THE EFFECT OF AN ACUTE DOSE OF BIOTIN AT A POST-IMPLANTATION STAGE AND ITS RELATION WITH FEMALE SEX STEROIDS IN THE RAT

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Summary An acute dose (10 mg/100 g body weight) of biotin at the post-implantation stage (day 14 and 15) inhibited the fetal and placental growth, and in few rats it also caused resorption of fetuses and placentae. The maintenance of pregnancy with normal fetal and placental growth was effected with estrogen therapy, but progesterone failed to correct the biotin-induced effect. The uterine and placental glycogen, RNA and protein levels, as well as, glucose-6-phosphate dehydrogenase activity in the ovary, liver and uterus showed a reduction following biotin treatment. Estrogen therapy under such conditions corrected these adverse effects of biotin overdose, while progesterone had no significant effects. The study suggests that the acute dose of biotin at an advance stage of pregnancy may cause adverse effects on the physiological regulation of gestation, possibly by creating deficiency of estrogen and gestagen. The possible role of estrogen in the fetal and placental growth and regulation of gestagen secretion is discussed.

The B group of vitamins are not known to cause hypervitaminosis. Nevertheless, recent studies show that an acute dose of biotin causes abnormalities in reproductive functions (1, 2). An acute dose of biotin at pre-implantation stage (day 1 and 2) causes conceptus resorption in association with reduction in hepatic and uterine glycogen and protein levels (3). The activity of glucose-6-phosphate dehydrogenase (G-6-PD) in the liver, uterus, ovary and adrenal also is reduced following biotin treatment (3). Estrogen therapy to these biotin-treated rats induces normal maintenance of pregnancy, and glycogen, protein and G-6-PD activity of the affected organs, except the adrenal, while progesterone supports only the maintenance of pregnancy. It is known that the requirement of biotin increases during the last days of pregnancy in the rat (4).

It was of interest, therefore, to study the effect of an acute dose of biotin at the advance stage of pregnancy, and to observe how estrogen or progesterone is involved in this relation.
MATERIALS AND METHODS

Colony bred Holtzman strain rats, approximately 3 months old were mated with the males of the same strain. Day 1 of pregnancy was determined in the morning the rat showed spermatozoa in its vaginal smear. Thus a total number of 34 pregnant rats were obtained. A dose of 10 mg D (+)-biotin (E. Merck) dissolved in 0.2 ml of 0.1 N NaOH per 100 g body weight was subcutaneously injected to 11 rats on day 14 and 15 of pregnancy. The next batch of 7 pregnant rats was identically treated with biotin and was also administered with 0.1 µg of 17-β-estradiol in 0.05 ml of olive oil subcutaneously daily up to day 21 of pregnancy starting from day 15. Yet another batch of 7 pregnant rats was treated with 4 mg of progesterone in 0.2 ml of olive oil following biotin treatment up to day 21. The last batch of 9 pregnant rats served as untreated controls.

The animals were housed in an air-conditioned room under a 10:14 hr of light-dark schedule, and were fed a standardized rat pellet commercially (Hind Lever, Ltd.) prepared from natural food sources. According to the manufacturer’s specification the food contained 24% protein, 50% carbohydrate and 4% fat with adequate amount of vitamin mixture. The caloric value of the food was 3,200 per kg.

Autopsies were performed in the morning of the 22nd day of the experiment. The liver, ovaries, uteri, placentae and fetuses were dissected out and weighed. The initial and final body weights of the pregnant rats were recorded. The final body weight was considered excluding fetuses, placentae and uterus.

Suitable pieces of the liver, uterus and placenta were processed for quantitative estimations of glycogen, RNA, DNA and protein. MONTGOMERY’s (5) method was used for glycogen estimation. Tissue RNA, DNA and protein were extracted according to the method of SCHMIDT and THANNHAUSER (6) and measured following the method of MERCHANT et al. (7), BURTON (8) and LOWRY et al. (9). The G-6-PD activity of the ovary, adrenal, liver, uterus and placenta was measured according to the method of GLOCK and McLEAN (10) as modified by HUGGINS and YAO (11).

RESULTS AND DISCUSSION

The results showed that the acute dose of biotin on day 14 and 15 of pregnancy caused resorption of fetuses and placentae in only 2 out of 11 rats (Table 1). Although the remaining 9 rats maintained pregnancy up to the term, the maternal body and uterus and the fetal and placental weights were significantly (P<0.01) lower than those of controls (Table 1). Estrogen therapy under such biotin-treated conditions restored the maternal body and uterus and the fetal and placental weights at levels which were comparable with those of controls. Progesterone therapy, on the other hand, was able to maintain maternal body and uterine weights at normal levels without having any stimulatory effect on the fetal and
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Table 1. Effects of an acute dose of biotin on pregnancy maintenance and on the body and organ weights, and its relation with estrogen and progesterone.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. implantation sites</th>
<th>No. fetuses</th>
<th>Fetal weight (g)</th>
<th>Placental weight (g)</th>
<th>Maternal body and organ weights</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated control run for 21 days</td>
<td>(9)* 9.2 ± 1.2</td>
<td>7.55 ± 1.45</td>
<td>6.02 ± 0.25</td>
<td>0.602 ± 0.153</td>
<td>B.W. gain (+/−) 4.06 ± 4.6 B.W./100 g, or loss (−) 17.1 ± 2.05</td>
</tr>
<tr>
<td>Biotin</td>
<td>(9)* 9.7 ± 1.3</td>
<td>7.5 ± 0.75</td>
<td>2.79 ± 0.13</td>
<td>0.409 ± 0.205</td>
<td>Liver 25.7 ± 5.3 B.W. 861.3 ± 18.0 B.W.</td>
</tr>
<tr>
<td>Biotin + 0.1 μg estradiol from day 15 to 21</td>
<td>(7)* 9.5 ± 2.04</td>
<td>9.5 ± 2.4</td>
<td>5.55 ± 0.14</td>
<td>0.593 ± 0.036</td>
<td>Uterus 47.0 ± 3.2 B.W. 1,364.1 ± 54.7 B.W.</td>
</tr>
<tr>
<td>Biotin + 4 mg progesterone from day 15 to 21</td>
<td>(7)* 11.5 ± 1.2</td>
<td>10.4 ± 3.4</td>
<td>3.00 ± 0.12</td>
<td>0.406 ± 0.042</td>
<td>Ovary 38.0 ± 3.0 B.W. 1,007.2 ± 26.5 B.W.</td>
</tr>
</tbody>
</table>

* The figures in the parentheses indicate number of rats. The animals were sacrificed on day 22 of pregnancy in the morning.

** 10 mg/100 g B.W. in 2 injections on day 14 and 15 of pregnancy.

*** 2 out of 11 rats resorbed their fetuses and placentae. Data obtained from these 2 rats are not included.

placental growth. The liver and ovarian weights were not significantly influenced by biotin or biotin plus estrogen or progesterone treatment (Table 1). Recently we (3) have reported that the acute dose of biotin at the pre-implantation stage causes resorption of fetuses and placentae in most of the rats which were put under the treatment. The pregnancy under such conditions is maintained with estrogen or progesterone therapy in association with an increase in maternal body and uterine weight, showing a superiority of estrogen over progesterone. Therefore, the present study on the effect of biotin at an advance stage of pregnancy confirms our earlier observations that an acute dose of biotin induces adverse effects on pregnancy by creating deficiency of estrogen and progesterone. The present report emphasizes that biotin can induce adverse effects on pregnancy at any stage. Furthermore, the study signifies that the maintenance of an adequate circulatory level of estrogen is an important factor for normal fetal and placental growth. It is an established fact that the ovarian gestagen secretion is indispensable for the maintenance of pregnancy in the rat. The maintenance of pregnancy in the biotin-treated rat with only estrogen, therefore, suggests a role of estrogen on endogenous gestagen secretion.

Biotin treatment after mid pregnancy caused a significant (P<0.01) reduction in the uterine and placental glycogen concentration (Table 2). Estrogen therapy under such conditions raised the glycogen in these organs to normal control levels. But progesterone therapy failed to improve glycogen levels of these organs. The liver glycogen and the blood glucose level did not show any significant change.
Table 2. Effects of an acute dose of biotin on tissue glycogen and blood glucose levels in the pregnant rat and its relation with estrogen and progesterone.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Glycogen (mg/100 mg tissue)</th>
<th>Blood glucose mg/100ml blood</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Uterus (with implantation site)</td>
</tr>
<tr>
<td>Untreated control run for 21 days</td>
<td>(9) 1.34±0.168</td>
<td>0.565±0.084</td>
</tr>
<tr>
<td>Biotin b</td>
<td>(9) 0.965±0.121</td>
<td>0.313±0.093</td>
</tr>
<tr>
<td>Biotin+0.1 μg estradiol from day 15 to 21</td>
<td>(7) 1.27±0.143</td>
<td>0.491±0.102</td>
</tr>
<tr>
<td>Biotin+4 mg progesterone from day 15 to 21</td>
<td>(7) 0.864±0.102</td>
<td>0.232±0.082</td>
</tr>
</tbody>
</table>

- The figures in the parentheses indicate number of rats. The animals were sacrificed on day 22 of pregnancy in the morning.
- 10 mg/100 g B.W. in 2 injections on day 14 and 15 of pregnancy.

The analysis of RNA and protein levels in the liver, uterus and placenta showed that biotin caused a significant ($P<0.01$) level of reduction only in the liver protein as compared with those of controls (Table 3). Estrogen therapy to

following biotin, or biotin plus estrogen or progesterone treatment (Table 2). It is well established that estrogen or pregnancy causes glycogen accumulation in the uterus, but progesterone exerts glycolytic effects in association with the production of hyperglycemia ($12$–$14$), yet is essential for the maintenance of pregnancy. The observed decline in the uterine and placental glycogen following biotin treatments, therefore, could be partially due to deficiency of estrogen which is glycogenic in these organs.
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these biotin-treated rats induced a significant \((P<0.01)\) increase in the protein level in these organs over only biotin-treated conditions, while RNA concentration was raised to a level which was significantly \((P<0.01)\) above the control values. Progesterone therapy failed to improve upon these biotin-induced effects. No significant influence on DNA concentration was noticed in the biotin or biotin plus estrogen or progesterone treated rats (Table 3). Our earlier report (3) shows that treatment with biotin at the pre-implantation stage significantly inhibits RNA and protein levels in the liver and uterus, and only estrogen therapy under such conditions is able to correct these adverse effects of biotin. Although estrogen therapy in the present study continued for a shorter period (day 15 to 21), it exhibits a similar nature of action. It is known that during normal pregnancy in the rodent, total RNA, DNA and protein increases in the liver, uterus and placenta \((15–17)\). Estrogen induces an increase in protein and nucleic acid synthesis in the uterus \((18–20)\) and liver \((21)\) of the nonpregnant rat. Therefore, it appears that the adverse effects of biotin on RNA and protein levels in the liver, uterus and placenta are primarily due to induction of estrogen deficiency by biotin through unknown means.

The ovarian, uterine and liver G-6-PD activity was significantly \((P<0.01)\) reduced following biotin treatment on day 14 and 15 of pregnancy, but no change in the activity of the enzyme was noticed in the adrenal and placenta (Table 4). Following estrogen therapy the G-6-PD activity of the affected organs of the biotin-treated rat became comparable with those of controls, but the enzyme activity in the adrenal reduced significantly \((P<0.01)\). Rat ovarian G-6-PD shows its highest activity during cycle, at parturition and after weaning, but during pregnancy its activity is low \((22)\). HUGGINS and YAO \((11)\) have observed that estrogen increases the enzyme activity in the liver of ovariectomized and adrenalectomized rats. On the other hand, adrenal G-6-PD activity is enhanced

Table 4. Effects of an acute dose of biotin on tissue G-6-PD activity in the pregnant rat and its relation with estrogen and progesterone.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>G-6-PD activity (nm TPNH/100 mg tissue/min at room temp.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ovary</td>
</tr>
<tr>
<td>Untreated control run for 21 days</td>
<td></td>
</tr>
<tr>
<td>Biotin(^b)</td>
<td>(9)</td>
</tr>
<tr>
<td>Biotin+1 μg estradiol from day 15 to 21</td>
<td>(7)</td>
</tr>
<tr>
<td>Biotin+4 mg progesterone from day 15 to 21</td>
<td>(7)</td>
</tr>
</tbody>
</table>

\(^a\) The figures in parentheses indicate number of rats. The animals were sacrificed on day 22 of pregnancy in the morning.

\(^b\) 10 mg/100 g B.W. in 2 injections on day 14 and 15 of pregnancy.
by ovariectomy and depressed by estrogen (23). It has recently been reported that estrogen is more potent than progesterone in increasing the ovarian, hepatic, uterine and placental G-6-PD activity in the rat treated with biotin at pre-implantation stage, while progesterone stimulates adrenal G-6-PD activity (3). Biotin-induced reduction of tissue G-6-PD activity probably indicates that the cellular synthetic mechanism regulated by pentose phosphate pathway is adversely affected. The ameliorative effect of estrogen supplementation in this enzyme activity seems to signify induction of estrogen deficiency by the acute dose of biotin.

REFERENCES