Nucleic Acid and Collagen Content of Mammary Glands Between 30 and 80 Days of Age in Normal and Ovariectomized Rats and During Pregnancy

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Abstract

Nucleic acid and collagen content of parenchymal and extraparenchymal fat pad portions of mammary glands were determined at 10-day intervals from 30 to 80 days of age in ovariectomized, sham-ovariectomized, and intact rats; and at 6-day intervals throughout pregnancy. Pubertal development of the mammary gland was characterized not only by an increase of cell numbers (deoxyribonucleic acid) but also by a substantial increase in connective tissue elements (collagen) which was shared both by parenchymal and fat pad segments of the mammary gland. Ovariectomy reduced (P < 0.01) epithelial cell numbers (DNA), protein synthesis (ribonucleic acid), and to a lesser extent collagen in the mammary gland parenchyma but produced (P < 0.01) net increases of these constituents in the mammary fat pad, suggesting that factors other than ovarian steroids also play an important role in collagen synthesis by the mammary gland.

On the other hand, during pregnancy, mammary growth was reflected only in the parenchymal portion and occurred at the cost of fat pad although, contrary to the earlier view, collagen continued to increase throughout pregnancy. The major part of the collagenous framework of the mammary parenchyma, however, was formed prior to major epithelial cell growth, suggesting that the collagenous framework of the gland may be necessary for epithelial cell growth.

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Introduction

Morphologically, the connective tissue framework of the mammary gland can be divided into three parts: cells, fibers, and ground substance. The primary fibrous component, collagen, is secreted by fibroblasts into the extracellular space where it becomes polymerized to form fibrils (4). This fibrous protein contains large amounts of hydroxyproline (14%) (5), and this amino acid is used to estimate the collagen content of tissue (10, 13). Ovarian steroids appear to regulate the amount of collagen synthesized by fibroblasts (2). In cattle, the hydroxyproline in mammary tissue is highest during the estrogenic phase of the estrous cycle (17).

Although collagen plays an important role in inflammation (7) and mammary parenchymal growth (9, 12), relatively little information is available regarding the collagen content of mammary tissue during early stages of mammary development or how collagen synthesis by the mammary gland is affected by ovarian steroids. Our study was designed to understand better growth of collagen in the mammary gland of virgin and pregnant animals and to determine the amount of collagen contributed by the parenchymal and extraparenchymal (fat pad) portions of the gland.

Materials and Methods

Female Sprague-Dawley rats, weaned at 20 days of age, were obtained from litters previously adjusted to six young per mother. After weaning, the young rats were reared 6 per cage with free access to feed and water.

At 20 days of age, groups of rats were either ovariectomized (OVX), sham-OVX or left intact. Ten rats from each group were killed at 10-day intervals from 30 through 80 days of age.

To determine growth of collagen in the mam-
MAMMARY NUCLEIC ACID AND COLLAGEN

Mammary gland during pregnancy, 80 day old primiparous rats were mated and killed at 6 day intervals throughout pregnancy. The day sperm were found in the vaginal smear was recorded as the first day of pregnancy.

At autopsy, the six abdominal-inguinal mammary glands were removed, and the parenchymal tissue was separated from the fat pad. This was achieved by placing the gland in 95% ethyl alcohol for at least 24 hr. This made the gland translucent, and the periphery of the parenchymal portion of the gland could be seen against a light source allowing one to trim away the fat pad tissue (16). The mammary lymph nodes were discarded.

The mammary gland parenchyma and fat pad were further extracted for lipids, then dried, weighed, and ground to a fine powder (16). The biochemical parameters measured in both portions were hydroxyproline (OHP) (13), a measure of collagen; desoxyribonucleic acid (DNA), an estimate of cell numbers; and ribonucleic acid (RNA), an index of protein synthetic activity (19).

Results and Discussion

Body weights (Table 1) of OVX, sham-OVX, and intact rats increased linearly (P < 0.01) from 30 to 80 days of age. Body weights of OVX rats were greater than sham-OVX and intact rats (collectively referred to as controls) (P < 0.01) at all ages studied. An increase in body weight as a result of ovariectomy has also been reported by others (3, 14). Since ovariectomy markedly increased body weights, the mammary gland characters measured were adjusted to 100 g of the body weight.

Compared to controls, parenchymal growth was retarded (P < 0.01) by ovariectomy and fat pad growth was increased (P < 0.01) (Table 1). These differences were apparent at 30 days of age, indicating that in control animals ovarian steroids began stimulating parenchymal and inhibiting fat pad growth before 30 days of age. By 80 days of age, OVX rats possessed 44% less parenchymal tissue and 136% more fat pad tissue than sham-OVX controls.

This inhibition of parenchymal growth and stimulation of fat pad growth was reflected in the nucleic acid content of the tissues (Table 2). Compared to controls, DNA and RNA (mg/100 g body wt) in the parenchyma were less (P < 0.01) for OVX groups decreasing to minimum values at Day 80. Controls, on the other hand, increased to maximum amounts at Day 60 and remained unchanged to Day 80. At Day 80, parenchymal tissue of OVX rats contained 50% less DNA and 50% less RNA than controls. These findings support histological whole mount results of others (3, 14) where marked inhibition of ductal and end bud formation occurred as a result of ovariectomy. Changes in DNA and RNA between days 30 and 80 were quadratic (P < 0.01) in nature for all three groups of animals.

Fat pad DNA and RNA of OVX rats were greater than controls (P < 0.01). The changes

![Table 1. Mean body weight and mammary parenchymal and fat pad weights of ovariectomized and control rats from 30 to 80 days of age.](image-url)

TABLE 1. Mean body weight and mammary parenchymal and fat pad weights of ovariectomized and control rats from 30 to 80 days of age.

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Body wt (g)</th>
<th>Parenchyma (mg/100 g body wt)</th>
<th>Fat pad (g)</th>
<th>Body wt (g)</th>
<th>Parenchyma (mg/100 g body wt)</th>
<th>Fat pad (g)</th>
<th>Body wt (g)</th>
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Standard error of treatment mean ±5 ±3 ±5 ±5 ±3 ±5 ±5 ±3 ±5

a Linear regression (P < 0.01) among days.
b Quadratic regression (P < 0.01) among days.
c Each value represents mean of 10 rats.
<table>
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<th>Age (days)</th>
<th>Ovariectomized</th>
<th></th>
<th></th>
<th>Sham-ovariectomized</th>
<th></th>
<th></th>
<th>Intact</th>
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<td>Fat pad</td>
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<tr>
<td></td>
<td>DNA</td>
<td>RNA</td>
<td>OHP</td>
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<td>1.1</td>
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<td>2.8</td>
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<td>2.5</td>
<td>1.4</td>
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<td>1.8</td>
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<td>1.0</td>
<td>2.3</td>
<td>1.5</td>
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<td>0.8</td>
<td>1.7</td>
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<td>0.8</td>
<td>1.9</td>
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<td>1.3</td>
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<td>1.6</td>
<td>2.0</td>
<td>1.3</td>
<td>3.0</td>
<td>4.9</td>
<td>1.3</td>
<td>2.6</td>
<td>2.8</td>
</tr>
<tr>
<td>80</td>
<td>0.6</td>
<td>1.4</td>
<td>2.2</td>
<td>1.5</td>
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<td>6.6</td>
<td>1.2</td>
<td>2.8</td>
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Standard error of treatment mean ±0.1 ±0.2 ±0.2 ±0.1 ±0.2 ±0.3 ±0.1 ±0.2 ±0.2 ±0.1 ±0.2 ±0.3 ±0.1 ±0.2 ±0.2 ±0.1 ±0.2 ±0.3

- \(^{a}\) Quadratic regression (P < 0.01) among days.
- \(^{b}\) Cubic regression (P < 0.01) among days.
- \(^{c}\) Linear regression (P < 0.01) among days.
- \(^{d}\) Each value represents a mean of 10 rats.
in fat pad DNA (mg/100 g body wt) of OVX rats between days 30 and 80 represented a quadratic pattern (P < 0.01). By 80 days of age, fat pads of OVX rats contained approximately 100% more DNA than controls. There was no significant increase in the fat pad DNA content of the controls (P > 0.05). Fat pad RNA of OVX rats changed cubically (P < 0.01) between 30 and 80 days of age but decreased linearly for controls. By Day 80, this represented as much as a twofold increase in protein synthetic activity in the fat pad tissue of OVX animals compared to controls.

The nucleic acid results indicated that ovariectomy reduced epithelial cell numbers and protein synthesis in the parenchymal portion of the gland but stimulated cell growth and protein synthesis in the fat pad portion of the gland.

Hydroxyproline (mg/100 g body wt) in the parenchymal portion of the gland was reduced (P < 0.01) by ovariectomy (Table 2). The regression curves between Days 30 and 80 were quadratic (P < 0.01) for all three groups. At 80 days of age, parenchymal tissue of OVX rats contained 33% less OHP than controls. Although ovariectomy retarded collagen synthesis by the parenchymal portion of the gland it did not completely inhibit it. This suggests that factors besides ovarian steroids are also involved in collagen synthesis in the mammary parenchyma. Parenchymal OHP (mg/100 g body wt) of controls increased in a linear manner to 60 days of age and increased only slightly thereafter. This indicated that pubertal growth of mammary collagen was essentially complete by 60 days of age.

To compare the rates of accumulation of collagen in the mammary gland parenchyma for OVX and sham-OVX rats, the logarithm of parenchymal hydroxyproline (uncorrected for body weight) was plotted against log (body wt)2/3 (body surface area) (Fig. 1). The equilibrium constant a, indicates whether an organ is growing faster than (allometry; a > 1) or at the same rate as (isometry; a = 1) body surface area (3). Slopes (2.6 for OVX rats and 1.8 for sham-OVX rats) were different from unity (P < 0.01) and also hydroxyproline between Days 30 and 80 represented quadratic (P < 0.01) regression curves for OVX, sham-OVX, and intact rats. By Day 80, ovariectomy increased OHP content of fat pad tissue by 106 and 74% compared to sham-OVX and intact rats. Thus, it appeared that the heightened protein synthetic activity in the fat pads of OVX rats was for the synthesis of fat pad collagen.

The regressions of fat pad hydroxyproline against log (body wt)2/3 for OVX and sham-OVX groups are in Figure 2. The slopes (2.5 for OVX rats and 1.8 for sham-OVX rats) were different from unity (P < 0.01) and also compared to controls, ovariectomy increased (P < 0.01) hydroxyproline in mammary fat pads (Table 2). The changes in fat pad hy-
### Table 3. Nucleic acids and hydroxyproline content of parenchymal and fat pad portions of mammary gland during pregnancy.

<table>
<thead>
<tr>
<th>Days pregnant</th>
<th>Body wt a,b (g)</th>
<th>Parenchyma</th>
<th>Fat pad</th>
<th>Total mammary gland</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wt c</td>
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<td>RNA b</td>
<td>OHPe</td>
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<tr>
<td>24</td>
<td>219</td>
<td>312</td>
<td>6.0</td>
<td>23.6</td>
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Standard error of treatment mean

| ±6 ±12 ±0.2 ±1.0 ±0.2 ±4 ±0.2 ±0.1 ±0.2 ±12 ±0.2 ±1.0 ±0.4 |

- a Does not include weight of fetuses and placentas.
- b Quadratic regression (P < 0.01) among days.
- c Linear regression (P < 0.01) among days.
- d Linear regression (P < 0.05) among days.
- e Quadratic regression (P < 0.05) among days.
- f Each value represents a mean of 10 rats.
significantly different from one another. The
values indicated that the increase in fat pad
hydroxyproline between 30 and 80 days for
OVX and sham-OVX rats was not only allo-
metric but also of a considerably faster rate
for OVX animals than sham-OVX.

During pregnancy (Table 3) parenchymal
hydroxyproline increased rapidly during the
early stages and then proceeded at a much
slower rate during late pregnancy (significant
linear regression) (P < 0.01). This indicated
that the collagenous framework of the mammary
gland parenchyma was formed prior to major
epithelial cell growth. This supports work of
Lasfargues (9) who reported that collagen
deposition is necessary for an early organiza-
tion of the mammary epithelium.

Fat pad hydroxyproline (Table 3) decreased
linearly (P < 0.05) throughout pregnancy.
Nevertheless, when parenchymal and fat pad
hydroxyproline were combined to obtain a total
mammary gland estimate, a linear increase
(P < 0.05) throughout pregnancy was ob-
served. This does not agree with the results of
Harkness and Harkness (6), who reported
that hydroxyproline in mammary tissue did not
change during pregnancy. Rather, the results
of this experiment indicate that hydroxyproline
is actively synthesized by the mammary gland
during pregnancy.

Several interesting observations emerged from
this study. Firstly, in agreement with earlier
reports (15, 18), the rapid phase of the
pubertal development of the mammary gland
in the intact rat plateaued around 60 days of age.
This growth consisted not only of an
increase in cell numbers (DNA), but also in
the connective tissue elements of the mam-
mary gland (OTIP). And, although a large
part of cell proliferation occurred in the
parenchymal portion, cells in the fat pad seg-
ment of the gland also multiplied.

Secondly, removal of the ovaries virtually
abolished any significant proliferation of paren-
chymal cells, and resulted in a spurt of signifi-
cant hyperplasia of fat pad cells. This in
turn, was reflected in a small increment in
parenchymal collagen and considerable en-
hancement of fat pad collagen. How a lack
of ovarian hormones helped growth of mam-
mary connective tissue remains obscure. It is
possible that lack of estrogen resulted in re-
duced prolactin (1) and enhanced growth hor-
monal (GH) secretion, and the hormone stimu-
lated fibroblast proliferation and collagen
formation (2). Enhanced pituitary growth
hormone in the rat following ovariectomy has
been reported (8), and the increase in the body
size of the OVX rats of this experiment and
others (3, 14) would also suggest enhanced
growth hormone secretion. This is not to state
that ovarian hormones suppress mammary col-
lagen growth. On the contrary, both during
the pubertal state and during pregnancy when
the mammary gland was under the influence
of ovarian hormones, parenchymal growth was
accompanied by increases in mammary collagen.
It only suggests that when the stimuli for
mammary parenchymal growth (ovarian hor-
mones and prolactin) are lacking, the col-
gogenous components continue to grow either
autonomously or under the stimulation of
enhanced GH.

Thirdly, there was a marked difference in the
pattern of mammary growth during the
pubertal state and during pregnancy. During
the pubertal phase, growth of the mammary
gland occurred both in the parenchymal and
fat pad portions as reflected by the weight,
DNA, and OHP. But during pregnancy,
growth occurred only in the parenchymal por-
tion, somewhat at the cost of the fat pad seg-
ment. Significantly, the decline in the fat pad
was attributed mostly to a loss of collagen
and not to a loss of cells (DNA). Nicoll and
Tucker (11) also did not notice any significant
difference in the fat pad DNA of virginity and
mid-lactational states of mice. It appears,
therefore, that the connective tissue cells of the
fat pad but not of the parenchymal segment
are rendered inactive during the rapid growth
phase of pregnancy. After termination of
pregnancy or lactation, these cells probably are
reactivated which results in the initiation of
involutionary changes and return of the mam-
mary gland to the virginal state. How the
fibroblasts of the parenchymal portion continue
to lay down collagen whereas the corresponding
cells of the fat pad area are unable to do so,
remains unknown.

Acknowledgment

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