THE EFFECT OF LOCALLY ADMINISTERED PROLACTIN ON THE RESPIRATORY ACTIVITY OF THE MAMMARY GLAND OF RABBIT AT THE CORRESPONDING STAGE TO THE ALVEOLAR FORMATION

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It has been shown by numerous workers that prolactin participates in the mammary gland growth. Previously, we showed that prolactin induced alveolar formation when it was injected intraductally in the rabbit mammary gland having a moderately developed duct system with few alveoli in the histological study (Mizuno et al., 1955) and in the study on the nucleic acids content (Mizuno and Naito, 1956). In this connection, it is of interest to investigate the effect of prolactin on the mammary respiratory activity at the corresponding stage to the alveolar formation.

MATERIALS AND METHODS

Animals

Japanese white virgin rabbits weighing from 1.8 to 2.3 kg were ovariectomized and the injection of prolactin was begun on the 6th postoperative day. Two pairs of abdominal mammary glands were used in each animal.

Prolactin in doses of 1, 3, 5 or 10 international units per day dissolved in 0.3 ml distilled water was injected every other day for a period of 10 days into a galactophore in each of three glands and a remaining gland was served as a control. The prolactin preparation used was "Luteotrophin Squibb" supplied by E. R. Squibb & Sons.

Manometric method

The animals were killed on the 2nd day after the last injection. The mammary tissues were dissected from injected sectors and placed into ice-cold Krebs-Ringer-Phosphate solution quickly. Since slicing techniques proved to be difficult to apply to the glands which seemed to be in the neighbourhood of 3D stage grouped in previous experiment (Mizuno et al., 1955), judging from the macroscopic observation, although in the present study the histological observation was not carried out, the tissue was cut into fine pieces with scissors. A batch of tissue pieces (approximately 100 mg) were blotted on the filter paper and weighed with a torsion balance.

The incubation was carried out for 1 hr. at 37.5°C in a 4 ml of K.R.P. buffer solution added with 0.3 % glucose. Oxygen consumption and R.Q. were measured by the direct manometric method. The reaction vessel was a 20 ml Warburg's ordinary flask with side arm and gas vent, containing 0.2 ml of 20 % KOH and fluted filter paper in the center well or 0.2 ml of 3N-H₂SO₄ in the side arm. The gas phase was 100 % oxygen.

As the activity reference base, both the initial wet weight and desoxyribonucleic acid were used. Oxygen consumption was expressed as μl per initial 100 mg wet weight and also as μl per μg of DNA-phosphorus for 1 hr.

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DNA determination  At the end of manometric experiment, tissue pieces were removed from flask together with the medium, and then homogenized with a glass homogenizer which was cooled in an ice-bath. DNA was extracted by the method of Schneider (1945), except that the phospholipid extraction was not carried out. DNA was determined by the method of Dische (1930) modified by Yagi (1951). The DNA was expressed as phosphorus content.

RESULTS

The results obtained were summarized in tables and figures.

Since we observed the significant increase of DNA in the prolactin injected gland (Mizuno and Naito, 1956) and it has been recommended to use DNA as the activity reference base especially when one wishes to compare various physiological state of the mammary glands, in this experiment the respiratory activity was expressed as well on the basis of wet weight and DNA. There was a fair parallelism between the trends of change of activity expressed on both basis.

The oxygen consumption of gland injected with prolactin was significantly higher than that of control gland. And it increased nearly linearly corresponding to the dose level of administered prolactin.

The carbon dioxide production did not follow the oxygen consumption and resulted in gradual decrease of R.Q. in the glands injected with prolactin ranging from 1 to 5 i. u. However, in the gland injected with 10 i.u., it increased in the similar trend to the oxygen consumption and then resulted that the R.Q. recovered to the comparable level with that of control gland.
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PROLACTIN ON MAMMARY ALVEOLAR GROWTH

Several of the tested animals showed the slight secretion in the glands receiving 5 or 10 i.u. of prolactin. However, both oxygen consumption and R.Q. in them were not outstandingly different from those of the other non-secreting glands, while R.Q. was always below unity.

The significance of differences in oxygen consumption and R.Q. between glands receiving different doses of prolactin in the same animal, was shown in Tables 1 and 2.

Table 1. Significance of differences in oxygen consumption between mammary glands receiving different doses of prolactin

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Table 2. Significance of differences in R.Q. between mammary glands receiving different doses of prolactin

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+ 5% level ** 1% level in wet weight base
* 5% level ** 1% level in DNA base
— non-significant

DISCUSSION

Hitherto, changes in gland composition, enzyme activity and metabolic activity reflecting lactogenic hormone action have been reported by numerous workers. However, most of them followed the changes in the physiological state of mammary gland, i.e., at pregnancy, lactation and weaning. Concerning the metabolic study, Folly and French (1949 a, b) found that mammary gland slices in vitro from pregnant rats, in the presence of glucose, metabolized with a R.Q. of less than one whereas slices from lactating gland showed a R.Q. greater than one, and suggested that a close relationship must exist between changes of the metabolism of mammary slice and hormonal problem. Hoover and Turner (1954) investigated the changes in respiration, aerobic glycolysis and "metabolic" carbon dioxide production on mammary tissue slices in vitro throughout the entire normal reproductive cycle of rats.

A few reports are concerned with the effects of prolactin administered experimentally. Balmain and Folley (1952) observed that prolactin did not affect the tissue from pregnant rats but affected markedly on lactating tissue. Fujii (1956) showed that prolactin stimulated oxygen consumption of mammary gland slice taken from lactating rats.

As far as we are aware, however, there is no available information on the effect of injected prolactin on the metabolic activity of the mammary gland at its early developmental stage. From the macroscopic inspection, as mentioned above, the animals used in the present study were considered to be in the neigh-
bourhood of 3D group of the previous experiment. Thus the changes of oxygen consumption and R.Q. obtained in this experiment seems to be closely associated with the alveolar formation.

Hoover and Turner (1954) reported that respiration on nitrogen basis increased about two-fold from the virgin state to mid- and late-pregnancy, and by many workers it has been observed that the lobule-alveoli develop and are well accomplished during this period. As to the changes of R.Q., Hoover and Turner (1954) observed that the metabolic carbon dioxide production from the tissues of virgin animals was in general much greater than for early pregnant animals, and this was responsible for the extremely high R.Q. of mammary tissue from the virgin non-estrous rat. During pregnancy the carbon dioxide production in general followed the trend of respiration and R.Q. was below unity throughout pregnancy. The result in the present study that the R.Q. of control gland was significantly higher than that of 3 or 5 i.u. prolactin injected gland seems to be somewhat reflected on the observation of Hoover and Turner (1954). And the tendency of increase of it in 10 i.u. prolactin injected gland may imply the change from early- to mid-pregnancy.

It was shown recently that the non-removal of milk lowered the respiratory activity of the lactating mammary gland (Mizuno and Chikamune, 1958). This might be a factor responsible for that the respiratory activity of gland in which slight secretion was observed did not significantly differ from that of non-secreting gland, although the circumstances were of course not the same in this experiment. Further it may be considerable that enzymic activities might be inhibited by the substances contained in the retained secretion. For example, on this problem Greenbaum and Slater (1957) discussed that abrupt increase of succinic oxidase activity at parturition might be in part due to the removal of colostrum, which, generally, had a much higher content of metal ions, potent inhibitors of this enzyme, than milk secreted in active lactation. Thus, the non-removal of secretion might also be a factor responsible for the R.Q. below unity during pregnancy. However, further study should be awaited to clarify this problem.

Greenbaum and Slater (1957) studied the correlation of succinic oxidase activity with mitochondrial fractions at various stages of lactation cycle of rat, and Williams and Turner (1956) reported that the most part of intraductally injected I\(^{131}\)-labeled prolactin became associated with the particulate bodies of cytoplasm, the mitochondria and microsomes. It will therefore be of interest to study further the effect of prolactin on the alveolar formation and mammary growth in this connection.

In conclusion, it may be possible to say that the changes of respiratory activity obtained in the present study are closely related to the alveolar proliferation, in consideration of the previous reports on histological results (Mizuno et al., 1955) and on the nucleic acids determination (Mizuno and Naito, 1956).

**SUMMARY**

The effect of locally administered prolactin on the respiratory activity was
studied on the mammary gland of rabbit at the corresponding stage to the alveolar formation. There was a fair parallelism between the trends of change of respiratory activity referring to both wet weight and DNA basis. The oxygen consumption of the gland injected with prolactin was significantly higher than that of the control gland. And it increased nearly linearly with the high dose level of administered prolactin. The carbon dioxide production did not follow the oxygen consumption and resulted in a gradual decrease of R.Q. in the glands injected with prolactin ranging from 1 to 5 i.u. However, in the gland injected with 10 i.u. it increased showing a similar trend to that of oxygen consumption and then resulted in the recovery of R.Q. to the level comparable with the control. The R.Q. was always below unity.

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REFERENCES