Proteoglycan metabolism in the connective tissue of pregnant and non-pregnant human cervix

An *in vitro* study

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Profound changes occur in the cervix during pregnancy. In particular, the connective tissue is remodelled. To elucidate the mechanisms behind this process, the metabolism of cervical connective tissue was studied using tissue cultures. Cervical biopsies from non-pregnant and pregnant women were incubated with [³⁵S]sulphate. The proteoglycans of the tissue specimens were purified by ion-exchange and gel chromatography and characterized by SDS/PAGE and by enzymic degradation. In the non-pregnant cervix, the incorporation of [³⁵S]sulphate into the proteoglycans was linear for 48 h. During the first 6 h of incubation the accumulation of chiefly one small labelled proteoglycan (apparent M_r 110 000) substituted with dermatan sulphate was recorded. This is in accordance with the known proteoglycan composition of non-pregnant cervical tissue. In addition, small amounts of two larger radioactive dermatan/chondroitin sulphate proteoglycans (apparent M_r values 220 000 and > 500 000) were recorded. After longer periods of incubation the proportion of heparan sulphate proteoglycans increased considerably. The pregnant tissue showed a clearly different composition of labelled proteoglycans. An increased accumulation of the two larger dermatan/chondroitin sulphate proteoglycans was seen in addition to the dominant small dermatan sulphate proteoglycan of the non-pregnant cervix. The rate of accumulation of these two proteoglycans was about 3 times higher in the pregnant tissue, whereas that of the small dermatan sulphate proteoglycan was only increased 2-fold. The fact that the concentration of proteoglycans in the pregnant cervix is approximately one-half of that in the non-pregnant cervix indicates that the turnover of proteoglycans in pregnant cervical tissue is significantly increased. The major effect of this profound change of metabolism was a 50 % decrease in proteoglycan content and a 2-fold increased proportion of a dermatan sulphate proteoglycan with an apparent M_r of 220 000.

INTRODUCTION

The spontaneous ripening of the cervix that normally occurs in pregnancy implies softening of the tissue and later dilatation of the cervical canal. This process is necessary for a subsequent vaginal delivery [1]. Cervical ripening can occur independently of uterine contractions and is due to an active process within the connective tissue [2,3]. More than 85 % of the human uterine cervix consists of fibrous connective tissue [4]. The main components of this connective tissue are collagen types I and III. In addition, the presence of proteoglycans and elastin has been documented [5,6]. The main proteoglycan in the non-pregnant human cervix is a small dermatan sulphate proteoglycan [5]. It has not been shown if this small proteoglycan is of the type that is substituted with one (PG-S2; decorin) or two (PG-S1; biglycan) dermatan sulphate chains [7]. Small amounts of heparan sulphate proteoglycans are also present, probably localized to the blood vessels and on the cell surfaces [8]. Furthermore, no demonstration of the large chondroitin/dermatan sulphate proteoglycan (PGL) has been reported. During pregnancy a remodelling of the cervical connective tissue takes place, with decreases in the concentrations of collagen and proteoglycans concomitant with an increase in the collagenolytic activity [9].

An important task of the proteoglycans is considered to be consolidation of the collagen fibrils in the connective tissue and

hydration of the tissue [10,11]. While collagen content decreases to about 30 % of the non-pregnant value in late pregnancy, the proteoglycan content only decreases to about 50 % [9]. This changed ratio might explain the altered qualities of the tissue in late pregnancy. The mechanisms behind these changes are unknown. Changes in the oestrogen/progesterone ratio, an activation of the fibroblasts and the accumulation of polymorphonuclear granulocytes may be involved [12].

The aim of this study was to investigate whether the profound remodelling of the cervix is achieved by an activation of the metabolic machinery to produce a new connective tissue with different mechanical properties.

EXPERIMENTAL

Subjects

Sixteen healthy women participated in the study. Six were non-pregnant, still menstruating, with a mean age of 42 years (range 33–49) and a mean parity of 2 (range 1–3). Ten were term pregnant with a mean age of 32 years (range 23–43), a mean parity of 1 (range 0–4), a mean gestational age of 38 weeks (range 36–40) and a mean cervical score of 3 (range 0–6) [13].

Cervical biopsies were taken from the non-pregnant women at hysterectomies carried out due to benign disorders not involving

Abbreviations used: PGL, large chondroitin sulphate proteoglycan; PGS, small dermatan sulphate proteoglycan, PG-S1 (biglycan), small dermatan sulphate proteoglycan substituted with two dermatan sulphate chains; PG-S2 (decorin), small dermatan sulphate proteoglycan substituted with one dermatan sulphate chain.

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the cervix. The pregnant women were undergoing elective caesarean section due to foeto-pelvic disproportion. Cervical biopsies were taken transvaginally by scissors and tweezers and directly immersed in Dulbecco's medium.

All women gave their informed consent and the study was approved by the local ethics committee of the Karolinska Hospital.

Materials

Dulbecco's medium was purchased from Gibco Limited (Paisley, Scotland, U.K.) and [35 S]sulphate came from Amersham International (Amersham, Bucks., U.K.). DEAE-cellulose (DE-52) was obtained from Whatman (Maidstone, Kent, U.K.); Superose 6 HR 10/30, Sephadex G-50 and Mono Q HR 5/5 ion-exchanger were from Pharmacia Fine Chemicals (Uppsala, Sweden). Guanidinium chloride (practical grade) and papain were from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Before use, 8 M-guanidinium chloride was treated with active charcoal and filtered in order to obtain an A_{280} below 0.13. Solutions of 8 M-urea were passed over a mixed ion-exchanger before use. Chondroitinases ABC (chondroitin ABC lyase; EC 4.2.2.4) and AC (chondroitin AC lyase; EC 4.2.2.5) were obtained from Miles Laboratories (Elkhart, IN, U.S.A.). All other chemicals were of analytical grade.

Incubation of biopsies

The biopsies were taken out of the medium, excess medium was blotted away using a filter paper, and the tissue was weighed and divided into 4–5 equal parts of about 30–40 mg each. A small piece of each specimen was frozen in liquid N_2 and then stored at -60°C for collagen analyses. Each specimen (around 30 mg) was then cut into 4–5 smaller pieces and equilibrated in 1 ml of Dulbecco's medium containing glutamine for 30 min at 37°C and 5% CO_2 . They were then moved to 1 ml of Dulbecco's medium containing 500 μCi of [35 S]sulphate and incubated for 1–48 h under the same conditions. The incubations were stopped by taking the specimens out of the medium and immediately immersing them in liquid N_2 . They were stored at -60°C until analysed.

Extraction and isolation of proteoglycans

The frozen biopsies were powdered in a Retsch ball mill for 1 min at full frequency during cooling with liquid N_2 . Proteoglycans were extracted for 12 h with 0.5 ml of 4 M-guanidinium chloride containing 0.05 M-acetate, pH 5.8, and proteinase inhibitors (10 mM-6-aminohexanoic acid, 10 mM-EDTA, 5 mM-benzamidine chloride and 5 mM-N-ethylmaleimide). After centrifugation at 10000 rev./min (r_{av} 7 cm) in a Sigma 101 M centrifuge for 10 min, the supernatant was diluted with 20 ml of 6 M-urea-containing 0.05 M-acetate buffer, pH 5.8, and proteinase inhibitors and applied to a column (1 cm $\times$ 1 cm) of DE-52. The pellet was re-extracted as above and the supernatant was applied to the same column. Hereafter the column was washed with 6 vol. of 0.05 M-acetate buffer, pH 5.8, containing 6 M-urea and proteinase inhibitors. The medium was applied directly to a column of DE-52 as above. The columns were eluted with 0.5 M-acetate buffer, pH 5.8, containing 6 M-urea and 5 μg of ovalbumin/ml. Proteoglycans were finally eluted with 0.05 M-acetate buffer, pH 5.8, containing 4 M-guanidinium chloride, 0.01 M-EDTA, 0.1% Triton X-100 and 5 μg of ovalbumin/ml. The isolated radioactively labelled proteoglycans were subjected to gel chromatography on a column (0.25 cm $\times$ 25 cm) of Superose 6 eluted with 4 M-guanidinium chloride containing 0.05 M-acetate buffer, pH 5.8, and 0.1% Triton X-100. The column was operated with an LKB h.p.l.c. system at a flow rate of 0.25 ml/min. In the experiment, where

the proteoglycan pattern was studied using non-labelled proteoglycans, chromatography on Mono Q ion-exchanger was performed before the chromatography on Superose. The Mono Q column (0.25 cm $\times$ 5 cm) was operated at 0.5 ml/min with 0.05 M-acetate buffer containing 0.1 M-NaCl and 6 M-urea. The column was first eluted for 5 min isocratically with starting buffer. The concentration of NaCl was then increased to 1.2 M during a period of 60 min using an LKB low-pressure gradient mixer. Fractions of 1 min were collected.

Degradatory methods

Chondroitinase ABC and AC digestions were performed as described earlier [5] and the degraded products were separated on a column (0.8 cm $\times$ 140 cm) of Sephadex G-50. After chondroitinase AC digestion, the amount of L-iduronic acid residues was calculated using a formula described by Malmström *et al.* [14]. Degradation with nitrous acid was performed as described by Shively & Conrad [15]. Heparan sulphate fragments were separated from dermatan/chondroitin sulphate by chromatography on columns (0.8 cm $\times$ 40 cm) of Sephadex G-50. Papain digestion of proteoglycans was performed as described [5].

Analytical methods

Collagen was measured as hydroxyproline by the method of Stegemann & Stalder [16]. To evaluate the degree of ripeness of the cervix, the collagen content was determined in an acetic acid extract, in a pepsin digest of the remaining tissue and in the residue obtained after pepsin digestion [9]. Glycosaminoglycans and proteoglycans were detected using 1,9-Dimethylmethylene Blue in microtitre plates [17]. The columns were monitored with an LKB 2151 variable-wavelength monitor at A_{280} and/or a Radiometric-Flow-One-Beta scintillation flow counter. In preparative separations, fractions were collected over 1.5 min and samples were counted for radioactivity in an LKB Redirac scintillation counter or analysed with Dimethylene Blue.

SDS/PAGE was carried out as described by Laemmli [18]. Gradient gels of 3–12% were used. The gels were stained with Coomassie Blue-Crocein Scarlet. Autoradiography was performed after staining/destaining [8]. Scanning of the gels was performed using a Joyce-Loebl gel scanner.

RESULTS

Characterization of proteoglycans formed during incubation of biopsies from non-pregnant cervix

Incubation of tissue from the non-pregnant cervix resulted in a linear incorporation of [35 S]sulphate into proteoglycans for 48 h (Fig. 1). To obtain these results it proved important to divide a biopsy of 30 mg into 4–5 pieces. Larger or smaller pieces resulted in non-linearity of sulphate incorporation. The proteoglycans obtained after 2 h of incubation were separated by gel chromatography on a column of Superose into one small peak in the void volume, comprising 7% of the total radioactivity, and a major peak at a K_{av} of 0.35 (Fig. 2a). The elution position of this latter peak is in accordance with the elution position of the proteoglycan isolated from the non-pregnant cervix in chemical amounts [5]. The pattern obtained for samples incubated for up to 6 h showed a very similar pattern on gel chromatography on Superose. After longer periods of incubation the composition of labelled proteoglycans gradually changed. After 48 h of incubation a completely different gel chromatographic pattern was obtained (Fig. 2b). More radioactivity (28%) was eluted in the void volume of the column. Furthermore, the position of the major (included) peak changed position from K_{av} 0.35 to 0.14.

Fig. 1. Accumulation of proteoglycans in the non-pregnant cervix with time

Cervical biopsies were incubated with [^{35}S]sulphate for different periods of time as described in the Experimental section. The proteoglycans were purified by ion-exchange chromatography. The total incorporation into proteoglycans (Δ) was obtained as ^{35}S radioactivity in proteoglycans purified by chromatography on DE-52. The activities of the large proteoglycans ($\circ$) and the small proteoglycans ($\bullet$) were obtained after chromatography on Superose 6 as described in the legend to Fig. 2.

Fig. 2. Gel chromatography on Superose 6 of proteoglycans obtained after (a) 2 h and (b) 48 h incubations of the non-pregnant cervix

The proteoglycans were purified by ion-exchange chromatography and then separated on a column of Superose 6 as described in the Experimental section. The radioactive material eluting in fractions 19–23 is a large proteoglycan and the material in fractions 24–36 is a mixture of various small proteoglycans.

Two fractions, one eluting in the void volume and one included (K_{av} , 0.061–0.49), were prepared by gel chromatography on a column of Superose. Both components were subjected to proteolytic digestion with papain. The resulting radioactivity of both large and small components chromatographed as free chains at K_{av} values of 0.40 and 0.35 respectively. The polysaccharide chains were treated with nitrous acid and chondroitinase in order to characterize their structure. The larger components contained less than 10% heparan sulphate during the entire experiment. The composition of the smaller dominant proteoglycans changed during the incubation. For incubation

Fig. 3. Gel chromatography of proteoglycans from non-pregnant (---) and pregnant (—) tissue after 2 h of incubation

The proteoglycans were purified by ion-exchange chromatography and then separated on a column of Superose 6 as described in the Experimental section. For experimental details, see the legend to Fig. 2. The proteoglycans were pooled as shown by the horizontal bars.

periods less than 6 h, approx. 10% heparan sulphate was detected, whereas for longer incubation periods the proportion of heparan sulphate increased to approx. 50% (obtained after 23 h of incubation). The remaining polysaccharide chains of the large and small proteoglycans were digested with chondroitinase AC followed by chromatography on Sephadex G-50. Using the formula described by Malmström *et al.* [14] the relative content of L-iduronic versus D-glucuronic acid was calculated to be around 60%, which is well in agreement with the copolymeric structure of the dermatan sulphate proteoglycan prepared from non-pregnant cervix [5]. The large proteoglycan contained few L-iduronosyl residues.

These data indicate that, during short incubations (up to 6 h), the tissue synthesizes proteoglycans that are representative of the native tissue. Even though the sulphate incorporation is linear for 48 h, considerable changes take place in the tissue. The proteoglycan composition gradually changes and proportionally larger amounts of both a large dermatan/chondroitin sulphate proteoglycan and a heparan sulphate proteoglycan are recorded.

Characterization of proteoglycans formed during incubation of biopsies from pregnant cervix

The collagen concentration and its organization are good indicators of cervical ripeness [9,19]. Therefore these parameters were determined in the non-pregnant and pregnant specimens used. The non-pregnant biopsies had a collagen concentration of $18.9 \pm 3.0 \mu\text{g}/\text{mg}$ wet wt., and only 44% was extractable by pepsin, indicating extensive cross-linking. The pregnant samples contained $12.5 \pm 0.9 \mu\text{g}/\text{mg}$ wet wt., and 76% was extractable by pepsin. This indicates a large amount of collagen with a different organization.

Using the established conditions, cervical biopsies from non-pregnant and pregnant women were incubated in medium containing [^{35}S]sulphate for periods of 1–4 h. The total incorporation of [^{35}S]sulphate into proteoglycan material (Fig. 3) from biopsies from pregnant subjects was on average 2–3-fold higher than in biopsies from non-pregnant women.

Proteoglycans from the non-pregnant cervix showed a pattern similar to that presented in Fig. 2, with large and small proteoglycans, but the relative amount of the large proteoglycan was somewhat higher (Fig. 3). The material from the pregnant cervix, however, showed a completely different pattern. The major findings were an increase in the proteoglycan material eluted in the void volume of the Superose column and an increase in a proteoglycan of intermediate size (K_{av} , 0.25; Fig. 3).

Fig. 4. SDS/PAGE of large (PGL) and small (PG-S1 and PG-S2) proteoglycans from non-pregnant (lanes a and c) and pregnant (lanes b and d) cervical tissue

Proteoglycans were isolated from biopsies (that had been incubated for 4 h) by ion-exchange and gel chromatography as described in the Experimental section. Radioactive material was pooled as shown in Fig. 3. PG-S1 and PG-S2 were pooled together. The proteoglycans were precipitated, in the presence of 25 µg of cervical dermatan sulphate proteoglycan, with 3 vol. of ethanol. After centrifugation and drying, the proteoglycans were dissolved in the sample application buffer. The polyacrylamide gels were gradients from 3 to 12%. S, start; F, front.

To characterize more thoroughly the different proteoglycans in the non-pregnant and pregnant cervix, the material eluting in the void volume (PGL) and the components with elution characteristics of PG-S1 and PG-S2 were prepared by gel chromatography and subjected to electrophoresis on 3–12% (w/v) polyacrylamide gels. PGL, obtained from the void volume of the Superose column, barely entered the gel (Fig. 4, lanes c and d). A slight contamination of the smaller proteoglycan of the PG-S1-type was also noticeable, especially in the samples from the pregnant cervixes. The smaller proteoglycans were separated into two major components which had the same electrophoretic mobilities as PG-S1 and PG-S2 (Fig. 4, lanes a and b). The PG-S1/PG-S2 ratio, obtained by scanning the gels, increased from 0.5 to 0.7 in the pregnant cervix. Both proteoglycans were somewhat larger in the pregnant tissue, possibly indicating larger side-chains.

The labelled proteoglycans in the medium from incubations of biopsies from both non-pregnant and pregnant women were also characterized by gel chromatography on Superose 6. The

amounts of labelled proteoglycans isolated from the incubation media were, at all incubation times, less than 10% of that isolated from the cervical biopsies, and the chromatographic patterns on Superose 6 were similar to those of the biopsies (results not shown). These components were therefore not further characterized.

The structure of the polysaccharide side-chains of the proteoglycans from non-pregnant and pregnant tissue was investigated by specific degradation using nitrous acid and chondroitinases AC and ABC. The large proteoglycan was contaminated with small amounts of heparan sulphate proteoglycan (Table 1). Digestion with chondroitinase ABC indicated that the remaining radioactive material was sulphated galactosaminoglycans. No sulphated glycoprotein was detected. Chondroitinase AC digestion converted the major part of the polysaccharides of PGL to disaccharides, indicating that this proteoglycan consists mainly of D-glucuronic-acid-containing disaccharides (Table 1). It can, therefore, be classified as a chondroitin sulphate proteoglycan. The two small proteoglycan fractions (one of intermediate size and one small) contained mainly galactosaminoglycan proteoglycans. Less than 20% of the total radioactivity was localized in heparan sulphate proteoglycans (Table 1). The two galactosaminoglycan-containing proteoglycans were both substituted with dermatan sulphate polysaccharide chains containing approx. 50–60% L-iduronic acid residues (Table 1). No difference was noted between non-pregnant and pregnant tissue regarding the relative content of iduronic acid in PG-S1 and PG-S2. The proportions of these proteoglycans in non-pregnant and pregnant cervix, however, differ considerably.

In order to estimate the differences between non-pregnant and pregnant cervix on a weight basis, the amount of proteoglycan accumulated per mg of tissue was calculated. The amount of radioactivity introduced into proteoglycans increased several-fold in the pregnant cervix (Fig. 5a). The large proteoglycan of the pregnant cervix was increased approx. 3 (±0.5)-fold compared with that of the non-pregnant cervix (Fig. 5b). The radioactivity incorporated into the dermatan sulphate proteoglycan of intermediary size increased approx. 2.8 (±0.7)-fold (Fig. 5c) compared with that in the non-pregnant tissue, while the small dermatan sulphate proteoglycan, typical of non-pregnant tissue, only showed a 1.9 (±0.3)-fold increase (Fig. 5d).

In order to investigate if these metabolic changes resulted in an accordingly changed connective tissue, the proteoglycan patterns of non-pregnant and pregnant tissue were studied. In Fig. 6, the chromatographic pattern of non-labelled proteoglycans in pooled samples from 5 different donors is presented. The proteoglycan pattern is only marginally changed compared with non-pregnant tissue. In order to more clearly identify the various types of

Table 1. Structural characterization of the side-chains of proteoglycans obtained after incubation of non-pregnant and pregnant cervical tissue for 4 h

The proteoglycans were purified and degraded as described in the Experimental section. The amount of heparan sulphate was calculated after nitrous acid degradation followed by gel chromatography on Sephadex G-50. The amount of iduronic acid in chondroitin/dermatan sulphate was calculated, using a formula described previously [14], after chondroitinase AC digestion and gel chromatography on Sephadex G-50. HS, heparan sulphate; CS, chondroitin sulphate; DS, dermatan sulphate; IdoA, iduronic acid.

Proteoglycan	Content (% of total)					
	Non-pregnant tissue			Pregnant tissue		
	HS	CS/DS	IdoA in CS/DS	HS	CS/DS	IdoA in CS/DS
PGL	13	87	0	19	81	0
PG-S1	27	73	55	12	88	56
PG-S2	14	86	62	19	81	53

Fig. 5. Accumulation of proteoglycans with time in non-pregnant (●) and pregnant (○) cervixes

The amount of labelled proteoglycan synthesized/mg of tissue was determined after ion-exchange and gel chromatography as indicated by the bars in Fig. 3. Incorporation into (a) total proteoglycans, (b) PGL, (c) a fraction mainly containing PG-S1 and (d) a fraction mainly containing PG-S2. Approximately the same relative increase was obtained when the fractions were analysed by SDS/PAGE followed by scanning.

Fig. 6. Gel chromatography of non-labelled proteoglycans from non-pregnant (---) and pregnant (—) cervixes

Proteoglycans were prepared from pooled cervixes and purified by ion-exchange chromatography on columns of DE-52 and Mono Q. They were then chromatographed on Superose 6 and analysed using Dimethylene Blue as described in the Experimental section.

Fig. 7. SDS/PAGE of small proteoglycans isolated from non-pregnant (lanes b, d, f and h) and pregnant (lanes a, c, e and g) cervixes

Proteoglycans were prepared as described in the legend to Fig. 6. They were then pooled as shown in Fig. 6 and recovered as indicated in the legend to Fig. 4. SDS/PAGE was performed in a 3–12% polyacrylamide gel. Fractions mainly containing PG-S1 are shown in lanes a and b, and chondroitinase ABC digestion of these fractions is shown in c and d. PG-S2 is shown in lanes e and f and chondroitinase ABC-digested PG-S2 in lanes g and h. S, start.

proteoglycans, the material was pooled as indicated by the bars in Fig. 6 and subjected to SDS/PAGE. The amount of PGL was too small to visualize on the gel, indicating that only small amounts of this proteoglycan are synthesized in both pregnant and non-pregnant cervixes. Two types of small proteoglycans were present in both non-pregnant and pregnant tissue. The dominant species was the smaller proteoglycan (PG-S2; Fig. 7, lanes e and f) with a 43000- M_r protein core (Fig. 7, lanes g and h). The larger component of the major peak in Fig. 6, designated PG-S1, contained mainly a larger proteoglycan with an apparent M_r of 220000 (Fig. 7, lanes a and b), which also contains a 43000- M_r core protein (Fig. 7, lanes c and d). By scanning the gels (Fig. 7), it could be shown that the proteoglycan migrating like PG-S1 constituted approx. 5% of the small proteoglycans in non-pregnant cervix. In the pregnant cervix this proportion was increased to 13%. Gel chromatography (Fig. 6) as well as electrophoresis indicated a slight increase in hydrodynamic size of the small proteoglycan in the pregnant tissue. This is most likely due to an increased size of the polysaccharide component.

DISCUSSION

It is well established that profound changes occur in the organization of the cervical connective tissue during pregnancy [9]. Most striking is the 70% decrease in the amount of collagen and the change in its organization with respect to both cross-linking and supermolecular organization [20]. The proteoglycan concentration decreases, but only to around 50% at term [9]. The changed ratio of proteoglycan to collagen is most likely influencing the organization of the collagen fibres in the pregnant tissue. The mechanisms behind these changes are unknown. The results from this study suggest that a changed metabolism correlates to a remodelling of the tissue, since the main findings are a 2–3-fold increase of ^{35}S incorporation into proteoglycans. Before any valid physiological conclusion can be drawn from *in vitro* experiments such as those presented here, careful examination of the experimental system tissue must be performed. It could be demonstrated, by the use of non-pregnant cervixes, that the two types of proteoglycans synthesized during the first 6–10 h were similar in structure and proportion to those of the native tissue. The incorporation of $^{35}\text{SO}_4$ was linear for 48 h but the pattern of synthesis changed to give an increased proportion of heparan sulphate. The reason for this metabolic change is unknown, but it is clear that to obtain some information on the

synthesis in the native tissue, it is important to incubate it for less than 10 h. Thus in all subsequent experiments the maximal observation period was 4 h or less.

The increase in labelled proteoglycans in the pregnant cervix is conspicuous. The pattern of labelled proteoglycans also differed from that of the non-pregnant tissue. Incorporation of $^{35}\text{SO}_4$ was mostly increased into a large glucuronic-acid-rich proteoglycan (PGL) and into a small dermatan sulphate proteoglycan, with presumably two side chains (PG-S1). Incorporation was also increased, but only 2-fold, into the small dermatan sulphate proteoglycan (PG-S2). It is remarkable that the rate of synthesis of proteoglycans in the pregnant cervix is much higher than that in the non-pregnant cervix and yet the concentration of proteoglycans is diminished by 50 % [9]. This means that the increased accumulation of labelled proteoglycans must be counterbalanced by an increased degradation, resulting in a much higher turnover of proteoglycans in the pregnant tissue. It is also noteworthy that, in spite of the large increase in proteoglycan turnover, and especially the increases in labelled PG-S1 and PGL, relatively small changes in the proteoglycan composition of the tissue were noted. The major change was an increase in PG-S1 from 5 to 13 % of the total. This may be explained by activation of the connective-tissue-synthesizing cells, which results in increases in all proteoglycan species. The turnover of the various proteoglycans differs, however, with a faster turnover of PGL and PG-S1. This means that the newly synthesized collagen is secreted into a slightly different proteoglycan milieu in the present state, with an increased proportion of PG-S1. This may have a bearing on collagen fibrillation, as PG-S2 has been shown to influence this process [11,21], whereas PG-S1 does not [22]. Furthermore, PGL, with its interaction with hyaluronan [23], should also influence this milieu.

Increased metabolism of proteoglycans may be caused by hormonal changes such as an altered oestrogen/progesterone ratio or an increased secretion of prostaglandins. An increased round-cell infiltration [12] may also play a role by secretion of cytokines such as interleukin-1 and transforming growth factor- β [24] or of degradatory enzymes such as collagenase. The increased vascularization which is associated with the presence of fibroblast growth factor, angiogenin and platelet-derived growth factor may also, directly or indirectly, induce increased metabolism in the cervical connective tissue [25]. Incubations performed *in vitro* with tissue from a pregnant ovine cervix have also showed an increased metabolic turnover [26]. This study did not, however, report a decrease in proteoglycan concentration or synthesis of new types of proteoglycans. Thus activation of cells takes place in the ovine cervix, but no remodelling, as found in the human tissue, could be detected. Instead, increased synthesis of sulphated glycoproteins was demonstrated [27].

Incubation of human cervical tissue *in vitro* opens up new possibilities in the study of the metabolism of various disorders in both spontaneously and pharmacologically induced cervical

ripening processes. In our opinion, the mechanism(s) responsible for the activation of the connective-tissue-producing cells must be recognized as the key issue in future research.

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