EFFECTS OF PROGESTERONE, OESTRADIOL AND/OR CLOMIPHENE ON LIVER GLYCOCEN AND BLOOD GLUCOSE IN INTACT AND ADRENALECTOMISED RATS DURING DELAYED IMPLANTATION

By

M. S. Sankaran and M. R. N. Prasad

ABSTRACT

Prolonged administration of progesterone alone caused significant changes in liver glycogen. Oestradiol-17β increased the liver glycogen 18 hours after the treatment. A single administration of clomiphene citrate on day 9 post-coitum (pc) inhibited the oestradiol or progesterone induced increase in hepatic glycogen. Bilateral adrenalectomy on day 3 pc abolished the changes in liver glycogen induced by progesterone, oestradiol and/or clomiphene.

Administration of progesterone, oestradiol or clomiphene caused a decrease in blood glucose levels in rats during delayed implantation. Although the effects of progesterone and oestradiol on blood glucose levels were abolished by adrenalectomy, clomiphene induced changes persisted in the adrenalectomised rats.

It is concluded that progesterone, oestradiol and/or clomiphene induced changes in liver glycogen are mediated through the adrenal glands. Changes in the blood glucose levels are discussed in relation to increased insulin level in the blood and also in relation to the increased glucocorticoid secretion following various treatments.

It is well known that the liver is the site of transformation and inactivation of hormones and is influenced directly or indirectly by drug induced metabolic interactions. Liver glycogen increases in rats during delayed implantation following prolonged treatment with progesterone (Sankaran et al. 1971) or oestra-
diol (Mohla & Prasad 1968, 1969; Sankaran & Prasad 1971). Sankaran et al. (1971) postulated that the progesterone induced increase in hepatic glycogen may be mediated through changes in adrenal steroidogenesis.

Clomiphene, a synthetic analogue of chlorotrianisene (TACE) inhibits the oestrogen or progesterone induced increase in liver glycogen (Mohla & Prasad 1968, 1969; Sankaran & Prasad 1971; Sankaran et al. 1971). It was, therefore, of interest to study the effect of adrenalectomy on progesterone, oestrogen and/or clomiphene induced changes in the liver glycogen and blood glucose in rats during delayed implantation. Delayed implantation in the rat caused by ovariectomy on day 3 pc and subsequent progesterone treatment is a condition comparable to the period of pre-implantation stages of pregnancy and is thus an ideal experimental design for the study of mechanisms of oestrogen-antioestrogen interaction (Sankaran & Prasad 1972).

**MATERIALS AND METHODS**

Colony bred, adult, virgin, female rats derived originally from the Holtzman strain, ranging in weight from 180–220 g were used. They were housed in air-conditioned rooms (25 ± 1°C) and fed a balanced diet. Tap water was freely available for drinking. Females in pro-oestrus were caged with males of proved fertility and left overnight. Mating was confirmed by the presence of sperm in the vaginal smear the next morning, which was considered as day 1 of pregnancy.

Delayed implantation was induced according to the method of Cochrane & Meyer (1957). Mated females were bilaterally ovariectomised by the dorsal approach on day 3 of pregnancy; on the day of ovariectomy and thereafter each rat was administered subcutaneously 4 mg of progesterone/day in 0.25 ml of olive oil till the termination of the experiment. Surgical procedures were carried out under light anaesthesia using semesterile conditions. Additional treatments were begun on day 9 pc which is also referred to as day 9 of delayed implantation.

**Experiment 1: Effects of oestradiol and/or clomiphene on liver glycogen in rats during delayed implantation**

A group of 96 rats were divided into 4 groups which were further divided into 4 subgroups each with 6 rats per subgroup. These animals were treated according to the following schedule.

**Group 1.** – Twenty-four rats in delayed implantation (treated with 4 mg/day of progesterone) were autopsied in groups, on days 9, 16, 24 and 39 pc and are referred to as delayed implantation controls.

**Group 2.** – Twenty-four rats in delayed implantation were administered a single dose of clomiphene citrate, 1-P (β-diethylaminoethoxy) phenyl 1–2, diphenyl-2-chloroethylene, 3 mg/kg body weight, by oral gavage in olive oil, on day 9 pc; they were autopsied in groups at various time intervals after clomiphene treatment namely, 6 h and 7, 15 or 30 days, corresponding to days 9, 16, 24 and 39 pc.
**Group 3.** Twenty-four rats in delayed implantation were treated subcutaneously with a single dose of 1 μg/rat of oestradiol-17β in 0.25 ml of olive oil on days 9, 16, 24 or 39 pc and were autopsied 18 h after oestradiol treatment at each time sequence.

**Group 4.** Twenty-four rats in delayed implantation were treated with a single dose of 3 mg/kg body weight of clomiphene citrate orally in olive oil on day 9 pc; such clomiphene treated rats were administered subcutaneously 1 μg/rat of oestradiol-17β in 0.25 ml of olive oil, 6 h, 7, 15 or 30 days after clomiphene treatment, corresponding to days 9, 16, 24 and 39 pc. They were autopsied, in subgroups of 6 rats each, 18 h after treatment with oestradiol-17β at each time sequence.

**Experiment II: Effect of adrenalectomy on liver glycogen following treatment with progesterone, oestradiol and/or clomiphene**

A group of 72 rats was bilaterally adrenalectomised and ovariectomised on day 3 pc and was administered 4 mg/day progesterone on the day of ovariectomy and thereafter till the termination of the experiment. These rats were divided into 3 groups each consisting of 4 subgroups (6 rats per subgroup). Adrenalectomised rats were provided with 0.9% sodium chloride in tap water for drinking, from the day of adrenalectomy till the termination of the experiment on day 24 pc. Additional treatments were begun on day 9 pc as described for experiment I. The groups representing day 39 pc were not duplicated with adrenalectomised rats since the liver glycogen values on day 24 and day 39 pc were similar to that seen in intact delayed rats studied earlier.

At autopsy (Experiments I, II) a piece of liver was quickly weighed to the nearest mg on a torsion balance, dropped in 30% KOH and hydrolysed in a boiling water bath for the estimation of glycogen. Glycogen was precipitated according to the procedure of Good et al. (1933) and estimated by the method of Montgomery (1957). Blood was collected simultaneously directly from the heart in a heparinised syringe and was transferred to heparinised tubes for the estimation of glucose (Nelson 1944). The data were analysed using Student's t-test.

**RESULTS**

**Liver glycogen**

Changes in liver glycogen following different treatments are shown in Table 1. Progesterone only (Group 1) caused a four-fold increase in liver glycogen on day 16 pc as compared with that on day 9 pc. The decreases in liver glycogen on days 24 and 39 pc were significantly higher than those of the delayed implantation controls on day 9 pc. The administration of oestradiol in delayed implantation rats (Group 3) caused a significant increase in liver glycogen on days 9, 24 and 39 pc over the respective delayed implantation controls (Group 1); however, there was a numerical but statistically insignificant increase in liver glycogen on day 16 over the delayed implantation controls. Clomiphene treatment (Group 2) caused a sharp increase in liver glycogen on the day of administration over the corresponding delayed implantation controls. This was followed by a marked, statistically significant, decrease as compared with that of the delayed implantation controls. Fifteen
Table 1.
Effects of progesterone*, clomiphene and/or oestradiol-17β on liver glycogen (mg/100 g) in rats during delayed implantation.

<table>
<thead>
<tr>
<th>Days after clomiphene treatment</th>
<th>Control (Progesterone-Group 1)</th>
<th>Clomiphene (Group 2)</th>
<th>Oestradiol (Group 3)</th>
<th>Clomiphene + Oestradiol (Group 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>371.2 ± 36.52**</td>
<td>1126.3 ± 116.89</td>
<td>1369.5 ± 52.00(^1)</td>
<td>1018.2 ± 69.33</td>
</tr>
<tr>
<td>(Day 9 post-coitum)</td>
<td>[]</td>
<td>[]</td>
<td>[]</td>
<td>[]</td>
</tr>
<tr>
<td></td>
<td>[P &lt; 0.01]</td>
<td>[P &lt; 0.001]</td>
<td></td>
<td>[P &lt; 0.001]</td>
</tr>
<tr>
<td>7</td>
<td>1283.5 ± 280.00</td>
<td>290.3 ± 40.01</td>
<td>1411.5 ± 56.44(^1)</td>
<td>283.3 ± 38.96</td>
</tr>
<tr>
<td>(Day 16 post-coitum)</td>
<td>[P &gt; 0.05]</td>
<td>[P &lt; 0.001]</td>
<td></td>
<td>[P &lt; 0.001]</td>
</tr>
<tr>
<td>15</td>
<td>788.3 ± 104.00(^1)</td>
<td>758.5 ± 84.00(^2)</td>
<td>1347.7 ± 69.46(^1)</td>
<td>826.0 ± 86.22</td>
</tr>
<tr>
<td>(Day 24 post-coitum)</td>
<td>[P &gt; 0.05]</td>
<td>[P &gt; 0.05]</td>
<td></td>
<td>[P &gt; 0.05]</td>
</tr>
<tr>
<td>30</td>
<td>898.5 ± 62.67(^1)</td>
<td>845.8 ± 57.33(^2)</td>
<td>1596.3 ± 124.89(^1)</td>
<td>671.3 ± 72.44</td>
</tr>
<tr>
<td>(Day 39 post-coitum)</td>
<td>[]</td>
<td>[]</td>
<td>[]</td>
<td>[]</td>
</tr>
</tbody>
</table>

* Progesterone: 4 mg/day/rat in 0.25 ml of olive oil. Rats were ovariectomised on day 3 pc and administered progesterone daily till the termination of the experiment.
Clomiphene: 3 mg/kg single, oral gavage in oil on day 9 pc.
Oestradiol: 1 µg/rat subcutaneously in oil and autopsied 18 h later.

** Mean ± se: 1) \[P < 0.001\] vs. control (Day 9 pc).
2) \[P < 0.01\] vs. clomiphene (7 days).
Table 2.
Effects of progesterone, clomiphene and/or oestradiol-17β on liver glycogen (mg/100 g) in adrenalectomised* rats during delayed implantation.

<table>
<thead>
<tr>
<th>Days after clomiphene treatment</th>
<th>Control (Progesterone only) (Group 1)</th>
<th>Clomiphene (Group 2)</th>
<th>Additional treatment</th>
<th>Clomiphene + Oestradiol (Group 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (Day 9 post-coitum)</td>
<td>606.7 ± 86.07**</td>
<td>574.4 ± 63.11</td>
<td>492.0 ± 39.18</td>
<td>673.7 ± 77.87</td>
</tr>
<tr>
<td>7 (Day 16 post-coitum)</td>
<td>528.7 ± 83.23</td>
<td>421.1 ± 37.59</td>
<td>377.4 ± 77.87</td>
<td>578.1 ± 103.28</td>
</tr>
<tr>
<td>15 (Day 24 post-coitum)</td>
<td>747.8 ± 123.66</td>
<td>491.4 ± 56.15</td>
<td>525.5 ± 59.00</td>
<td>579.0 ± 72.54</td>
</tr>
</tbody>
</table>

* Rats were bilaterally adrenalectomised on day 3 pc along with bilateral ovariectomy. Description of treatments is given in Table 1.
** Mean ± se.

Numerical differences in the values are not statistically significant ($P > 0.05$).
and 30 days after clomiphene treatment the liver glycogen values were significantly higher than in those 7 days after clomiphene administration. The liver glycogen values 15 and 30 days after clomiphene treatment were not statistically significantly different from those of their respective delayed implantation controls. The administration of oestradiol to the clomiphene treated rats (Group 4) did not cause any significant increase in liver glycogen values at any of the time intervals studied.

Changes in the concentration of liver glycogen in adrenalectomised rats undergoing delayed implantation and following different treatments are shown in Table 2. The administration of progesterone (Group 1), clomiphene (Group 4) did not cause any statistically significant change in liver glycogen in the adrenalectomised rats, although there were considerable numerical differences in the values in the different groups.

Blood glucose
Changes in blood glucose following different treatments are shown in Table 3. There was no statistically significant change in blood glucose on day 9 and 16 pc following the administration of 4 mg progesterone/rat/day (Group 1). However, there was a statistically significant reduction in blood glucose levels on day 24 pc in the progesterone treated, delayed implantation controls, the level being maintained till the termination of the experiment on day 39 pc. The administration of 3 mg/kg clomiphene citrate on day 9 pc did not cause any statistically significant change in blood glucose on the day of administration. However, on day 16 pc, i.e. 7 days following a single administration of clomiphene, there was a marked, statistically significant reduction in blood glucose and the same level was maintained till day 39 pc, i.e. 30 days after the administration of clomiphene. The administration of 1 μg/rat of oestradiol-17β on days 9, 16, 24 or 39 pc did not cause any statistically significant change in blood glucose levels as compared to the respective controls, 18 h after oestradiol treatment. The administration of oestradiol-17β on days 9, 16, 24 or 39 pc to rats treated with clomiphene citrate on day 9 pc did not cause any change in blood glucose levels as compared to those of rats treated with clomiphene only. However, blood glucose levels on day 24 and 39 pc following oestradiol and/or clomiphene treatment were not significantly different from those of the controls at the same time sequence.

Changes in blood glucose levels following different treatments in adrenalectomised rats during delayed implantation are shown in Table 4. The administration of progesterone did not cause any statistically significant change in blood glucose at any of the time intervals studied in the adrenalectomised rats (Group 1) as compared with that of the delayed implantation rats with intact adrenals (Table 3, Group 1). The administration of clomiphene on day 9 pc to adrenalectomised rats caused a significant reduction in blood
### Table 3.
Effects of progesterone, clomiphene and/or oestradiol-17β ond blood glucose (mg/100 ml) in rats during delayed implantation.

<table>
<thead>
<tr>
<th>Days after clomiphene treatment</th>
<th>Control (Progesterone only) (Group 1)</th>
<th>Clomiphene (Group 2)</th>
<th>Additional treatment</th>
<th>Clomiphene + Oestradiol (Group 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (Day 9 post-coitum)</td>
<td>78.4 ± 14.27*</td>
<td>97.2 ± 13.82</td>
<td>80.0 ± 13.67</td>
<td>95.2 ± 14.19</td>
</tr>
<tr>
<td></td>
<td>*P &gt; 0.05</td>
<td>*P &lt; 0.05</td>
<td></td>
<td>*P &gt; 0.05</td>
</tr>
<tr>
<td>7 (Day 16 post-coitum)</td>
<td>103.2 ± 5.94</td>
<td>56.0 ± 10.37</td>
<td>84.0 ± 10.19</td>
<td>61.2 ± 15.93</td>
</tr>
<tr>
<td></td>
<td>*P &lt; 0.001</td>
<td></td>
<td>*P &lt; 0.01</td>
<td></td>
</tr>
<tr>
<td>15 (Day 24 post-coitum)</td>
<td>17.2 ± 2.06</td>
<td>36.8 ± 10.02</td>
<td>15.6 ± 2.18</td>
<td>50.4 ± 20.57</td>
</tr>
<tr>
<td></td>
<td>*P &gt; 0.05</td>
<td>*P &gt; 0.05</td>
<td>*P &lt; 0.01</td>
<td>*P &lt; 0.05</td>
</tr>
<tr>
<td>30 (Day 39 post-coitum)</td>
<td>23.6 ± 7.66</td>
<td>39.2 ± 6.18</td>
<td>45.2 ± 8.87</td>
<td>37.2 ± 2.18</td>
</tr>
</tbody>
</table>

*Mean ± se. Description of treatments is given in Table 1.
**Table 4.**
Effects of progesterone, clomiphene and/or oestradiol-17β on blood glucose (mg/100 ml) in adrenalectomised* rats during delayed implantation.

<table>
<thead>
<tr>
<th>Days after clomiphene treatment</th>
<th>Control (Progesterone only) (Group 1)</th>
<th>Clomiphene (Group 2)</th>
<th>Additional treatment</th>
<th>Clomiphene + Oestradiol (Group 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>115.7 ± 4.78**</td>
<td>74.7 ± 3.55</td>
<td>123.7 ± 6.39</td>
<td>72.7 ± 5.21**</td>
</tr>
<tr>
<td>(Day 9 post-coitum)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>97.3 ± 12.56</td>
<td>53.0 ± 5.48</td>
<td>95.0 ± 12.89</td>
<td>63.0 ± 8.03**</td>
</tr>
<tr>
<td>(Day 16 post-coitum)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>103.3 ± 5.97</td>
<td>49.7 ± 5.84</td>
<td>77.3 ± 7.10</td>
<td>54.0 ± 7.53**</td>
</tr>
<tr>
<td>(Day 24 post-coitum)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Rats were bilaterally adrenalectomised on day 3 pc along with bilateral ovariectomy. Description of treatments is given in Table 1.

** Mean ± se. 1) *P < 0.02 vs. Control (Day 24 pc)*
2) *P < 0.001 vs. Control (Day 9 pc)*
3) *P < 0.05 vs. Control (Day 16 pc)*
4) *P < 0.001 vs. Control (Day 24 pc)*
glucose 6 h after the administration of clomiphene and this low level was maintained till day 39 pc, i.e. 30 days after clomiphene treatment. The administration of oestradiol-17β to adrenalectomised rats undergoing delayed implantation on day 9 or 16 pc did not cause any change in the blood glucose levels 18 h after treatment with oestradiol, as compared with that of the respective progesterone treated control rats. However, there was a significant reduction in the levels of blood glucose on day 24 pc as compared with those of the control rats. Blood glucose levels following the administration of oestradiol on days 9, 16 or 24 pc to adrenalectomised rats treated with clomiphene on day 9 pc, were not statistically different from rats treated either with clomiphene alone or with oestradiol alone. However, the blood glucose levels in this group (Group 4) were significantly lower as compared with the respective delayed implantation controls.

**DISCUSSION**

Progesterone, oestradiol and/or clomiphene have various effects on hepatic glycogenesis. The administration of progesterone alone increased the liver glycogen by day 16 pc; oestradiol caused an increase in liver glycogen at all time intervals studied. The administration of a single dose of clomiphene citrate inhibited the oestradiol or progesterone induced increase in liver glycogen. Bilateral adrenalectomy on day 3 pc along with bilateral ovariectomy abolished the progesterone, oestradiol or clomiphene induced changes in liver glycogen.

The increase in liver glycogen following prolonged administration of progesterone is in agreement with our earlier observations (Sankaran et al. 1971). Although the present sets of data do not explain the mechanism(s) involved in progesterone induced increase in liver glycogen, the data concerning the adrenalectomised rats clearly points to a possible adrenal intervention. Thus the progesterone induced increase in liver glycogen may be due to alterations in the adrenal steroidogenesis resulting in an increased production and secretion of glucocorticoids, or to its direct action on the liver carbohydrate metabolism (Sankaran et al. 1971). Progesterone causes an increase in liver weight, hepatic demethylation, hepatic microsomal protein and excretion of ascorbic acid in adult female rats (Fahim & Hall 1970); progesterone also functions as a hepatic enzyme inducer (Fahim et al. 1971).

The increase in liver glycogen following a single administration of oestradiol-17β is in agreement with our earlier findings (Mohla & Prasad 1968, 1969; Sankaran & Prasad 1971; Sankaran et al. 1971). The abolition of oestradiol induced increase in hepatic glycogen by adrenalectomy supports Ingle's hypothesis (Ingle 1959) that adrenal cortical hormones play a role in oestrogen
induced changes in liver carbohydrate metabolism. The mechanism(s) involved in the oestradiol-corticoid interaction in increasing liver glycogen is not clear (see Sankaran et al. 1971, for a full discussion). It is possible that oestrogens increase the binding ability of plasma for corticoids and influence the availability of corticosterone or cortisol to the liver cells (Avdalovic 1971) or enhance adrenal steroidogenesis (Bohus et al. 1963; Kitay 1963).

The administration of progesterone alone reduced the blood glucose levels to nearly 1/5th of the initial control levels, by day 24 pc. The mechanism(s) by which progesterone causes changes in blood glucose levels is not clear. It may be that prolonged administration of progesterone may have caused an increased synthesis and secretion of insulin (Haist 1961; Beck 1969).

The administration of clomiphene once on day 9 pc induced an inhibition of the progesterone induced increase in liver glycogen on day 16 pc and inhibited the oestradiol induced increase in hepatic glycogen at all time intervals studied. Though the mechanism(s) of action of clomiphene on the liver is not clear, the possibility that clomiphene may have caused an inhibition of carbohydrate metabolism by a direct action on the liver cannot be ruled out (Fahim et al. 1971). However, it is interesting to note that the glycogenic effect of clomiphene, 6 h after its administration, is abolished following bilateral adrenalectomy, although it did not affect the blood glucose levels.

Our results clearly indicate that the increase in liver glycogen results from an interplay of ovarian as well as adrenocortical hormones. As has been shown previously oestrogens and progestogens contained in the oral contraceptive pills influence and cause changes in carbohydrate metabolism in the liver (Spellacy 1969). An understanding of the acute and long-range hepatic response to the oral antifertility drugs (progestins and oestrogens) may lead to the development of new compounds with minimal deleterious side effects for use in contraceptive practice.

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