Effects of Growth Hormone and/or Prolactin on the Function of the Mammary Glands of Guinea Pigs in the Declining Phase of Lactation (II)

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In their previous paper\(^1\), the authors mentioned that prolactin (PL) retarded the involution of the mammary gland histologically and that growth hormone (GH) ameliorated the metabolism in addition to retarding the involution. The results, however, were not always definite, and the reasons might probably be due to the following causes: (1) dosages of the hormones too small for the purpose, (2) injection started too late, and (3) large individual differences. Then, in the succeeding experiment\(^1\), the local injection method was employed in earlier stages of lactation in order to exclude these probable causes. Nevertheless, the results were hardly affected.

The present study was carried out to determine more precisely the effects of these hormones on the function of the mammary gland in the declining phase.

**Materials and Methods**

The guinea pigs used had been bred in the authors' department. All of them were in their first lactation. The number of young was adjusted to two for each animal soon after delivery. The young were nursed naturally up to the 7th day of lactation. Milk yield was measured by the authors' method\(^2\) from the 8th to the 14th day. On these days, timed nursing was done twice instead of four times, a day. Immediately before each nursing, the mother was injected peritoneally with 1.0 I.U. of oxytocin (Atonin-O, a product of the Teikoku Pharmaceutical Company) to induce complete milk ejection.

According to the indications of the National Institute of Health (NIH), GH* and PL** were dissolved to a required concentration in 0.3 ml of medium. The animals of each group were injected with one of the following doses, twice daily for four days beginning on the 11th day of lactation: 0.9% saline for the control group, 8 mg of GH for group A, 75 I.U. of PL for group B, and 8 mg of GH plus 75 I.U. of PL for group C. Saline and GH were injected subcutaneously and PL was intramuscularly.

All animals were killed on the 15th day of lactation. The techniques of measuring dry weight, nucleic acids contents (DNA-P, RNA-P), and oxygen consumption (\(-\text{Qo}_2\)) of the mammary gland were essentially the same as described previously\(^1\), excepting that the Krebs-Ringer phosphate buffer solution, instead of Ringer III solution, was used as incubation medium. Respiratory quotient (R.Q.) was also measured with the manometer.

For histological observation, a block from the mammary gland of each animal was fixed in Bouin's solution, embedded in paraffin, cut at 5 \(\mu\), and stained with Mayer's hemalum and eosin. The number of alveoli per field was counted and the diameters of the modal and the largest alveoli were measured by the same methods as mentioned in the previous paper\(^1\).

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* NIH-GH-B (1.53×U.S.P. Reference Standard)
** NIH-P-S-3 (15 I.U./mg)
Results

The results concerning lactation are shown in Fig. 1. No group exhibited any increase in milk yield as seen in the previous experiment after injection even with those large doses which seemed to be fairly over the physiological ones even in a stage of lactation earlier than that adopted in the previous experiment.

The results of determination of mammary gland weight, nucleic acids, oxygen consumption, and respiratory quotient are presented in Table 1. There were no significant differences among the treated groups in the fresh and dry weights of the mammary gland, which, on the other hand, were apparently higher in group A than in the control (P<0.05). The total DNA-P was higher in group A than in the control (P<0.05). It was also higher in groups A and C than in group B (P<0.05). Furthermore, the difference between group C and the control group was apparently large, though it was not significant. No differences existed among the groups in \(-Qo2/DNA-P\) and \(RNA-P/DNA-P\). The total \(-Qo2\), however, was significantly higher in group A than in the control (P<0.05). It was also higher in group C than in the control group, though the difference between the two groups was not significant. The total RNA-P was significantly higher in groups A and C than in group B and the control (P<0.05, 0.01).

Table 1. Results of determination of mammary-gland weight, DNA-P, \(-Qo2\), RQ, and RNA-P

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of animals</th>
<th>Mammary gland wt. (g)</th>
<th>Total dry wt. (g)</th>
<th>Dry wt. percentage (%)</th>
<th>Total DNA-P (mg)</th>
<th>(-Qo2/hr)</th>
<th>R.Q.</th>
<th>RNA-P</th>
<th>total RNA-P (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5</td>
<td>7.49± 1.45± 19.26± 3.01±</td>
<td>1.71± 0.07± 1.32±</td>
<td>1.91± 5.78±</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GH 8mg×2×4 (A)</td>
<td>6</td>
<td>9.66± 1.95± 20.12± 4.58±</td>
<td>1.66± 7.45± 1.40±</td>
<td>2.06± 9.31±</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PL 75 IU. ×2×4 (B)</td>
<td>4</td>
<td>8.65± 1.78± 20.52± 3.14±</td>
<td>1.85± 5.64± 1.41±</td>
<td>2.03± 6.26±</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(GH 8 mg + PL 75 IU.) ×2×4 (C)</td>
<td>3</td>
<td>9.02± 1.75± 19.35± 4.29±</td>
<td>1.59± 6.54± 1.22±</td>
<td>2.06± 8.69±</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

* GH: growth hormone, PL: prolactin.
Table 2. Histometric results

<table>
<thead>
<tr>
<th>Treatment*</th>
<th>No. of animals</th>
<th>No. of alveoli per field</th>
<th>Modal alveolar diameter (µ)</th>
<th>Largest alveolar diameter (µ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5</td>
<td>106.7±8.0</td>
<td>65.6±2.8</td>
<td>1111~154</td>
</tr>
<tr>
<td>GH 8 mg x 2 x 4 (A)</td>
<td>4</td>
<td>107.1±5.3</td>
<td>69.3±3.0</td>
<td>140~165</td>
</tr>
<tr>
<td>PL 75 I.U. x 2 x 4 (B)</td>
<td>6</td>
<td>99.2±4.6</td>
<td>74.0±5.3</td>
<td>111~140</td>
</tr>
<tr>
<td>(GH 8 mg + PL 75 I.U.) x 2 x 4 (C)</td>
<td>3</td>
<td>131.1±8.5</td>
<td>55.0±2.1</td>
<td>194~151</td>
</tr>
</tbody>
</table>

* See Table 1.

In the present experiment, the values of \(-Q_O2/DNA-P\) were smaller than those given in the previous report, in spite of the earlier stages of lactation in all groups. This might be due to the difference in the sort of medium used for incubation.

Respiratory quotient was higher in group A than in group C (P<0.05).

The results of histological observations are presented in Table 2 and Figs. 2~13. In group C, the number of alveoli per field was considerably larger and the diameter of the modal alveolus significantly shorter than in any other group (P<0.05). These results would indicate that the mammary gland was composed of alveoli large in number but small in size in group C. Variation in the structure of the gland was observed among different parts within the same individual, as well as among individuals. In general, however, histological sections of the gland from the control showed some involuted alveoli with tongue-shaped cells. On the other hand, in group A, most part of each section was occupied by comparatively large alveoli having functional and cuboidal cells. In group B, large alveoli with functional cells were found in greater parts, although the effects of the hormones seemed to be very slight on alveoli already involuted. In group C, sections showed such phenomena as seen in group A or B, though alveoli were smaller in size.

Discussion

In spite of the doses fairly higher than the physiological ones and administered stages earlier, GH and PL, alone or in combination, did not increase the milk yield. These results were the same as those obtained from the previous experiment\(^1\). It is, therefore, distinct that these hormones are no limiting factors for the maintenance of lactation or galactopoiesis itself at least in the guinea pig.

It has been demonstrated that GH has some effect on the alveolar growth of the mammary gland in the normal or hypophysectomized rat or mouse\(^3~5\). On the other hand, GH has not any synergistic effect with estrogen and progesterone on the mammary-gland growth in the goat\(^7\). In the present experiment, GH maintained the glandular parenchyma or at least retarded the involution of it, as shown in the DNA content and histological findings of group A. MOON\(^8\) recognized the effect of GH on the mammary-gland growth in an ovariectomized rat which had been administered with this hormone in combination with estrogen and thyroxine. He attributed this effect to the synergistic action of the three hormones with lactogen, the production and secretion of which was induced in the pituitary by GH. In the present experiment, while GH and PL were effective on retardation of involution of the glandular parenchyma as seen in the DNA content of group C, it was not different from that of group A.
Accordingly, it may well be that GH plays a more important role on the maintenance or retardation of involution of the glandular parenchyma than PL in the declining phase of lactation in this species. Such antagonistic actions of these hormones as seen previously\(^1\) were not found in the present experiment.

The metabolic and synthetic functions of the whole mammary gland was higher in the animals administered with GH and GH plus PL than in the control. This might have been due to the effect of GH on the maintenance or the retardation of involution of the glandular parenchyma, because differences in function per cell were little among the groups. On the other hand, it was reasonable to assume from the previous experiment\(^1\) that GH may increase the metabolic function per cell, but that it may have no effect on the maintenance of the glandular parenchyma. It is far from clear whether GH had these different effects on the gland according to the dose and stage employed.

As Folley\(^9\) and Folley and McNaught\(^10\) reported, GH had no marked effect on lipogenesis. PL was not effective on the metabolic and synthetic functions of the gland even at the doses used, but it fairly retarded the involution of the gland histologically. These results agree well with those of the previous experiment\(^1\).

From the results of the present experiment, it is concluded that neither GH nor PL is a main factor for the increase of milk yield itself, which is the consolidated result of various actions of the mammary gland, though the two hormones play some role in the maintenance of the structure of the gland and also have some effects on the function of the gland.

### Summary

Succeeding to the previous study, the effects of growth hormone (GH) and prolactin (PL) on the mammary gland of the guinea pig were investigated in the declining phase of lactation. It was concluded that these hormones had little effects on the maintenance of lactation or galactopoiesis itself, although they played some role on the maintenance of the structure of the mammary gland and, in addition, GH on the amelioration of the metabolic and synthetic functions of the gland.

### Acknowledgments

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### References

4) Univ. of Calif. (Berkeley) Publication of Zoology, No. 65: 1, 1959.
Explanation of Figures

Fig. 2. Control (×100)  Epithelial cells are tall and tongue shaped. Alveoli are small.
Fig. 3. " (×200)
Fig. 4. " (×400)
Fig. 5. GH 8 mg×2×4 (×100)  Epithelial cells are cuboidal and very functional. Alveoli are large.
Fig. 6. " (×200)
Fig. 7. " (×400)
Fig. 8. PL 75 I.U.×2×4 (×100)  Epithelial cells are compact and most of them functional.
Fig. 9. " (×200)
Fig. 10. " (×400)
Fig. 11. (GH 8 mg+PL 75 I. U.)×2×4 (×100)  Epithelial cells are functional. Alveoli are a little smaller.
Fig. 12. " (×200)
Fig. 13. " (×400)
Growth Hormone and Prolactin on Lactation (II)