OESTROGEN-INDUCED GLYCOGENESIS
IN THE UTERI OF FASTED AND INSULIN-TREATED HYPOGLYCAEMIC RATS

By
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ABSTRACT

Uterine glycogen was found to be markedly resistant to the stress of both prolonged fasting and insulin treatment, even though blood glucose levels were drastically lowered. Under these hypoglycaemic conditions the uterus was found to maintain its glycogen synthetic response, and oestradiol administration caused increases in both uterine glucose and glycogen. Insulin-induced decreases in blood glucose were partially restored by the oestrogen treatment.

High levels of glucose in the blood influence the concentration of glucose and glycogen in the uterus. Uterine glucose and glycogen increased in rats made diabetic with alloxan (Swigart et al. 1961; Leonard & Shane 1965; Bitman & Cecil 1967; Bitman et al. 1967). Oestradiol administration has been found to further increase this concentration (Swigart et al. 1961; Bitman et al. 1967).

In recent experiments in our laboratory, we have found that uterine glucose and glycogen increased after the induction of hyperglycaemia by stomach tube feeding or intraperitoneal injections of glucose.

Since we had suggested that the mechanism of oestrogen action might involve control of uterine glucose (Bitman & Cecil 1967) we conducted the present experiments to determine the effect of very low levels of circulating glucose on uterine glycogenesis.

MATERIALS AND METHODS

Virgin female Wistar rats were ovariectomized 10 days prior to the beginning of fasting. Blood glucose was lowered by, A) fasting for 8 days or B) insulin ad-
ministration to rats fasted for 40 h. Rats fed ad libitum were also studied as controls. In preliminary studies insulin dosages and timing were varied to achieve a treatment which would reduce blood glucose to below 50 mg/100 ml without causing coma or death. The treatment schedule adopted for ovariectomized rats, selected for uniformity in weight (± 15 g), consisted of two doses of 0.25 units insulin (Iletin® – Eli Lilly & Co., Indianapolis), 1/2 h before and 3 h after oestradiol administration. All rats received water ad libitum. 17β-Oestradiol (0.2 μg) was injected subcutaneously in 0.2 ml of 10% ethanol 6 h prior to killing.

The rats were killed by decapitation. Samples of blood were analyzed for glucose, and diaphragm samples were taken for water, glycogen, and glucose determinations. The uterus was excised and divided by two transverse cuts through both horns. The ovarian ends were used for glucose, the central portion for glycogen, and the cervical end for water. Per cent water was determined after drying overnight in vacuo at 100°C. Glycogen analysis was by the method of Seifter et al. (1950), and glucose by the glucose oxidase method (as described in Manual No. 11:75. Worthington Biochemical Corp., Freehold, N. J.). Control experiments showed that no glucose was measurable when samples of pure glycogen were carried through the entire glucose oxidase procedure. Similarly, mixed samples of glucose and glycogen yielded amounts equivalent only to their glucose content. Samples of glycogen added to tissue and subjected to the glucose oxidase method were also negative in contributing to additional yields of glucose.

Statistical comparisons were made by Student's t test with correction for unequal group size.

RESULTS

Hypoglycaemia produced by prolonged fasting

The prolonged fast of 8 days lowered blood glucose from 135 mg/100 ml to 88 mg/100 ml (Fig. 1). Uterine glucose reflected this, declining ca. 25% (P < .005). There were no differences in uterine wet weight, or in water content between fed and fasted rats, but uterine glycogen was significantly increased in the fasted group (P < .010). When the fasted rats were given oestrogen, highly significant increases in uterine weight, water, glucose and glycogen occurred, demonstrating that the general metabolic response to oestrogen was maintained in these starved rats. However, fasting significantly restricted the oestrogenic response in uterine weight (P < .05), glucose (P < .001) and glycogen (P < .025).

The fasting treatment induced a decrease of about 60% in diaphragm glycogen, 1225 vs. 492 μg/100 mg for control and fasted rats respectively (P < .001). In contrast to this, uterine glycogen was remarkably resistant to the fasting procedure (Fig. 1), and had even shown an increase during the prolonged fast, thus preserving the carbohydrate reserve of the biologically important reproductive tissue.
Blood glucose levels and uterine changes in response to oestrogen in normal rats and rats fasted for 8 days. (N equals 11 or 12 for all groups, + E = Oestradiol 0.2 µg subcutaneously 6 h before killing).

**Fig. 1.**

**Hypoglycaemia induced by insulin**

Insulin treatment caused blood glucose to decrease from 86 mg/100 ml to 39 mg/100 ml (Table 1). Uterine glucose reflected this decrease in circulating glucose level. When oestrogen was given, increases in uterine water, glucose and glycogen content occurred and the blood glucose level was partially restored. The water increase was somewhat restricted, being +1.4 % as compared to +3.0 % in rats not receiving insulin. Similarly, uterine glucose increased but the increase was less with oestrogen in insulin treated fasted rats ($P < .025$) than in those not receiving insulin ($P < .001$). The oestrogen-induced increase in uterine glycogen, however, was as large as that observed in rats not receiving insulin.

Insulin, a hormone which stimulates glycogen synthesis in many tissues (Krahl 1961), did not stimulate glycogenesis in the uterus (Table 1). Further, it did not affect the oestrogen stimulated increase in glycogen synthesis. Reduction in blood glucose and the stimulation of glycogen synthesis in the diaphragm demonstrated that insulin was active in these rats. Diaphragm is
Table 1.
Effect of insulin on the blood glucose and uterine responses to oestrogen in rats fasted for 40 h (mean ± S. E.).

<table>
<thead>
<tr>
<th>Insulin</th>
<th>Blood glucose mg/100 ml</th>
<th>Uterus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Oestrogen</td>
</tr>
<tr>
<td>—</td>
<td>86 ± 3</td>
<td>90 ± 3</td>
</tr>
<tr>
<td>+</td>
<td>39 ± 3</td>
<td>58 ± 4</td>
</tr>
</tbody>
</table>

| % change | —55 | —36 | +0.1 | —1.5 | —46 | —53 | —1.3 | —4.9 |

N = 31 for the control groups given insulin, and 34 for all other groups.
Insulin dose = 2 × 0.25 units at 6½ and 3 h before killing.
Oestadiol dose = 0.2 µg 6 h before killing.
known to be extremely sensitive to insulin action and has been widely used in insulin studies (Krahl 1961). Insulin produced a 92% increase in diaphragm glucose concentration (131 vs. 252 μg/100 mg dry weight, \( P < .001 \)) and a 266% increase in glycogen concentration (365 vs. 1337 μg/100 mg dry weight, \( P < .001 \)). Oestrogen had no effects upon the insulin stimulation of glycogenesis in the diaphragm.

**DISCUSSION**

We found that oestrogen was able to stimulate uterine glycogen synthesis in conditions of low circulating glucose levels, produced both by fasting or insulin treatment, to an extent very similar to that observed in fed animals. This supports the work of Walaas (1952) who extensively studied uterine glycogen. He reported that fasting for 24 or 48 h had no effect in either ovariectomized rats or ovariectomized rats given oestrogens. Bo & Atkinson (1953), using semi-quantitative histochemical evaluation, also reported that neither the deposition of uterine glycogen nor the glycogen response to oestrogen were affected by starving rats for 48 h.

Swigart et al. (1962), using chemical methods, concluded that insulin did not affect the glycogen concentration of the uterus or the glycogen response to oestrogen of non-diabetic rats. They administered insulin 48 h after oestrogen treatment, when glycogen synthesis had already occurred, and found little change in glycogen content. Our results demonstrate that insulin is without effect upon the initial stages of oestrogen-stimulated glycogenesis, even though uterine glucose is markedly lowered. It appears that the level of glucose in the blood does not affect the ability of the uterus to synthesize glycogen. The net increase in uterine glycogen which occurred in the 6-h period would only require a removal of about 0.2% of the blood glucose per hour, an amount the glycogen synthetic system was apparently easily able to accumulate. Even when uterine glucose was lowered by insulin treatment to half of its normal level, glycogen synthesis in response to oestrogen remained unaffected.

These findings do not provide direct support for our previous suggestion that the mechanism of oestrogen action might involve control of uterine glucose. In the present experiments, a lowered pool of uterine glucose did not limit glycogenesis. In the presence of oestrogen an increase in uterine glucose occurred, although the magnitude of the increase was much smaller than formerly reported (Bitman et al. 1967). In this earlier work, great expansions of the glucose space were noted in uteri of normal and hyperglycaemic rats given oestrogen, as well as increased glycogen synthesis with high circulating glucose in alloxan diabetic rats. In this connection, several workers (Leonard 1958; Williams & Provine 1966; Bo et al. 1967) have biochemically demonstrated changes in
phosphorylase activity and increased UDPG-glycogen synthetase, enzymatic mechanisms that could rapidly »turnover« and utilize free glucose, thereby accelerating glycogen accumulation in response to oestrogen. Additional studies by histochemical assessment of enzyme activities have been made by Bo & Smith (1964) and Hall (1965). It seems probable that such enzymic actions are more important in the regulation of uterine glycogen metabolism than the factor of glucose availability.

Fowler et al. (1963), have found that insulin, given 3 h after oestradiol, did not reduce the 6-h uterine water imbibition in fed rats. In contrast, water uptake was restricted in our experiments when insulin was present during the entire period of oestrogen action.

Our experiments indicate that oestrogen apparently has a counteraction on the effect of insulin, since the blood glucose of rats that received insulin and oestrogen was significantly higher (P < .001) than in those given insulin alone. Support for this action can be found in reports of other workers. Ingle (1941), found that stilboestrol increased blood glucose and glycosuria in depancreatized and normal force-fed rats. Rodriguez (1950, 1954), reported that administration of oestrogen to 95% depancreatized, normal force-fed or alloxan-diabetic rats had a biphasic effect. First there was an elevation of blood glucose for about a month followed by a permanent return to normal levels. Haist (1961), in a review of the effects of steroids on the pancreas, cites other work showing that oestrogens are diabetogenic in partially depancreatized, alloxan or force-fed rats during the first month of continuous administration.

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