INHIBITION OF OESTROGEN-INDUCED INCREASE IN HEPATIC AND UTERINE GLYCOCEN BY PROGESTERONE IN THE RAT

By

P. K. Paul and P. N. Duttagupta

ABSTRACT

The effects of progesterone in two different doses (2 mg and 4 mg/rat/day) given alone and in combination with 17β-estradiol (0.5 μg/rat/day) for 21 days, on ovariectomized rat liver and uterine glycogen were investigated. The food intake and blood glucose level were also studied under these experimental conditions. The liver and uterine glycogen and weight increased during the oestrous stage of the normal rat and following oestrogen treatment of the ovariectomized rat. Progesterone when given in combination with oestrogen inhibited the oestrogen-induced increase in glycogen of these organs and also the weight of the uterus proportionally to the doses. Moreover, progesterone by itself reduced both the concentration and total uterine glycogen of the ovariectomized rat. The food intake and blood glucose level of ovariectomized animals increased after the administration of progesterone alone or in combination with oestrogen. Nevertheless, progesterone alone was more effective in increasing the food intake than in combination with oestrogen, while it was less effective in raising the blood glucose level. It can be concluded from this study that progesterone inhibits the glycogenic effects of oestrogen in the liver and uterus of the ovariectomized rat. Moreover, progesterone by itself inhibits uterine glycogen accumulation in these animals. This glycogenolytic effect of progesterone occurs in the presence of hyperphagia and hyperglycaemia. A possible involvement of the adrenal medulla in these conditions is discussed.

Oestrogen can cause an increase in the weight and glycogen content of the liver and uterus in experimental animals (Song & Kappas 1968; Villar-Palasi 1968; Gregory et al. 1967). Nevertheless, recently Paul (1971, 1972) reported
that continued oestrogen treatment for 21 days reduces the liver and uterine glycogen in the intact rat, but these effects of oestrogen are not observed in the ovariectomized rat. Based on these observations we have suggested that oestrogen-induced ovarian progesterone secretion probably antagonizes the glycogenic effect of oestrogen in these organs. On the contrary, during normal pregnancy in the rat the hepatic and uterine glycogen increase (Paul 1971, 1972), when the maternal progesterone level remains high (Csapo & Wiest 1969). However, there are no reports on the effects of progesterone alone or in combination with oestrogen on the liver glycogen in intact or spayed animals. Hall (1965) has reported that progesterone in intact or oestrogen-treated ovariectomized mice induces glycogenolysis in the uterine longitudinal muscle, but that it produces an opposite effect in the circular muscle.

It was of interest, therefore, to study the effects of progesterone alone or in combination with oestrogen on the hepatic and uterine glycogen of the ovariectomized rat. The food intake and blood glucose level have also been studied under these experimental conditions.

MATERIALS AND METHODS

Colony bred adult female rats of the Holtzman strain 3 to 4 months old were housed in an air-conditioned temperature controlled \((22\pm1^\circ\text{C})\) room on a 14:10 hours light and dark schedule. The animals were fed a standardized “Hind’Liver” rat feed in the form of pellets. The rats were selected for experiment after studying their vaginal smears for at least three normal oestrous cycles. The thirty six rats thus selected were bilaterally ovariectomized and divided into six groups of six rats each. One of these groups served as untreated control. The remaining groups were given 17β-oestradiol and progesterone (dissolved in olive oil) separately and in combination subcutaneously daily for 21 days in the following manner:

- Group 1 ovariectomized control,
- Group 2 0.5 µg oestradiol,
- Group 3 0.5 µg oestradiol plus 2 mg progesterone,
- Group 4 0.5 µg oestradiol plus 4 mg progesterone,
- Group 5 2 mg progesterone and
- Group 6 4 mg progesterone.

Another batch of 40 rats constituting four groups (10 rats in each) served as normal controls at different stages of the oestrous cycle, viz., pro-oestrous, oestrous, metaoestrous and dioestrous respectively. The daily food intake and the gain or loss of body weight of all the animals were recorded. The rats were sacrificed on the morning following the termination of the treatment after feeding.

At autopsy the liver and the uterus were dissected out and weighed. Suitable amounts of the liver and uterus were estimated for glycogen, according to the method of Montgomery (1957). The blood was collected through heart puncture and estimated for glucose according to the method of Nelson (1944).
Fig. 1.
Daily food intake during oestradiol and/or progesterone treatment. There were 6 rats in each group.
RESULTS

The average food intake of normal controls was 15.8 g per rat per day (Fig. 1 A1). It was found that the food consumption was highest during the dioestrous stage and lowest during oestrous. The ovariecotomized controls did not show any significant change in food intake as compared with those of the normal controls (Fig. 1 A2). However, the food intake was reduced significantly following oestrogen treatment in the ovariecotomized rat, while progesterone counteracted the effect of oestrogen (Fig. 1 B). Moreover, progesterone was

Table 1.
Effects of 17β-oestradiol and/or progesterone on body and organ weights.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Body weight (B.W.) gain (g)</th>
<th>Liver weight</th>
<th>Uterine weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Absolute (g)</td>
<td>Relative mg/100 g B.W.</td>
</tr>
<tr>
<td>Normal controls run for 21 days through the oestrous cycle:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pro-oestrous</td>
<td></td>
<td>7.64±1.30</td>
<td>3.39±0.51</td>
</tr>
<tr>
<td>Oestrous</td>
<td></td>
<td>7.02±1.62</td>
<td>3.54±0.19</td>
</tr>
<tr>
<td>Metaoestrous</td>
<td>17.0±1.1</td>
<td>7.78±1.32</td>
<td>3.39±0.11</td>
</tr>
<tr>
<td>Dioestrous</td>
<td></td>
<td>7.05±1.24</td>
<td>3.71±0.19</td>
</tr>
<tr>
<td>Ovariectomy + Oe2 for 21 days</td>
<td></td>
<td>17.0±2.4</td>
<td>8.23±0.66</td>
</tr>
<tr>
<td>Ovariectomy + Oe2 + 2 mg P2 for 21 days</td>
<td></td>
<td>32.0±5.5</td>
<td>9.77±1.1</td>
</tr>
<tr>
<td>Ovariectomy + Oe2 + 4 mg P for 21 days</td>
<td></td>
<td>7.5±1.2</td>
<td>8.33±0.34</td>
</tr>
<tr>
<td>Ovariectomy + 2 mg P for 21 days</td>
<td></td>
<td>53.0±3.8</td>
<td>8.80±0.25</td>
</tr>
<tr>
<td>Ovariectomy + 4 mg P for 21 days</td>
<td></td>
<td>46.0±6.5</td>
<td>9.47±0.38</td>
</tr>
</tbody>
</table>

Ten rats were used for each of the stages of the oestrous cycle and 6 rats in the remaining groups. 1 = 0.5 µg 17β-oestradiol/rat/day. 2 = progesterone.
most effective in increasing food intake proportionately to the doses used (Fig. 1 C).

All the animals in these investigations gained in body weight irrespective of the treatment given to them, although the degree of weight gain was dependent on the type of treatment (Table 1). In this respect progesterone alone at a dose level of 2 mg proved to be most effective while a combination of 4 mg of progesterone with oestrogen was least effective in the ovariectomized rat. The liver weight (absolute or in relation to body weight) after oestrogen and progesterone treatment (separately or in combination) to the spayed rat showed a slight increase above the normal and ovariectomized controls (Table 1). The uterine weight (absolute or in relation to body weight) was highest during the oestrous stage of the cycle and it was lowest in the dioestrous stage of the normal controls (Table 1). Ovariectomy alone significantly \( P = < 0.001 \) further

![Graph showing effects of progesterone and oestradiol on liver glycogen](image-url)

**Fig. 2.** Effects of oestradiol and/or progesterone given for 21 days on the liver glycogen. Ten rats were used at each of the stages of the oestrous cycle and 6 rats in the remaining groups.

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reduced the uterine weight to a level lower than the dioestrous level of the normal rat after 21 days. Oestrogen, however, induced a maximum gain in uterine weight of the spayed rat, while progesterone counteracted this effect of oestrogen proportionately to the doses used.

A significant ($P < 0.01$) increase in the concentration and total liver glycogen was observed during the oestrous stage of the normal rat in comparison with the other stages of the cycle (Fig. 2). Twentyone days after ovariectomy the liver glycogen maintained a level comparable with those of the prooestrous level of the normal controls. Oestrogen treatment in these spayed rats induced a marked increase in the concentration and total glycogen content of the liver. On the other hand, progesterone exerted an antagonistic effect on the oestrogen-induced increase in hepatic glycogen proportionately to the doses used. Progesterone as such, however, irrespective of the doses given, was unable to alter the hepatic glycogen from the ovariectomized control value.

The blood glucose level of the normal controls increased in the dioestrous stage in comparison with the other stages of the cycle (Fig. 3). Twentyone days after ovariectomy, the blood glucose level was lower than at any of the stages of the oestrous cycle of the normal rat. Oestrogen treatment to the spayed rats, although it caused an increase in the blood glucose level above that of the ovariectomized controls, it was still significantly ($P < 0.01$) lower than normal.

\[ Oe_2 = 17\beta - \text{Oestradiol} \]
\[ P^* = \text{Progesterone} \]

**Fig. 3.** Effects of oestradiol and/or progesterone given for 21 days on the blood glucose level.

Ten rats were used at each of the stages of the oestrous cycle and 6 rats in the remaining groups.

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than that during the dioestrous stage of the normal controls. On the other hand, progesterone alone or in combination with oestrogen, irrespective of the doses used, was more potent than oestrogen only in increasing the blood glucose level in the spayed rat. Nevertheless, the higher dose (4 mg) of progesterone either independently or in combination with oestrogen was less effective as compared with its lower dose (2 mg) respectively. In fact 2 mg progesterone and oestrogen in combination exhibited a higher degree of synergism in this respect.

The concentration and total uterine glycogen were higher during the oestrous stage of the cycle than at any of the other stages of the cycle of normal rats (Fig. 4). Twentyone days after ovariectomy the concentrations of the uterine glycogen was comparable with the dioestrous level of the normal rat, but the total glycogen was lower than at any stages of the oestrous cycle. Oestrogen treatment of the spayed rat induced a marked increase in uterine

\[ Oe_2 = 17\beta-Oestradiol \]
\[ P = \text{Progesterone} \]

![Graph showing effects of oestradiol and progesterone on uterine glycogen](image)

*Fig. 4.* Effects of oestradiol and/or progesterone given for 21 days on uterine glycogen. Ten rats were used at each of the stages of the oestrous cycle and 6 rats in the remaining groups.

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glycogen, while progesterone inhibited this oestrogen-induced increase in uterine glycogen proportionately to the doses used. Moreover, progesterone alone reduced the uterine glycogen of the spayed rat, indicating a dose-dependent response.

**DISCUSSION**

The results of the present study show that in oestrogen-treated ovariectomized rats the liver glycogen and blood glucose level increase despite a reduction in food intake. On the other hand, when progesterone is given to these rats in combination with oestrogen, the hepatic glycogen level is reduced proportionately to the doses of progesterone administered, although the food intake and blood glucose level are increased under these conditions. Recently Paul (1972) has suggested that the oestrogen-induced secretion of some ovarian factor(s), probably progesterone, antagonizes the deposition of hepatic glycogen. It has also been reported that the liver glycogen is reduced following Enovid (norgestrel plus mestranol) treatment for a month (Paul 1969). The present investigation, therefore, provides direct evidence that progesterone is antagonistic to the glycogenic effects of oestrogen in the liver. It has been postulated by Beck (1969) that progesterone may be a diabetogenic factor in pregnancy, since it produces similar changes in carbohydrate metabolism as in pregnancy. Yang (1970) has shown that a single dose of progesterone (6 mg/100 g body weight) increases the blood glucose level within 30 to 60 minutes and after five hours of the injection in the intact rat, but this effect is not observed in adrenal demedullated rats. Yang (1970) concludes that the adrenal medulla is probably involved in the hyperglycaemic effect of progesterone. It is well known that adrenaline exerts its hyperglycaemic action by increasing the rate of glycogenolysis in the liver and muscle. Danforth (1965) has reported that adrenaline inhibits glycogen synthesis by converting glycogensynthetase from its D to I form. Hence, it is tempting to suggest that the suppression of the oestrogen-induced increase in liver glycogen by progesterone as reported here, may be due to the involvement of the adrenal medulla.

The present investigation also shows that oestrogen induces growth and glycogen accumulation in the uterus of the ovariectomized rat, but that progesterone counteracts these effects of oestrogen. Moreover, progesterone by itself causes depletion of uterine glycogen in the ovariectomized rat. It has been shown by a histochemical study that oestrogen causes glycogenolysis in the uterine longitudinal muscle fibres and that it has an opposite effect on the circular fibres of the intact or oestrogen-injected ovariectomized mice (Hall 1965). Leonard (1962) has reported that the glycogen concentration is increased in the uterus after administration of oestradiol to spayed rats, but that adre-
naline causes an opposite effect and counteracts the action of oestradiol. It is possible, therefore, that progesterone-induced depression of uterine glycogen as described here, is mediated through the secretion of adrenaline from the adrenal medulla.

In general, it can be concluded that oestrogen is glycogenic both in the liver and the uterus, while progesterone has an inhibitory effect on the glycogenic effects of oestrogen in these organs of the spayed rat. Furthermore, progesterone exerts hyperphagic and hyperglycaemic effects in these animals.

REFERENCES


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