INFLUENCE OF THE FOETUS, PLACENTA AND OVARY ON THE MAMMOTROPHIC ACTIVITY OF PREGNANT RAT SERUM

By

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ABSTRACT

Serum mammotrophic activity was assayed using an organ culture technique with histological endpoints. Using sera of 13 days pregnant rats with 1 to 18 conceptuses, activity was detectable even in the presence of 1 conceptus, but the activity with 1-4 conceptus(es) tended to be less than with 6-18. Serum of rats with 1-3 conceptus(es) was approximately 2-4 times less active than serum of rats with 14 conceptuses. Removal of the conceptuses on day 15 caused loss of mammotrophic activity of the serum, tested 4 days later. When the foetuses, the ovaries or both the foetuses and ovaries were removed on day 15 of pregnancy, mammotrophic activity was present in the serum collected 4 days later. Differences in activity between the treated groups were small. The mammotrophic activity was comparable to the activity of serum of untreated 15 days pregnant rats or 19 days sham-operated pregnant rats. Explanted single fragments of a 19 days pregnant rat placenta released activity into the medium. The placenta retained this capacity even when the foetus had been removed 4 days previously.

The presence of mammotrophic activity in the serum of pregnant rats from day 8-9 till parturition, has been demonstrated by a radioreceptor assay (Kelly et al. 1975) as well as by organ culture of mammary gland (Peters et al. 1976).


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It has been assumed (Kelly et al. 1975) that this mammotrophic activity originates in the placenta.

In the present investigation mammotrophic activity was assessed using an organ culture technique (Peters et al. 1976). The importance of the presence of the placenta, the foetus and the ovaries was determined by studying the result of their removal on the mammotrophic activity of the serum. The effect of the number of conceptuses on the level of mammotrophic activity was also studied. Finally the placental origin of the mammotrophic activity was demonstrated by first culturing placental explants and then testing the medium, primed by the placenta culture, for the presence of mammotrophic activity in an organ culture of the rat mammary gland.

**MATERIALS AND METHODS**

**Serum.** – Serum was collected from rats of a Wistar strain (Cpb: WU(WI), Centraal Proefdieren Bedrijf, TNO, Zeist, The Netherlands). Young adult nulliparous rats were mated. The day on which the vaginal smear showed the presence of spermatozoa was taken as day 0 of pregnancy. The collection and preparation of the serum samples have been described elsewhere (Peters et al. 1976). In some instances the exsanguination of the rat was followed by weighing the placentas.

**Medium.** – To the various samples a chemically defined medium (t8) was added in concentrations up to 100%. The medium was modified from Trowell's T8, but simplified. It contained salts, but no amino acids, vitamins or phenol red. Glucose was added to the medium so that the final concentration was approximately 4 mg/ml, except in exp. 2 (3 mg/ml). Bovine insulin (Organon, Oss, The Netherlands) was added to the medium in a final concentration of 50 μg/ml (23.6 IU/mg).

**Culture.** – The mammotrophic activity of the samples was assayed using mammary gland explants of 13 days pregnant rats in 3 days cultures. The technique has been described elsewhere (Peters et al. 1976). In each experiment the mammary glands of 4 rats were used.

**Operation.** – When foetectomy was performed the abdominal wall was opened under light trichloroethylene anaesthesia and the uterus incised (about 3 mm) opposite the placental implantation. After cutting the umbilical cord the foetuses were removed and the uterus closed. The foetuses were removed from both sides. When both foetus and placenta were removed a slightly larger incision (about 5 mm) was made. The foetuses were removed first and thereafter the placenta was detached from the uterine wall. In case of haemorrhage some thrombine (46 NIH-U/mg, Hoffman-La Roche, Basel, Switzerland) was applied locally. Ovariectomy was performed using a midline abdominal incision.

**Histology and grading** of the results have been described elsewhere (Peters et al. 1976). The parameters important in this study were the degree of development, mitotic activity, cytoplasmic opalescence, secretion and pyknosis. Differences between gradings were tested for statistical significance using the distribution – free test of Wilcoxon
(Wabeke & van Eeden 1970). The differences between incidences were tested with the chi-square test with Yates' correction, between means with Student's t-test. (Croxton 1959). A level of significance of 5 or 1 % was chosen.

Experiments

The following samples were assayed:

Exp. 1a: Serum collected from 13 days pregnant rats with a varying number of conceptuses. – The number of conceptuses per rat varied from 1 to 18. Twenty-four sera were assayed individually. Virgin rat serum served as a control. The serum collected was added to the medium in a concentration of 20 %.

Exp. 1b: Serum collected from 13 days pregnant rats with 1–3 and with 14 conceptus(es) and assayed at various concentrations. – Serum which was saved from exp. 1a and had been collected from 6 rats with 1–3 conceptus(es) and from 2 rats with 14 conceptuses was pooled separately and added to the medium in concentrations of 0, 1.6, 3.1, 6.3, 12.5, 25 and 50 %. Serum of virgin rats was added to make a final serum concentration of 50 % serum.

Exp. 2: Serum collected from rats after removal of the conceptuses. – On day 15 of pregnancy the conceptuses were removed from 3 rats. Four days after the operation the serum was collected. Sera of virgin rats, and sera of 15 and 19 days pregnant rats (n = 3) served as controls. The number of foetuses ranged from 7 to 14 per rat. The sera were assayed individually in a concentration of 25 %.

Exp. 3: Serum collected from rats after removal of the foetuses and/or ovaries and assayed at various concentrations. – On day 15 of pregnancy the foetuses were removed from 5 rats, the ovaries from 5 rats and both the foetuses and ovaries from another 5 rats. Four days after the operation the serum was collected. Sera of virgin rats (n = 5) and of 15 days (n = 5) and 19 days sham-operated pregnant rats (n = 5) served as controls. The number of conceptuses ranged from 2 to 15 per rat, but in each of the 5 experiments in which one set of sera was tested the number of the conceptuses was matched as far as possible. Sera were assayed individually and added to the medium in concentrations of 0, 1.6, 3.1, 6.3 and 12.5 %.

Exp. 4: Medium collected after a culture of placenta explants. – Placentas were obtained from a 19 days pregnant rat and from another rat 4 days after foetectomy (day 15). Six explants from each placenta were cultured individually on 100 % Troowell’s T8. After 3 days of culture the media were collected separately under sterile conditions. To test these media for mammotrophic activity they were added to a culture medium for mammary gland explants in a concentration of 25 %. This latter medium was supplemented with 25 % virgin rat serum.

RESULTS

1. The mammary gland of 13 days pregnant rats

The degree of alveolar development reached grade 4–5. The cytoplasm of the acini was opalescent. Traces of secretion and vacuolization were seldom observed. Mitotic figures were infrequent.

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Fig. 1.
The effect of serum collected on day 13 of pregnancy from rats with 1 to 18 conceptus(es), on degree of alveolar development (median grade, ○) and presence of mitotic activity (% of explants, □) plotted for every rat separately. Sera of individual rats (20% of medium) were assayed in a medium supplemented with insulin; 3 days cultures; 11–12 mammary gland explants were used per rat. Arrows indicate response using virgin rat serum.

2. Effect of the variation in the number of conceptuses on the level of mammotrophic activity in the serum (exp. 1 a)

Mammotrophic activity in the serum of pregnant rats was significantly increased in comparison with virgin rat serum (degree of alveolar development: at 5% level). Fig. 1 shows that one conceptus was sufficient to obtain a high level of activity as judged by the degree of alveolar development and mitotic activity in the mammary gland explants. In one case serum from a rat with a single conceptus gave results comparable to those produced by serum of a rat with 18 conceptuses. However, the degree of development or mitotic activity with sera from rats with 1 to 4 conceptuses seemed more often to be less than with 6 to 18 conceptuses. The low median grade 4 for alveolar development
occurred significantly more frequently (at 5% level) in the first group than in the second group.

3. The effect of various concentrations of serum of rats with 1–3 and with 14 conceptuses (exp. 1 b)

Fig. 2 shows the dependence of the alveolar development and mitotic activity on the concentration (0–50%) of 13 days pregnant rat serum in the medium. Approximately two to four times the amount of serum from rats with 1–3 con-

Fig. 2.
The relationship between the concentration of serum collected on day 13 of pregnancy from rats with 1–3 (▼—▼) and with 14 conceptuses (○—○), on the degree of alveolar development and mitotic activity. Fifty per cent virgin rat serum in the medium was replaced by increasing concentrations of pooled serum of pregnant rats. Medium supplemented with insulin; 3 days culture; 12 mammary gland explants used for each value recorded. Arrows indicate response with 50% virgin rat serum.

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Table 1.
The effect of rat serum on degree of alveolar development (median grade) and mitotic activity (% of explants). The following sera were collected from individual rats: F Pi: conceptuses removed on day 15 and bled 4 days later; P15: 15 days pregnant; P19: 19 days pregnant; N-P: non-pregnant (virgin). Serum was added to the medium in a concentration of 25%; 3 days culture with addition of insulin.

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Treatment</th>
<th>Number of foetuses</th>
<th>Degree of alveolar development (grade)(^1)</th>
<th>Mitotic activity (%(^2))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F Pi</td>
<td>12</td>
<td>2</td>
<td>17</td>
</tr>
<tr>
<td>2</td>
<td>F Pi</td>
<td>14</td>
<td>2</td>
<td>17</td>
</tr>
<tr>
<td>3</td>
<td>F Pi</td>
<td>12</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>4</td>
<td>P15</td>
<td>6</td>
<td>3-4</td>
<td>83</td>
</tr>
<tr>
<td>5</td>
<td>P15</td>
<td>14</td>
<td>5</td>
<td>100</td>
</tr>
<tr>
<td>6</td>
<td>P15</td>
<td>14</td>
<td>3</td>
<td>50</td>
</tr>
<tr>
<td>7</td>
<td>P19</td>
<td>6</td>
<td>4-5</td>
<td>100</td>
</tr>
<tr>
<td>8</td>
<td>P19</td>
<td>11</td>
<td>5</td>
<td>92</td>
</tr>
<tr>
<td>9</td>
<td>P19</td>
<td>11</td>
<td>4</td>
<td>83</td>
</tr>
<tr>
<td>10</td>
<td>N-P</td>
<td>2</td>
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<td>0</td>
</tr>
<tr>
<td>11</td>
<td>N-P</td>
<td>2</td>
<td>2</td>
<td>17</td>
</tr>
<tr>
<td>12</td>
<td>N-P</td>
<td>2</td>
<td>2</td>
<td>8</td>
</tr>
</tbody>
</table>

1) Median value of semiquantitative grading.
2) Per cent of explants showing one or more mitotic figures; No. of explants per serum: 12 per group.

ceptus(es) was needed to obtain results comparable with those obtained with serum from rats with 14 conceptuses. However the differences between the results obtained with the two types of serum, tested for each concentration separately, were not statistically significant.

4. The effect of the removal of the conceptuses (exp. 2)

Table 1 shows that after removal of the conceptuses on day 15 of pregnancy, the degree of alveolar development and mitotic activity obtained with the sera collected from these rats four days later, were at the level of virgin rat serum. In comparison with the results of 15 and 19 days pregnant rat serum the median values of the alveolar development and the percentages of explants showing mitotic activity were significantly (at 5% level) decreased in the serum collected after removal of the conceptuses.
The relationship between the concentration of serum and the occurrence of cytoplasmic opalescence, pyknosis, mitotic activity and secretion (% of explants). Each point represents the average of the 5 values obtained in 5 sets of experiments, each experiment performed with 12 explants per different concentration. The following rat sera were collected: 1 = virgin, 2 = 15 days pregnant, 3 = 19 days pregnant, 4 = foetecotomized, 5 = ovariectomized, 6 = ovariectomized plus foetecotomized. The animals of group 4, 5 and 6 were operated, and of group 3 sham-operated, on day 15 and bled 4 days later. Medium supplemented with insulin; 3 days culture; mammary gland explants of 13 days pregnant rats. Arrows indicate response with 100 % Trowell's T8.

5. The effect of various concentrations of serum after removal of the foetuses and/or ovaries (exp. 3)

Fig. 3 shows the average results of 5 different sets of experiments. Pyknosis decreased with increasing serum concentration, including the serum of virgin rats. Presence of cytoplasmic opalescence, mitoses or traces of secretion increased with increasing concentrations of all types of serum except the serum of virgin rats. The serum concentration necessary to obtain opalescence and
traces of secretion (3.1–6.3 %) was generally lower than that necessary to obtain mitotic activity (6.3–12.5 %). Of all the active sera the serum of foetectomized rats seemed the least active, but the latter activity was not significantly less than that of 15 days pregnant rats. In comparison with the serum after foetectomy, ovariectomy in addition resulted in serum that stimulated the secretory activity slightly more, the difference being significant (5 % level) at 6.3 % serum. In comparison with 19 days pregnant rat serum, ovariectomy had no effect on the mammotrophic activity of the serum.

Similarly the alveolar development was related to the serum concentration in the medium. Differences between the virgin rat serum and the other sera were not clear-cut at concentrations lower than 12.5 %. With 12.5 % alveolar development was less with virgin rat sera, than with the other sera, the differences between the other five active types of serum were not consistent.

Table 2 shows the placental weights. The weight was significantly decreased (at the 5 % level) after foetectomy in comparison with the weight on day 15 of pregnancy. The placental weight, however, was significantly increased after both foetectomy and ovariectomy in comparison with the two groups. At day 19 of pregnancy placental weight was not changed by ovariectomy four days earlier.

6. Appearance of mammotrophic activity in the medium after culture of the placenta (exp. 4)

Table 3 shows that placental fragments 1, 2, 3, 7, 8 and 9 released a considerable amount of activity into the medium. Fragments 6, 11 and 12 did not release activity, while the effects of fragments 5 and 10 were intermediate.

Table 2.
Placental weight (mean ± SEM) of 15 days (P15), 19 days sham-operated pregnant (P19), foetectomized (F), foetectomized and ovariectomized (FO) and ovariectomized rats (O). The animals were operated on day 15 and the placentas weighed on day 19.

<table>
<thead>
<tr>
<th>Type of serum</th>
<th>No. of rats</th>
<th>No. of placentas</th>
<th>Placental weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P15</td>
<td>7</td>
<td>85</td>
<td>206 ± 4.60</td>
</tr>
<tr>
<td>P19</td>
<td>7</td>
<td>75</td>
<td>383 ± 12.19</td>
</tr>
<tr>
<td>F</td>
<td>7</td>
<td>76</td>
<td>177 ± 3.46</td>
</tr>
<tr>
<td>FO</td>
<td>7</td>
<td>64</td>
<td>250 ± 8.10</td>
</tr>
<tr>
<td>O</td>
<td>6</td>
<td>64</td>
<td>385 ± 8.85</td>
</tr>
</tbody>
</table>

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Table 3.
The effect of medium, in which placental fragments had been cultured individually during 3 days, on the degree of alveolar development and mitotic activity in subsequently exposed mammary gland explants of 13 days pregnant rats. Placental fragments which were obtained from a 19 days pregnant rat and a rat foetectomized on day 15, were cultured in 100% t8. An aliquot of this primed medium was then added in a concentration of 25% to a fresh medium in which the mammary gland explants were cultured during 3 days; supplementation with insulin; No. of mammary explants per placenta fragment cultured: 6.

<table>
<thead>
<tr>
<th>Placenta 19 days pregnant rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>No foetectomy</td>
</tr>
<tr>
<td>-----------------------------</td>
</tr>
<tr>
<td>Fragment No.</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>5</td>
</tr>
<tr>
<td>6</td>
</tr>
</tbody>
</table>

1) Median value of semiquantitative grading.
2) Per cent of explants showing response indicated.

DISCUSSION

The present results confirm the assumption that the placenta releases mammosrophic activity. The factor involved is probably identical to rat chorionic mammotrophin (rCM) or its synonym rat placental lactogen (Kelly et al. 1975). The latter name implies a resemblance to human placental lactogen (hPL) in action and origin. In the case of hPL, serum levels seem to correlate with the total functional placental mass (Spellacy 1972). Serum hPL levels in women bearing twins seem to be approximately twice the levels found in women with only one child (Sullivan & Knaff 1974). A correlation between total placental mass and serum levels of rCM could not be demonstrated for the rat. The level of rCM activity determined by either radioreceptor assay (Kelly et al. 1975) or organ culture (Peters et al. 1976) does not show a consistent increase from day 8 to 21 of pregnancy. In the present study no relationship could be de-
monstrated between the number of conceptuses and the level of mammotrophic activity of 13 days pregnant rat serum. Only with extremely low numbers of conceptuses did mammotrophic activity seem to be decreased in comparison with a greater number of conceptuses. Nevertheless, the mammotrophic activity in the serum is controlled by the placenta. Removal of the conceptuses results in the disappearance of activity in the maternal serum, removal of only the foetuses having no such effect. There is further evidence that the placenta itself is the most likely source of the mammotrophic activity in that placental fragments released activity in vitro. A similar release of hPL in vitro has been demonstrated by Suwa & Friesen (1969).

The factors which control the placental secretion of rCM could not be elucidated, but some possibilities could be excluded. The foetus has no major direct control. Nor are the ovaries of major importance, alone or in combination with the foetuses. These observations agree with the findings for hPL; administration of oestrogens or progesterone (Spellacy 1972) and of other steroids (Lauritzen 1975) does not affect hPL levels in pregnant women.

In the present experiments, when foetectomy was combined with ovariectomy, there was evidence of a slight change in the characteristics of the mammotrophic activity in the maternal serum when foetectomy was combined with ovariectomy. This type of serum produced slightly more secretion in the mammary gland explants than serum obtained after foetectomy alone. This, however, does not necessarily imply a change in the level of the placental hormone since the organ culture technique measures overall mammotrophic activity.

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REFERENCES


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