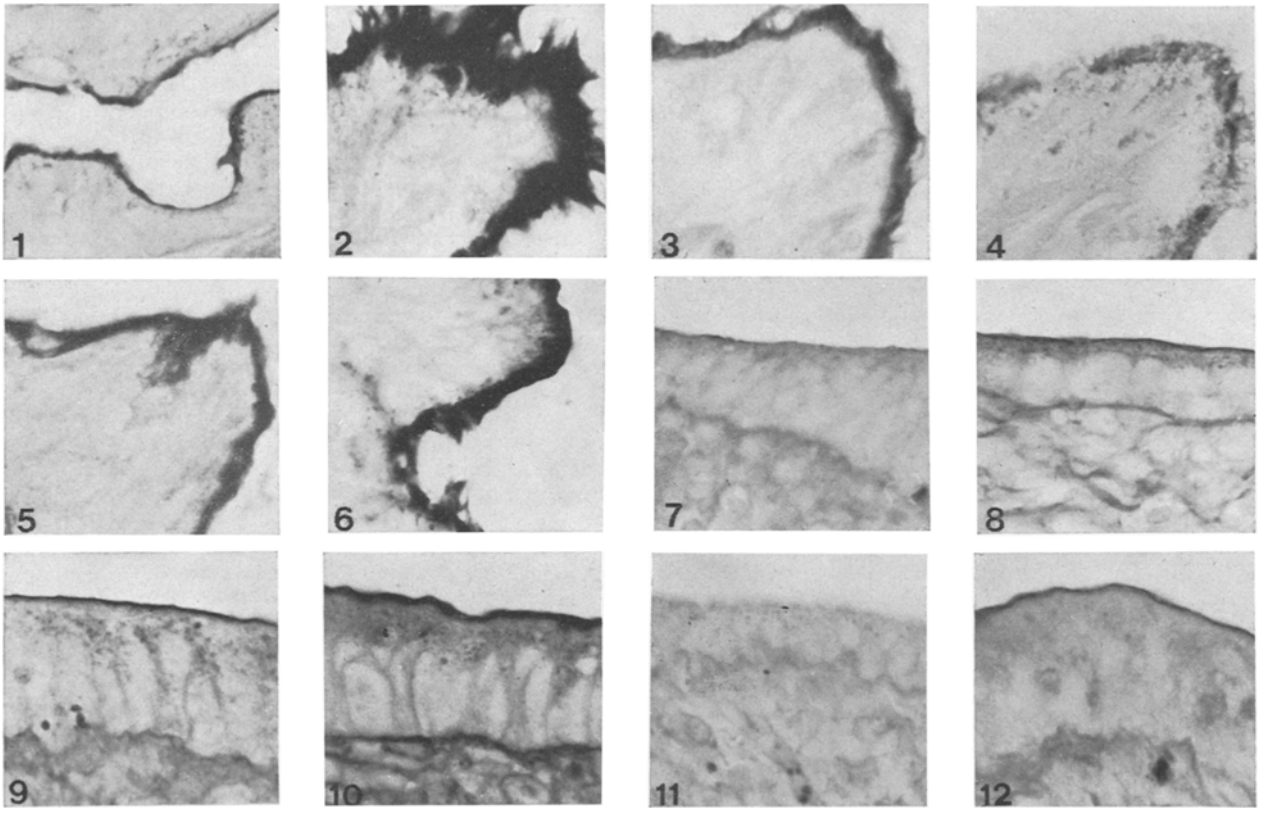


Fig. 1–6. Correspond to the ampulla of the rat oviduct, whereas Figures 7–14 correspond to rat uterus. All pictures are at 760 × magnification. 1. Prepuberal rat (10th day). A thin surface coat is seen (AB-PAS). 2. Metestrus. The surface coat as well as the apical aspect of the cytoplasm are stained (PAS). 3. Metestrus. The surface coat is very developed, but no cytoplasmic staining is seen (AB pH 2.5). 4. Castrated rat (13th day). The surface coat is little apparent (AB-PAS). 5. Castrated rat treated for 15th days with estradiol benzoate. The surface coat is well developed. Compare with Figure 4 (AB-PAS). 6. Castrated rat treated for 15 days with progesterone. Both lumenal cell surface and apical cytoplasm are intensely stained (AB-PAS). 7. Prepuberal rat (10th day). No surface coat is seen (AB-PAS). 8. Proestrus. The coat is more apparent (AB-PAS). 9. Estrus. The surface coat is more developed than in diestrus and proestrus (AB-PAS). 10. Metestrus. The coat is most apparent (AB-PAS). 11. Castrated rat (15th day). The coat is little apparent (AB-PAS). 12. Castrated rat treated for 15 days with estradiol benzoate.

Table II. Histochemical reactions in the lumenal surface coats of the rat oviducts and uterus at metestrus

| Organ | AB pH 1.0 | AB pH 2.5 | AB pH 1.0-PAS | AB pH 2.5-PAS | AB-Safranine | PA-p-Diamine | Colloidal iron | Alcian Blue $MgCl_2$ 0.1 M | 0.2 M | 0.5 M | 0.8 M | 1.0 M | Azure A pH 1.0 | pH 2.0 | pH 3.0 | pH 4.0 | pH 5.0 | Meth 37 °C AB pH 2.5 | Meth 60 °C AB pH 2.5 | Meth 37 °C-Sap. AB pH 2.5 | Meth 60 °C-Sap AB pH 2.5 | Sialidase AB pH 2.5 | Sialidase control |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Oviducts (fimbriated-end) | B1 | B1 | B1 | B2 | B2 | 0 | B2 | B2 | B2 | B1 | B1 | B1 | V1 | V1 | V1 | V1 | V2 | 0 | 0 | B1 | B1 | B1 | B1 |
| Oviducts (ampulla and isthmus) | B3 | B4 | PV to PR3 | PV to PR4 | B3 | BR3 | B4 | B3 | B3 | B2 | B1 | B1 | V1 | V1 | V1 | V2 | V2 | B2 | B1 | B2-3 | B2-3 | B4 | B4 |
| Uterus | B2 | B3 | B to PV2 | B to PV3 | B3 | Gr Br2 | B3 | B3 | B3 | B2 | B2 | B1 | V1 | V1 | V2 | V2 | V2 | B2 | B1 | B2 | B2 | B3 | B3 |

R, red; B, blue; V, violet; PV, purple violet; PR, purple red; BR, brown; GrBr, grayish brown.

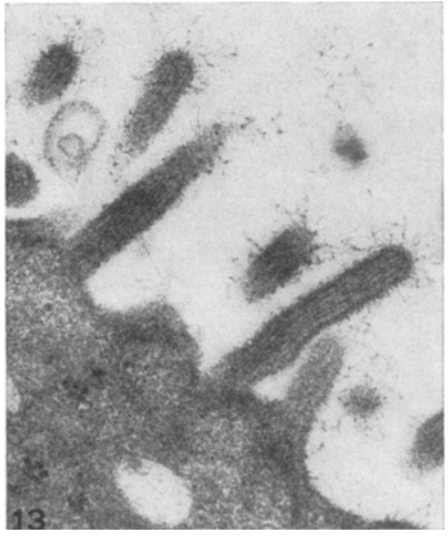

Fig. 13. Uterus. Glutaraldehyde and osmium tetroxide fixation. The surface coat is made up of barely visible filaments.

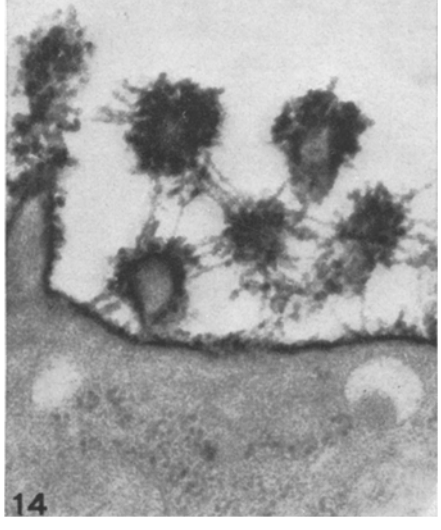

Fig. 14. Like Figure 13, but fixatives contained ruthenium red. The coat appears now made up by thick filaments and globular structures.

periodic acid-*p*-diamine procedure[9]; periodic acid-phenylhydrazine-Schiff[10]; alcian blue with various concentrations of magnesium chloride (0.1, 0.2, 0.5, 0.8 and 1.0 *M*)[11,12]; azure A at pH 1.0, 2.0, 3.0, 4.0 and 5.0[10]; methylation at 37 °C and 60 °C[13,14]; methylation-saponification sequence[10] and sialidase digestion and AB procedure[15].

*Electron microscopy.* Blocks of the uterus at the level of the uterine horns were fixed overnight at 4 °C in a 3% glutaraldehyde in 0.2 *M* cacodylate buffer 7.4 followed by 1.5% osmium tetroxide in 0.2 *M* cacodylate buffer 7.4 for 2 h. Tissues were dehydrated and embedded in epoxy resins. Ultrathin sections were stained with uranyl acetate and with lead citrate. Other tissue blocks were processed likewise but the glutaraldehyde and the osmium tetroxide solutions contained ruthenium red (1 mg/ml)[16].

*Results and discussion.* PAS-positive material, non-digestible with diastase, was found on the cell surfaces of the rodent fallopian tube and uterine horn[1,2,4,5]. In our data, the lumenal surface coats of the ampulla and the isthmus of the oviduct and the uterine horns were stained by the PAS procedure and the alcian blue (Table I, Figures 1–12). Color reactions were little visible in prepuberal rats (Figures 1 and 7) and in castrated rats (Figures 4 and 11). The coat was more apparent at estrus and metestrus (Figures 2, 3, 8–10), as well as in pseudopregnancy, and in castrated rats receiving sex hormones (Figures 5 and 12). The cell coat of castrated rats receiving estrogens stained with the PAS and the AB procedures as at estrus (Figure 5). In castrated rats receiving progesterone, the PAS and the AB stainings were more intense, resembling the color reaction seen at metestrus (Figure 6).

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The surface coat of the 8th day pseudopregnant rats reacted like at metestrus whereas at day 17th, the cell coat resembled that seen in proestrus and estrus.

Methylation decreased alcianophilia, which was restored incompletely by saponification. This indicated the presence of both sulfate and carboxyl groups in cell surface components with a preponderance of sulfate groups as indicated by the metachromasia with azure A, pH 1.0, and persisting alcianophilia at pH 1.0 which withstood the effect of 1.0 *M* $MgCl_2$. The alcianophilia was reduced by methylation at 60 °C and partially restored by saponification. Similar results were noted with the colloidal iron and the AB-safranin procedure and the azure A pH 1, which showed metachromasia. With the PA-*p*-diamine, the fimbriated end was negative whereas in the ampulla and the isthmus, the glycocalyx stained a deep brown. With this procedure in the uterine horns, the coat was grayish brown (Table II).

Electron microscopy of the uterus at metestrus revealed a surface coat made up of filamentous and globular structures measuring up to 550 nm which were distinctly stained by ruthenium red and which appeared surrounding the microvilli (Figures 13 and 14).

Our data seemed to indicate that the lumenal cell coat is a differentiation of the lumenal plasma membrane of the epithelial cells of the accessory organs of the rat female genital tract with peculiar histochemical ultrastructural characteristics. Some of the qualitative and semi-quantitative changes observed by histochemical techniques suggested that the hormones of the ovaries may control the changes noted[2, 3, 17, 18].

The present results are in keeping with previous data on the glycocalyces of the male accessory organs of the rat, suggesting that they were under endocrine control. Bilateral orchidectomy was followed by a significant decrease of the content of sialic acid of the glycocalyx of rat epididymis[19]. The content of sialic acid of homogenates of whole epididymis of castrated rats was significantly lower than in the intact control rats[20].

It has been suggested that estrogens might control the synthesis of mucins in the fallopian tubes of the rabbit, whereas progesterone would be required for the releasing of the mucous secretion[21]. The secretion of the oviduct is increased by estrogen administration[22]. This seemed supported by the observation of cyclic variation of PAS positive substances in mouse uterus[4] and of the changes in the size of the Golgi body induced by estrogens[17, 18]. This corresponded with data showing that the Golgi body was larger in estrus[23].

The functional role of the complex carbohydrates of cell surfaces is unknown. It has been proposed that complex carbohydrates of the lumenal surface coat of certain epithelial were released into the corresponding biological fluids[19]. It can be proposed that glycocalyx components of the oviduct might be required for the nutrition of the egg. In the uterus, substances of the lumenal cell surface might have a role during implantation.

It can be concluded that surface coat materials characterized in this study are chemically heterogeneous. The alcian blue positive substances were of early appearance and varied little in the present experimental conditions, whereas PAS material appeared at or near puberty and showed changes in these various conditions[24].

*Summary.* The lumenal surface coat of the rat oviducts and uterine horns have been histochemically characterized at prepuberty, estrous cycle, castration, hormone replacement and pseudopregnancy. Under the EM, the coat was made up of filamentous and globular structures. Histochemical variations suggested that coat components are under endocrine control.

I. A. Parlanti and B. Monis

*Instituto de Biología Celular, Facultad de Ciencias Médicas, Universidad Nacional de Córdoba, Casilla Postal 362, Córdoba (Argentina), 24 February 1975.*

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## Properties of Glutamine Aminohydrolases in Subcellular Fractions of Liver of Tumour Bearing Mice

The intracellular localization of glutamine aminohydrolase in tumours and host tissues has been found to be different[1] from that reported earlier in normal tissues[2–4]. It has also been indicated[1] that there may be a shift in the site of synthesis of glutaminase from mitochondria to the supernatant fraction of the host liver and kidney, due to the presence of tumour in the body of the animal. This led us to investigate the time and exact location of the shift of the enzyme in the liver after the transplantation of tumour into the animals. An attempt has also been made to see if the enzyme obtained from the two sources are in any way different from each other.

*Materials and methods.* Ehrlich ascites cells (EAC) were maintained in our laboratory by serial i.p. transplantation in Swiss mice. Liver from both normal and EAC-bearing mice were taken out, washed and then homogenized in 0.25 *M* sucrose (1:10 w/v) in cold using Potter Elvehzem homogenizer. Cell fractionation was done according to the method of de Duve et al[5]. The incubation mixtures for the total homogenate and mitochondrial fractions were 0.1 *M* $NaH_2PO_4$ (pH 7.4), 0.25 *M* *Tris* buffer (pH 7.4), 0.04 *M* glutamine and 0.1 ml of tissue fraction and that for microsomal and supernatant fractions were 0.2 *M* $NaH_2PO_4$ (pH 8.6), 0.25 *M* *Tris* (pH 8.6), 0.1 *M* glutamine and 0.1 ml of the cell fractions in a total volume of 3.0 ml. All incubations were carried out at 37 °C for 20 min and ammonia produced was estimated

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