STUDIES IN HISTOCHEMISTRY*

V. THE VITAMIN C CONCENTRATION OF THE CORPUS LUTEUM
WITH REFERENCE TO THE STAGE OF THE ESTROUS CYCLE
AND PREGNANCY†

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The singularly high concentration of vitamin C in certain
physiologically related endocrine organs is striking. Of all animal
tissues studied, the adrenal cortex, corpus luteum, and anterior
lobe and pars intermedia of the hypophysis are the richest sources;
and of these, the pars intermedia contains vitamin C in the great-
est concentration (4).

The present paper continues our earlier histochemical studies of
vitamin C distribution in mammalian endocrine organs (2, 4). The correlation between the vitamin C concentrations of function-
ally related portions of the adrenal and hypophysis (4) may be
extended to the corpus luteum, which is physiologically related to
the anterior lobe of the hypophysis and contains vitamin C in
approximately the same concentration.

That this parallelism between vitamin C concentration and func-
tion is not merely a coincidence seems to be borne out by the work
of Kramer, Harmon, and Brill (3) who have reported degeneration
of both follicles and corpora lutea in scorbutic guinea pigs, as well
as failure to become pregnant or to deliver normal young. Ingier
had shown that on scorbutic diets in early pregnancy the animals
were born dead or prematurely (6). In this connection there
should be mentioned a recent note by Bourne (7) who reported

* For the previous papers of this series see (1-4) respectively.
† Included in a paper presented before the vitamin symposium at the
meeting of the American Chemical Society at San Francisco, September,
1935.

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that pregnant guinea pigs remain normal on a scorbutic diet. As will be pointed out later, there may be a connection between the presence of vitamin C and the corpus luteum hormone, progesterone.¹

Vitamin C in the corpus luteum has been reported by Bessey and King (9) who found 1.39 mg. per gm. in this body in the sow, and Huszák (10) who reported that an early corpus luteum in the pig had a concentration of 0.74 mg. per gm., while an involuted body had only 0.19 mg. per gm.; Huszák also reported other corpora lutea of pigs varying from 1.02 to 1.50 mg. per gm., and one from a cow with 1.19 mg. per gm. Giroud, Leblond, and Giroux (11), using the silver nitrate staining method for the identification of vitamin C, found a strongly positive reaction for corpora lutea of estrus and pregnancy. However, as previously mentioned, the unreliability of the staining reaction is noteworthy (1, 2).

Except for the two pig corpora lutea of Huszák referred to above, no attempt was made in any of this previous work to correlate the vitamin concentration with the exact stage of the cycle of the corpus luteum.

In our earlier work on the histochemical distribution of vitamin C in endocrine organs (2, 4) we determined the concentration of the vitamin, as well as the numbers of each type of cell in the various portions of the organ in question, so that the vitamin content per cell might be found. In the present case, however, this was not done. Since the corpus luteum is not a layered body but contains a homogeneous mixture of several types of cells, no cell counts were made, since counts of several types of cells could contribute nothing to the estimation of the vitamin concentration in any one type of cell. Hence only the concentration of vitamin C in corpora lutea as a whole at various stages of estrus and pregnancy was determined.

EXPERIMENTAL

Because of their convenient size, availability of the normal tissue in the fresh state, and for consistent comparison with the other bovine endocrine glands studied, corpora lutea of the cow

¹ The recently approved term "progesterone" will be used throughout this paper (8).
were employed. Another advantage derived from using the bovine corpus luteum is that it resembles the human more than does that of the pig, sheep, and other animals, since only one corpus luteum is formed during each estrous cycle. The ovaries were removed from the freshly killed animals, stored at \(-5^\circ\) as previously described (2), and used for vitamin C titration within 1 to 2 days.

Though macroprocedure could have been employed for the vitamin C estimation in the corpora lutea, the micromethod previously employed (1) was used instead, since it offered the advantage that many analyses could be performed on each corpus luteum and enough tissue for histological study still be left. A further benefit was conservation of reagents and time. As many as 50 separate titrations could be performed in the course of 2 to 3 hours by this method. Each c.mm. of the standard 2,6-dichlorophenol indophenol solution employed for the titration was equivalent to 0.130 microgram of vitamin C.

The sampling and sectioning of the tissue were conducted in a manner similar to that previously employed (2, 4). Fig. 1 demonstrates the manner in which the samples were removed. The stiffly frozen ovary was first cut in half along the horizontal middle dotted line, then the cork borer, which had an internal diameter of 4.2 mm., was pushed through the corpus luteum in the manner indicated. The cylinder of tissue thus removed was placed at once on a freezing rotary microtome, a drop of normal

![Fig. 1. Method of sampling corpus luteum in bovine ovary (approximately actual size).](image-url)
saline, rather than water (2), being used to make firm contact between the tissue and the freezing block. In order to obtain representative sections (30 μ thick) of tissue for vitamin C extraction and titration, two adjacent sections were removed for two separate analyses, after which 50 sections were discarded, and then two more taken for vitamin estimation, 50 again discarded, and so on through the entire cylinder of tissue. The homogeneity of the corpus luteum is emphasized by the consistent titration results obtained. Usually ten titrations were performed on each sample of tissue; i.e., duplicate determinations in five regions 1500 μ apart. The block of tissue surrounding the hole left by the cork borer was fixed in Bouin's fluid, mounted in paraffin, sectioned, and stained with iron hematoxylin, acid fuchsin, and light green.

The approximate age of the corpus luteum could be estimated from the gross and histological appearance. In the case of corpora lutea of pregnancy, the size of the fetus was used as the chief criterion of the age of the corpus luteum and the duration of pregnancy. The relation of the age of a fetus to its size was obtained according to Hammond (12).

Changes Occurring in Corpus Luteum—Since it was impossible for us to have cows killed at known stages of their estrous cycles, it became necessary to determine the stage by macroscopic and microscopic examination of the organs. The following is a résumé of the changes that occur.

The macroscopic and microscopic changes have been described by Hammond (12). When the Graafian follicle bursts and discharges its ovum, the granulosa cells of the lining of the collapsed follicle rapidly become transformed into large pigmented luteal cells which resemble the cells of the adrenal cortex. The luteal cells enlarge, and the corpus luteum as a whole increases in size until it reaches a maximum diameter of about 2.0 cm. (in pregnancy the size is a little larger). This maximum is reached approximately 6 to 8 days after ovulation. In the normal estrous cycle of 21 days, involution sets in after the 15th to 17th day and the corpus regresses and steadily diminishes in size until it finally leaves a pigmented scar in the ovary. At the time of the next ovulation the size of the corpus has decreased to about half the maximum size. In pregnancy the maximum size and full function are maintained up to parturition. Atrophy is delayed until about 30 days after parturition.
**Table I**

Vitamin C Content of Bovine Corpus Luteum at Various Stages of Estrous Cycle

<table>
<thead>
<tr>
<th>Corpus luteum No.</th>
<th>24</th>
<th>26</th>
<th>31</th>
<th>10</th>
<th>3</th>
<th>14</th>
<th>32</th>
<th>13</th>
<th>33</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size, cm.</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>1.9</td>
<td>2.0</td>
<td>1.6</td>
<td>1.7</td>
<td>1.6</td>
</tr>
<tr>
<td>No. of sections analyzed</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>9</td>
<td>11</td>
<td>10</td>
<td>8</td>
<td>13</td>
<td>10</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Average vitamin C per section, microgram</th>
<th>0.58</th>
<th>0.58</th>
<th>0.54</th>
<th>0.50</th>
<th>0.48</th>
<th>0.44</th>
<th>0.41</th>
<th>0.23</th>
<th>0.16</th>
<th>0.17</th>
<th>0.14</th>
<th>0.13</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum deviation from mean, vitamin C per section, microgram</td>
<td>+0.06</td>
<td>+0.06</td>
<td>+0.09</td>
<td>+0.03</td>
<td>+0.11</td>
<td>+0.07</td>
<td>+0.00</td>
<td>+0.08</td>
<td>+0.02</td>
<td>+0.02</td>
<td>+0.02</td>
<td>+0.01</td>
</tr>
<tr>
<td>Average concentration, mg. per gm.</td>
<td>1.4</td>
<td>1.4</td>
<td>1.3</td>
<td>1.2</td>
<td>1.2</td>
<td>1.1</td>
<td>1.0</td>
<td>0.56</td>
<td>0.50</td>
<td>0.41</td>
<td>0.34</td>
<td>0.31</td>
</tr>
</tbody>
</table>

**Table II**

Vitamin C Content of Bovine Corpus Luteum in Various Stages of Gestation

<table>
<thead>
<tr>
<th>Corpus luteum No.</th>
<th>22</th>
<th>23</th>
<th>21</th>
<th>19</th>
<th>25</th>
<th>20</th>
<th>18</th>
<th>20</th>
<th>17</th>
<th>28</th>
<th>27</th>
<th>30</th>
<th>16</th>
<th>11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size, cm.</td>
<td>2.0</td>
<td>2.0</td>
<td>2.2</td>
<td>2.0</td>
<td>1.8</td>
<td>2.0</td>
<td>2.1</td>
<td>2.2</td>
<td>2.3</td>
<td>2.3</td>
<td>1.7</td>
<td>1.8</td>
<td>2.1</td>
<td>2.4</td>
</tr>
<tr>
<td>No. of sections analyzed</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>7</td>
<td>11</td>
<td>9</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>9</td>
<td>7</td>
<td>8</td>
<td>12</td>
<td>13</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Average vitamin C per section, microgram</th>
<th>0.68</th>
<th>0.74</th>
<th>0.90</th>
<th>0.91</th>
<th>0.90</th>
<th>0.68</th>
<th>0.67</th>
<th>0.71</th>
<th>0.62</th>
<th>0.75</th>
<th>0.79</th>
<th>0.83</th>
<th>0.73</th>
<th>0.46</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum deviation from mean, vitamin C per section, microgram</td>
<td>+0.11</td>
<td>+0.06</td>
<td>+0.01</td>
<td>+0.06</td>
<td>+0.09</td>
<td>+0.03</td>
<td>+0.03</td>
<td>+0.03</td>
<td>+0.05</td>
<td>+0.02</td>
<td>+0.02</td>
<td>+0.07</td>
<td>+0.04</td>
<td>+0.03</td>
</tr>
<tr>
<td>Average concentration, mg. per gm.</td>
<td>1.6</td>
<td>1.8</td>
<td>2.2</td>
<td>2.2</td>
<td>1.6</td>
<td>1.6</td>
<td>1.7</td>
<td>1.5</td>
<td>1.8</td>
<td>1.9</td>
<td>2.0</td>
<td>1.8</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td>Approximate age, mos.</td>
<td>1.6</td>
<td>2</td>
<td>2</td>
<td>2.2</td>
<td>2.2</td>
<td>2.6</td>
<td>2.8</td>
<td>3.8</td>
<td>4.4</td>
<td>4.4</td>
<td>6</td>
<td>6.6</td>
<td>7.2</td>
<td>8</td>
</tr>
<tr>
<td>Size of fetus, cm.</td>
<td>4</td>
<td>5</td>
<td>5</td>
<td>6</td>
<td>6</td>
<td>10</td>
<td>12</td>
<td>22</td>
<td>30</td>
<td>30</td>
<td>50</td>
<td>60</td>
<td>70</td>
<td>80</td>
</tr>
</tbody>
</table>
During regression the luteal cells degenerate, the blood sinusoids increase in size and number, the walls of the blood vessels thicken, and filaments of fibrous tissue increase to thick bands.

**Results**

The corpora lutea of estrus were divided into four age groups. Group A was composed of organs just forming (up to 5 or 6 days old), Group B at the maximum development (from 5 or 6 to 16 or 17 days), Group C at the beginning of involution (from 16 or 17 to 23 or 24 days), and Group D with further involution and atrophy (all corpora older than 24 days). No corpora lutea could be obtained from non-pregnant cows that we could be sure fitted into Group A.

The ovarian tissue surrounding a corpus luteum (No. 1, Table I) was analyzed for vitamin C and found to contain 0.32 mg. per gm.

**DISCUSSION**

It may be seen from Table I that the vitamin C content of the corpus luteum is at a maximum when the organ itself is fully mature, and decreases as the corpus involutes and atrophies. Since this body is in full function throughout pregnancy, it is not surprising that the vitamin concentration is at a rather constantly high level during the course of gestation (Table II). It is interesting to note that the corpus luteum of pregnancy has a concentration of vitamin C practically 50 per cent higher than the mature corpus in the non-pregnant animal.

A comparison of old atrophic corpora lutea with normal ovarian tissue shows that the two have practically the same vitamin C concentration, the former having 0.31 to 0.39 mg. per gm., while the latter contains 0.32 mg. per gm.

Vitamin C appears to be unrelated to the female sex hormone, estrin, since follicular fluid is very poor in the vitamin (9, 10) and the estrin content of corpora lutea of gestation falls off almost to zero towards the end of the pregnancy ((13) p. 417), though it has been shown in the present paper that the vitamin C content remains at a high level. On the other hand, vitamin C may be related to progesterone. The variations in the vitamin content of the corpus luteum that we have found seem to parallel the
The necessity for the presence of a corpus luteum for proper embryo implantation and continuance of gestation depends upon the progesterone it produces (13, 15). When it is recalled that vitamin C deficiency produces degeneration of corpora lutea and failure of normal gestation in guinea pigs (5), it would appear at least reasonable to suggest that the vitamin is necessary for the normal production of the hormone, either by maintaining the integrity of the structures responsible for its formation or more directly by influencing the chemical reactions involved in the progesterone synthesis. Bourne (16) has suggested that vitamin C is associated non-specifically with the production of the corpus luteum hormone.

**SUMMARY**

The vitamin C content of corpora lutea of cows was determined in various stages of the estrous cycle and gestation. The concentration of vitamin varied with the degree of development of the corpus luteum of estrus, being at a maximum of 1.4 mg. per gm. of tissue when the organ was most fully developed and falling off to 0.3 mg. per gm. with regression.

The vitamin level in gestation remained at from 1.5 to 2.2 mg. per gm. for the first 7 months, decreasing to 1.1 mg. per gm. in the 8th month.

The relation of vitamin C to progesterone was indicated.

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**BIBLIOGRAPHY**

STUDIES IN HISTOCHEMISTRY: V. THE VITAMIN C CONCENTRATION OF THE CORPUS LUTEUM WITH REFERENCE TO THE STAGE OF THE ESTROUS CYCLE AND PREGNANCY

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