Sugar residues content and distribution in atrophic and hyperplastic post-menopausal human endometrium: lectin histochemistry

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Summary. A lectin histochemical study was performed to investigate the glycoconjugate saccharidic moieties of the human postmenopausal endometrium (14 atrophic and 15 hyperplastic). For this purpose a battery of seven horseradish peroxidase-conjugated lectins (PNA, SBA, DBA, WGA, ConA, LTA and UEA I) was used. No differences in lectin binding between atrophic and hyperplastic endometria were observed. This investigation allowed us to provide a basic picture of the oligosaccharidic distribution in postmenopausal endometria. The data on the saccharidic distribution at the postmenopausal endometria showed a large amount of sugar residues at all the investigated sites, i.e. the lining and glandular epithelium, the stroma and the vessels (capillary and large vessels). Furthermore, at the endometrial lining epithelium, at the glands and at the wall of the blood vessels of some postmenopausal women the presence of α-L-fucosyl residues which bind via α (1-6) linkage to penultimate glucosaminyl residues and/or difucosylated oligosaccharides was demonstrated for the first time.

Key words: Sugar residues, Lectins, Postmenopausal endometrium

Introduction

Lectins are plant or animal proteins or glycoproteins which have been extensively employed in biological assays because they bind specifically with terminal sugar residues of glycoconjugates (glycoproteins or glycolipids) allowing their identification (Danguy et al., 1994). The importance of the sugar residues is well known in cell to cell adhesion, cellular recognition and proliferation in normal and pathological tissues (Damjanov, 1987; Zanetta et al., 1994) as well as during

embryonic differentiation (Gheri et al., 1992, 1993a,b; Gheri Bryk et al., 1994).

Using lectin histochemistry the oligosaccharidic distribution of the glycoconjugates has been widely investigated both in proliferative and/or secretive phases in fertile women at the lining epithelium and, in particular, at the glandular endometrial epithelium, (Bychov and Toto, 1986; Söderström, 1987; Duncan et al., 1988; Aoki et al., 1989; West and Cope, 1989; Klentzeris et al., 1991; Noci et al., 1996).

On the contrary, only scanty data are available on the oligosaccharidic content of menopausal and postmenopausal endometria. In such a perspective Söderström (1987) examined 4 postmenopausal women, but the number of investigated subjects seems to be too small to give reliable results, and, furthermore, the histochemical observations performed on these postmenopausal patients are limited to the lining and glandular epithelium. Other endometrial components, such as stroma and blood vessels, have been completely ignored.

The aim of the present research was to investigate the oligosaccharidic moieties of the glycoconjugates present in the lining epithelium, glandular epithelium, stroma and blood vessels of postmenopausal endometria of 29 women (14 atrophic and 15 hyperplastic endometria). For this purpose a battery of seven HRP-conjugated (horseradish peroxidase-conjugated) lectins was used.

Materials and methods

Twenty-nine healthy asymptomatic postmenopausal women were recruited from patients who, affected by uterine prolapse without vaginal bleeding, underwent a vaginal hysterectomy at the Gynecologic and Obstetric Department of Florence University in the period January-May 1995. The mean age of these subject was 65, ranging from 58 to 79. Mean menopausal duration was 21 years, ranging from 5 to 43. The uteri were

removed and endometrial specimens were taken, fixed in buffered formalin and embedded in paraffin. The samples were processed routinely and 5 μm-thick paraffin sections were obtained. Some sections were stained with Hematoxylin-Eosin according to conventional histological examination.

Lectin histochemistry

After hydration, sections were treated with 0.3% hydrogen peroxide for 10 min (to inhibit the endogenous peroxidase), rinsed in distilled water and washed with 1% bovine serum albumin (BSA) (Murata et al., 1983) in 0.1M phosphate-buffered saline (PBS), pH 7.2. The sections were then incubated for 30 min at room temperature in horseradish peroxidase-conjugated lectins (HRP-conjugated lectins) dissolved in phosphate-buffered saline (0.1M PBS pH 7.2, 0.1M each of NaCl, 0.1mM $CaCl_2$, $MgCl_2$ and $MnCl_2$) and then rinsed three times in PBS. The optimal concentration for each lectin (Sigma Chemical Co., St. Louis, MO) which allowed maximum staining with minimum background was as follows: DBA (*Dolicus biflorus*, binding specificity α-D-GalNAc) 25 μg/ml; PNA (*Arachis hypogea,* binding specificity D-Gal (ß1-3)-D-GalNAc) 25 μg/ml; SBA (*Glycine max* binding specificity α/ß-D-GalNAc > D-Gal) 20 μg/ml; WGA (*Triticum vulgare* binding specificity $(\alpha$-D-GlcNAc$)_n$ and sialic acid) 20 μg/ml; ConA (*Canavalia ensiformis* binding specificity α-D-Man>α-D-Glc) 50 μg/ml; LTA (*Lotus tetragonolobus* binding specificity α-L-fucose) 25 μg/ml; UEA I (*Ulex europaeus* binding specificity α-L-fucose) 25 μg/ml. Staining of the sites containing bound lectin-HRP was obtained by incubating the slides with PBS (pH 7.0), containing 3,3'-diaminobenzidine (DAB) (25 mg/100 ml) and 0.003% hydrogen peroxide, for 10 min at room temperature. Specimens were rinsed in distilled water, dehydrated using graded ethanol solutions, cleared in xylene and mounted in Permount. Controls for lectin staining included: 1) substitution of unconjugated lectins for lectin-HRP conjugates; 2) exposure to HRP and substrate medium without lectin; 3) oxidation with 1% periodic acid for 10 min prior to lectin staining; 4) exposure of sections to 10-20 μg/ml of each lectin-HRP conjugate containing 0.1M D-galactose, D-glucose, D-mannose, L-fucose, N-acetyl-D-galactosamine, N-acetyl-D-glucosamine and methyl-α-mannopyranoside.

Sialidase digestion

In some experiments sialic acid was removed by pre-treating the sections for 18 hr at 37 °C in a solution of sodium acetate buffer 0.25M, pH 5.5, containing 0.1 unit/ml sialidase (neuraminidase Type X from *Clostridium perfrigens* (Sigma Chemical. Co., St. Louis, MO), 5.0mM $CaCl_2$ and 154mM NaCl, prior to staining with lectin-HRP conjugates. Controls containing the sialidase buffer without the enzyme were also prepared.

Results

Morphological remarks (Scheme 1)

The conventional staining with Hematoxylin-Eosin showed two basic pictures in he postmenopausal endometria, atrophic and hyperplastic. The endometrial atrophy was observed in 14 patients, whereas in he other 15 mainly a simple hyperplasia without atypia was noted.

Lectin histochemistry

Lectin binding at postmenopausal endometrium revealed a similar distributional pattern in the two groups of the examined specimens for all the lectins employed, i.e. hypotrophic and hyperplastic endometria.

The following results, if not otherwise specified, concern all the specimens belonging to the two groups.

PNA

The apical portion of the cells of the lining epithelium reacted with PNA in 11 subjects. Reactivity was also observed at the cytoplasm, basolateral and apical surface of the glandular cells (Fig. 1). Neuraminidase treatment increased PNA glandular reactivity only in some specimens. The stroma, as well as the wall of the arteries and veins and the endothelium of the capillaries reacted with the lectin only following neuraminidase digestion (Fig. 2).

ConA

ConA lectin strongly reacted with the cytoplasm, and the basolateral and apical surface of the cells of the lining and glandular epithelium. A strong reaction was observed at the stroma, in particular at the level of the stromal cell surface. In 16 subjects, mastocytes filled with granules which strongly reacted with the lectin were seen (Fig. 3). The vascular wall appeared intensely stained. 11 patients showed strong reactivity with ConA at the endothelium of the capillaries.

SBA

This lectin reacted with the cytoplasm and the apical and lateral surface of the cells of the lining and glandular epithelium, and with the endometrial stroma. SBA reactivity was also detected at the endothelium of the arteries, veins and capillaries (Fig. 4).

LTA

The apical surface of the cells of the lining epithelium reacted with LTA in 10 subjects. In 22 cases, at the same cellular site, lectin reactivity was observed at the glandular epithelium (Fig. 5). Eight specimens showed LTA reactivity at the level of thin granular

material found within the tunica media of the arteries and veins. In 11 subjects weak or moderate reactivity was observed at the capillary endothelium.

DBA

DBA reacted with the cytoplasm of the lining and glandular epithelial cells in about one third of the examined specimens. Reactivity was also observed at the apical plasmalemma in the glandular epithelium (Fig. 6). Reactivity at both the lining and the glandular epithelium did not always coincide in the same subject. The capillary endothelium showed a weak reactivity with DBA only in those patients (12) belonging to A and AB blood groups (Fig. 6).

UEA-I

This lectin strongly reacted with the cytoplasm and the apical portion of the cells of the lining and glandular epithelium. Intense reactivity was observed at the stroma and at the endothelium of the arteries, veins and capillaries (Figs. 7, 8).
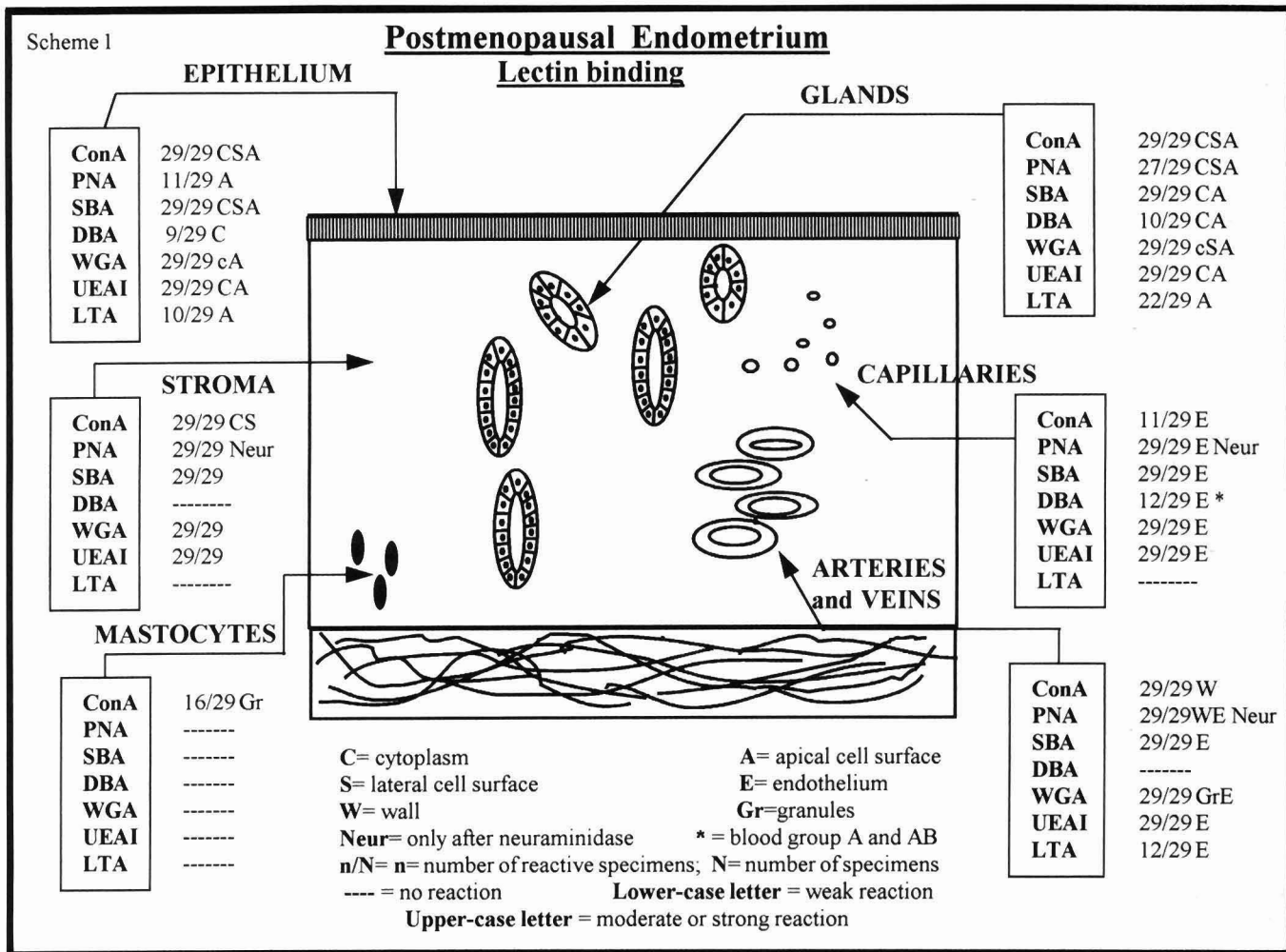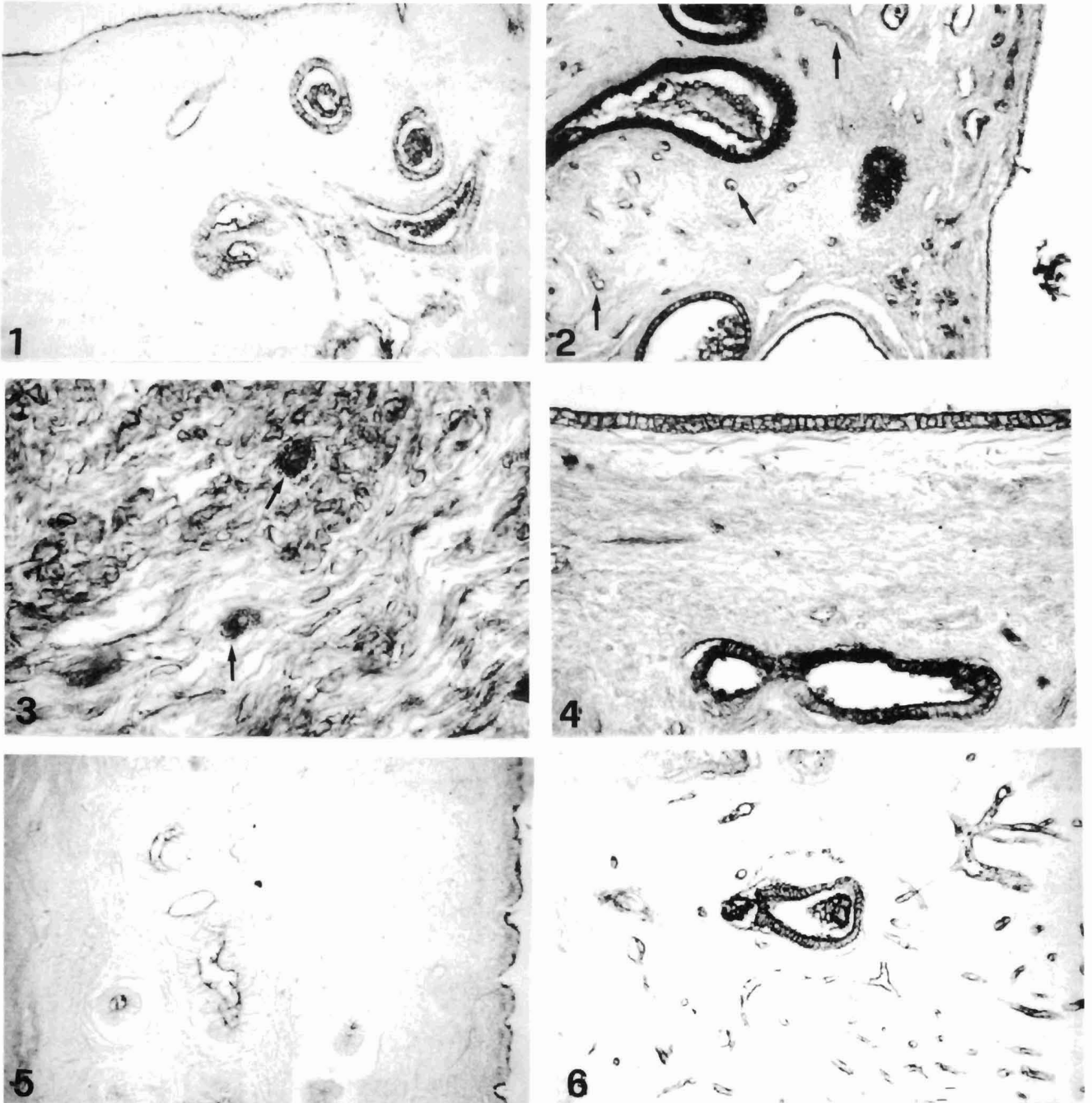
WGA

At the lining and glandular epithelium strong WGA reactivity was observed at the apical cell surface and, sometimes, at the cytoplasm. Intense positivity was seen in the stroma. The tunica media of the wall of the arteries and veins was characterized by reactive thin granular material. The endothelium of the arteries, veins and capillaries intensely reacted with WGA (Figs. 9, 10). Following neuraminidase digestion, WGA reactivity slightly decreased at the above mentioned sites.

Cytochemical controls

When the sections were stained with lectins in the presence of the haptenic sugars pertinent to each lectin, the above described positive reactions were completely abolished or highly reduced. No staining was evident in

Scheme 1. Postmenopausal Endometrium — Lectin binding

EPITHELIUM

ConA	29/29 CSA
PNA	11/29 A
SBA	29/29 CSA
DBA	9/29 C
WGA	29/29 cA
UEAI	29/29 CA
LTA	10/29 A

GLANDS

ConA	29/29 CSA
PNA	27/29 CSA
SBA	29/29 CA
DBA	10/29 CA
WGA	29/29 cSA
UEAI	29/29 CA
LTA	22/29 A

STROMA

ConA	29/29 CS
PNA	29/29 Neur
SBA	29/29
DBA	--------
WGA	29/29
UEAI	29/29
LTA	--------

CAPILLARIES

ConA	11/29 E
PNA	29/29 E Neur
SBA	29/29 E
DBA	12/29 E *
WGA	29/29 E
UEAI	29/29 E
LTA	--------

MASTOCYTES

ConA	16/29 Gr
PNA	-------
SBA	-------
DBA	-------
WGA	-------
UEAI	-------
LTA	-------

ARTERIES and VEINS

ConA	29/29 W
PNA	29/29 WE Neur
SBA	29/29 E
DBA	-------
WGA	29/29 GrE
UEAI	29/29 E
LTA	12/29 E

C= cytoplasm
S= lateral cell surface
W= wall
Neur= only after neuraminidase
A= apical cell surface
E= endothelium
Gr= granules
* = blood group A and AB
n/N= n= number of reactive specimens; N= number of specimens
---- = no reaction
Lower-case letter = weak reaction
Upper-case letter = moderate or strong reaction

Scheme 1.

Postmenopausal uterus: sugar residues

Fig. 1. PNA-HRP. Endometrial hyperplasia (A.Z., 75 years old). Strong reactivity is seen at the apical surface of the cells of the lining epithelium. Strong reactivity with PNA is also shown by the glandular cells. x 230

Fig. 2. Neuraminidase-PNA-HRP. Endometrial hyperplasia (A.Z., 75 years old). Following the enzymatic treatment, there is also reactivity at the stroma and the endothelium of the capillaries (arrows). x 230

Fig. 3. ConA-HRP. Endometrial hyperplasia (S.G., 76 years old). The surface and the cytoplasm of the stromal cells and the surface of the collagen fibers show reactivity. The granules of the mastocytes (arrow) are strongly reactive with ConA. x 900

Fig. 4. SBA-HRP. Endometrial atrophy (G.C., 62 years old). SBA strongly reacts with the lining and glandular epithelium. Stromal cells and fibers show a weaker reactivity. x 230

Fig. 5. LTA-HRP. Endometrial atrophy (L.F., 70 years old). The apical surface of the cells of the lining and glandular epithelium are strongly reactive. x 230

Fig. 6. DBA-HRP. Endometrial hyperplasia (R.P., 76 years old, blood group AB). Reactivity is observable at the level of the glandular cells and of the capillary endothelium. x 230

sections exposed to substrate medium alone or to unconjugated lectins. Pre-treatment of sections with periodic acid abolished the affinity of the histological sites for the lectins.

Discussion

As far as the glycoconjugate content and distribution are concerned no difference was found between atrophic and hyperplastic endometria. When lectin reactivity of some endometrial structure was observed in a limited number of cases, such cases appeared uniformly distributed in the two groups.

Our data on the sugar residue content and distribution at the endometrial structures in postmenopausal patients cannot be compared with the numerous observations performed on fertile menstruating women, because of the absence of the of hormonal stimuli alternance in postmenopausal endometria. On the other hand, differences in lectin binding with respect to proliferative and secretive phases are considered as a possible consequence of hormonal stimulation (Aoki et al., 1989).

The uniform distribution of the glycoconjugate sugar residues as revealed by our research could be the consequence of the absence of progesterone and extremely low estrogenic stimulation in postmenopausal endometria. Our data are consistent with the recent un-published findings of Noci et al. who have shown that no statistically significant differences exist in hormonal levels (steroids and gonadotropins) and in estrogen and progesterone receptors between atrophic and hyperplastic endometria.

The pattern of the sugar residue distribution at the
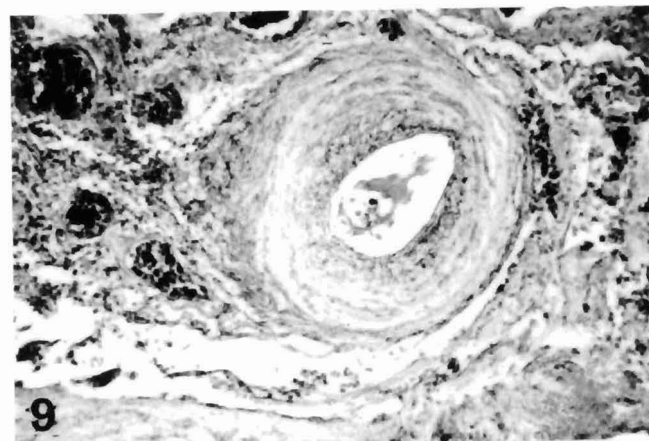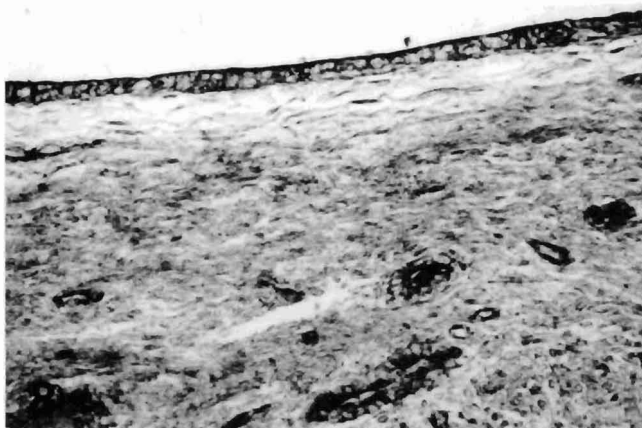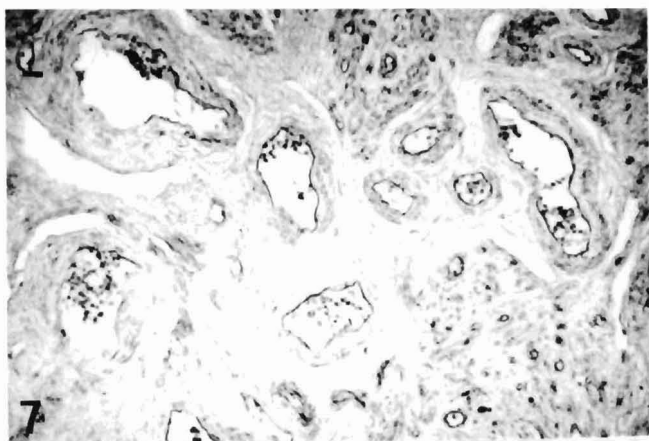
Fig. 7. UEA I-HRP. Endometrial atrophy (G.C., 62 years old). The endothelium of the capillary vessels and that of the arteries and veins are reactive. x 230

Fig. 8. UEA I-HRP. Endometrial atrophy (G.C. 62 years old). The lining and glandular epithelium as well as the stromal cells and fibers are reactive. x 370

Fig. 9. WGA-HRP. Endometrial atrophy (LF., 70 years old). Granular material reactive with WGA is visible within the wall of an artery. x 230

Fig. 10. WGA-HRP. Endometrial atrophy (G.C., 62 years old). Strong reactivity with WGA is shown by the glandular cells. The stroma shows a moderate reactivity. x 230

level of the various endometrial components are reported in Scheme 1. Some particular features must be pointed out:

1- An extreme abundance of capillary vessels, whose endothelium is characterized by sialic acid, D-galactose-(ß1-3)-N-acetyl-D-galactosamine, N-acetyl-D-galactosamine, N-acetyl-D-glucosamine and α-L-fucose reactivity in all the examined specimens, as revealed by neuraminidase -PNA, SBA, WGA and UEA I reactivity. In some specimens the capillary endothelium also reacted with ConA and DBA, thus revealing the presence of α-D-mannose and confirming the presence of N-acetyl-D-galactosamine. Concerning the acetyl-D-galactosaminyl residues, SBA and DBA lectins show the same sugar residue specificity, SBA for α/ß acetyl-D-galactosaminyl residues and DBA for α-N-acetyl-D-galactosaminyl residues (Schulte et al., 1985). Therefore, in all the specimens the endothelium of the capillaries is characterized by the presence of α-N-acetyl-D-galactosamine while the ß anomer was detected only in the subjects belonging to the blood group A or AB.

2- At the level of the lining and glandular epithelium and at the wall of the blood vessels, α-L-fucose was detected. For the fucosyl residue detection we have employed two different types of lectins, UEA I and LTA. UEA I reactivity was observed in all the examined specimens, while LTA reactivity in a lesser number of specimens was also detected. With respect to the fucosyl residues, reactivity with LTA suggests the presence of reactive sites containing α-L-fucose which bind via α (1-6) linkage to penultimate glucosaminyl residues and/or difucosylated oligosaccharides (Goldstein and Poretz, 1986) and reactivity with UEA I indicates the presence of L-fucose bound via ß1,2 linkage to penultimate D-galactose-(ß1-4)-N-acetyl-D-glucosamine residues, in agreement with the observations of Debray et al. (1981), Schulte and Spicer, (1983) and Foster et al. (1991). Thus, L-fucose bound via ß1,2 linkage to penultimate D-galactose-(ß1-4)-N-acetyl-D-glucosamine residues seems to be a finding which is characteristic of the endometrial epithelium and of the blood vessel wall in all the postmenopausal women. α-L-fucose wich bind via a (1-6) linkage to penultimate glucosaminyl residues and/or difucosylated oligosaccharides is present at the same sites only in a limited number of patients. This seems to be the first demonstration of two different possibilities of linkage by α-L-fucose at the endometrial structures. It is to be noted that in a previous investigation performed on fertile women in progesteronic phase we never observed endometrial structures reacting with LTA (Noci et al., 1996). Our data show that UEA I and LTA endometrial reactivity does not seem to be correlated to a particular blood group, although Ito and Hirota (1992) reported an affinity for these lectins to individuals belonging to the H-blood group.

3- At the stroma, α-D-mannose, sialic acid and D-galactose-(ß1-3)-N-acetyl-D-galactosamine, ß-N-acetyl-D-galactosamine, N-acetyl-D-glucosamine and α-L-fucose bound via ß1,2 linkage to penultimate D-galactose-(ß1-4)-N-acetyl-D-glucosaminyl residues were present. α-D-mannose was mainly detected at the cytoplasm and at the surface of the stromal cells. Mastocytes, whose granules strongly reacted with ConA, were observed in 16 patients.

In conclusion, although hyperplastic and atrophic endometria are different from a morphological point of view, no appreciable differences exist between the two types of endometria concerning the oligosaccharidic moieties of the glycoconjugates. The aim of our research was to draw a basic picture of the oligosaccharidic distribution in postmenopausal endometria. The knowledge of the normal oligosaccharidic distribution in postmenopausal endometria might be of some relevance concerning the egg-donation programs, in order to obtain pregnancies in late postmenopausal women. The practice in this field is permitted in some countries, while in others has been interrupted for ethical and legal problems. It is to be noted that for some authors (Duncan et al., 1988; Klentzeris et al., 1991; Horvat, 1993) oligosaccharides might play an important role in favouring egg implantation.

Although our data on the saccharidic distribution at the postmenopausal endometria are hardly comparable with those of normally menstruating women, both in proliferative and secretive phases, it is amazing to observe in «silent» endometria such a large amount of sugar residues as well as of blood capillaries.

Is the postmenopausal endometrium really a «silent» structure?

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