Detection and synthesis of a progestagen-dependent protein in human endometrium

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Summary. Immunological and biochemical methods were employed to demonstrate the presence of progestagen-dependent proteins in human endometrium. Cytosols were prepared from proliferative and secretory phase endometria of cycling women, from decidua and decidua-rich tissues of women in early pregnancy and from decidua of tubal pregnancy. Antisera were raised in rabbits against the antigens of decidua of tubal pregnancy and decidua-rich tissues. Immunoelectrophoresis, Ouchterlony's immunodiffusion test and polyacrylamide gel electrophoresis using native gels revealed 2 antigenic proteins, designated antigens A and B, in secretory endometria, decidua-rich tissues, decidua, and in decidua of tubal pregnancy. However, only 1 antigenic protein was detected by SDS-gel electrophoresis: antigens A and B may therefore be two different proteins or two forms of a single protein. The antigens could not be detected in non-pregnancy sera or in term placentae. Double isotopic labelling (incubation of tissues with [3H]- and [14C]leucine) followed by protein fractionation methods were used to compare the in-vitro synthesis rates of antigens in proliferative tissues with those in decidua or secretory endometria. The rate of synthesis of antigens A and B was markedly higher in the decidua and secretory endometria than in the proliferative endometria. We conclude, therefore, that during progestagen-dependent transformation of proliferative phase endometria into secretory endometria and decidua in women, there is a selective stimulation of at least one species of pregnancy-associated protein.

Introduction

In most mammalian species, the development of the endometrium in early pregnancy is influenced mainly by progestagens which are secreted by the corpora lutea. Such development is characterized by the onset of a secretory change in the glands and a decidual change of the stromal cells. Proteins of both the glandular secretions and the decidualized tissues have been studied in an effort to identify pregnancy-associated or pregnancy-specific proteins which may be important in early pregnancy. Studies of uterine secretions have led to the detection of uteroglobin (or blastokinin) in the rabbit (Krishnan & Daniel, 1967; Beier, 1968) and a purple basic protein and acidic protein in the pig (Squire, Bazer & Murray, 1972) and a pregnancy-specific secretory protein in the cow (Laster, 1977). Examination of endometrial cytosols has disclosed a decidual protein (Denari, Germino & Rosner, 1976) and a tissue-specific decidual antigen in the rat (Yoshinaga, 1972). In cyclic baboons the pattern of synthesis of soluble tissue proteins in the oestrogen-dominated proliferative-phase endometrium is qualitatively different from that in the progestagen-dominated secretory-phase tissue (Joshi,

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1974). The present paper is part of a similar study of the proteins of the progestational-phase human endometrium. Preliminary details have been presented elsewhere (Joshi, 1977).

Materials and Methods

Specimens

Tissue and blood samples were obtained through the co-operation of attending gynaecologists and patients of Albany Medical Center Hospital. Part of each tissue specimen was submitted to the Department of Pathology for histological examination. The following tissues were used in the study. Endometrial tissues were obtained from 20–45-year-old non-pregnant women who had undergone abdominal hysterectomy for benign pelvic diseases, and from young women who were subjected to dilatation and curettage for the diagnosis of dysfunctional uterine bleeding. Histological examination of haematoxylin and eosin-stained sections permitted broad classification of the tissue as proliferative phase or secretory phase. Decidua-rich tissues of early pregnancy were isolated from menstrual induction specimens that were obtained from women undergoing elective termination of early pregnancy (5–8 weeks). Such tissues were unavoidably contaminated with blood and fetal elements. They were therefore immediately processed (see below) to minimize the concentration of these contaminants. Histological examination of such tissues showed a preponderance of decidual cells and hypersecretory glands, with some residual contamination by placental villi and blood. Decidua of early pregnancy were obtained immediately after curettage of women undergoing termination of early pregnancy by menstrual induction. Tissues examined histologically were relatively free of fetal elements. One sample of endometrium (decidua) was procured by curettage at the time of surgical intervention for a tubal pregnancy. Placental tissues were obtained after normal vaginal delivery at term. Blood samples were obtained from women in early pregnancy, from several healthy men, and from non-pregnant women aged 20–45 years.

All the tissues except decidua-rich tissues and placentae were washed thoroughly in cold (4°C) physiological saline (9 g NaCl/l), blotted on pieces of filter paper to remove blood clots and excess fluid, and weighed. Decidua-rich tissues were repeatedly washed with saline to remove excess blood, then suspended in saline and dissected free of fetal membranes and trophoblast-rich white alveolar tissue before being blotted on filter paper and weighed. For the placentae, pieces of the fetal tissue were excised, dissected free of fetal membranes, and washed thoroughly in saline. The tissues were blotted on filter paper, and weighed.

Those tissues to be utilized for biosynthetic studies were used without further delay, whereas the remainder were stored in glass vials at −20°C until needed.

Sera were prepared from clotted blood by centrifugation and were stored in glass vials at −20°C in the presence of merthiolate (1:10 000, final concentration) until used. Also, samples of quality control female serum and quality control male serum were generated by pooling several serum samples from non-pregnant women and men respectively, and were stored in aliquots at −20°C.

Immunological studies

Soluble antigenic constituents in tissues and sera were analysed, using rabbit antisera against decidua-rich tissues and decidua of tubal pregnancy.

Several decidua-rich tissue samples were thawed and approximately equal portions were pooled and weighed. The pooled tissue (about 20 g) and a 12 g sample of decidua of tubal pregnancy were separately homogenized in an equal volume of cold, 0-01 M-Tris—HCl, pH 7.4. After high-speed centrifugation of the homogenates (110 000 g, 90 min at 4°C), the supernatants, hereafter referred to as tissue extracts, were aspirated and saved for immunization.
of rabbits. Aliquots of the extracts of decidua-rich tissues and decidua of tubal pregnancy were emulsified with equal volumes of Freund's complete adjuvant and injected into adult, male New Zealand rabbits. Each animal received a primary injection of antigen (≈2 mg protein) at multiple subdermal sites on the back. Starting about 3 weeks later each rabbit was given booster injections (≈1 mg protein) at about 2-week intervals. Rabbits were bled through the ear vein 1 week after each booster injection and the sera were tested for the presence of antibody by immunoassays. All antisera were stored at −20°C in the presence of merthiolate (1:10 000 final concentration) until they were absorbed.

Samples of antisera containing precipitating antibodies were pooled and absorbed with a freeze-dried extract of pooled human term placenta and/or quality control male serum. The placental extract was prepared as follows. Approximately equal amounts of several placentae were pooled and homogenized in an equal volume of 0-1 M-Tris–HCl, pH 7-4. The homogenate was centrifuged at 800 g for 20 min, and the supernatant was freeze-dried. Immunoelectrophoretic analysis of the placental extract, using goat antiserum to whole human serum (Antibodies Inc., Davis, California), demonstrated that the extract was grossly contaminated with blood plasma components.

For absorption, a sample of antiserum was mixed with a predetermined amount of the freeze-dried placental extract or quality control male serum and was incubated at room temperature (about 22°C) for 1 h, and then at 4°C for about 72 h. After centrifugation (110 000 g, 90 min) the clear supernatant was stored at −20°C. The amount of the placental extract or quality control male serum which was required to absorb a given antiserum was determined by mixing increasing amounts of the placental extract or male serum with a fixed amount of the antiserum and incubating as described above. Complete absorption was ascertained by immunoassays, and 1 ml antiserum and 50 mg placental extract or male serum were usually sufficient.

Antigens were analysed by Ouchterlony’s two-dimensional immunodiffusion test, immuneelectrophoresis, or by polyacrylamide gel electrophoresis. In the gel electrophoretic studies, acrylamide gels were cast in absence or presence of sodium dodecyl sulphate (native gels or SDS gels), and the location of antigen(s) in the gels was determined by immunodiffusion.

Tissue samples for immunoassay were homogenized in approximately 2 volumes of 0-1 M-Tris–HCl buffer, pH 7-4, and the homogenates were centrifuged (110 000 g, 90 min) at 4°C. The supernates were adjusted to a protein concentration of 8–10 mg/ml by dilution with Tris–HCl buffer or by ultrafiltration using Amicon PM-10 membranes (Amicon Corporation, Lexington, Massachusetts).

**Immunodiffusion and immunoelectrophoresis**

Aliquots (3–10 µl) of tissue extract and of serum were tested at 1:1, 1:2 and 1:4 dilution with buffer. Immunodiffusion was carried out in 1% agarose in veronal buffer (pH 8-6, ionic strength 0-05), containing sodium azide (0-1% w/v final concentration), using templates to cut wells (about 10 µl capacity) 3-5 mm edge to edge. Immunoelectrophoresis was carried out on glass slides (2-5 x 7-5 cm) or plates (8-2 x 10 cm), which were pre-coated with 1% agarose in veronal buffer. Wells for antigen solutions were cut 1 cm apart and were filled with test solutions. The samples were electrophoresed at room temperature for 45 min at a constant voltage of 8–10 V/cm. Troughs (0-15 cm-wide, 5 cm long) were then cut midway between the two antigen wells and filled with the antiserum (about 120 µl). After diffusion, the immunoprecipitates were washed with saline and stained with Coomassie brilliant blue.

**Polyacrylamide gel electrophoresis**

Samples containing 5–7 mg protein were supplemented with sucrose (10% final concentration) and Bromophenol blue (tracking dye) and electrophoresed on an Ortec premade
Gradipore gel (native gel) slab (4–26% gradient) in the Ortec electrophoresis apparatus (model 4200) with pulsed-constant power supply (model 4100, Ortec Inc., Oak Ridge, Tennessee). Tris–glycine, pH 8-4, was used as the tank buffer. The voltage (350 V) and the capacitance (1 µF) were kept constant throughout and the increase in the power output was achieved by advancing the pulse rate, according to the manufacturer’s instructions. Electrophoresis was continued until the tracking dye had migrated to about 6 cm from the origin. After electrophoresis, two vertical gel strips (about 0.5 cm wide) were cut from the slab. One strip was used for the staining of protein bands with Coomassie brilliant blue and the other was utilized for the location of antigenic proteins by immunodiffusion, using the absorbed antiserum to decidua-rich tissue.

When SDS-gels were used, samples (250–300 µg protein) were prepared by heating at 100°C for 10 min and then at 65°C for 30 min in the presence of SDS (1% final concentration) and β-mercaptoethanol (0-4% final concentration), as suggested by Lebond-Larouche, Morais, Nigam & Karasaki (1975), and electrophoresed on 10% acrylamide gels containing SDS, according to the method of Weber & Osborn (1969). After electrophoresis, each gel was cut longitudinally into two strips of approximately equal width. One strip was stained with Coomassie blue for the location of protein bands, and the other was used for the location of antigens by immunodiffusion.

To locate the antigens, the strip was carefully placed on the surface of a solidified bed containing 1% agarose in veronal buffer (pH 8-6) in a Petri dish. A trough was cut parallel to the length of the acrylamide gel strip and was filled with the absorbed antiserum to decidua-rich tissue. After incubation at room temperature for 26–72 h, the positions of the acrylamide gel strip and the precipitation arcs were marked. The precipitate was washed thoroughly with saline and stained with Coomassie blue. The acrylamide gel strip was removed and replaced with the gel strip containing stained protein bands, and the migration distances of the protein bands containing antigen(s) were measured for the determination of the relative mobilities ($R_F$) of the antigens.

**Biosynthesis studies**

Differences in the patterns of the synthesis of soluble proteins by endometrial tissues were examined by the conventional double-labelling technique. The labelled proteins were fractionated by polyacrylamide gel electrophoresis, or by an immunoprecipitation method as follows.

Nine experiments were conducted with 5 specimens of proliferative endometrium, 1 specimen of secretory endometrium and 8 specimens of decidua. In each experiment, freshly excised tissues (150–200 mg) of two different specimens were isotopically labelled by incubation for 2 h in 1 ml Eagle’s minimal essential medium (without leucine, Grand Island Biological Co., Grand Island, New York) containing 10 µCi $[^3H]$leucine ([4,5-$^3$H]leucine, sp. act. 52 Ci/mmol: New England Nuclear Corporation, Boston, Massachusetts; diluted to sp. act. of 311 mCi/mmol before use) for one specimen and 10 µCi $[^14C]$leucine ([U-$^14$C]leucine, sp. act. 311 mCi/mmol: New England Nuclear Corporation) for the other, at 37°C under 5% CO$_2$–95% O$_2$ with constant agitation. In each of Exps 1–4, a sample of proliferative endometrium and of decidua were labelled with $[^14C]$- and $[^3H]$leucine, respectively. In Exp. 5, a sample of proliferative endometrium and of secretory endometrium were labelled with $[^3H]$- and $[^14C]$leucine respectively. In each of Exps 6–9, a freshly excised decidua specimen was divided into two portions, and these were similarly incubated to incorporate $[^3H]$- and $[^14C]$leucine labels.

After incubation, both samples in each experiment were thoroughly washed, homogenized in a buffered sucrose solution (0.25 M-sucrose–0.05 M-Tris–HCl, pH 7.4) and cytosols were prepared by centrifugation (110 000 g, 90 min). The cytosols were dialysed against 0.1
m-phosphate buffer, pH 7, and aliquots were taken for determination of total protein and of acid-insoluble radioactivity, immunoelectrophoresis and fractionation of leucine-labelled proteins.

In each experiment, for comparison of the pattern of radioleucine-labelled proteins in the cytosols, aliquots which contained approximately equal amounts of protein were combined. Each of these mixtures was divided into three portions, which were used for the immunoprecipitation of antigenic proteins by antiserum to decidua-rich tissue, protein fractionation by polyacrylamide gel electrophoresis and for determination of acid-insoluble radioactivity.

For the immunoprecipitation of antigens, the sample was mixed in a 12 ml centrifuge tube with a non-radioactive, glycoprotein fraction of decidua-rich tissue cytosol (see later). A predetermined amount of the absorbed antiserum was added and the mixture was incubated first at room temperature for 1 h, and then at 4°C for about 48 h to precipitate antigens. The tube was then centrifuged at 800 g for 30 min. The supernatant was aspirated and saved, and the immunoprecipitate was washed three times with cold 0·1 m-phosphate buffer, pH 7. The completeness of immunoprecipitation was confirmed by subjecting an aliquot of the supernatant to immunoelectrophoresis, using the absorbed antiserum to decidua-rich tissue. Radioactivity in the precipitate and an aliquot of the supernatant was determined by liquid scintillation spectrometry of NCS (Amersham Corporation, Arlington Heights, Illinois)-solubilized samples.

In some experiments immunoprecipitates were obtained from individual cytosols (3H or 14C labelled) rather than from cytosol mixtures. The labelled antigens in these precipitates were analysed as follows. Each precipitate was washed with and suspended in 0·01 m-phosphate buffer (pH 7) containing 0·01 m-dithiothreitol and 1% SDS. The suspension was heated at 90°C for 5 min to dissociate the antigen–antibody complexes. After cooling to room temperature, the suspension was centrifuged at 10000 g for 5 min at room temperature. An aliquot of the supernatant was removed for the determination of total radioactivity and two additional aliquots were fractionated on two different acrylamide gels containing SDS, as described earlier. After electrophoresis, one gel was sectioned into slices (about 1·1 mm thick) and the radioactivity in the slices was determined by the method of Basch (1968). The other gel was used for the location of immunoreactive proteins by immunodiffusion as described above.

For electrophoretic resolution of leucine-labelled proteins two aliquots of the same mixture of [14C]- and [3H]leucine-labelled proteins were electrophoresed on separate gels. When the native gels were used, the two samples were electrophoresed on gel discs containing 8% and 6% acrylamide in the separating gel, and 4½% acrylamide in the stacking gel, as described elsewhere (Joshi & Ebert, 1976). When SDS gels were used, the samples were treated with SDS and β-mercaptoethanol and subjected to electrophoresis on 10% acrylamide gels, as described earlier. After electrophoresis, one of the two gels was used for the location of antigens by immunodiffusion (see above), and the other gel was sectioned into about 1·1 mm thick slices and radioactivity in the slices was determined by the method of Basch (1968).

The amount of radioactivity was expressed as disintegrations per minute (d.p.m.).

The non-radioactive glycoprotein fraction of decidua-rich tissue that was used as carrier for immunoprecipitation of labelled antigens was prepared by passing the tissue cytosol through a Concanavalin A–Sepharose column (Pharmacia, Piscataway, New Jersey) which had been pre-equilibrated with 0·5 m-Tris–HCl, pH 7·4, containing 1 m-NaCl and 1 mm each of MgCl2, MnCl2, and CaCl2 (starting buffer). After washing the column with the starting buffer, the bound glycoproteins were eluted with starting buffer supplemented with α-methylglucopyranoside (10% w/v). The eluate was concentrated by ultrafiltration and dialysed against 0·1 m-phosphate buffer, pH 7.

The colorimetric method of Lowry, Rosebrough, Farr & Randall (1951) was employed to determine total proteins, using bovine serum albumin as reference standard.
Text-fig. 1. Immunoelectrophoresis of the extract of decidua-rich tissue of pregnancy. DR = extract of the decidua-rich tissue; Ab-DR = rabbit anti-DR exhaustively absorbed with the extract of term placenta; Ab-DT = antiserum against decidua of tubal pregnancy exhaustively absorbed with the extract of term placenta; A,B = immunoprecipitate lines of Antigens A and B.

Text-fig. 2. Immunoelectrophoretic analysis of antigens in tissues and sera. DR = extract of the decidua-rich tissue; SE = cytosol of the secretory phase endometrium; PE = cytosol of the proliferative phase endometrium; NPS = non-pregnancy serum; PS = pregnancy serum; PL = extract of the term placenta; Ab-DR = rabbit anti-DR exhaustively absorbed with the extract of term placenta; A, B = the immunoprecipitate lines of Antigens A and B.
Results

Immunological studies

Five rabbits were immunized against the soluble antigens of decidua-rich tissues. In addition, 1 rabbit was challenged with the soluble antigens derived from decidua of tubal pregnancy. Precipitating antibodies against decidua-rich tissue and decidua of tubal pregnancy were detected in the peripheral blood of all 6 rabbits. Although these antibodies were detected as early as 6–8 weeks after the primary antigen injection, booster injections were continued and the animals were terminally bled 80–100 days after the primary injection. The antisera were analysed before and after absorption with quality control male serum or with placental extract, as described earlier.

The unabsorbed antisera reacted with the extracts of decidua-rich tissue, quality control female serum and the placental extracts to give complex precipitin patterns which were difficult to interpret. However, most of the precipitin bands were abolished after the antisera were absorbed with the quality control male serum or placental extract. Immunelectrophoresis showed that the absorbed antisera reacted with the extract of decidua-rich tissue by formation of only two parallel precipitin lines in the same location (Text-fig. 1), suggesting at least two antigens, A and B, with identical electrophoretic mobility. The line corresponding to Antigen B, which was located near the antigen source, was invariably diffuse. The formation of the sharp Antigen A precipitin line was dependent on the amount of antigen (at least 3 times the amount of tissue extract required to demonstrate the presence of Antigen B) and on the duration of

Text-fig. 3. Analysis of antigens in tissues and sera by an immunodiffusion test. DR = extract of the decidua-rich tissue; SE = cytosol of the secretory phase endometrium; PE = cytosol of the proliferative phase endometrium; NPS = non-pregnancy serum; PL = extract of the term placenta; PS = pregnancy serum; Ab-DR = rabbit anti-DR exhaustively absorbed with the extract of term placenta; A, B = the immunoprecipitate lines of Antigens A and B.
Analysis of soluble antigens in the decidua-rich tissues by an immunodiffusion test combined with polyacrylamide gel electrophoresis using (a) native gels and (b) SDS-containing gels. About 5–7 mg (a) and 300 μg (b) of soluble proteins of the decidua-rich tissues were electrophoresed (4–26% acrylamide gradient in (a) and on 10% gels containing SDS in (b)). After electrophoresis, the proteins were allowed to diffuse on an agarose bed as described in the text. Ab-DR = rabbit anti-DR thoroughly absorbed with the extract of term placenta. The immunoprecipitate lines containing Antigens A and B are shown separately in (a) and by an arrow in (b). DF = dye front.

immunodiffusion (> 24 h). Also, the line was visible only after staining the immunoprecipitates with Coomassie brilliant blue. Further analyses of the absorbed antisera (Text-figs 2 and 3) showed that they reacted with the cytosols of the secretory endometria, but not with those from proliferative endometria or placental extracts. These antisera also failed to react with 20 samples each of serum from pregnant or non-pregnant women and term placentae.

The possibility that the decidua-rich tissues contain more than two antigens which may not be adequately resolved by electrophoresis or by simple diffusion on an agarose bed was investigated by fractionating proteins by acrylamide gel electrophoresis. No greater resolution was obtained. Only 2 precipitin lines (Text-fig. 4a) were seen when native gels were used and only one diffuse precipitin line (Text-fig. 4b) was obtained when SDS-gels were used. The results (not shown) for the secretory endometrial proteins were similar.

A summary of the detection of Antigens A and B in various endometria samples is given in Table 1.

**Table 1. Immunoelectrophoretic analysis of soluble tissue antigens in human endometrium (see ‘Methods’)**

<table>
<thead>
<tr>
<th>Tissues</th>
<th>No. of samples Studied</th>
<th>Containing Antigens A and B (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proliferative tissue (cycle)</td>
<td>32</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Secretory tissue (cycle)</td>
<td>38</td>
<td>18 (47)</td>
</tr>
<tr>
<td>Decidua-rich tissue</td>
<td>42</td>
<td>42 (100)</td>
</tr>
<tr>
<td>Decidua</td>
<td>8</td>
<td>8 (100)</td>
</tr>
<tr>
<td>Decidua (with tubal pregnancy)</td>
<td>1</td>
<td>1 (100)</td>
</tr>
</tbody>
</table>
Table 2. Immunoprecipitation of Antigens A and B from cytosol mixtures of endometrial proteins labelled with \([3H]\)leucine or \([14C]\)leucine (see 'Methods')

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Labelled tissue*</th>
<th>Radioactivity in original mixture</th>
<th>Radioactivity in immunoprecipitates</th>
<th>Isotope ratio†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>d.p.m.</td>
<td></td>
<td>d.p.m. precipitated (% of total in original mixture)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Isotope ratio†</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>(^3H)-D(_1)</td>
<td>(2.58 \times 10^5)</td>
<td>(1.07 \times 10^4(4.1))</td>
<td>3.15</td>
</tr>
<tr>
<td></td>
<td>+ (^{14}C)-P(_1)</td>
<td>(2.07 \times 10^5)</td>
<td>(0.34 \times 10^4(1.6))</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>(^3H)-D(_2)</td>
<td>(3.97 \times 10^5)</td>
<td>(1.06 \times 10^4(2.7))</td>
<td>6.24</td>
</tr>
<tr>
<td></td>
<td>+ (^{14}C)-P(_2)</td>
<td>(1.82 \times 10^5)</td>
<td>(0.17 \times 10^4(0.9))</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>(^3H)-D(_3)</td>
<td>(5.16 \times 10^5)</td>
<td>(1.15 \times 10^4(2.2))</td>
<td>6.05</td>
</tr>
<tr>
<td></td>
<td>+ (^{14}C)-P(_3)</td>
<td>(2.08 \times 10^5)</td>
<td>(0.19 \times 10^4(0.9))</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>(^3H)-D(_4)</td>
<td>(2.87 \times 10^5)</td>
<td>(0.60 \times 10^4(2.1))</td>
<td>2.31</td>
</tr>
<tr>
<td></td>
<td>+ (^{14}C)-P(_4)</td>
<td>(3.13 \times 10^5)</td>
<td>(0.26 \times 10^4(1.3))</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>(^{14}C)-S</td>
<td>(3.13 \times 10^5)</td>
<td>(0.44 \times 10^4(1.4))</td>
<td>1.10</td>
</tr>
<tr>
<td></td>
<td>+ (^{3H})-P(_3)</td>
<td>(5.03 \times 10^4)</td>
<td>(0.40 \times 10^4(0.8))</td>
<td></td>
</tr>
</tbody>
</table>

* D\(_1\)–D\(_4\) = decidua of pregnancy; P\(_1\)–P\(_4\) = proliferative endometria; S = secretory endometrium.
† \(^3H\):\(^{14}C\) for Exps 1–4; \(^{14}C\):\(^3H\) for Exp. 5.

Text-fig. 5. Analysis of the immunoprecipitate containing leucine-labelled soluble antigens of the proliferative-phase endometrium. Cytosol antigens labelled with \([3H]\)leucine were precipitated by the absorbed antiserum as described in the text. The immunoprecipitate was treated with SDS in the presence of dithiothreitol and subjected to electrophoresis on 10% acrylamide gel containing SDS. The arrows indicate fractions containing Antigens A and B, as determined separately by an immunodiffusion test, using the absorbed antiserum. DF = dye front.
Double labelling studies

The results of the immunoprecipitation studies, summarized in Table 2, show that an average of 1.1% of the acid-insoluble radioactivity that was associated with proteins of the proliferative endometrial cytosol was precipitated but the percentage was much higher (average 2.5%) for the immunoprecipitates derived from cytosol of secretory endometrial tissues or decidua. These differences were reflected by higher ratios of $^3\text{H}/^{14}\text{C}$ (Exps 1–4) or $^{14}\text{C}/^3\text{H}$ (Exp. 5) in the immunoprecipitates than in the original mixtures containing the leucine-labelled proteins. Analysis of the immunoprecipitates containing leucine-labelled antigens by electrophoresis on SDS-gels showed that about 40% of the acid-insoluble radioactivity was associated with one major peak of immunoreactive protein (Fractions 19–26, Text-fig. 5).

The results of the studies in which leucine-labelled tissue proteins were fractionated on native or SDS gels are shown in Text-fig. 6. Co-electrophoresis of $^{14}\text{C}$- and $^3\text{H}$-labelled soluble proteins in Exp. 1 resulted in the appearance of several protein species (Text-fig. 6a). The ratio of $^3\text{H}/^{14}\text{C}$ was markedly higher in only one segment of the gel (Fraction 38–40) and only that segment

![Text-fig. 6](https://example.com/figure6.png)

**Text-fig. 6.** Co-electrophoresis of soluble proteins of proliferative phase endometrium (O) and decidua (●) labelled with $[^{14}\text{C}]$leucine and $[^3\text{H}]$leucine, respectively. Radioactivity distributions are shown in (a) and (c), and $^3\text{H}:^{14}\text{C}$ ratios in (b) and (d) for analyses on native gels (a, b) and SDS-containing gels (c, d). The arrows indicate the fractions containing Antigens A and B, as determined separately by an immunodiffusion test using the absorbed antiserum. DF = dye front.
contained Antigens A and B (Text-fig. 6b). The differences in the incorporation of radio-leucine were even more pronounced when the proteins (Exp. 1) were fractionated on SDS gels (Text-figs 6c and 6d). The ratio of $^{3}H/^14C$ was increased in at least 4 gel areas, Fractions 1, 8 and 9, 23 and 24, and 42-44. An immunodiffusion test indicated that only Fraction 24 contained the antigen(s). In the other 4 experiments the pattern of radioactivity distribution and changes in isotope ratios were qualitatively similar to those described above. However, the magnitude of change in isotope ratios in different gel fractions varied from experiment to experiment (results not shown). In the control experiments (Nos 6–9) in which $^{14}C$- and $^{3}H$-labelled proteins of the decidua-rich tissues were co-electrophoresed on native gels (Text-fig. 7a) or SDS gels (Text-fig. 7b), the $^{3}H/^14C$ ratios in different protein fractions were more or less constant, indicating that the variations in the ratios shown in Text-figs 6(b) and 6(d) were not artefacts.

**Text-fig. 7.** Co-fractionation of labelled proteins from decidua by polyacrylamide gel electrophoresis using (a) native gel and (b) SDS-containing gel to show $^{3}H/^14C$ ratios. The arrows indicate the protein fractions containing Antigens A and B. DF = dye front.

**Discussion**

The presence of two tissue antigens (designated Antigens A and B) has been demonstrated in progestagen-dominated endometrial tissues of cyclic and pregnant women by using antisera against soluble antigens of pregnancy endometria. Furthermore, studies on incorporation of labelled leucine into the endometrial proteins which are precipitated by the antiserum clearly demonstrate that the two antigens are synthesized within the endometrial tissue and are not sequestered from peripheral blood. Based on the amounts of acid-insoluble radioactivity which are precipitated from cytosols of decidual or secretory endometrial tissues by the addition of antiserum, it is estimated that, at the most, 2-5% of the soluble proteins present in those tissues is represented by Antigens A and B (Table 2). Although these antigens were not detected in proliferative endometrium by immunoelectrophoresis (Table 1), leucine-incorporation studies indicated that as much as 1-1% of the acid-insoluble radioactivity in that tissue was precipitated by the antiserum, and about 40% of this radioactivity is associated with the antigens. These findings suggest that proliferative endometrium also synthesizes the two antigens, but in amounts below the sensitivity limit of immunoelectrophoresis. Quantitative differences in the synthesis of the two antigens in the 3 positive tissues were therefore studied. Decidual and secretory-phase endometria synthesized significantly greater amounts of Antigens A and B than did proliferative-phase endometrium. We believe, therefore, that variable amounts of Antigens A and B are synthesized during both phases of the menstrual cycle and during early pregnancy, and that synthesis is regulated mainly by progesterone. Undoubtedly, oestrogens may facilitate progestagen-dependent endometrial synthesis of Antigens A and B by maintaining high levels of
progesterone receptors in the endometrium (Milgrom, Thi, Atger & Baulieu, 1973). In the present studies, the increased synthesis of Antigens A and B in secretory-phase and decidual endometrium is to be expected because secretion of progesterone is known to rise dramatically after ovulation and during early pregnancy. However, equally important are the observations of Moghissi, Syner & Evans (1972) that, in cyclic women, progesterone is detectable in the serum even during the proliferative phase of the menstrual cycle. The stimulus provided by this preovulatory progesterone may account for the observed ability of proliferative-phase endometrium to incorporate radiolabelled-leucine into Antigens A and B. We are studying the occurrence of Antigens A and B in endometrial samples in relation to serum levels of oestradiol and progesterone.

Progestagen-dependent uterine proteins of the human and non-human primates have not been studied as extensively as those in non-primate species. Much attention has been focussed on uteroglobin. This protein was first detected in the rabbit and its synthesis in the uterus was shown to be dependent on progestagens (Beier, 1968). However, Feigelson, Noske, Goswami & Kay (1977) have shown that it is present in both reproductive and non-reproductive tract tissues, and its synthesis in the uterus and the oviduct is differentially controlled by oestrogens and progestagens. Furthermore, Feigelson et al. (1977) have demonstrated the presence of an immunoreactive uteroglobin-like material in the luteal-phase uterine washings of women. However, Voss & Beato (1977) and Beier, Kirchner & Mootz (1978) did not detect uteroglobin in human uterine secretions or tissue homogenates. Our studies indicate that the human Antigens A and B are not related to uteroglobin (Joshi, Ebert & Smith, 1980). Tseng & Gurpide (1975) have shown that, in the human endometrium, activity of oestradiol dehydrogenase is induced by progestagens. It remains to be determined whether Antigens A and B represent oestradiol dehydrogenase, or are related to the protein demonstrated to incorporate radiolabelled leucine after exposure of the human endometrium to progesterone in vivo and in vitro (Shapiro & Forbes, 1978).

The present study does not clarify whether Antigens A and B are two different proteins, or two different forms of a single protein. Analysis of tissue antigens by immunoelectrophoresis (Text-figs 1 and 2), immunodiffusion (Text-fig. 3), and by polyacrylamide gel electrophoresis using native gels (Text-figs 4a, 6a and 6b) gave two precipitin patterns, corresponding to Antigens A and B. However, analysis by SDS-gel electrophoresis, in which tissue proteins were dissociated at 90 or 100°C in the presence of a reducing agent (Text-figs 4b, 5, 6c and 6d) gave only one precipitin pattern. The observed difference in the results obtained by native and SDS gel electrophoresis may be due to effect of heat on Antigens A and B, although other studies have demonstrated that the immunoreactivity of the two antigens is not destroyed by heating at 85°C for 30 min (Joshi et al., 1980). These observations, when considered along with the finding that the analysis of radiolucine-labelled tissue proteins by electrophoresis using either the native or SDS-containing gels yields only one major radioactive peak or immunoreactive protein, strongly indicate that Antigens A and B are two different forms of a single protein. The nature of the two antigens is being further studied.

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References


Progestagen-dependent protein in human endometrium


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