The Effect of Follicle-stimulating Hormone on the Biosynthesis in Vitro of Estradiol-17β from Acetate-1-C14 and Testosterone-4-C14*

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The conversion of C14-testosterone to C14-labeled estrogens was shown by Heard (1) to take place in the pregnant mare. Raggett et al. (2) and Wotiz et al. (3) have demonstrated this same conversion in human ovarian slices. The conversion of C14-acetate to C14-estradiol and estrone in dog ovary slices and homogenates has been described by Rabinowitz and Dowben (4).

The present study concerns the action of FSH,3 administered in vivo or in vitro, on the conversion of C14-acetate and C14-testosterone to C14-estradiol-17β in dog ovary slices.

EXPERIMENTAL

Ovaries were obtained from two groups of mongrel female dogs. One group was designated anestrous. In this group the vulvae were atrophic, vaginal smears showed no cells, the uterus was small, compact, and whitish, and ranged in weight from 0.4 to 0.9 gm. The dogs of the second group were treated with 6 mg. of FSH2 per day per dog for 11 days before they were killed. At death these animals had greatly swollen vulvae, vaginal smears showed abundant cornified cells, and the uterus was enlarged. The ovaries were large, had numerous follicles filled with yellowish fluid, and ranged in weight from 1.1 to 1.3 gm. Before the dogs were killed they were anesthetized with Nembutal, after which the ovaries were removed and placed immediately in a beaker of cracked ice. The capsules of the ovaries were then removed and with a Stadie slicer the ovaries were sliced to a thickness of approximately 0.5 mm. 400 to 500 mg. of tissue were transferred to Warburg vessels each of which contained 1 ml. of dog serum, and 1.5 ml. of bicarbonate-phosphate buffer at pH 7. The composition of the buffer was 0.065 M K2HPO4, 0.040 KH2PO4, 0.006 M MgCl2, 0.006 M KHCO3, 0.030 M neotnamide, 0.0008 M diphosphopyridine nucleotide, 0.0029 M adenosine-5'-phosphate. In some of the incubations in which anestrous ovaries were used, there was, in addition, 1 mg. of FSH in the vessels. The atmosphere was 5 per cent CO2 in oxygen, and the mixture was incubated in a 37.5° water bath for 3 or 4 hours with shaking. At the end of the incubation 1 mg. of estradiol-17β was added as carrier to each vessel.

The liquid phase was diluted with 10 volumes of physiological saline and extracted four times with 50 ml. of methylene chloride. The tissue was dried in acetone, frozen in liquid nitrogen, pounded to fine flakes in a metal mortar, and extracted with the methylene chloride used to extract the corresponding liquid. The remainder of the extraction procedure is outlined in Diagram 1. After extraction, the tissue was dried further in a vacuum desicator and weighed.

The phenolic fraction was chromatographed on Schleicher and Schuell paper in a formamide-o-dichlorobenzene system (5). The estradiol strip was located and eluted with 95 per cent ethanol. The chemical estimation of estradiol was determined by a modification of the colorimetric reaction described by Lieberman and Tagnon (6). After determination of the specific activity of the isolated carrier, a 24 transfer countercurrent separation was done on the estradiol fraction with the use of a 50 per cent methanol-CCl4 system. After removal of aliquots for analysis, additional estradiol was put in and the mixture was recrystallized from ethyl acetate-petroleum ether. The specific activity of both the crystals and the mother liquor was ascertained.

RESULTS

In the samples stimulated by FSH, the formation of estradiol was 0.6 per cent of the C14-testosterone initially added. The radioactivity of the estradiol was indicated by a constant specific activity obtained through paper chromatography, countercurrent separation, and recrystallization, as shown in Table I. In addition, the estradiol, as determined by the colorimetric method, and the corresponding radioactivity, followed the theoretical curve for the 24 transfer countercurrent separation in all the experiments, one of which is shown in Fig. 1.

The transformation of testosterone-C14 to estradiol by ovarian slices is accelerated by FSH whether this is administered by injection into the animal or is added in vitro. Table II is a
FSH Effect on Estradiol-17β Biosynthesis

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TABLE I

Specific activity of estradiol

Experiments 1 and 2 both involved the use of ovaries of dogs treated with FSH. Additional estradiol was put in for counter-current distribution and again for recrystallization, but the specific activities are corrected for comparison.

<table>
<thead>
<tr>
<th>Method</th>
<th>System</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paper chromatography</td>
<td>Foramide-o-dichlorobenzene</td>
<td>5000</td>
<td>4800</td>
</tr>
<tr>
<td>24 transfer counter-current</td>
<td>50% methanol-CCl₄</td>
<td>4800</td>
<td>4600</td>
</tr>
<tr>
<td>Recrystallization</td>
<td>Ethyl acetate-petroleum ether</td>
<td>4800</td>
<td>5100</td>
</tr>
</tbody>
</table>

Mean ± s.e.

<table>
<thead>
<tr>
<th>Group I</th>
<th>Group II Anestrous and FSH in vitro</th>
<th>Group III FSH in vivo</th>
<th>Group IV FSH in vivo and HCG* in vitro</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.p.m.</td>
<td>c.p.m. mg. FSH c.p.m. mg. FSH</td>
<td>c.p.m. mg. FSH</td>
<td>c.p.m. mg. FSH</td>
</tr>
<tr>
<td>11.8</td>
<td>51.5</td>
<td>10.0</td>
<td>95.4</td>
</tr>
<tr>
<td>21.8</td>
<td>85.5</td>
<td>0.5</td>
<td>63.2</td>
</tr>
<tr>
<td>10.9</td>
<td>24.6</td>
<td>1.0</td>
<td>50.0</td>
</tr>
<tr>
<td>11.5</td>
<td>52.8</td>
<td>1.0</td>
<td>73.8</td>
</tr>
<tr>
<td>9.8</td>
<td>42.5</td>
<td>1.0</td>
<td>12.8</td>
</tr>
<tr>
<td>14.4</td>
<td>13.5</td>
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<td>55.5</td>
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<tr>
<td>23.1</td>
<td>26.0</td>
<td>1.0</td>
<td>15.0 ± 4.2</td>
</tr>
<tr>
<td>18.8</td>
<td></td>
<td></td>
<td>44.3 ± 8.9</td>
</tr>
</tbody>
</table>

* HCG, human chorionic gonadotropin.

summary of the effect of FSH on this conversion. The results are given in terms of dry weight of ovarian tissue. The effect of the FSH added in vitro seems to be maximal in these studies since no difference in conversion was noted between duplicate vessels containing 0.5 mg. and 10 mg. of FSH. FSH added in vitro to ovaries from FSH-treated dogs produced no additional stimulation and no inhibition.

When chorionic gonadotropin instead of FSH was added in vitro to anestrous ovary slices, there was a similar stimulation of activity.

Carboxyl-labeled Acetate C¹⁴ as Precursor—Carboxyl-labeled

⁴ Acetate-¹⁴C, specific activity (a) 1 mc. per mmole and (b) 5 mc. per mmole, was obtained from Nuclear Chicago Corporation.
acetate was studied in the incubation system described above. The extraction and the isolation of the estradiol was identical. No activity could be found in the estradiol in the experiments in which acetate was used as the precursor. A total of 42 experiments was performed with both FSH and chorionic gonadotropin administered to the dogs or added to the incubation vessel. Some experiments were performed with acetate that had a specific activity of 5 mc. per mmole when acetate of 1 mc. per mmole afforded no evidence of conversion. Increasing the volume of acetate in the incubation vessel to 65 \( \mu \text{g} \) of acetate with a specific activity of 5 mc. per mmole did not affect the result. In two negative experiments, the cholesterol isolated as the digitonide was found to be radioactive, with a specific activity that indicated the conversion of approximately 0.1 per cent of the acetate to cholesterol.

**DISCUSSION**

The failure of the data to show incorporation of C\(^{14}\)-acetate into estradiol-17\( \beta \) under any of our conditions does not imply that this pathway does not exist in the ovary. On the contrary, the evidence that a C\(^{14}\) compound is a precursor of estradiol reinforces the role of acetate as an eventual carbon source. Acetate, however, enters into a multiplicity of reactions of which steroidogenesis is but a minor path. It is possible that experiments performed with an even higher specific activity of acetate-C\(^{14}\), or with an inhibitor to the alternate pathways of acetate utilization, would show the incorporation of acetate into estradiol. The repeated isolation of nonradioactive estradiol from acetate incubation mixtures of high radioactivity afforded evidence that the estradiol purification was adequate. The isolation of radioactive cholesterol from such mixtures indicated that the tissue was metabolically active.

By contrast, in all experiments there was evidence of the conversion of testosterone-4-C\(^{14}\) to estradiol-17\( \beta \). The radio-purity of the product was indicated by the constant specific activity of estradiol through paper chromatography, counter-current separation, and recrystallization. The administration of FSH to dogs increased the extent of conversion of testosterone to estradiol. This experiment showed that a rate-limiting reaction involved in this conversion is stimulated by FSH. The response of anestrus ovaries to FSH in vitro was evidence that this reaction is prompt and is not the result of increased enzyme synthesis due to growth. The fact that stimulation in vitro of ovaries from FSH-treated animals did not lead to further incorporation suggests that the tissue was already stimulated to the maximal degree.

FSH and human chorionic gonadotropin have long been known to increase estrogen production. The implication of gonadotropin in the aromatization of testosterone suggests that this reaction is of physiological importance in estrogen biosynthesis.

**SUMMARY**

1. The conversion in vitro of testosterone-4-C\(^{14}\) to C\(^{14}\)-estradiol-17\( \beta \) in dog ovary slice is stimulated by follicle-stimulating hormone administered in vivo or in vitro.
2. The conversion of acetate-1-C\(^{14}\) to C\(^{14}\)-estradiol-17\( \beta \) could not be demonstrated.
3. The role of follicle-stimulating hormone is estrogen biosynthesis is discussed.

**REFERENCES**

The Effect of Follicle-stimulating Hormone on the Biosynthesis in Vitro of Estradiol-17β from Acetate-1-C\textsuperscript{14} and Testosterone-4-C\textsuperscript{14}
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