THE METABOLISM OF PROGESTERONE BY LIVER TISSUE IN VITRO

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Although the administration of progesterone to human beings gives rise to the excretion of pregnanediol in the urine (1), the course and sites of the metabolism of progesterone have not been established. The uterus and ovaries are not essential for the reduction of progesterone, since a rise in urinary pregnanediol has been demonstrated in men (2, 3) and in hysterectomized women (4, 5) who were injected with progesterone. However, the liver would appear to be important in metabolizing the hormone. It has been shown in animals that when progesterone is implanted in the spleen (6), mesentery (7), or stomach (8) or injected into the portal vein (9) its biological potency is much lower than when administered subcutaneously. The anesthetic action of orally administered progesterone is greatly enhanced by partial hepatectomy (10, 11). Pregnanediol has been isolated from the bile of human beings after progesterone was given parenterally (12). In spite of all of these observations on the rôle of the liver in the metabolism of progesterone, experiments in vitro have indicated that progesterone is not inactivated by the liver (13, 14). By chemical techniques for the quantitative estimation of steroids, it has been found in this laboratory (15) that testosterone is destroyed by liver tissue in vitro. It was also shown (16) that the \( \alpha,\beta \)-unsaturated ketone group of progesterone underwent changes in the presence of rat liver mince. This did not appear competitive with the metabolism of ring A of testosterone at the concentrations used. We have therefore carried out more detailed experiments on the metabolism of progesterone and have attempted to determine the conditions which affect the rate of reaction.

Methods and Materials

Rat and rabbit liver tissues were used in these experiments. The rats used were killed by decapitation, and the rabbits by intravenous injection of air. The liver tissue was removed immediately after death and homo-

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genized in a phosphate buffer, pH 7.4; in a Potter homogenizer. 25 ml.
of the homogenate, equivalent to 0.50 gm. of liver, were introduced into
each 125 ml. incubation flask containing 2 μM of progesterone dissolved in
0.2 ml. of propylene glycol. After being filled with a gaseous mixture of
95 per cent oxygen and 5 per cent carbon dioxide, the flasks were incubated
for 1 hour on a rotating drum in a water bath at 37.4°C.

The analytical procedure employed was a modification of that originally
designed by Samuels (17). The contents of the flasks were boiled to stop
enzymic action and extracted five times with 25 ml. of a mixture of 4 parts
of ether to 1 part of chloroform. After evaporation to dryness, these ex-
tracts were dissolved in 20 ml. of hexane and chromatographed on alumi-
num oxide. The following fractions were eluted: Fraction A, 30 ml. of
hexane; Fraction B, 15 ml. of a mixture of hexane and chloroform (95:5);
Fraction C, 50 ml. of hexane and chloroform (80:20); Fraction D, 50 ml.
of chloroform. Progesterone appeared in Fraction C.

Fraction C was evaporated to dryness, dissolved in 20 ml. of hexane,
and shaken with an equal volume of 70 per cent ethanol. The ethanol was
drained off and the extraction repeated twice with 15 ml. portions of 70
per cent ethanol. The ethanol solutions were combined, diluted with 20
ml. of distilled water, and extracted three times with 20 ml. of chloroform.
The combined chloroform fractions were evaporated to dryness and dis-
solved in 4 ml. of absolute ethanol. Dilutions of this solution were used
for determination of the ultraviolet absorption spectrum. The spectrum
was measured on either a Beckman or Cary spectrophotometer over the
range of 220 to 300 μm. The maximum absorption at 240 μm was used
to measure the unsaturated ketone structure in ring A. Blanks on liver
without progesterone were run with each series and gave a small absorption
over the whole spectrum, gradually decreasing toward the longer wave-
lengths. Corrections were applied to all results by subtraction of such
tissue blanks run concurrently.

To test the validity of the method, some samples containing progesterone2
were not incubated but boiled immediately after the homogenate was
washed into the flasks, and others were boiled both before and after in-
cubation. Recoveries in all of these were 90 ± 5 per cent of the proges-
terone. Zimmermann reactions were run by the method of Callow, Cal-
low, and Emmens (18).

Results

The effects of rat liver homogenate on progesterone are shown in Table
I. With the buffer solution only, there was approximately 28 per cent

1 0.005 M KCl, 0.002 M MgCl2, 0.08 M NaCl, 0.032 M Na2HPO4, and 0.007 M NaH2PO4.
2 The progesterone was procured through the courtesy of Dr. D. A. McGinty of
Parke, Davis and Company, and of Dr. Percy Julian of The Glidden Company.
TABLE I

Effect of Various Compounds on Metabolism of Progesterone by Rat Liver Homogenate

<table>
<thead>
<tr>
<th>Incubation medium</th>
<th>Progesterone recovered, average* per cent</th>
<th>Progesterone destroyed per gm. tissue per hr. μM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boiled control</td>
<td>93.5</td>
<td>1.11</td>
</tr>
<tr>
<td>Phosphate buffer</td>
<td>65.7</td>
<td>1.18</td>
</tr>
<tr>
<td>Buffer + DPN</td>
<td>64.0</td>
<td>2.67</td>
</tr>
<tr>
<td>&quot; + citrate</td>
<td>24.5</td>
<td>2.76</td>
</tr>
<tr>
<td>&quot; + DPN + citrate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boiled control</td>
<td>101.7</td>
<td></td>
</tr>
<tr>
<td>Phosphate buffer</td>
<td>83.5</td>
<td>0.72</td>
</tr>
<tr>
<td>Buffer + citrate</td>
<td>76.5</td>
<td>1.02</td>
</tr>
<tr>
<td>&quot; + cysteine</td>
<td>59.2</td>
<td>1.70</td>
</tr>
<tr>
<td>&quot; + cyanide</td>
<td>45.4</td>
<td>2.25</td>
</tr>
</tbody>
</table>

* All samples were run in triplicate.

TABLE II

Effect of Various Compounds on Metabolism of Progesterone by Rabbit Liver Homogenate

<table>
<thead>
<tr>
<th>Incubation medium</th>
<th>Gaseous phase</th>
<th>Progesterone recovered, average* per cent</th>
<th>Progesterone destroyed per gm. tissue per hr. μM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boiled control</td>
<td>O₂-CO₂</td>
<td>91.5</td>
<td></td>
</tr>
<tr>
<td>Phosphate buffer</td>
<td></td>
<td>83.5</td>
<td>0.32</td>
</tr>
<tr>
<td>Buffer + citrate</td>
<td></td>
<td>49.0</td>
<td>1.70</td>
</tr>
<tr>
<td>&quot; + succinate</td>
<td></td>
<td>83.0</td>
<td>0.34</td>
</tr>
<tr>
<td>&quot; + α-ketoglutarate</td>
<td></td>
<td>85.7</td>
<td>0.23</td>
</tr>
<tr>
<td>&quot; + ATP</td>
<td></td>
<td>82.3</td>
<td>0.37</td>
</tr>
<tr>
<td>&quot; + AMP</td>
<td></td>
<td>82.7</td>
<td>0.35</td>
</tr>
<tr>
<td>Boiled control</td>
<td>O₂-CO₂</td>
<td>87.0</td>
<td></td>
</tr>
<tr>
<td>Buffer + citrate</td>
<td></td>
<td>74.5</td>
<td>0.50</td>
</tr>
<tr>
<td>&quot; + isocitrate</td>
<td></td>
<td>75.5</td>
<td>0.46</td>
</tr>
<tr>
<td>&quot; + cis-aconitate</td>
<td></td>
<td>74.5</td>
<td>0.50</td>
</tr>
<tr>
<td>Boiled control</td>
<td>N₂</td>
<td>92.0</td>
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<tr>
<td>Phosphate buffer</td>
<td></td>
<td>88.0</td>
<td>0.16</td>
</tr>
<tr>
<td>Buffer + citrate</td>
<td></td>
<td>67.3</td>
<td>1.09</td>
</tr>
</tbody>
</table>

* All samples were run in triplicate. The variability of the results obtained in the different series when citrate was used in an atmosphere of oxygen and carbon dioxide was due to the quality of liver tissue used, since all other conditions were constant.
destruction of the conjugated double bond system of the steroid; this was not increased by the addition of 6 \( \mu M \) of diprophosphopyridine nucleotide (DPN). Following addition of 0.001 M citrate about 68 per cent of the progesterone disappeared. Similarly, with rabbit liver homogenate, there was less than 10 per cent destruction of the \( \alpha,\beta \)-unsaturated ketone in the buffer solution, but about 42 per cent disappearance in the presence of 0.001 M citrate (Table II).

To determine what other compounds might affect the system, the following substances were used: succinate, \( \alpha \)-ketoglutarate, cis-aconitate, and isocitrate in 0.001 M concentration and 12 \( \mu M \) of adenosinetriphosphate (ATP) or adenosine-5-phosphate (AMP). Isocitrate and cis-aconitate seemed to have a similar effect to citrate, but the two other members of the Krebs cycle used, succinate and \( \alpha \)-ketoglutarate, as well as ATP and AMP, were not active.

One experiment in which 0.01 M and 0.1 M citrate was used resulted in slightly increased destruction of progesterone with increasing concentration of citrate. It appeared, however, that the action of citrate was almost maximal at 0.001 M concentration.

To study the effect of oxygen in the reaction, flasks were filled with nitrogen prior to incubation. There was a similar disappearance of progesterone in the presence of 0.001 M citrate.

Since all tricarboxylic acids seemed to accelerate the reaction at low concentrations, even in the absence of oxygen, it was thought that they might exert their effect by forming a complex with an inhibiting metal ion. Two other metal-binding compounds, 0.001 M cysteine and 0.005 M cyanide, unrelated to the Krebs cycle, were therefore tried. Both increased the destruction even more than 0.001 M citrate. It seems, then, that the effect of the tricarboxylic acids of the Krebs cycle is probably due to removal of an inhibiting metal ion.

Zimmermann reactions performed on several samples from the chromatograms in each series failed to reveal the presence of 17-ketosteroids. The side chain is apparently not split off.

**DISCUSSION**

The data presented indicate that progesterone is metabolized by an enzyme system in liver tissue under the conditions used in these experiments. The marked acceleration of the reaction in the presence of chelating agents indicates that the reaction can be inhibited by some di- or trivalent metal. The lack of effect of ATP and DPN indicates that high energy phosphate bonds are probably not directly involved. The reaction

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2 In flasks containing DPN, niacin was added to the buffer solution to give a final concentration of 0.04 M.
is probably a reduction process, since the absence of oxygen does not inhibit the disappearance of the \(\alpha,\beta\)-unsaturated ketone structure in ring A.

The failure of Zondek (13) and of Engel (14) to demonstrate inactivation of progesterone by liver tissue in vitro probably stems from the fact that no agent causing metal complexes, such as citrate, was added. Under these circumstances, our results indicate that the amount of progesterone destroyed would have been too small to detect by the Clauberg test, by which those investigators assayed the quantity of hormone present.

It has been shown (19) that testosterone, in a similar system, is converted to 17-ketosteroids in the presence of DPN; methyltestosterone, however, does not behave in this way. This is also apparently the case with progesterone. The side chain on carbon 17 is apparently not readily split off under these conditions. The destruction of the \(\alpha,\beta\)-unsaturated ketone group in ring A of testosterone and methyltestosterone is also accelerated by 0.001 M citrate. The enzyme system is apparently different, however, since elimination of oxygen markedly reduced the destruction. Unpublished results demonstrate that cysteine and cyanide do not increase the metabolism of methyltestosterone. Apparently there are two enzymes acting on the conjugated double bond system in ring A, a metal-inhibited one acting on the C-20 substituted C\(_{21}\) steroids, and one not so affected which reacts with steroids having oxygen on C-17.

**SUMMARY**

1. The \(\alpha,\beta\)-unsaturated ketone structure in ring A of progesterone disappears on incubation with liver tissue in vitro.
2. The process is accelerated by the tricarboxylic acids citric, isocitric, and cis-aconitic, and by other metal-binding agents. It appears, therefore, to be inhibited by metal ions.
3. The reaction seems to be a reduction of ring A, since it proceeds at normal rates under very low oxygen tensions.
4. In this type of preparation the side chain of progesterone does not appear to be split from the nucleus.
5. The enzyme system involved appears to be different from that acting on ring A of testosterone and methyltestosterone.

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**BIBLIOGRAPHY**

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