EFFECT OF INSULIN AND ALLOXAN ON MAMMARY GLAND GROWTH IN RATS

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ABSTRACT

The synergistic effect of insulin upon mammary gland growth has been studied in adult ovariectomized rats stimulated with estradiol benzoate (EB) and progesterone (P) for 19 days. When one, two, and three units of insulin were added, mean DNA's/100 g body weight (bw) were increased 8%, a non-significant increase; 17%, a significant increase; and 32%, a highly significant increase, respectively, when compared with the control group. A group of 21 rats were ovariectomized, treated with alloxan, then 2 μg EB + 6 mg P were administered daily to stimulate growth of the lobule-alveolar system of the mammary gland. The mean DNA of the glands was 4.5 mg/100 g bw, a reduction of 18%, a nonsignificant decrease, below the DNA of a control group without alloxan. A second group of 27 similar ovariectomized rats were treated with alloxan. They were injected with the ovarian hormones plus three units of insulin for the same period. The mean DNA of this group was 7.45 mg/100 g body weight, a highly significant increase of 66% in comparison with the group without insulin. Thus, the important role of insulin has been shown by its deficiency and by replacement therapy.

Two techniques are available for the study of various hormones on mammary gland growth in an intact animal. One method involves the administration of a given hormone to normal animals to determine whether the hormone will stimulate growth in excess of that stimulated in the control animals. The second method involves removal of the endocrine gland, to determine the effect of its absence upon gland growth and of replacement therapy.

The mean total DNA of 6.06 mg/100 g body weight was found (3) in mammary glands of ovariectomized rats treated with 2 μg EB + 6 mg P daily for 19 days. The influence of thyroxine (T₄) on mammary gland growth in rats has been studied in ovariectomized rats treated with the ovarian hormones (9). It was observed that the mean DNA was increased 26% above that of the controls treated with EB and P alone. When ovariectomized rats were treated with EB and P plus T₄, the addition of growth hormone (GH) stimulated a significant increase in the mean DNA of the mammary glands (8). In ovariectomized rats, in synergism with ovarian hormones, bovine and ovine GH stimulated a nonsignificant increase of DNA of 9 and 5% (5).

Upon ovarieetomy, the rat mammary gland fails to grow. By replacement therapy with ovarian hormones gland growth comparable to that observed in rats at the end of pregnancy may be produced experimentally by 1 or 2 μg EB + 3 or 6 mg P administered daily for 19 days.

In ovari-adrenalectomized rats, it has been observed that rats maintained on 1% saline drinking water and injected with 1 μg EB + 3 mg P showed a mean DNA of 4.64 mg, in comparison to a mean of 7.19 mg/100 g bw of normal controls, a reduction of 36% (1). Since adrenalectomy decreased normal voluntary feed intake by 27% (4), it is not clear whether decreased mammary gland growth was due to decreased feed intake or to lack of the adrenal hormones.

It is possible to produce rats with a marked insulin deficiency with alloxan, which destroys the beta cells of the pancreas. The object of this study was to determine the effect of insulin in normal and diabetic rats upon their mammary gland growth and of the growth stimulated by replacement therapy in such animals.

MATERIALS AND METHODS

Groups of adult ovariectomized rats of the Sprague-Dawley-Rolfsmeyer strain were maintained in a room at 78 ± 1°F. Purina Lab Chow with an energy value of 4.41 calories per gram and 23.4% total protein was fed during control and experimental period. Estra-
diol benzoate and P were dissolved in sesame oil (USP) and injected daily subcutaneously in 0.2 ml of oil for 19 days. The protamine zinc insulin was diluted in physiological alkaline saline.

The animals were injected as follows: 1) 16 rats received 2 μg EB + 6 mg P + 0.5 ml of alkaline saline, 2) 21 rats received 2 μg EB + 6 mg P + 1 unit of insulin, 3) 21 rats received 2 μg EB + 6 mg P + 2 units of insulin, and 4) 24 rats received 2 μg EB + 6 mg P + 3 units of insulin.

The other group of ovariecctomized rats were given a single intraperitoneal injection of alloxan (reagent) at a level of 15 mg/100 g bw, to induce a chronic state of diabetes (2). The rats were allowed 2 wk to recover from the treatment, to insure a state of chronic diabetes (6). Blood glucose levels were estimated by enzymatic determination of true glucose by the method of Saifer and Gerstenfeld (10). Rats with a glucose level of at least 200 mg per 100 ml of blood were used in the experiment.

The chronic diabetic rats were injected for 19 days subcutaneously, once daily, approximately at the same time as follows: 5) 21 controls were injected with 2 μg EB + 6 mg P + 0.3 ml physiological alkaline saline solution. 6) 27 rats were injected with ovarian hormones plus three units of protamine zinc insulin. The rats were sacrificed one day after the last injection and six abdominal-inguinal glands removed and DNA determined by the Webb and Levy method (11). The amount of DNA/100 g bw was used as an index of extent of mammary gland development.

**RESULTS**

The administration of 2 μg EB + 6 mg P alone stimulated a mean DNA of 5.47 ± 0.19 mg/100 g body weight (Table 1). When one, two, and three units of insulin were added to the ovarian hormones the mean DNA’s were increased to 5.90 ± 0.24, a nonsignificant increase of 8%; to 6.41 ± 0.29, a significant increase of 17% (P < 0.01); and to 7.22 ± 0.54; a highly significant increase of 32% (P < 0.005), respectively, over the control group.

The mammary glands of a group of 21 rats in which a chronic diabetes had been induced with alloxan, then stimulated with the ovarian hormones showed a mean of 4.50 ± 0.27 mg/100 g bw, a nonsignificant reduction of 18% below the normal control group.

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<table>
<thead>
<tr>
<th>No. of animals</th>
<th>Body weight</th>
<th>Treatment</th>
<th>Total DNA mean ± S.E.</th>
<th>Increase control Si</th>
<th>Significance level</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>Control, 2 μg EB + 6 mg P</td>
<td>30.90 ± 0.34</td>
<td>0.04</td>
<td>N.S.*</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>21</td>
<td>16 μg EB + 6 mg P</td>
<td>30.40 ± 0.34</td>
<td>0.01</td>
<td>0.4 &gt; P &lt; 0.2</td>
<td>P &gt; 0.001</td>
</tr>
<tr>
<td>31</td>
<td>16 μg EB + 6 mg P + 1 unit of insulin</td>
<td>30.18 ± 0.34</td>
<td>0.01</td>
<td>0.4 &gt; P &lt; 0.2</td>
<td>P &gt; 0.001</td>
</tr>
<tr>
<td>31</td>
<td>16 μg EB + 6 mg P + 2 units of insulin</td>
<td>30.18 ± 0.34</td>
<td>0.01</td>
<td>0.4 &gt; P &lt; 0.2</td>
<td>P &gt; 0.001</td>
</tr>
<tr>
<td>24</td>
<td>16 μg EB + 6 mg P + 3 units of insulin</td>
<td>27.25 ± 0.34</td>
<td>0.01</td>
<td>0.4 &gt; P &lt; 0.2</td>
<td>P &gt; 0.001</td>
</tr>
</tbody>
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*Supplied by Eli Lilly & Company, Indianapolis, Indiana.
Replacement therapy with three units of insulin with the ovarian hormones in 27 alloxan treated rats stimulated a mean DNA of 7.45 ± 0.81 mg/100 g body weight, a highly significant increase of 66% in comparison with a similar group without insulin. The mean DNA of this group is slightly higher than the DNA of the normal ovariectomized group.

**DISCUSSION**

Study of the hormones which stimulate mammary gland growth has been aided greatly by estimation of the DNA content of the glands. It has been shown that pregnant rats at the end of pregnancy and ovariectomized rats stimulated with estrogen and progesterone show great variation in regard to total DNA/100 g body weight. The theory has been advanced that part of the variation in the total DNA of the glands of individual rats is due to variation in the secretion rates of various hormones which may influence mammary gland growth.

Since estrogen and progesterone in the ovariectomized rats will stimulate total DNA comparable to that observed at the end of pregnancy, it is believed that these two ovarian hormones are of primary importance. However, preliminary data indicate that thyroxine and growth hormone may synergize (5) with the ovarian hormones and in rats secreting less than optimal amounts of these two hormones may limit growth of the mammary glands.

The present study, indicating that two and three units of insulin added to the ovarian hormones will increase DNA 17 and 32% respectively, suggests that less than optimal amounts of this hormone may also limit gland growth.

It has been shown that alloxan causes a destruction of the beta cells of the pancreas which secrete insulin, thus causing diabetes. In such rats stimulated with an ovarian hormones, the mean DNA of their mammary glands was 4.5 mg/100 g body weight, a nonsignificant reduction of 18% below the normal control group. Thus, the absence of insulin in the body was shown to depress gland growth. By replacement therapy with three units of insulin a similar group of alloxan-treated rats showed a mean DNA of 7.45 mg/100 g body weight, a highly significant increase of 66% in comparison with the group without insulin. The mean DNA of this group was slightly higher than the normal group to which 3 units of insulin had been administered. Thus, both by its presence in optimal amount and by its absence, the role of insulin in mammary gland growth is clearly indicated.

In the absence of adrenal glands, it was shown that mammary gland growth was significantly inhibited. However, it has been shown that adrenalectomy markedly depresses feed consumption (4). As a result, it is not clear whether the absence of the adrenal hormones plays specific roles in mammary gland growth or whether the reduced growth is due to restricted feed intake.

In the case of alloxan-treated rats, it has been shown that the feed consumption of these animals gradually increased to a level 56% above their consumption prior to treatment (6). Thus, the reduced mammary gland growth was not due to reduced feed intake. On the other hand, it has been shown in normal rats that the administration of increasing levels of insulin also stimulates an increased feed intake (7). For this reason, it is possible that the favorable effect of insulin on mammary gland growth in normal animals may be due, in part, to increased feed consumption.

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**REFERENCES**

