Slices of rabbit liver metabolize testosterone to \( \Delta^4 \)-androstene-3,17-dione, epitestosterone, and other unidentified metabolites (2). The same reactions have been shown to take place with homogenates of the liver and also of the kidneys of the rabbit.

Procedure

The liver and kidneys were removed aseptically from non-fasted adult male rabbits, weighing 2.7 to 4.5 kilos, which were anesthetized with Dialurethane. The homogenate\(^1\) of the tissues was prepared by mixing the tissue, testosterone,\(^2\) and 50 ml. of the Ringer's phosphate buffer in the Waring blendor for 2 minutes. The mixture was transferred to a Fernbach flask with another 50 ml. of buffer and incubated at 37° for 2\( \frac{1}{2} \) hours. The entire procedure was carried out with sterile precaution. The buffer and glassware were chilled in the refrigerator prior to use.

The extraction and isolation procedures for the steroids were as previously described (2).

Results

Control Experiments—Three 8 gm. portions of liver were homogenized and each incubated with 100 ml. of the buffer solution for 2\( \frac{1}{2} \) hours. At

* This investigation was supported by a grant from the American Cancer Society on recommendation of the Committee on Growth of the National Research Council. Part of the data was reported at the meetings of the Federation of American Societies for Experimental Biology (1).

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‡ Part of the data was taken from a thesis submitted by J. Gongora in June, 1949, to the Graduate School of The University of Rochester in partial fulfilment of the requirements for the degree of Master of Science.

\(^1\) It should be noted that the break-up of tissue in the Waring blendor is more drastic than by the now accepted method of preparation of homogenates (3), but intact cells occasionally are observed on examination under the microscope.

\(^2\) The steroids used in this study were generously supplied by Ciba Pharmaceutical Products, Inc.
the end of the incubation period 100 mg. of testosterone, dissolved in 1 liter of alcohol, were added to each flask and the mixture carried through the detailed extraction and isolation procedure (2). The ketonic fraction yielded 278 mg. of testosterone, m.p. 151–152°, which represented a recovery of 93 per cent. The acetate and oxime were prepared. The non-ketonic fraction yielded 160 mg. of cholesterol, m.p. 143–145°. The acetate was prepared. Melting points and mixed melting points confirmed the identity of these compounds.

No other steroids were isolated or indicated by the color tests (4, 5).

**Table I**

<table>
<thead>
<tr>
<th>Metabolism of Testosterone by Rabbit Liver Homogenate</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Incubation</strong></td>
</tr>
<tr>
<td>Tissue</td>
</tr>
<tr>
<td>Testosterone</td>
</tr>
<tr>
<td>Steroids isolated</td>
</tr>
<tr>
<td>Testosterone</td>
</tr>
<tr>
<td>Δ4-Androstene-3,17-dione</td>
</tr>
<tr>
<td>Epitestosterone</td>
</tr>
<tr>
<td>Cholesterol</td>
</tr>
<tr>
<td>Steroids isolated</td>
</tr>
</tbody>
</table>

* The tissue and testosterone in each experiment were mixed in the Waring blender with 50 ml. of sterile distilled water and transferred to the Fernbach flask with double strength Ringer's phosphate buffer.

**Liver**—The metabolites formed were Δ4-androstene-3,17-dione and epitestosterone (Table I). The recovered testosterone plus the metabolites accounted for 83 to 97 per cent of the added testosterone.

Variations in the ratio of hormone to wet weight of tissue in the incubation from 1:66 to 1:100 or the use of water (Experiment 3) instead of buffer in the preparation of the homogenate did not alter the results. The lower yield of metabolites in Experiment 4 was very likely due to a greater loss of these products during the final isolation procedure. A number of oily fractions gave color tests for the 17-keto (4) and the 17-hydroxyl groups (5) but failed to yield crystalline materials.

The non-ketonic fractions yielded no metabolites. The naturally occurring cholesterol was readily isolated and in Experiment 4 a few mg. of testosterone were found in this fraction.

**Kidney**—The results with kidney homogenates (Table II) were identical...
with those obtained with liver homogenate. The failure to find epitestosterone in Experiment 1 was due to the small amount of starting material. The incubation of a large amount of testosterone (Experiment 2) resulted in the formation of an isolable amount of epitestosterone.

**Table II**

*Metabolism of Testosterone by Rabbit Kidney Homogenate*

<table>
<thead>
<tr>
<th>Incubation</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue</td>
<td>10,000 per cent</td>
<td>5 x 16,700 per cent</td>
</tr>
<tr>
<td>Testosterone</td>
<td>150</td>
<td>5 x 200</td>
</tr>
<tr>
<td>Steroids isolated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Testosterone</td>
<td>136 91</td>
<td>786 79</td>
</tr>
<tr>
<td>Δ4-Androstene-3,17-dione</td>
<td>11 7</td>
<td>49 5</td>
</tr>
<tr>
<td>Epitestosterone</td>
<td>73</td>
<td>12 1</td>
</tr>
<tr>
<td>Cholesterol</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Discussion**

The intact cell is not necessary for the metabolism of testosterone by rabbit liver and kidney. The metabolites formed by incubation of testosterone with the homogenates of either rabbit liver or kidney were not only the same as those obtained with rabbit liver slices (2), but the amounts formed were essentially the same. Δ4-Androstene-3,17-dione was the main metabolite and epitestosterone was the other metabolite. Thus, both the oxidative enzyme system necessary for the conversion of testosterone to Δ4-androstene-3,17-dione and the reductive enzyme system for reversing the reaction were not destroyed by homogenization in the Waring blender. The recovery of testosterone and its metabolites in quantities equivalent to the testosterone recovered in the control experiment indicated that no other metabolites in appreciable quantity were formed. As the non-ketonic fraction yielded no metabolites, it appears that the α,β-unsaturated 3-ketone group is quite resistant to both oxidation and reduction.

**Experimental**

The incubation and extraction procedures were as described previously (2, 6). The ketonic and non-ketonic fractions of the control, liver Experiments 1 to 3, and kidney Experiment 1 were chromatographed with magnesium silicate-Celite (7) and the following eluting solvents: carbon tetrachloride, benzene, ethyl ether, methyl alcohol. In liver Experiment 4 and kidney Experiment 2, washed alumina (Harshaw) was used as the adsorbent and carbon tetrachloride with progressively increasing amounts of absolute alcohol as the eluting solvents.
The Holtorff-Koch (4) modification of the Zimmermann color test was used at 20° for detection of the 17-keto group. The Kagi-Miescher tests (5) were used for detection of the 17-hydroxyl and the 17α-hydroxyl groups. Final confirmation of the identity of all of the isolated steroids was obtained by infra-red analysis.3

_Purification of Testosterone_—The testosterone was further purified by recrystallization from carbon tetrachloride, m.p. 156–158°.

_Identification of Δ4-Androstene-3,17-dione_—The Δ4-androstene-3,17-dione isolated in each experiment was identified by melting point and mixed melting point with an authentic specimen. Furthermore, ultraviolet spectra3 were obtained for the Δ4-androstene-3,17-dione isolated in liver Experiment 4 and kidney Experiment 2 and optical rotation4 on the metabolite obtained from kidney Experiment 2. The physical constants of the product obtained in kidney Experiment 2 are as follows: m.p. 173–175°; mixed m.p. 168–176° with an authentic sample of m.p. 172–175°; λ_max. 239, ε = 15,100; [α]_D^22 = +198° (CHCl3).

_Identification of Epitestosterone_—The melting point and mixed melting point of the metabolite and also those of its oxime agreed with those of authentic samples. Thus, the epitestosterone isolated in liver Experiment 2 gave a melting point of 219–222°; oxime 222–225°; no depression of mixed melting points was observed. The epitestosterone gave the expected color tests (4, 5).

**SUMMARY**

Testosterone is metabolized by homogenates of rabbit liver and kidney to Δ4-androstene-3,17-dione and epitestosterone. The recovered testosterone plus the metabolites accounted for 83 to 97 per cent of the original testosterone.

**BIBLIOGRAPHY**


3 These analyses were kindly performed by the Lederle Laboratories Division, American Cyanamid Company, through the courtesy of Dr. S. Bernstein and Dr. J. W. Williams.

4 This analysis was generously performed by Ciba Pharmaceutical Products, Inc., through the courtesy of Dr. A. St. André.
METABOLISM OF TESTOSTERONE BY HOMOGENATES OF RABBIT LIVER AND KIDNEY
Charles D. Kochakian, José Gongora and Nicholas Parente


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