Immunochemical detection and characterization of pregnancy-associated endometrial α_1 - and α_2 -globulins secreted by human endometrium and decidua

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Summary. Antisera raised against the soluble antigens of the endometrium of early pregnancy detected two antigenic proteins of α_1 and α_2 mobility in extracts of this tissue and were termed antigens A and B. Neither antigen was detected in pregnancy sera or extracts of proliferative endometrium, but antigen B was detected in extracts of secretory endometrium and both were present in amniotic fluid and medium from in-vitro incubations of pregnancy endometrium. Fractionation of radiolabelled medium on ion-exchange chromatography demonstrated that antigens A and B co-eluted with the proteins from which EP14 and EP15 were derived and which were the major secretory polypeptides of pregnancy endometrium *in vitro*. Further biochemical purification revealed that EP14 (M_r 32 000) was derived from a protein of native molecular weight 36 000 which existed in two forms, whereas EP15 (M_r 28 000) was derived from a dimeric glycoprotein of native molecular weight 56 000. Immunochemical studies demonstrated that antigens A and B are identical to these two secretory proteins and have been termed pregnancy-associated endometrial α_1 - and α_2 -globulins (α_1 - and α_2 -PEG).

Introduction

Under the influence of progesterone the human uterine endometrium exhibits marked differentiation with the appearance of glandular epithelial secretion and decidualization of the stroma, although in some species additional stimuli are required for the latter phenomenon. Our understanding of the possible roles of these alterations in the establishment of pregnancy may be facilitated by the identification of associated secreted endometrial and decidual products (Bell, 1983a, b, 1985). However, direct studies of the protein composition of human uterine secretions are difficult to interpret due to the possible differential transudation of serum proteins during the cycle.

A number of studies have examined the in-vitro synthesis of cytosolic proteins by the endometrium during the cycle and early pregnancy and during in-vitro exposure to steroid hormones. Consistently these studies have demonstrated the synthesis of a protein, of molecular weight under reducing conditions of 50–55 000, associated with the secretory phase endometrium or in-vitro exposure of the endometrium to progesterone (Hirsch, Ferguson & King, 1977; O'Grady, Bell, Govan & Black, 1978; Shapiro & Forbes, 1978; Iacobelli *et al.*, 1981). Examination of the synthesis of cytosolic proteins of pregnancy endometrium has also detected a "progesterone-dependent endometrial protein" ("PEP": Joshi, Ebert & Swartz, 1980a) and although secretion of this or the former protein has not been demonstrated the presence of immunochemically reactive PEP in amniotic fluid (Joshi, Smith & Stokes, 1980b) and blood (Joshi, Bank & Szarowski, 1981) suggests that PEP is secreted.

Fewer studies have been directed toward the specific identification of proteins secreted by the endometrium. Prolactin is a secretory product of the decidualized endometrium although this represented less than 0.5% of the total secretory products (cited in Riddick, Daly & Walters, 1983). Strinden & Shapiro (1983) have identified a number of polypeptides synthesized and secreted by the proliferative endometrium, the secretion of 5 of which is altered by in-vitro exposure to progesterone. As part of a programme to generate markers of endometrial differentiation, in a previous study (Bell, Hales, Patel, Kirwan & Drife, 1985) we identified by SDS-gradient polyacrylamide gel electrophoresis and fluorography 17 polypeptides synthesized and secreted by the pregnancy endometrium during in-vitro incubation with [35 S]methionine. In the present study rabbit antisera have been raised to soluble extracts of pregnancy endometrium to determine the relationship of these antigens to the endometrial polypeptides secreted *in vitro*.

Materials and Methods

Specimens

Tissues were obtained from patients attending the Leicester General Hospital and Leicester Royal Infirmary and portions of all tissues were forwarded to the University Department of Pathology for routine histological examination. Endometrium was isolated from specimens obtained from women undergoing therapeutic termination of pregnancy (6–12 weeks). Dissection to remove fetal material and placental villi was performed in physiological saline (9 g NaCl/l) and the endometrium, which was obtained in sheets, was rinsed to remove excess blood. Histology was performed to confirm absence of placental contamination. The specimens (decidua parietalis) were normally composed of an upper layer of decidualized endometrium (decidua compacta) and a lower layer of secretory glands (decidua spongiosa), but some specimens were obtained, especially between weeks 10 and 12, that consisted only of decidua compacta. Endometrial tissues were also obtained from non-pregnant women undergoing dilatation and curettage for the diagnosis of dysfunctional uterine bleeding and from women undergoing abdominal hysterectomy for benign pelvic disease. Placental tissues were obtained after normal vaginal delivery at term. Samples of amniotic fluid were obtained from women who underwent amniocentesis for diagnosis of genetic disease, and were centrifuged at 1000 *g* for 20 min and stored at -20°C . Blood samples were obtained from women in early pregnancy, male volunteers and from non-pregnant women.

Antisera

Soluble extracts of pregnancy endometrium were prepared by homogenization of 20 g tissue (collected from at least 5 specimens and with both histological types referred to above represented) in an equal volume of ice cold 0.01 M-Tris-HCl, pH 7.4. After centrifugation at 100 000 *g* for 60 min at 4°C the supernatant was assayed for protein and stored at -20°C . The yield of soluble protein ranged from 12 to 21 mg/g wet weight tissue (6 preparations). Adult male rabbits (New Zealand White) received 3 intramuscular injections of 5 mg soluble extract emulsified in equal volumes of Freund's complete adjuvant (Difco Labs Ltd, East Molesey, Surrey) at 2-week intervals. Rabbits were subsequently immunized intraperitoneally with 5 mg soluble extract emulsified in equal volumes of Freund's incomplete adjuvant (Difco) at 2-week intervals. Test blood samples were obtained from the ear veins 7 days after immunization. Samples containing precipitating antibodies were absorbed with freeze-dried aliquants of pooled male serum by incubation at 37°C for 1 h and then 4°C for 72 h, centrifuged at 100 000 *g* for 1 h and stored. Complete absorption was determined by immunodiffusion and crossed immunoelectrophoresis. Other tissue extracts to be tested were prepared by the procedure described for pregnancy endometrium extracts.

Immunochemical techniques

A double-diffusion technique (Ouchterlony, 1958) was employed using 1% (w/v) agar in 0.06 M-barbital buffer (pH 8.6) (Sigma, Poole, Dorset). Crossed immunoelectrophoresis was carried out as described by Crowle (1977) except that electrophoresis in the first dimension was performed with a voltage of 250 V for 60 min. Rocket immunoelectrophoresis (Laurell, 1972) was performed in 1% (w/v) agarose in 0.06 M-barbital buffer (pH 8.6) with a voltage gradient of 100 V for 18 h on 10 × 10 cm plates. Precipitin lines were stained with Coomassie Brilliant Blue G250 after washing with distilled water.

Incubating and radiolabelling of secreted endometrial protein

Dissected pregnancy endometrium (4.25 ± 1.75 g per specimen at 9–10 weeks, mean $\pm$ s.d., $n = 28$) was cut into 2 mm² pieces and 100–150 mg tissue were placed in vials containing 2 ml Trowell's T-8 (Gibco, Paisley, U.K.) supplemented with penicillin (100 U/ml), streptomycin (100 µg/ml) and fungizone (0.25 µg/ml). Vials were preincubated for 2 h at 37°C in an atmosphere of 95% O₂–5% CO₂, and the medium was replaced. For each specimen 40 µCi [³⁵S]methionine (sp. act. 800 Ci/mmol; Amersham International, Bucks, U.K.) was added to one or two vials. Incubations were continued for 10–20 h then incubation media from labelled and unlabelled vials were removed, combined, centrifuged at 1000 g for 10 min and stored at –20°C. Routinely incubation media from 10–20 cultured specimens were combined (total volume 400–600 ml) and concentrated 10-fold by ultrafiltration.

Biochemical separation techniques

Ion-exchange chromatography. Aliquants of [³⁵S]methionine-labelled incubation medium concentrated 10-fold by ultrafiltration were dialysed against 0.1 M-Tris-HCl, pH 8.0, and applied to a column (1.6 × 20 cm) of DE52 (Whatman, Maidstone, Kent). After washing with two column volumes of 0.1 M Tris-HCl, pH 8.0, buffer proteins were eluted with a 0–0.2 M-NaCl gradient prepared in the same buffer.

Gel filtration. Samples (<5 ml) were applied to a column (1.6 × 20 cm) of Sephadex G150 SF (Pharmacia, Milton Keynes, U.K.) equilibrated and eluted in 0.1 M-Tris-HCl, 0.5 M-NaCl, pH 8.0. The column was calibrated with protein standards of known molecular weight (Sigma).

Concanavalin A-sepharose chromatography. Aliquants of incubation media were dialysed against 0.5 M-Tris-HCl, pH 7.4, containing 1 M-NaCl on a concanavalin A-sepharose (Pharmacia) column (1 × 15 cm). Unbound material was removed and a gradient of 0–0.2 M- α -D-methylmannoside (in 0.5 M-Tris-HCl, 1 M-NaCl, pH 7.4 buffer) was applied to remove glycoproteins.

Chromatofocussing. Aliquants of incubation media were dialysed against 0.025 M-imidazole, pH 7.4, and applied to a column (1 × 15 cm) of polybuffer exchanger PBE 94 (Pharmacia). Proteins were eluted by application of 12.5 column volumes of polybuffer 74-HCl, pH 4 (Pharmacia). Further removal of bound material was achieved by elution with one column volume of 1 M-NaCl. The pH of all fractions was monitored continuously and the pH readjusted to 7.4 within 30 min of elution.

Fractions from columns were monitored at 280 nm, assayed for [³⁵S]methionine and the nature of unlabelled and labelled proteins present was assessed by SDS-gradient polyacrylamide gel electrophoresis followed by protein dye binding and fluorography.

SDS-gradient polyacrylamide gel electrophoresis. For one-dimensional SDS-gradient gel electrophoresis up to 50 µl volumes of column fractions and media were mixed with an equal volume of buffer containing β -mercaptoethanol (10%), SDS (5%), glycerol (20%), Trizma (0.125 M, pH 6.8) and bromophenol blue (0.002%). Samples were heated at 100°C for 3 min and analysed on 1.5 mm thick 5–15% acrylamide slab gel (containing 0.26–0.82% bisacrylamide) with a stacking gel

(containing 0.12% bisacrylamide), both containing 0.1% SDS. Gels were stained for 1–2 h with Coomassie blue G (0.04% in 3.5% perchloric acid–20% methanol). Gels to be processed for fluorography were soaked in 1 M-sodium salicylate (Chamberlain, 1979), dried and exposed to Kodak X-Omat X-ray film using Harmer intensifying screens. Gels were calibrated with proteins of known molecular weight (Sigma).

Protein estimation

The colorimetric method of Bradford (1976) was used to determine total protein content using human serum albumin as a reference standard. Incorporation of [^{35}S]methionine into proteins was performed by trichloroacetic acid precipitation as previously described (Bell *et al.*, 1985). To determine elution of radiolabelled proteins from columns, aliquants of fractions were added to 0.5 ml 0.2 M-NaOH and 5 ml scintillation fluid (Fisofluor 1, Fisons Ltd, Loughborough, U.K.) and counted in an LKB Beta-Rac scintillation counter.

Results

Detection of antigens A and B with antisera raised against pregnancy endometrium

Five rabbits were immunized against the soluble cytosolic antigens of decidualized endometrium of early pregnancy (6–12 weeks) and precipitating antibodies were detected from 6 weeks in all animals. Booster injections were continued and animals terminally bled at 100–120 days. The antisera were analysed by immunodiffusion and crossed immunoelectrophoresis before and after absorption with male serum.

Unabsorbed antisera produced complexes of precipitin arcs with the soluble extracts of pregnancy endometrium and male and female serum in immunodiffusion tests. However, absorption with male serum resulted in antisera from 3 rabbits which produced only 2 parallel precipitin arcs with pregnancy endometrial extracts. These arcs appeared identical to those obtained by Joshi *et al.* (1980a), i.e. one arc being diffuse and located nearest the antigen well, termed antigen B, and the other being sharp and visible only after Coomassie brilliant blue staining termed antigen A. However, in contrast to the previous report in which the antigens appeared physicochemically inseparable and were suggested to represent variants of the same protein, crossed immunoelectrophoresis indicated that antigens A and B were distinct species of proteins of different mobilities, i.e. α_1 and α_2 (Pl. 1, Fig. 1). In crossed immunoelectrophoresis and subsequent rocket immunoelectrophoresis precipitin arcs due to antigens A and B could be distinguished by their different appearance and the fact that the arc due to antigen A required protein staining to be visible (Pl. 1, Fig. 2).

Neither antigen was detectable by rocket immunoelectrophoresis in soluble extracts of proliferative phase endometrium (6 specimens, Days 8–14) and only antigen B in soluble extracts of secretory phase endometrium (4/6 specimens, Days 18–24). Antigens A and B were both detectable in samples of amniotic fluid collected between 15 and 23 weeks of pregnancy.

Relationship between antigens A and B and secreted endometrial proteins

Absorbed antisera also formed precipitin lines with concentrated aliquants of culture medium from short-term incubations of pregnancy endometrium, which did not appear to be due to serum contamination since pregnancy sera did not react. It had previously been shown by in-vitro radiolabelling with [^{35}S]methionine that first trimester pregnancy endometrium synthesized and secreted 17 polypeptides, 2 of which accounted for approximately 60% of the incorporation of methionine into secreted proteins (Bell *et al.*, 1985). Experiments were performed to determine whether cytosolic antigens A and B were related to the proteins from which these secreted polypeptides were derived.

A pool of medium from short-term incubations of several pregnancy endometrium specimens, cultured in the presence of [^{35}S]methionine, was fractionated by anion-exchange chromatography (see 'Materials and Methods'; Text-fig. 1). Fluorographic examination of one-dimensional SDS-gradient polyacrylamide gels of β -mercaptoethanol-reduced column fractions revealed that the two main peaks of radioactivity were due to radiolabelled endometrial polypeptide (EP) 15, of subunit molecular weight 28 000 (peak III), and EP14, of subunit M_r 32 000 (peak IV) (Pl. 2, Figs 3 & 4). Rocket immunoelectrophoresis revealed that antigen B co-eluted with EP15 and antigen A co-eluted with EP14, also demonstrating the complete physicochemical separation of antigens A and B. Peaks containing EP14 and EP15 were not pure as assessed by examination of the Coomassie blue stained SDS-gradient polyacrylamide gel (Pl. 2, Fig. 3) and, therefore, further purification of antigens A and B, and the proteins from which EP14 and EP15 were derived, was attempted.

Text-fig. 1. Elution of [^{35}S]methionine-labelled secreted endometrial proteins from ion-exchange chromatography. A 40 ml aliquant (10-fold concentration of original medium) of medium obtained from incubation of pregnancy endometrium with [^{35}S]methionine (see 'Materials and Methods') was applied to a DE52 column and eluted with an NaCl gradient (arrow marks application of gradient). Fractions were monitored for protein at 280 nm (unmarked line, absorbance in arbitrary units) and for [^{35}S]methionine (●) (see 'Materials and Methods'). The presence of antigens A and B was determined by rocket immunoelectrophoresis and is indicated by + or -. Arrowheads refer to fractions which were analysed by one-dimensional SDS-gradient polyacrylamide gel electrophoresis (see Pl. 2, Figs 3 & 4). Horizontal bars indicate fractions that were pooled, concentrated and subjected to further purification.

Peak III (Text-fig. 1) containing labelled EP15 was subjected to Sephadex G150 (superfine) chromatography and a peak of radiolabelled protein was eluted at an apparent M_r of 56 000 (Text-fig. 2a). This was confirmed to represent a protein derived from EP15 by fluorographic analysis of β -mercaptoethanol fractions on SDS-gradient polyacrylamide gels. Further purification was obtained by concanavalin A-Sepharose chromatography whereby radiolabelled EP15 was detected in the fraction bound and eluted with 0.035 M- α -D-methylmannoside (Text-fig. 2b). This fraction was then passed through a chromatofocussing column and EP15 was eluted over a wide pH range (peak at pH 4.6) with a profile suggesting pI polymorphism (Text-fig. 3a,b). The purity of this preparation was 83% as assessed by scanning stained SDS-gradient gel tracks with a densitometer. Throughout this purification schedule the absorbed antisera reacted with EP15 containing fractions, resulting in precipitin lines in rocket immunoelectrophoresis of antigen B type (Pl. 1, Fig. 2).

Text-fig. 2. Elution of antigens A and B from (a) gel filtration and (b) concanavalin A-sepharose columns. Fractions from ion-exchange chromatography containing antigen A, corresponding to Peak IV, and antigen B, corresponding to peak III, of [35 S]methionine-labelled proteins (see Text-fig. 1) were applied to a Sephadex G150SF columns and eluted (see 'Materials and Methods'). Fractions were monitored for protein (unmarked line) and [35 S]methionine ($\bullet$) and the horizontal bar represents detection of the antigens. These fractions were combined, dialysed against buffer, and fractionated on a concanavalin A-sepharose column (see 'Materials and Methods'). Fractions were monitored for protein radioactivity and presence of antigens as described.

PLATE 1

Fig. 1. Crossed immunoelectrophoresis using soluble extract of pregnancy endometrium in the first dimension against rabbit anti-pregnancy endometrium extract absorbed with male serum in the second dimension. This demonstrates the detection of two immunoprecipitates, antigens A and B of α_1 and α_2 mobility.

Fig. 2. Rocket immunoelectrophoresis using the absorbed dispecific antiserum shown in Fig. 1, and (a) concentrated medium from in-vitro incubations of pregnancy endometrium, (b) partly-purified antigen B (containing EP15) and (c) partly-purified antigen A (containing EP14) at a series of double dilutions. Antigen A is revealed only after Coomassie Brilliant Blue G staining.

PLATE I

PLATE 2

Text-fig. 3. Elution of antigens A and B from (a) chromatofocussing and (b) gel filtration columns. Fractions from concanavalin A-sepharose chromatography containing antigens A and B were concentrated, dialysed and applied to a chromatofocussing column and eluted (see 'Materials and Methods'). Fractions containing the antigens were concentrated, dialysed, reappplied to Sephadex G150 5F columns and eluted. Protein, radioactivity and the antigens were monitored in column fractions as described in 'Materials and Methods' and Text-fig. 1.

Peak IV (Text-fig. 1), containing labelled EP14, was eluted at an apparent M_r of 36 000 on a Sephadex G150 (superfine) column (Text-fig. 2). No binding of this fraction was apparent on concanavalin A-sepharose and labelled EP14 was eluted in the unbound fraction with albumin (Text-fig. 2), suggesting a low carbohydrate content for this protein. Two forms of the protein from which EP14 was derived were revealed by chromatofocussing, with one being eluted at pH 4.25 whereas the second required elution with 1 M-NaCl (Text-fig. 3). Both peaks were combined and concentrated for gel filtration. Subsequent gel filtration on Sephadex G150 to remove the

PLATE 2

Fig. 3. SDS polyacrylamide gel electrophoresis of fractions from ion-exchange chromatographic separation of ^{35}S -labelled secreted endometrial proteins. Aliquants of fractions from DE52 column chromatography of medium obtained from incubations of pregnancy endometrium were subjected to SDS-gradient polyacrylamide gel electrophoresis under reducing conditions (numbered tracks correspond to arrowed fractions in Text-fig. 1). The gel was stained with Coomassie brilliant blue (see 'Materials and Methods') and then processed for fluorography (see Pl. 2, Fig. 4). EP14 and EP15 are observed in tracks 11–14 and 8–10 respectively. Molecular weights ($\times 10^{-3}$) of marker proteins in the left-hand track are referred to in the axis on left-hand side of Pl. 2, Fig. 4.

Fig. 4. Fluorograph of SDS-gradient polyacrylamide gel. The radioactive bands corresponding to EP14 and 15 are marked.

polybuffer (Text-fig. 3) produced a preparation which was 95% pure as assessed by densitometer scanning of a SDS-gradient polyacrylamide gel.

Partly purified proteins from which EP14 and EP15 were derived exhibited single non-confluent arcs with the absorbed antiserum which were typical of the antigens A and B, and rockets of antigen A and B type (Pl. 1, Fig. 2).

Discussion

By means of conventional immunochemical techniques two antigens (termed A and B) have been detected in soluble extracts of early pregnancy endometrium and appear to represent the most immunogenic soluble antigens specific to this tissue. A previous investigation had demonstrated that during *in-vitro* incubation of this tissue with [³⁵S]methionine at least 17 polypeptides were synthesized and secreted, although about 60% of the radiolabel was incorporated into only two, EP14 and 15 (Bell *et al.*, 1985), which represented the major secreted polypeptides of pregnancy endometrium *in vitro*. Analysis of this radiolabelled incubation medium with the antisera has now revealed that antigens A and B are identical to the proteins synthesized and secreted *in vitro* from which the polypeptides EP14 and EP15 are derived, therefore it is likely that the latter are also synthesized *in vivo*. In the previous study EP14 and 15 were detected on fluorographs of SDS-gradient polyacrylamide gels (carried out under reducing conditions) as polypeptides of molecular weights of 32 000 and 28 000 and could have represented subunits of a single protein. It is apparent from the present study, however, that they are derived from distinct proteins, and EP14 polypeptide representing an α_1 -globulin of native M_r 36 000, and the EP15 polypeptide a subunit of a dimeric α_2 -globulin of M_r 56 000. Since antigens A and B were detected by using the same antiserum it is possible that the two antigens and hence EP14 and 15 are derived from the same protein, i.e. antigen A/EP14 a deglycosylation product of antigen B/EP15. However, this is considered unlikely since pregnancy endometrial tissues have been obtained which preferentially synthesize and secrete either EP14 or EP15. Both proteins exhibit charge polymorphism which is very apparent for the α_1 -globulin for which two forms were separable. In consideration of the demonstration of the *de-novo* synthesis and secretion of these proteins by the pregnancy endometrium we have termed the proteins pregnancy-associated endometrial α_1 - and α_2 -globulins (α_1 -PEG and α_2 -PEG).

The only well characterized protein demonstrated to be synthesized by pregnancy endometrium is PEP (Joshi *et al.*, 1980a) although secretion of this protein has not been reported. PEP was identified in soluble extracts of pregnancy endometrium by immunochemical procedures and existed as two biochemically inseparable antigenic forms, A and B. In the present study similar procedures were used to raise antisera and two antigens were revealed, but they do not correspond to antigens A and B of Joshi *et al.* (1980a) since we have demonstrated their association with two distinct proteins. If, however, PEP is a major secretory protein of the endometrium, then the α_2 -globulin most closely resembles PEP in its physicochemical properties, both being dimeric glycoproteins of similar subunit molecular weight, i.e. 28 000 for α_2 -PEG and 27 000 for PEP (Joshi, Ebert & Smith, 1980c).

The relationship between α_1 -PEG and α_2 -PEG and two proteins isolated from first trimester placental tissue and also identified in amniotic fluid must be considered (Tatarinov, 1982). These two proteins, chorionic α_1 -microglobulin (CAG-1) or placenta-specific α_1 -microglobulin (PAMG-1) and chorionic α_2 -microglobulin (CAG-2) or placenta-specific α_2 -microglobulin (PAMG-2) (Petrinin, Gryaznova, Petrunina & Tatarinov, 1978; Tatarinov, Kozljaeva, Petrunin & Petrunina, 1980), have been demonstrated to exhibit immunochemical identity with placental protein 12 (PP-12) and placental protein 14 (PP-14), two proteins isolated from term placenta (Bohn, Kraus & Winkler, 1982). Proteins CAG-2/PAMG-2 (Petrinin, Kozljaeva, Mesrjankina & Shevchenko, 1980) and PP-12 (Rutanen *et al.*, 1984) have been detected immunochemically in soluble extracts of the endometrium, but it is not known whether the placenta or the endometrium synthesizes these

proteins. Whether the major secretory proteins of the endometrium we have identified are related to these placental proteins is uncertain although their physicochemical properties suggest that they are dissimilar. For example the molecular weight of the α_1 -microglobulin (CAG-1/PAMG-1) has been reported as 20 000 and that for α_2 -microglobulin (CAG-2/PAMP-2) as 25 000 (Petrinin *et al.*, 1978), both very different from the molecular weights of α_1 - and α_2 -PEG. However, although CAG-1/PAMP-1 and CAG-2/PAMG-2 exhibit immunochemical identity with PP12 and PP14 respectively, totally disparate molecular weights and physicochemical properties have been reported for these proteins (see Bohn, Kraus & Winckler, 1982). If these proteins are similar it raises the possibility that these placental proteins actually represent secreted endometrial proteins that are sequestered by cells within the placenta, their possible cellular site of action. The proteins α_1 -PEG and α_2 -PEG do not appear to be synthesized by the placenta since first trimester chorionic villi during in-vitro incubation do not secrete the radiolabelled proteins (S. C. Bell, unpublished observations). Pregnancy-associated plasma protein-A (PAPP-A), a presumed placental protein because of immunohistological evidence of placental localization, has also been detected in extracts of the endometrium (Duberg *et al.*, 1982) although it is not known whether the amounts in blood of non-pregnant women (Bersinger & Klopfer, 1984) and in placentae arise from endometrial synthesis and secretion.

Endometrial α_1 - and α_2 -globulins are the major secretory proteins of pregnancy endometrium *in vitro* and their individual rates of synthesis and secretion are dependent upon the time of pregnancy at which endometrium is obtained (Bell *et al.*, 1985), perhaps reflecting different functions of the endometrium during pregnancy. It is suggested that α_2 -PEG secretion is associated with secretory glandular epithelium, a tissue prominent in secretory-phase endometrium and pregnancy endometrium (decidua parietalis) specimens in which secretory glands are found in the non-decidualized decidua spongiosa layer and exhibit maximum secretion of this protein. However, α_1 -PEG appears to be secreted by the decidualized decidua compacta layer of pregnancy endometrium and is the major secretory protein by weeks 15–16 of pregnancy (Bell *et al.*, 1985). Whether the amounts of the proteins detected in amniotic fluid parallel the in-vitro rate of synthesis and secretion during pregnancy remains to be determined. We are at present developing more sensitive assays to determine whether these endometrial proteins can be detected in peripheral blood since their assay may provide insights into the function of the endometrial compartment during implantation and early pregnancy (O'Grady & Bell, 1977; Bell, 1983a,b) and complement the clinical value of placentally synthesized proteins. Further studies are in progress to determine the cellular site of synthesis of these proteins and in-vitro organ and cell culture systems are being established to examine the mechanisms involved in the regulation of their synthesis.

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