EFFECT OF OESTROGENS ON THE GLYCOGEN CONTENT OF THE RAT LIVER

BY

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Several workers have shown that oestrogens influence the carbohydrate metabolism of rats. Thus, Ingle (1941) reported that stilbestrol exerted a diabetogenic effect in forcefed normal and partially depancreatized rats as well as in alloxandiabetic rats (Ingle, 1947). Rodriguez (1950), however, reported that this diabetogenic effect was temporary. In his experiments on subtotally depancreatized rats under force feeding and stilbestrol treatment, the condition returned to normal after 4—5 weeks in spite of continued injections, while non-injected controls developed diabetes. Simultaneously hypertrophy of the Langerhans' islets were found in stilbestrol treated animals. Further, Lewis, Foglia & Rodriguez (1950) observed that several oestrogens decreased the incidence of diabetes which follows subtotal pancreatectomy in the rat. The reason for an initial diabetogenic action followed by a permanent protective action produced by oestrogens on carbohydrate metabolism is not clear. Again, as far as an oestrogenic effect on the glycogen content of the liver is concerned, contradictory results have been obtained. Decreased liver glycogen content after oestrogen administration has been found by Bokelmann, Dickmann, Kaufmann & Scheringer (1931), Gulick, Samuels & Deuel

More recent work, however, has shown an increased glycogen content of the liver after the administration of oestrogens. 

Gilder & Philips (1939) reported an increased liver glycogen after the injection of oestradiol monobenzoate. Moreover, increased liver glycogen content after stilbestrol treatment in fasting rats were found by Janes & Nelson (1940) and after diethylstilbestrol, oestriol and oestradiol by Long (1942). After the administration of large doses of stilbestrol an increased liver glycogen and blood sugar was found by Fry, Miller & Long (1942). Griffiths, Marks & Young (1941) reported increased liver glycogen after implantation of pellets of oestrogen.

In the present work the glycogen content of the rat liver has been investigated after the administration of oestrogens. The significance of different experimental conditions on the hormone effect has been studied. Thus the effect has been investigated after various hormonal doses, after feeding and fasting of the animals and in rats of different ages.

**EXPERIMENTAL**

Adult rats of about 200 gm. and immature rats of 80 to 100 gm. were used for the experiments. The procedure was otherwise as described in a preceding paper (Walaas, 1952). The following crystalline hormones were used for injection:

- Oestradiol monobenzoate, Schering Co.
- Oestrone, Nyegaard & Co.
- Dioxydiethylstilbestrol, Nyegaard & Co.

The animals were killed by a blow on the head and decapitation. Small pieces of 50 to 100 mg. of liver tissue were rapidly removed with scissors from the anterior lobe of the liver, frozen between pieces of CO₂ ice, weighed and immersed in KOH. The glycogen method of Good, Kramer & Somogyi (1933) as modified by the author for work on a micro scale and described in the preceeding paper was used. The pieces of liver tissue were removed and frozen 2 min. after killing. In control experiments it was shown that the glycogen content of the liver was not significantly reduced within the first 4 min. after killing the animal. The micro procedure used was compared with the usual macro procedure for glycogen determination and agreement within 2 per cent was found. Further, the distribution of
glycogen in the liver was studied on small pieces of tissue from the different lobes. In these experiments the standard error was ± 4 per cent of the glycogen content. It is, therefore, concluded that the procedure used is satisfactory for the determination of the glycogen content of the liver.

RESULTS

In Table I the glycogen content of liver during the oestrous cycle of the rat is shown. When the rats were fed ad libitum no significant differences between the groups were found.

Table I.
Glycogen content of liver during the sexual cycle in the rat.
Animals fed at libitum.

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of experiments</th>
<th>Glycogen expressed in Micromoles glucose equivalents per gm. wet tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prooestrus</td>
<td>7</td>
<td>56 ± 9*)</td>
</tr>
<tr>
<td>Early oestrus</td>
<td>11</td>
<td>49 ± 12</td>
</tr>
<tr>
<td>Late oestrus</td>
<td>10</td>
<td>72 ± 12</td>
</tr>
<tr>
<td>Metoestrus</td>
<td>11</td>
<td>89 ± 16</td>
</tr>
<tr>
<td>Dioestrus</td>
<td>9</td>
<td>71 ± 15</td>
</tr>
</tbody>
</table>

Test for significance of difference in glycogen content:
Prooestrus versus Metoestrus: P: 0.10—0.20.
Early oestrus versus Metoestrus: P: 0.06.

Table II includes experiments on immature rats. In these animals food restriction did not significantly decrease liver glycogen. This is in contrary to the findings in grown rats, where 48 hours of fasting reduced the glycogen content by 95 per cent. Administration of 50 micrograms oestradiol monobenzoate had no effect on the liver glycogen content in immature rats. In Table III the effect of oestrogenic substances on the glycogen content of the liver in adult spayed rats is shown. In these experiments where the animals were fed, the mean values were increased after oestrone and oestradiol mo-
Table II.
Glycogen content of liver after the administration of oestradiol monobenzoate to (E. B.) immature rats.

<table>
<thead>
<tr>
<th>Experimental</th>
<th>No. of experiments</th>
<th>Glycogen expressed in Micromoles glucose equivalents per gm. wet tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immature, fed ad libitum</td>
<td>6</td>
<td>91 ± 14</td>
</tr>
<tr>
<td>Immature, 48 hours fasting</td>
<td>19</td>
<td>67 ± 7</td>
</tr>
<tr>
<td>Immature + inj. of 50 micrograms E. B., 48 hours fasting</td>
<td>17</td>
<td>52 ± 9</td>
</tr>
</tbody>
</table>

Test for significance of difference in glycogen content:
- Immature, fed ad libitum *versus* immature, 48 hours fasting. P: 0.10.
- Immature *versus* immature injected with E. B. (Both groups 48 hours fasting). P: 0.10—0.20.

The highest values were found after large doses of hormone, but the increase was of marginal statistical significance (P: 0.09 — 0.05). In Table IV similar experiments on fasting rats are presented. After fasting for 24—48 hours the glycogen content of the liver was reduced to a very low level. Here the glycogen content of the liver was not increased after a single injection of 50 micrograms oestrone or oestradiol monobenzoate. However, after the administration of 400 micrograms oestradiol monobenzoate, the glycogen content of the liver was nearly trebled, a highly significant increase. Further, it was found that pregnancy was without any effect on the liver glycogen in rats which had fasted for 24 hours.

**DISCUSSION**

The present work has shown that increased glycogen formation in rat liver can be induced by the administration of oestrogens. However, this effect highly depends on the ex-
Table III.
Glycogen content of liver after administration of oestrone and oestradiol monobenzoate (E. B.) to castrated rats fed ad libitum.
Autopsy 48 hours after last injection.

<table>
<thead>
<tr>
<th>Experimental</th>
<th>No. of experiments</th>
<th>Glycogen expressed in Micromoles glucose equivalents per gm. wet tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Castrates, controls</td>
<td>13</td>
<td>82 ± 15</td>
</tr>
<tr>
<td>Castrates, inj. with 25 micrograms</td>
<td>12</td>
<td>67 ± 12</td>
</tr>
<tr>
<td>dioxydiethylstilbestrol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Castrates, inj. with 50 micrograms</td>
<td>10</td>
<td>108 ± 14</td>
</tr>
<tr>
<td>oestrone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Castrates, inj. with 300 micrograms</td>
<td>6</td>
<td>123 ± 16</td>
</tr>
<tr>
<td>oestrone*)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Castrates, inj. with 400 micrograms</td>
<td>10</td>
<td>121 ± 10</td>
</tr>
<tr>
<td>Oe. B.**)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Test for significance of difference in glycogen content:
Castrates versus inj. with 25 micrograms dioxydiethylstilbestrol 0.20—0.30.
Castrates versus inj. with 50 micrograms oestrone. P: 0.20—0.30.
Castrates versus inj. with 300 micrograms oestrone*).
Castrates versus inj. with 400 micrograms E. B. P: 0.05.

Experimental conditions. Large doses of oestrogens and food restriction were necessary to obtain a significant increase in the liver glycogen content. This may explain the discrepancies found in earlier investigations.

The fact that the effect has also been found in fasting animals, excludes the possibility that the increased glycogen content of the liver is due to increased caloric intake after the injection of oestrogen. It seems of interest that the stimulation of glycogen formation in the liver by oestrogens, when compared with the stimulating oestrogenic effect on uterine glycogen content, is of a quite different character. This last mentioned effect, described in the preceding paper, occurred during the physiological changes in the oestrogen level in the

*) 50 micrograms injected daily for 6 days.
**) 50 micrograms injected daily for 8 days.
Table IV.
Glycogen content of liver after administration of oestrone and oestradiol monobenzoate (E. B.) to castrated rats. Fasting conditions. Autopsy 48 hours after last injection.

<table>
<thead>
<tr>
<th>Experimental</th>
<th>Fasting</th>
<th>No. of experiments</th>
<th>Glycogen expressed in Micromoles glucose equivalents per gm. wet tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Castrates, controls</td>
<td>24 hours</td>
<td>5</td>
<td>4.0 ± 0.6</td>
</tr>
<tr>
<td>Castrates, inj. with 50 micrograms oestrone</td>
<td>24 hours</td>
<td>12</td>
<td>2.1 ± 0.4</td>
</tr>
<tr>
<td>Castrates, Pregnancy 21 day</td>
<td>24 hours</td>
<td>6</td>
<td>2.7 ± 0.1</td>
</tr>
<tr>
<td>Castrates</td>
<td>48 hours</td>
<td>12</td>
<td>6.4 ± 1.7</td>
</tr>
<tr>
<td>Castrates, inj. with 50 micrograms E. B.</td>
<td>48 hours</td>
<td>16</td>
<td>7.0 ± 2.4</td>
</tr>
<tr>
<td>Castrates, inj. with 400 micrograms E.B.*)</td>
<td>48 hours</td>
<td>12</td>
<td>17.4 ± 1.8</td>
</tr>
</tbody>
</table>

Test for significance of difference in glycogen content:
Castrates versus castrates inj. with 400 micrograms E. B.
P < 0.001.

Oestrus cycle and was largely independent of the physiological conditions of the animals. Thus, the oestrogenic effects on the uterus and liver glycogen occur quite independently and the mechanism may be different.

The general effects of oestrogens on carbohydrate metabolism of rats are glycogen formation in the liver and initially increased blood sugar. No effect of oestrogens on muscle glycogen content has been found in rabbits (Schumann, 1940) or in rats (Walaas, 1952). The explanation of these effects may be an upset of the hormonal balance in the organism. The oestrogenic effect on liver glycogen content is probably transmitted through the pituitary-adrenal system. Indications have been obtained by Fry, Miller & Long (1942) that these glands are involved. These workers did not find any glycogen formation in the liver after oestrogen injection in hypophysectomized rats.*

*) 50 micrograms. E. B. injected daily for 8 days.
or adrenalectomized rats. Hence, the changes in carbohydrate metabolism after oestrogen administration may be explained by an increased anterior pituitary and adreno-cortical secretion. In support of this view it has been shown that purified growth hormone from the anterior pituitary, in addition to diminishing the utilization of carbohydrate (Houssay & Anderson, 1949), also increased glycogen storage in the tissues (Milman & Russell, 1950). Similar effects have been obtained by the administration of adreno-cortical hormones (Ingle, 1950).

**SUMMARY**

1. Determination of the glycogen content of rat liver has been made following the administration of oestrogen under different experimental conditions.
2. No significant changes in the liver glycogen content were found during the oestrous cycle. No effect was observed with a single injection of oestrogen in immature or adult spayed rats.
3. Prolonged administration of oestrone and oestradiol monobenzoate produced increased liver glycogen content in adult spayed rats. In fasting animals this effect was highly significant.

**REFERENCES**

Brunelli, B.: Arch. internat. de pharmacodynam. et de thérap. 49, 212.
Griffiths, M., Marks, H. P. & Young, F. G.: Nature 147.
Ingle, D. J.: Endocrinology 29, 838, 1941.