Uterine protein synthesis during the early stages of pregnancy in the rat

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Summary. The synthesis of uterine-soluble proteins during early pregnancy in the rat has been examined by means of dual-isotope labelling techniques and subsequent electrophoretic analysis. A protein of similar electrophoretic mobility to the uterine oestrogen-induced protein was observed, and synthesis of this 'presumptive induced protein' was maximal on Day 4 and Day 6 of pregnancy but low on Day 5. Pregnancy-associated protein synthesis was observed in many regions on polyacrylamide gels, including the β-lipoprotein, α1-macroglubulin, post-transferrin and albumin regions. Synthesis of the post-transferrin species rapidly increased from Day 4 to reach a maximum on Day 6 in the implantation tissue. The temporal pattern of synthesis of post-transferrin protein and of 'presumptive induced protein' suggests involvement in the processes of cell proliferation and decidualization.

Introduction

During investigations of biochemical mechanisms underlying implantation in the rat, it was found that there were considerable changes in the protein metabolism of rat uterine tissue during the preimplantation stages of pregnancy (Reid & Heald, 1970, 1971). The rate of uptake of [3H]leucine into uterine proteins increased between Days 2 and 3 of pregnancy and continued to rise until Day 5. After implantation, protein synthesis remained elevated in areas of implantation but decreased in the remaining areas of tissue. Previous attempts, using dual-isotope labelling and column chromatographic techniques, to determine whether the increase involved changes in the pattern of protein synthesis proved unsuccessful (Heald, 1973).

In the present experiments the problem has been re-examined using techniques which have demonstrated the induction of a new protein fraction ('induced protein') in the uteri of oestrogen-treated immature and normal cyclic rats (Notides & Gorski, 1966; Iacobelli, 1973; Katzenellenbogen, 1975).

Materials and Methods

Holtzman-derived Sprague–Dawley rats, weighing between 220 and 260 g, were used. They were kept in windowless rooms at a mean temperature of 22°C and with the lights on from 06.00 to 20.00 h. Food (Oxoid Breeders Diet) and water were always available. The rats were paired with males of proven fertility and the morning on which spermatozoa were found in the vagina was designated Day 1 of pregnancy. Animals, at least 4 in each group, were killed between 09.30 and 10.30 h on Days 1 to 6 of pregnancy. Mature rats, anaesthetized by a single injection of 2% tribromoethanol (Avertin: Winthrop Laboratories, Surbiton, U.K.) in 0.9% NaCl solution at a dose of 1 ml/100 g body weight, were ovariectomized by the technique described by Waynforth (1969), and killed at least 10 days later. Excised uteri were stripped of adhering fat and mesentery, slit longitudinally to remove excess intraluminal fluid and cut into squares with sides of about 3 mm.

Double isotope labelling of uterine proteins and preparation of uterine soluble proteins. From each rat, approximately 150 mg uterine tissue (wet) were incubated in 2 ml bicarbonate saline (Krebs &
Henseleit, 1932), containing 10 mM-glucose and the radioactive amino acid (Radiochemical Centre, Amersham, U.K.), at 37°C for 2 h under an atmosphere of 95% O₂ and 5% CO₂. Uterine proteins from ovariectomized mature rats were labelled by incubation with 10 μCi L-[U-14C]leucine (sp. act. 330 μCi/mmole)/ml and from pregnant rats by incubation with 25 μCi L-[4, 5-3H]leucine (sp. act. 51 Ci/mmole)/ml. After incubation the tissues were rinsed three times in ice-cold Na₂EDTA (0-05% w/v) and homogenized in 1 ml EDTA solution. Homogenates were centrifuged at 4°C for 60 min at 100,000 g and the supernatant frozen until required.

**Electrophoresis.** Aliquots of the supernatant fractions of uteri from the pregnant and ovariectomized rats were mixed and the mixture subjected to electrophoresis in polyacrylamide gels (B.D.H. Chemicals Ltd, Poole, U.K.) and on cellulose acetate strips (Cellogel: Reeve Angel Scientific Ltd, London, U.K.). In the polyacrylamide gel system (Ornstein & Davis, 1961) the separating gel (7 cm) was 7-5% (w/v) acrylamide in 0-075 M-tris–HCl, pH 8:8–9:0. The stacking gel (0-45 cm) was 2-5% (w/v) acrylamide photopolymerized in the sample buffer (0-05 M-tris–HCl, pH 6:7). The reservoir buffer was tris–glycine (0-025 M-tris, 0-192 M-glycine; pH 8-3) and the running time was 2–2.5 h at 3 mA/tube. Bovine serum albumin (Sigma Chemical Co., London, U.K.) was included as a reference marker. Gels were fixed and stained with 1% (w/v) naphthalene black 10B (BDH Ltd, Poole, U.K.) in 7% (w/v) acetic acid and destained with acetic acid–methanol–water (7:30:63 by vol.). Proteins were also separated on Cellogel strips (17 × 2-5 cm) as described by Somjen, Somjen, King, Kaye & Lindner (1973). Strips were fixed and stained with 0-5% (w/v) naphthalene black in acetic acid–methanol–water (10:45:45 by vol.) and destained with acetic acid–methanol–water (10:95:95 by vol.).

**Measurement of radioactivity.** Gels and strips were first fixed in 5% (w/v) trichloracetic acid. Gels were then frozen with solid CO₂, sectioned into 1-45 mm discs with a macrotome gel slicer (Yeda Research and Development Co. Ltd, Rehovot, Israel) and each disc placed in a vial containing 0-5 ml Soluene 350 (Packard Instrument Co., Inc., Illinois, U.S.A.). The vials were capped and gently shaken at 50°C for 6 h before addition of 10 ml scintillation solution (6 g 2,5-diphenyl oxazole/litre toluene; Koch-Light Laboratories, Colnbrook, U.K.). Cellogel strips were cut into 2-mm sections, placed in vials containing 0-5 ml Soluene 350 and left standing overnight before addition of 10 ml scintillation fluid. All vials were left at 2°C for 6 h in the dark before counting in a Nuclear Chicago Isocap 3000 scintillation spectrophotometer. Quench correction was determined with an external standard and d/min were calculated using the Scintpol programme (Seaton, 1973).

**Calculation of protein synthesis.** The relative rate of specific protein synthesis was determined by the methods of Mayol (1975).

**Results**

Examination of the ³H:¹⁴C ratios obtained from Cellogel strip separations of the uterine proteins revealed that a peak of increased ratio (see Text-fig. 1) was consistently present from Days 3 to 6 of pregnancy. This peak was of similar electrophoretic mobility to that of 'induced protein' and was designated 'presumptive induced protein'. A small peak of ¹⁴C incorporation in this region on Cellogel (Text-fig. 2) was also observed in some extracts from uterine horns of ovariectomized animals. A similar finding has been recorded by Barnea & Gorski (1970) and Dupont-Mairesse & Galand (1975) and is possibly due to increased secretion of adrenal oestrogen in ovariectomized animals (Alam, 1975).

The relative rate of 'presumptive induced protein' synthesis was determined after separation of proteins in the Cellogel system. Fewer proteins migrate with 'induced protein' in this system than in the polyacrylamide system (Somjen et al., 1973) and the ³H:¹⁴C ratios are therefore higher. There was very little synthesis on Days 1 and 2 of pregnancy but an increase on Days 3 and 4 (Table 1). After decreasing significantly on Day 5, synthesis again increased on Day 6.

Further analyses of rates of synthesis were made by the method of Mayol (1975) after separation of proteins on acrylamide gels. Although this method has a much better resolving power than the Cellogel strip, there is a loss of 2–3% of radioactivity which remains in the stacking gel and of 8–12%
Text-fig. 1. Representative ratio plots obtained after co-electrophoresis on Cellogel (see 'Materials and Methods') of soluble proteins synthesized in vitro by uteri of rats during early pregnancy (labelled with $[^{3}H]$-leucine) and by uteri from ovariectomized rats (labelled with $[^{14}C]$leucine). The arrow shows the position of bovine serum albumin.

Text-fig. 2. The counts of $[^{3}H]$ or $[^{14}C]$ activity after co-electrophoresis on Cellogel strips of soluble proteins synthesized in vitro by uteri of rats on Day 4 of pregnancy ($[^{3}H]$ counts, ○) and by uteri from ovariectomized rats ($[^{14}C]$ counts, ▲).

Table 1. Rate of synthesis of 'presumptive induced protein' in rats during early pregnancy

<table>
<thead>
<tr>
<th>Day of pregnancy</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Delta^{3}H$ (d/min $\times 10^{-2}$)</td>
<td>0.22</td>
<td>0.04</td>
<td>0.75</td>
<td>2.34</td>
<td>0.47</td>
<td>2.65</td>
</tr>
<tr>
<td>±0.02</td>
<td>±0.05</td>
<td>±0.21</td>
<td>±0.80</td>
<td>±0.10</td>
<td>±0.85</td>
<td></td>
</tr>
</tbody>
</table>

Each value is the mean (±S.E.M.) determination from 4 individual experiments, each experiment utilizing one pregnant rat uterus and duplicate samples.

Differences significant by Student's $t$ test were: Day 2 versus Day 3, 0.001 $< P <$ 0.01; Day 2 versus Day 4, $P < 0.0001$; Day 3 versus Day 5, 0.05 $< P <$ 0.01; Day 4 versus Day 5, 0.01 $< P <$ 0.02; Day 5 versus Day 6, 0.01 $< P <$ 0.02.
in the first slice of the running gel. As shown by the representative example in Text-fig. 3, the plots revealed complex patterns of protein synthesis and attention was concentrated only on those fractions in which the synthesis represented a substantial proportion of the total. Increased protein synthesis was consistently observed in regions of mobility corresponding to \(\beta\)-lipoprotein, \(\alpha_2\)-macroglobulin, post-transferrin and albumin. The synthesis of the post-transferrin fraction was apparently related to the stage of pregnancy, being low on Days 1–4 and increasing rapidly between Days 5 and 6: rates (relative to Day 6 = 100) were, Days 1–3 = 20, Day 4 = 24, Day 5 = 42. Separation of tissue on Day 6 into implantation and inter-implantation tissue showed that synthesis of this fraction in the inter-implantation tissue was reduced to 34\% of that of the implantation tissue.

![Text-fig. 3](image)

**Text-fig. 3.** The rate of synthesis of proteins associated with uterine implantation tissue on Day 6 of pregnancy in the rat. Soluble proteins from implantation tissue on Day 6 of pregnancy (labelled with \(^{3}\text{H}\))-leucine) and from ovariectomized rats (labelled with \(^{14}\text{C}\))leucine) were co-electrophoresed on 7.5\% polyacrylamide gel, and the data obtained were plotted according to the \(^{3}\text{H}\) method of Mayol (1975). 1 = \(\beta\)-lipoprotein; 2 = \(\alpha\)-macroglobulin; 3 = post-transferrin; 4 = albumin; 5 = prealbumin.

**Discussion**

Although double-isotope techniques provide a very sensitive means of detecting changes in synthesis of certain individual proteins in cells and tissues, the technique does not distinguish between synthesis of a protein *de novo* and the increased synthesis of an existing protein (Mayol, 1975). It is therefore not possible to state at this time that the increased synthesis described in the present study represents *de novo* appearance of ‘presumptive induced protein’ and post-transferrin proteins. Even so, the timing of the increased synthesis is of considerable interest especially if, as seems likely, the ‘presumptive induced protein’ is similar to the ‘induced protein’ described in other uterine systems. Synthesis of this protein is induced by oestradiol-17\(\beta\) and the extent of the synthesis is correlated with the dose of oestrogen (Katzenellenbogen & Gorski, 1972; Katzenellenbogen, 1975). Similar correlations have been found during the oestrous cycle in the mature rat (Iacobelli, 1973; Katzenellenbogen, 1975).
In the pregnant rat oestradiol secretion increases markedly between 22.00 h on Day 3 and 04.00 h on Day 4 of pregnancy (Watson, Anderson, Alam, O'Grady & Heald, 1975) and the increase in synthesis of 'presumptive induced protein' on Day 4 and the decline on Day 5 are explicable in terms of a response to a temporarily increased oestradiol level. The increase in 'presumptive induced protein' synthesis in implantation tissue on Day 6 is less readily explained, because the levels of circulating oestradiol at that time are as low as those on Days 1 and 2 of pregnancy (Watson et al., 1975), but it may be related to the intense stromal mitosis occurring on that day (Lobel, Levy & Shelesnyak, 1967; Dupont-Mairesse & Galand, 1975).

A similar relationship can also be suggested for the post-transferrin protein fraction, marked synthesis of which does not apparently commence until Day 5 when the synthesis of RNA (including the synthesis of new species) is already proceeding at an elevated rate (Heald, O'Grady, O'Hare & Vass, 1975; O'Grady et al., 1975) and the decidual response is undetectable. The increase in protein synthesis in the decidualizing tissue but not in the inter-implantation areas is interesting, and work is continuing to examine the relationship between these events more closely.

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References


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