THE METABOLISM OF TESTOSTERONE

by

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THESIS

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I

Introduction

A. Historical Introduction.

The functional significance of the testis has been known since the time of Aristotle (121) who ascribed post-pubertal changes to the function of the gonads, as such manifestations were absent in castrates -- both in man and in birds. Accurate knowledge of the nature and metabolism of the androgenically active substance secreted by the testis has, however, been acquired only during the past decade.

It was not until the first half of the Nineteenth Century that the gonads were recognized as being ductless glands, and capable of secreting a substance into the blood-stream.

That the testis is normally the main source of the androgenic substance in man and other animals was first indicated experimentally by Berthold in 1849 (4). He removed the testis of four roosters and replaced one testis in an unusual abdominal position in each of two capons. He killed these two capons six months later and found that because of the testicular grafts, "these animals remained male in regard to voice, reproduction, instinct, fighting spirits, and growth of comb and wattles". Berthold further suggested that the testicle exerted its influence upon the organism via the blood, and this viewpoint is now the generally accepted one. Recently, Denby (35) reported that bull's blood contains less than 1 ml. of androgens per litre, but that 500 cc. of oxygenated blood -- which had been
perfused through a surviving bull testis for 0.5 to 4.5 hours at 40 to 90 mm. Hg. pressure -- increased the concentration to the order of 16 to 46 I.U. per litre, without seriously lowering the androgenic activity of the testis itself, indicating that the testis synthesized an androgenically active substance(or substances) which it then secreted into the blood. It was not until 1927, however, that the first potent testicular extracts which gave reproducible results were obtained from bull testis by McGee and his colleagues (114). Various other investigators (114) made attempts to obtain such extracts prior to 1927, but these extracts were crude and far from satisfactory.

Among the other possible sources of androgenically active substances (and of steroid metabolites generally) in ram and experimental animals are blood and urine, and considerable attention has in fact been paid to urinary steroid excretion products. Studies dealing with the excretion of urinary steroids have been very extensive, much more so than similar studies dealing with the steroids in the blood. This may be so because the early investigators found it technically easier to analyze urine than to analyze blood. Furthermore, it was desirable at first to characterize the steroid metabolites, and urinalysis is a more convenient tool for this purpose than is blood analysis.

Thus it was that in 1939, Funk, Harrow and Lejwa (62-64) -- using the capon comb test worked out by Gallagher and Koch (65) -- showed that the urine of normal young men contained androgenically active substances. Shortly thereafter, Butenandt and his colleagues isolated Androsterone (20) and Dehydroisoandrosterone (21) from the urine of normal men. The isolation of Testosterone, however, was not accomplished until 1935, when David, Lacqueur et al (37) isolated it from bull's
testicular extracts. Since that time, a considerable body of evidence has been accumulated to suggest that Testosterone is the male sex hormone secreted by the testis of man and experimental animals, but it must be pointed out that as yet there is no absolute proof for this theory. At any rate, Testosterone is the most active androgen thus far isolated.

A knowledge of the metabolism of Testosterone is not only of academic interest, but is also of clinical importance, since it has been shown by many investigators that the excretion of substances believed to be metabolites of Testosterone (and excretion of substances closely related to these metabolites) varies in different pathological conditions (40). Notable among these pathological conditions are diseases of the adrenal cortex (2, 39, 82, 162) of the testis (82), of the anterior pituitary (2, 35) and of the thyroid (58). Among the urinary excretion products which are in use as an index of the activity of the aforementioned glands are the 17-ketosteroids. These compounds are characterized by the presence of a ketonic group \((\text{CO})\) at the \(C_{17}\) position. Typical 17-ketosteroids which have been isolated are those illustrated in Figure 1. Urinary 17-ketosteroids fall into two classes -- phenolic and non-phenolic ones. Estrone -- an estrogenually active steroid with a benzenoid Ring A -- is a phenolic 17-ketosteroid. The ketonic metabolites of Testosterone and related 17-ketosteroids are members of the non-phenolic (or neutral 17-ketosteroid) class. The present discussion will deal with the latter compounds.

B. Urinary 17-Ketosteroids.

The neutral 17-ketosteroids which have been isolated from the urine fall into three classes -- those which have been isolated from
**17-Ketosteroids**

- **Androsterone**

- **Etiocholanol-3(2)-one-17**  
  (Etiocholanolone)

- **Δ² Androstenone-17**

- **Dehydroisoandrosterone**

- **Δ⁴ Androstenediol-3(2)-one-17**

- **3-Chloro Dehydroisoandrosterone**

---
(i) normal and (ii) pathological urine, and those (iii) which have been isolated following the exogenous administration of a steroid compound, for example, Testosterone.

Normal Urine:

The 17-ketosteroids (Table 1) which have been isolated from normal human urine are Androsterone (20, 29, 30, 40, 127), Etiocholanolone (29, 30, 40, 127), $\Delta^2$ Androstenone-17 (40, 127), $\Delta^3:5$ Androstadienone-17 (19, 40, 127), Isoandrosterone (127), Androstanediol-3(17), 11-one-17 (110), 3-Chloro-Dehydroisoandrosterone (40), $\Delta^2$ Androstenol-3(17)-one-17 (51, 123, 165) and Dehydroisoandrosterone (21, 27, 29, 54, 77). The excretion of these compounds in mg./litre is listed in Table 1.

It will be noted from Table 1 that Androsterone and Etiocholanolone are excreted in equal amounts and that they are the 17-ketosteroids found to the greatest extent in normal human urine. The fact that the excretion of these two steroids does not rise greatly in hyperadrenalism (for example, Virilism, Eirsutism, Adrenocortical carcinoma, Basophilism) - Table 1 - indicates that in males Etiocholanolone and Androsterone are normally derived from testicular secretion rather than adrenocortical steroid precursors. There is no evidence available at present to indicate that ovarian steroids can act as precursors of Androsterone or Etiocholanolone or any of the steroids listed in Table 1.

The data presented in Table 1 suggests that Dehydroisoandrosterone is normally a product of adrenocortical secretion. This point will be discussed later. (See page 9)
TABLE 1

Urinary excretion of 17-ketosteroids considered to be metabolites of Testosterone (and of substances closely related to these metabolites) in patients with various forms of hyperadrenalism.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Reference</th>
<th>Excretion in Adrenal Hyperfunction Range of Yield mg./l.</th>
<th>Excretion in Normal Human Urine mg./l.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dehydroisoandrosterone</td>
<td>29,30,35,109</td>
<td>2.00 - 134.0</td>
<td>0.20 (#127)</td>
</tr>
<tr>
<td></td>
<td>117,137,165</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Androsterone</td>
<td>40,75,79,109</td>
<td>1.50 - 9.0</td>
<td>1.00 approx. (#127)</td>
</tr>
<tr>
<td></td>
<td>117,165</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Etiocholanolone</td>
<td>26,40,75,79,109</td>
<td>0.85 - 13.0</td>
<td>1.00 approx. (#127)</td>
</tr>
<tr>
<td></td>
<td>109,165</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isoandrosterone</td>
<td>26,124</td>
<td>0.05 - 0.5</td>
<td>0.20 (#127)</td>
</tr>
<tr>
<td>(\Delta^2) Androstenone-17</td>
<td>40,124</td>
<td>0.01</td>
<td>0.01 - 0.02 (#127)</td>
</tr>
<tr>
<td>(\Delta^X) Androstenol-3(\alpha)-one-17</td>
<td>51,123,165</td>
<td>4.80 - 8.0</td>
<td>---</td>
</tr>
<tr>
<td>(\Delta^{3:5}) Androstadienone-17</td>
<td>19,40,165</td>
<td>13.00 - 25.0</td>
<td>Qual, Detm'd. (#127)</td>
</tr>
<tr>
<td>3-Chloro-Dehydroisoandrosterone</td>
<td>40</td>
<td>----</td>
<td>Qual, Detm'd. (#127)</td>
</tr>
<tr>
<td>Androstenediol-3(\alpha)-11-one-17</td>
<td>109</td>
<td>0.25 - 2.1</td>
<td>0.30 (#110)</td>
</tr>
</tbody>
</table>

\#127 = Reference 127
\#110 = Reference 110
Isoandrosterone has been isolated only from normal human
female urine (123, 124), and its presence in normal human male urine
is indicated (123, 124). Its relation to the hormone secreted by the
testis, on the basis of available evidence (127), is that of an end-
product of a less important metabolic pathway of male sex hormone
metabolism than that associated with Androsterone and Etocholanolone.

As for Androstanediol-3(α)-11-one-17, the presence of the
C11 hydroxyl group suggests that this 17-ketosteroid is of adreno-
cortical origin. Δ² Androstenol-3(β)-one-17 (51) is also believed to
be of adrenocortical origin. The relation of these latter steroids
to the metabolism of Testosterone (and the male sex hormone) is not
clear.

The remaining compounds listed in Table 1 - Δ² Androsterone-17,
Δ³:⁵ Androstadienone-l7 and 3-Chloro-Dehydroisoandrosterone - are
considered to be artifacts of urinary steroids, for it is known that
they can arise during acid hydrolysis of the urine. The subject of
artifacts will be discussed under the section on "Conjugation of
Urinary Steroids".

In conclusion, it may be stated that Androsterone, Eto-
cholanolone, and possibly Isoandrosterone, are metabolites of the
male sex hormone occurring in normal human male urine.

Pathological Urine:

It has been previously pointed out that the excretion of
17-ketosteroids can be used as an index of the activity of the adrenal
cortex, testis, etc. Analysis of the urine in dysfunction of these
glands indicates that changes in the urinary excretion of 17-ketosteroids
is of a quantitative and possibly qualitative (40) nature. Table 1
**TABLE 2**

Urinary excretion of 17-Ketosteroids of a patient with an interstitial cell tumour of the testis (85).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Yield: mg./24 hr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Androsterone</td>
<td>27.1 - 6</td>
</tr>
<tr>
<td>Etiocholanolone</td>
<td>62</td>
</tr>
<tr>
<td>$\Delta^2$ Androstenone-17</td>
<td>6.1</td>
</tr>
<tr>
<td>Isoandrosterone</td>
<td>38.1</td>
</tr>
<tr>
<td>Dehydroisoandrosterone</td>
<td>----</td>
</tr>
</tbody>
</table>
illustrates the quantitative changes that can occur in cases of adrenal hyperfunction (Virilism, Eosinophilism, Hirsutism, Adrenal Cortical Tumours). It will be noted that the compounds found in normal urine are also found in adrenal hyperfunction urine, and that the greatest changes in urinary 17-ketosteroid excretion occurred in the case of Dehydroisoandrosterone and its artifact, \( \Delta^3:5 \) Androstadienone-17. These two steroids were excreted in greater than normal amounts.

Hence, as Mason and Kepler (109) pointed out, Dehydroisoandrosterone is probably derived chiefly from the adrenal cortex, whereas Androsterone and Etiocholanolone are probably derived chiefly from the testicular secretion. Seffman et al (25, 159) have provided further support for this theory. They have reported the isolation of Androsterone, Etiocholanolone, \( \Delta^2 \) Androstenone-17 and Isoandrosterone from the urine of a patient with an interstitial cell tumour of the testis. The data in Table 2 indicates that the greatest increase in urinary 17-ketosteroids isolated occurred for those compounds which are considered to be normal metabolites of the male sex hormone (Androsterone and Etiocholanolone), and confirms the theory that Isoandrosterone is probably such a metabolite. \( \Delta^2 \) Androstenone-17 is presumed to be an artifact of Androsterone. The fact that no Dehydroisoandrosterone was isolated supports the theory that it is of adrenocortical origin. Additional evidence is that the excretion of Dehydroisoandrosterone by ovariectomized women (79) is 0.13 mg./l. -- and, therefore, of the same order of magnitude for that of normal men (0.2 mg./l.).

Mason and Kepler (110), on the basis of their study of the metabolism of Dehydroisoandrosterone following its administration
(as the acetate) to one male with pituitary insufficiency, and to one female having Addison's Disease, suggest that Dehydroisoandrosterone is a primary product of the adrenal cortex -- from which, in the female, urinary Androsterone and Etiocholanolone are derived. However, Callow and Crooke (30) have reported one case of Virilism whose urine did not yield any Dehydroisoandrosterone. However, the urinary 17-ketosteroid value was 79-100 mg./24 hrs. At any rate, Mason and Karler's hypothesis requires further substantiation before it can be accepted unequivocably.

Dobriner et al (40) have investigated the pattern of excretion of urinary 17-ketosteroids in various pathological conditions, for example, myeloid and lymphatic leukemia, and carcinoma of the larynx and the prostate. The results indicate that the pattern of excretion of 17-ketosteroids is abnormal, but no conclusions can be drawn as to whether these abnormalities are specific for the particular disorders studied. The pattern of excretion of some thirty crystalline steroids isolated -- of which only eight are found in normal human urine -- indicates that the above pathological states are accompanied by abnormal function of the gonads or adrenals or both; or possibly a disturbed metabolism of the products of these glands.

It is apparent, therefore, that steroid metabolism is modified by pathological conditions, but except for the relation between Dehydroisoandrosterone with adrenocortical carcinoma, and Androsterone and Etiocholanolone with testis interstitial cell tumour, it is not possible to relate any urinary 17-ketosteroid (or 17-ketosteroids) with any specific pathological disorder or glandular
dysfunction.

**Urinary 17-ketosteroids following Testosterone administration:**

The experimental evidence thus far presented does not provide much definite information concerning the metabolism of Testosterone in man, since it has not been absolutely proven that Testosterone is the male sex hormone, and since there is no satisfactory information concerning the rate of excretion of the male sex hormone by the testis. Several investigators have, therefore, attempted to elucidate the metabolism of Testosterone in man by exogenous administration of this steroid.

In man, the 17-ketosteroids which have been isolated from the urine following the oral or intramuscular administration of Testosterone (either as crystalline Testosterone or Testosterone-propionate) are Androsterone (28, 43, 45, 136), Isoandrosterone (28, 45, 136) and \( \Delta^2 \) Androstenone-17 (136). A study of the metabolism of Testosterone has not been limited to man, however, and after subcutaneous administration in the guinea pig, it gave rise to Isoandrosterone (48). In the male chimpanzee (61), Androsterone, Etiocholanolone and \( \Delta^2 \) Androstenone-17 have been isolated following oral Testosterone administration. The pregnant Rhesus monkey (87) has also converted exogenous Testosterone to Androsterone. Details of the above experiments are summarized in Table 3.

It is noteworthy that urinary Dehydroisoandrosterone has not yet been isolated in amounts greater than normal following the administration of Testosterone to man and experimental animals. This is, therefore, further proof for the theory that Dehydroisoandrosterone is of adrenocortical origin.
<table>
<thead>
<tr>
<th>Experimental Conditions</th>
<th>Reference</th>
<th>Androsterone mg/l</th>
<th>Estradiolamine mg/l</th>
<th>Isoandrosterone mg/l</th>
<th>Δ² Androsterone-17 mg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>600 mg T.-P. orally (501 mg T.-) - gonadal deficient male</td>
<td>43</td>
<td>3.33</td>
<td>8.0</td>
<td>1.10</td>
<td>9.0</td>
</tr>
<tr>
<td>360 mg T.-P. intramuscularly (300 mg T.) - gonadal deficient male</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>640 mg T.-P. subcutaneously (530 mg T.) - male guinea pig</td>
<td>48</td>
<td></td>
<td></td>
<td></td>
<td>2.8</td>
</tr>
<tr>
<td>800 mg T.-P. subcutaneously (600 mg T.) - male guinea pig</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.4</td>
</tr>
<tr>
<td>300 mg T.-P. orally (250 mg T.) - castrate</td>
<td>45</td>
<td>6.0</td>
<td>1.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>360 mg T.-P. intramuscularly (300 mg T.) - hypogonadal male</td>
<td>49</td>
<td></td>
<td></td>
<td></td>
<td>2.9 mg./day 11.6</td>
</tr>
<tr>
<td>1.2 gm T.-P. intramuscularly (1 gm T.) - normal adult woman</td>
<td>136</td>
<td>6.50</td>
<td>14.6</td>
<td>3.50</td>
<td>7.7</td>
</tr>
<tr>
<td>800 mg T.-P. intramuscularly (667 mg T.) - hypogonadal male</td>
<td>28</td>
<td>8.00</td>
<td>9.0</td>
<td>7.70</td>
<td>8.3</td>
</tr>
<tr>
<td>3 gm T.-P. orally (2.5 gm T.) - male chimpanzee</td>
<td>61</td>
<td>2.40</td>
<td>2.1</td>
<td>0.10</td>
<td>0.3</td>
</tr>
<tr>
<td>1,200 mg T.-P. intramuscularly (1 gm T.) - pregnant monkeys</td>
<td>87</td>
<td>1.60</td>
<td>2.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*T.-P. = Testosterone-propionate  
\( T. \) = Testosterone
and Δ₇ Androstenedione-3α-ol-17 (Table 1) are 17-ketosteroids which are believed to arise from adrenocortical precursors, and their presence in urine has also not been reported following Testosterone administration.

One may conclude, therefore, that Androsterone, Etiocholanolone and Isoandrosterone are metabolites of exogenous Testosterone in man and experimental animals. These results agree with those previously reported for normal human male urine, and, therefore, support the theory that Testosterone may be the male sex hormone. As for Δ₇ Androsterone-17, it is believed to be an artifact of Androsterone, and evidence for this theory will be presented on page 26. Suffice it to say at the moment that it is not considered to be a true urinary metabolite of Testosterone.

C. Urinary Hydroxy-non-ketonic Steroids.

The aforementioned experiments -- in which only the ketonic fraction of the urine has been investigated -- provide an incomplete picture of the metabolism of Testosterone. Theoretically, Testosterone can be reduced to a large number of non-ketonic alcoholic steroids, both saturated and unsaturated. The importance of these carbinols clinically is potentially equal to or greater than that of the 17-ketosteroids, but the information available about these carbinol metabolites is meager. Furthermore, only a few carbinols of the Androstane series have been isolated.

In contrast to carbinol metabolites of the male sex hormone, carbinol metabolites (and their closely related isomers) of the Luteoid (Figure 2) and Folliculoid (Figure 3) hormones have often been
isolated from animal urine, and in some cases have proven to be of clinical interest. The information about to be quoted will show that non-ketonic steroids are in fact excreted in normal and pathological human (and animal) urine, and suggests, therefore, that carbinol metabolites of the Androstane and Etocholane series might reasonably be expected in the urine. Furthermore, these carbinol metabolites may be either saturated or unsaturated.

**Carbinols of the Pregnane Series (Figure 2):**

Thus, for example, Harrian (104) first isolated Pregnanediol-3(α) 20(α) (Figure 2) from human pregnancy urine in 1929. This compound is believed to be the major urinary metabolite of the corpus luteum hormone, Progestrone, in man. Allopregnanediol-3(α) 20(α) has been isolated from human pregnancy urine by Hartman and Locher (70), and from pregnant cow's and mare's urine (101) and non-pregnant human urine (100) by Marker et al. Marker et al have also isolated Allopregnanediol-3(β) 20(α) from the same sources (101, 102). These two compounds are stereoisomers of Pregnanediol-3(α) 20(α). Butler and Harrian have reported the isolation of Pregnanetriol-3(β) 17,20(25) and its isomer, Pregnanetriol-3(α) 17,20(26) from the urine of a woman with adrenocortical hyperplasia. The latter compound has also been isolated recently by Mason and Kepler (109) from both adrenocortical tumour and hyperplasia urine. Schiller et al (137) have isolated Δ⁵ Pregnanediol-3(β) 20(α) from the urine of a woman with an adrenocortical carcinoma. The saturated isomer, Pregnanediol-3(β) 20(α), has been isolated by Mason and Kepler (109) from the urine of patients with adrenal hyperplasia.
FIGURE 2

Carbinol Derivatives: Pregnan and Allopregnan Series

Pregnanediol-3(β)20(α)

Allopregnanediol-3(α)20(α)

Pregnanetriol-3(α)17,20

Pregnanetriol-3(β)17,20

Pregnanediol-3(α)20(α)
However, the relation of the latter compounds to adrenocortical hyperfunction and to adrenocortical steroid metabolism has not yet been clarified.

Carbinols of the Estrane Series (Figure 3): As for the Estrogenic carbinols, Estriol was first isolated by Marrian (105) and Doisy (42) in 1930 from human pregnancy urine. It has also been isolated by Browne (14) from human placenta extracts. \( \gamma \)-Estradiol is believed to be the female sex hormone and was first isolated from human pregnancy urine by Smith et al (148). Both Estriol and \( \alpha \)-Estradiol have since been isolated from several other sources (127). An isomer of \( \alpha \)-Estradiol, \( \beta \)-Estradiol, was first isolated from mare's pregnancy urine by Hirschmann et al (76). Its metabolic relation to \( \alpha \)-Estradiol is apparently not the same for man and the rabbit, since Heard and Hoffman (71) were not able to isolate any urinary \( \beta \)-Estradiol after the intramuscular administration of 250 mg. of \( \alpha \)-Estradiol to a normal man, whereas Fish and Dorfman (60) observed that the rabbit does excrete \( \beta \)-Estradiol after administration of \( \alpha \)-Estradiol.

Carbinols of the Androstane and Etioclan Series (Figure 4): (a) Unsaturated Carbinols. The first proof that unsaturated non-ketonic alcohols of the Androstane series are excreted in human urine was presented by Hirschmann (80) in 1943, when he reported the isolation of \( \Delta^5 \) Androstenetriol-3(\( \beta \)) 16,17 from the urine of a seven-year old boy with an adrenocortical carcinoma. Since then, Marrian (106) has announced its isolation in minute amounts from the urine of normal men, and Mason and
Kepler (109) have isolated it from patients with adrenal tumours. FIGURE 3

An isomer of this compound — differing in the spatial configuration of C₁₅ and C₁₉ hydroxyl groups — has been synthesized by Stodola et al. (151) and Butenandt et al. (84).

Hirschmann (31) has also reported the isolation of Carbinols of the Estrane Series Δ⁵ Androstanediol-17β, 17α-s. From the facts mentioned above, Mason and Kepler (109) and Schiller et al. (137) have likewise isolated this diol from adrenocortical tumour urine. This compound may be related metabolically to Dehydroisoandrosterone, and Mason and Kepler (109) were able to isolate small amounts of it from the urine of a pituitary deficient subject following the administration of Dehydroisoandrosterone sulfate. The converse reaction was demonstrated by Miller and Dorfman (115) who administered Δ⁵ Androstanediol-3(β)-17(α)-diacetate subcutaneously to adult male guinea pigs and isolated small amounts of Dehydroisoandrosterone from the urine. The extent of conversion in Mason and Kepler's experiment was 0.6% and in Miller and Dorfman's experiment it was 0.25%. Hence, it is not probable that the action of Dehydroisoandrosterone are normally closely related metabolically.

One might note that Hoffman et al. (65) have reported that after the administration of 4 mg. of Dehydroisoandrosterone to male rabbits, 90 mg. (2.25% conversion) of Δ⁵ Androstanediol-3(β)-17(β) (Figure 4) were isolated from the urine. This compound is an isomer of Hirschmann's dial and the experiment indicates that men and the rabbit do not metabolize Dehydroisoandrosterone in the same fashion.
Kepler (109) have isolated it from patients with adrenal tumours. An isomer of this compound--differing in the spatial configuration of C16 and C17 hydroxyl groups--has been synthesized by Stodola et al (151) and Butenandt et al (24).

Hirschmann (81) has also reported the isolation of $\Delta^5$ Androstenediol-3($\beta$) 17 ($\alpha$) from the source mentioned above. Mason and Kepler (109) and Schiller et al (137) have likewise isolated this diol from adrenocortical tumour urine. This compound may be related metabolically to Dehydroisoandrosterone, since Mason and Kepler (107) were able to isolate small amounts of it from the urine of a pituitary deficient male following the administration of Dehydroisoandrosterone acetate. The converse reaction was demonstrated by Miller and Dorfman (116) who administered $\Delta^5$ Androstenediol-3($\beta$) 17 ($\alpha$)-diacetate subcutaneously to adult male guinea pigs and isolated small amounts of Dehydroisoandrosterone from the urine. The extent of conversion in Mason and Kepler's experiment was 0.6% and in Miller and Dorfman's experiment it was 0.25%. Hence, it is not probable that the diol and Dehydroisoandrosterone are normally closely related metabolically.

One might note that Hoffman et al (83) have reported that after the administration of 4 mg. of Dehydroisoandrosterone to male rabbits, 90 mg. (2.5% conversion) of $\Delta^5$ Androstenediol-3($\beta$) 17 ($\beta$) (Figure 4) were isolated from the urine. This compound is an isomer of Hirschmann's diol and the experiment indicates that man and the rabbit do not metabolize Dehydroisoandrosterone in the same fashion.
Carbinols of the Androstane and Etiocholane Series

\[ \Delta^5 \text{Androstenetriol-3(\beta)16,17} \]

\[ \text{Androstanediol-3(\alpha)17(\alpha)} \]

\[ \Delta^5 \text{Androstanediol-3(\beta)17(\alpha)} \]

\[ \Delta^5 \text{Androstanediol-3(\beta)17(\beta)} \]

\[ \text{Etiocholaneol-3(\alpha)17(\beta)} \]
The carbinols, $\Delta^5$ Androstenetriol-3(\(\beta\)) 16,17 and $\Delta^5$ Androstenediol-3(\(\beta\)) 17 (\(\alpha\)), have been isolated from adrenal cortical tumour urines in varying amounts -- none of which was less than the yield per litre reported by Harriin (106) and in three reports (80, 81, 137) in very much greater yield. These carbinols are, therefore, probably derived from adrenocortical precursors whose nature is at the moment unknown, rather than from the testicular secretions. The role of these unsaturated carbinols in the metabolism of Testosterone is not clear, since Testosterone has never been isolated from adrenal cortical extracts and there is no evidence of its secretion by the adrenal cortex.

(b) Saturated Carbinols. The isolation of non-ketonic alcohols of the Androstane and Etiocholane series from human urine or the urine of experimental animals has not kept pace of the unsaturated homologues.

Butenandt et al (22) were the first to report the isolation of an Etiocholandirol from normal human urine. As Butenandt et al pointed out, however, this compound -- Etiocholandirol-3(\(\alpha\)) 17(\(\alpha\)) (Figure 4) -- may be an artifact which arose during the fractionation of the urine, since the urinary extract was absorbed on amalgamated Aluminum and reduction of ketone groups may have occurred during this procedure. Butenandt and Tschernin were able to isolate Androsterone (20) from normal male urine, but could not isolate any Etiocholanolone. Since subsequent work by various investigators has shown that Androsterone and Etiocholanolone are normally excreted in approximately equal amounts in human urine, it is probable that the Etiocholandirol-
3(\alpha') 17(\alpha') isolated was derived from urinary Etiocholanolone. Butenandt et al suggested such a ketonic Etiocholane steroid as the probable precursor of the diol.

In 1939, Dorfman et al (43) isolated from the non-ketonic steroid fraction of human urine small amounts of impure crystals whose identity could not be conclusively established. The authors suggested that the crystals were isuere Etiocholandiol-3(\alpha') 17(\alpha'), since their melting point was 214-17°C, and upon admixture with authentic Etiocholandiol-3(\alpha') 17(\alpha') (\%It. = 232°C), the mixed melting point was 234-27°C. However, the above facts can hardly be classified as conclusive evidence for the presence of Etiocholandiol-3(\alpha') 17(\alpha') and the occurrence of this diol in normal human urine has yet to be established unequivocally.

Schiller et al (12) have reported that after the administration intramuscularly of 1.2 mg. of Testosterone-croionate to a normal woman, 3 mg. of digitonin-non-precipitable crystals -- as the acetate -- were obtained from the non-ketonic steroid fraction of the urine. The melting point of the acetylated crystals was 15.5-58.5°C, and the mother liquor yielded crystals melting at 151-53°C, androstanediol-3(\alpha') 17(\alpha') diacetate melts at 159-63°C, according to several groups of workers who have synthesized it (14). After hydrolysis, the crystals melted at 217-21°C, and upon admixture with authentic androstanediol-3(\alpha') 17(\alpha') (\%It. = 221-23°C), there was no depression in the melting point (215-20°C). It is probable therefore that the compound isolated was indeed Androstanediol-3(\alpha') 17(\alpha'). Since the diol represents only about 0.25% of the administered Testosterone-croionate, it is
probably not a significant urinary metabolite of Testosterone.

Various investigators have isolated minute amounts of crystals from the non-ketonic steroid fractions of adrenal hyperfunction urine, but these crystals have not been further identified.

The evidence cited above indicates, therefore, that the number of known carbinol metabolites of Testosterone is small, and that the available information about these metabolites is very limited.

D. Microbiological Oxidation and Reduction.

It has already been indicated that Testosterone may probably be converted to saturated carbinols in man. Work with yeast and bacteria carried out by Læmoli et al has indicated conclusively that Testosterone can be reduced biologically not only to Etiocholandiol-3,17 (95,97,98) but also to Androstane-diol-3(\(\Delta^4\)) 17 (97). This, therefore, strengthens the theory that the same reactions can occur in man and experimental animals.

It is noteworthy that studies with micro-organisms have not been limited to carbinols, but have been extended to those compounds which are considered to be urinary 17-ketosteroids. Results of such studies are the following:

Læmoli et al (97) have demonstrated the bacterial conversion of \(\Delta^4\) Androstenedione-3,17 to Androsterone and Isoandrosterone in yields of 12.5 and 9.3\% respectively. The conversion of Testosterone to \(\Delta^4\) Androstenedione-3,17 by bacteria has been demonstrated in good yield (49\%) by Turfitt (155).
Lamoli (99) quotes the converse biochemical reaction carried out by fermenting yeast. Lamoli (99) also reports the conversion of \( \Delta^4 \) Androstenedione-3,17 to 7-tiocholandione-3,17 by putrefied stallion testis extract. Experiments by Lamoli and his coworkers have demonstrated the conversion of Androstenedione-3,17 to Androstanediol-3(\( \beta \)) 17 by fermenting yeast (161) and by yeast brei (98).

It will be noted that the above reactions carried out by yeast and bacteria are those involving hydrogenation of the compound added to the micro-organism. That dehydrogenation can also be carried out by such micro-organisms is indicated by the work of Lamoli and Vercellone (93, 94, 96) who reported the conversion of Dehydroisoandrosterone to \( \Delta^4 \) Androstenedione-3,17 (156) and of \( \Delta^5 \) pregneneol-3(\( \alpha \))-one-20 to frogosterone (99). Furthermore, Turfitt (156) has reported that \( \Delta^1 \) Androstenediol-3(\( \alpha \)) 17(\( \chi \)) is converted to Testosterone (42\( \beta \)) and to \( \Delta \) Androstenedione-3,17 (6\( \beta \)) by Proactinomyces spp.

The importance of these findings in deducing the probable pathways for the biochemical conversion of Testosterone to urinary 17-ketosteroids will be discussed later. (See see 68)

E. Conjugation of Urinary Steroids.

Urinary Steroid Conjugates:

Before discussing the role of the liver in the inactivation of steroids and the metabolism of Testosterone, mention must be made of the excretion of steroid conjugates in the urine. The fact that all of the aforementioned urinary steroids, including
those which are metabolites of Testosterone, have been isolated only after acid hydrolysis of the urine indicates that they were excreted in conjugated form.

Funk et al (2) and Koteck (26) first suggested this possibility, and Adler (1) noted that boiling acidified normal human urine greatly increased its androgenic activity. In 1938, Peterson et al (125) observed that the Glucuronic Acid concentration of the urine of normal men varied somewhat with its androgenic activity, and suggested, therefore, that the androgenic steroids may be conjugated in part at least with Glucuronic Acid. Recently, Hoffman (194) has noted that the Glucuronic Acid concentration of the urine of male rabbits increased markedly after the administration of Dehydroisoandrosterone. However, no conjugates of Glucuronic Acid with metabolites of Testosterone (or of endogenous testicular secretion) have been isolated as yet.

Attempts to isolate conjugates of Sulfuric Acid with androgenic urinary steroids have been more successful. Thus, Androsterone-sulfate (159) has been isolated from testis interstitial cell tumour urine, and Dehydroisoandrosterone-sulfate has been isolated from the urine of normal men (112) and adrenocortical tumour patients (109). They are the only conjugates of androgenically active substances whose isolation has been reported. However, the possibility that the metabolites of Testosterone are excreted in conjugation with both Glucuronic Acid as well as Sulfuric Acid is not yet excluded.

Dobriner (41) has suggested that conjugation of the adrenal steroids may also occur with both of these acids. The
advent of isolation techniques more refined than those in use at present will probably provide further information regarding the conjugation of the above steroids.

It is of interest that urinary conjugates of the metabolites of Progesterone and \( \alpha \)-estradiol have been isolated. Thus, Sodium-pregnanediol-3(\( \alpha \)) 20(\( \alpha \))-glucuronide (158) and Estriol-glucuronide (34) have been isolated from human pregnancy urine, while Estrone-sulfate (135) has been isolated from pregnant mare's urine, and Stilbestrol-glucuronide (113) has been isolated from rabbit urine after the intramuscular injection of 7 \( \mu \)g of Stilbestrol. Mason and Kepler (109) have reported the isolation of Sodium-pregnanetriol-glucuronide from the urine of patients with adrenal hyperplasia, while Klyne and Barrian (89) have recently isolated a new sulfate, \( \Delta^{16} \) Alloprogreneneol-3(\( \beta \))-one-\( \beta \)-sulfate, from pregnant mare's urine.

It is believed (160) that the liver is the site of conjugation of the steroids.

**Artifacts of Urinary Steroids:**

Studies on the conjugation of steroids employing the methods of hydrolysis currently in use have resulted in the detection of certain urinary steroids which are believed to be artifacts. These artifacts have been found only after acid hydrolysis of the urine. It is obviously of importance to be able to differentiate between true urinary steroids and artifacts thereof. One must, therefore, mention the following artifacts whose presence has been detected in the urine of man and animals:
(a) $\Delta^2$ Androstenone-17. During the discussion of the neutral 17-ketosteroids it was noted that $\Delta^5$ Androstenone-17 was considered to be an artifact arising during acid hydrolysis of urine. This compound has been isolated by Hirschmann (78) from the urine of ovariectomized women, and by Pearlman (124) and Dobriner et al (40) from the urine of men and women having adrenocortical carcinoma. In 1942, Venning et al (159) reported that $\Delta^2$ Androstenone-17 arose after acid hydrolysis of Androsterone-sulfate, which had been isolated from the urine of a man with generalized metastasis of an interstitial cell tumour of the testis. They thereby confirmed Hirschmann's suggestion that the former compound was an artifact of "Androsterone (or Isandrosterone)" arising during acid hydrolysis of the urine.

The position of the ethylenic bond in the structure of $\Delta^2$ Androstenone-17 has never been definitely established, but the work of Butenandt et al (21) -- who first prepared it by treating 3-($\Delta^5$) Chlororandrostanone-17 with Potassium Acetate at 180°C -- indicates that the double bond is at the $\Delta^2$ or $\Delta^8$ position. The variety of melting points reported for Androstenone-17 (102-110°C) may be due to the fact that a mixture (the exact composition depending upon the method of preparation) of $\Delta^1$ and $\Delta^5$ Androstenone-17 is present.

It is interesting to note that the corresponding homologue of Etiococholanolone, $\Delta^2$ Etiococholenone-17, has never been isolated from urine. This may be due to the fact that Androsterone and Etiococholanolone are excreted in different conjugated forms, but there is no factual information on this point.
(b) \( \Delta^{3:5} \text{Androstadienone-17} \). It has previously been noted that \( \Delta^{3:5} \text{Androstadienone-17} \) was considered to be an artifact of Dehydroisoandrosterone. The evidence for this theory follows.

Burrows et al (19) first isolated \( \Delta^{3:5} \text{Androstadienone-17} \) from the urine of a man with an adrenal tumour. This compound has also been isolated by Dobriner et al (40) from the urine of normal men. Burrows et al (19) prepared it by dehydration of Dehydroisoandrosterone using Copper Sulfate at 200°C, and in the rabbit (43) Dehydroisoandrosterone has in fact given rise to considerable amounts of urinary \( \Delta^{3:5} \text{Androstadienone-17} \). It appears also from spectroscopic evidence (39) that acid hydrolysis of a urine previously devoid of any detectable amounts of the dienone, but to which Dehydroisoandrosterone has been added, results in the production of a certain amount of the dienone -- and thereby provides proof for the theory that \( \Delta^{3:5} \text{Androstadienone-17} \) is an artifact of Dehydroisoandrosterone.

(c) 5-Chlorodehydroisoandrosterone. This compound has likewise been proved to be an artifact of Dehydroisoandrosterone, arising during hydrolysis of urine with Hydrochloric Acid.

F. Role of the Liver in the Metabolism of Testosterone and other Steroids.

Site of Steroid Metabolism:

In discussing the urinary steroids -- both ketonic and non-ketonic -- no mention was made of the site at which the metabolic reactions which resulted in the excretion of these metabolites took place. Knowledge of this phase of the problem is not only of theoretical importance, but may also have important
clinical applications. It has consequently been the object of recent experiments.

In the experiments carried out by several investigators (Table 3), Testosterone has been administered orally to the experimental animal. In these experiments, the Testosterone must first pass through the liver before passing into the general circulation. Testosterone has also been administered subcutaneously and intramuscularly by other investigators (Table 3) and, as will be seen from Table 2, the urinary metabolites obtained after oral administration of the hormone are the same as those obtained after intramuscular and subcutaneous injection of the hormone. The Testosterone administered by the latter methods must eventually pass through the liver. Consequently, the role of the liver in the metabolism of Testosterone must be evaluated.

(a) Experimental Animals. The work of several investigators indicates conclusively that in the rat and the rabbit, the liver is a major site of inactivation of both endogenous (15) and exogenous (5, 8, 18, 50, 143, 144) Androgens. This action of the liver on the steroid hormones has also been demonstrated for Estrogens (5, 9, 55, 56, 73, 74, 126, 140, 142, 143, 144, 164), Progesterone (57, 112, 143, 144), Desoxycorticosterone acetate (17, 59, 143, 144) and endogenous adrenal secretions (16). One feature common to all of these experiments is that after administration of the hormone such that they must first pass through the liver before reaching the general circulation, the physiological actions normally associated with these compounds has been either greatly diminished or absent. Hence the liver must be responsible, in part
at least, for their metabolism.

In the rat, Testosterone, Methyl-Testosterone (5, 18, 143, 144) and Testosterone-propionate have all been shown to be inactivated by the liver, and these studies indicate incidentally that the increased oral activity of Methyl-Testosterone is due to a lesser degree of inactivation of this compound than is observed for Testosterone or Testosterone-propionate.

The work of Burrill and Greene (15) in which Testosterone and Methyl-Testosterone pellets were implanted subcutaneously and in the mesentery and spleen of castrated male rats showed that although the liver is responsible for the inactivation of the aforementioned compounds, there is still a significant residual inactivation for which one must account. The site of this residual inactivation is not known. Szego and Roberts (152) have recently reported that in eviscerated, ovariectomized, adult rats, large intravenous or subcutaneous doses of Estrogens are incapable of causing an increase of the water content of the uterus. The effect of evisceration on other indices of estrogen activity (e.g. vaginal smears) was not investigated. It is apparent, at any rate, that the role of the viscera in the metabolism of the Estrogens is more complex than hitherto supposed. The specific role of the viscera in the above experiment could not be determined. The role of the viscera in the metabolism of Testosterone has not been investigated as yet.

Paschikis et al (122) have demonstrated that exogenous Testosterone, Methyl-Testosterone, and Androsterone are excreted to a limited extent in the bile of dogs, but the significance of this observation is not clear. A similar excretion has been noted
for exogenous and endogenous Estrogens (31, 32, 122, 130).

(b) Man. It is apparent from the work of several investigators that the liver is an important site of steroid hormone metabolism in man. For example, Dorfan and Hamilton (44) noted that the oral administration of Testosterone-propionate to a castrate and a eunuch did not result in relief of symptoms. Glass et al (67) have reported that in patients with cirrhosis of the liver there is gynecomastia, and clinically, menorrhagia and metrorrhagia have often been observed in the course of liver cirrhosis. Furthermore, Rekoff et al (130) have noted that the Estrogenic activity of blood disappears less rapidly in patients with cirrhosis and hepatitis than in normal humans after the intramuscular or subcutaneous injection of Δ-estradiol. Glass et al (67) have also reported that men with liver cirrhosis excrete approximately 80% of injected Estrone and Estradiol in the urine, largely in conjugated form. Normal men (72) excrete only about 10% of injected Estradiol in the urine. This is further proof for the role of the liver in the metabolism of the steroid hormones in man.

Influence of Dietary Factors on Steroid Metabolism:

(a) Experimental Animals. In 1942, Biskind and his colleagues (6, 7) published reports which indicated that certain dietary factors are necessary for the inactivation of Estrogens by the liver, and thereby bared another phase of the metabolism of the steroid hormones. Biskind et al have shown that the Vitamin B Complex is necessary for the inactivation of exogenous Estrone and endogenous Estrogen by the liver of the rat. The Segaloffs
(141) and Clark and Kochakian (23) have shown that it is Vitamin B1 and Vitamin B2 which are specifically needed. Singher et al (147) and Shipley and Gyorgy (146) have confirmed these findings. However, the Biskinds (8) have shown that the same may not be true for Androgens, since the presence or absence of the Vitamin B Complex in the diet has no effect upon the inactivation of Testosterone-propionate in the rat.

Drill et al (52, 53) have questioned the validity of the findings reported by the Biskinds. Drill et al have repeated their experiments using paired feeding techniques, and the results obtained suggest that the Vitamin B Complex affects the inactivation of Estrogen only through the concomitant inanition produced. Drill et al also noted that supplements of methionine were without effect. However, Singher et al (147) and Unna et al (157) have shown that reducing the B1 and B2 contents of the liver below a certain critical level will inhibit the ability of liver slices to inactivate Estradiol. Liver damage was not reported by these authors. Also, Segaloff (141) did use a modified form of paired feeding technique. Hence one may conclude that members of the B Complex of Vitamins have an as yet undetermined role in the inactivation of Androgens by the liver. As suggested by Singher et al (147) vitamins B1 and B2 may be essential components of an oxidative system.

Drill et al (52, 53) have indicated that the effect of the B Vitamins may be modified by various factors. Indeed, Shipley and Gyorgy (146) have shown that in the castrated rat, a low protein-high fat diet will result in cirrhosis of the liver and this will result in continued estrus, indicating the inability
of the liver to inactivate exogenous Estrone. In these experiments, large doses of yeast exerted a curative effect -- much more so than the synthetic B-Vitamin supplements. The factors responsible for the greater activity of the yeast are not known.

Gyorgy (69) has reported that "cirrhosis" of the liver resulting from a specific dietary hepatic injury -- deficiency of methionine or a low protein diet -- will be manifested by the inability of the liver of ovariectomized rats to inactivate exogenous Estrone pellets, and that five-fold increases in the daily Vitamin B supplements will have no effect on the state of estrus of the rats. The addition of methionine or protein hydrolysates to the diet will, however, restore the ability to inactivate Estrone.

Whether these lipotropic factors act by reversing the hepatic changes, or by specifically taking part in the Estrone inactivating mechanism was not determined. The degree of hepatic injury observed in Gyorgy's rats was not clear. If the hepatic injury was moderate, then the probable mechanism is the former. If the liver was very fibrotic, then a therapeutic agent of great importance has been discovered and the mechanism of action may be a combination of the two possibilities listed above. Unna et al. (157) has noted that a low protein diet over a period of three months will derelte the liver of Riboflavin and this will be manifested by inability to inactivate Estradiol. Hence it is apparent that not only the B-Vitamins, but also Methionine and adequate dietary protein are essential for inactivation of Estrogens. Similar findings have not yet been
reported for Testosterone.

(b) Men. In men, Biskind et al (10) have indicated that dietary insufficiency will result in menorrhagia and retro-

rrhagia, and other related disorders, since these disorders have been corrected by the administration of large doses of the B Vitamins and liver extract. Also, Ashworth and Sutton (3) have reported that Estrogens will increase the demand for utilization of the B Complex in three female alcoholics and one woman with amenorrhia and one with menopausal symptoms. Hence it is apparent that in man as in the rat, the B Vitamins and other dietary factors are associated with the metabolism of Estrogens. The relation between these dietary factors and Testosterone metabolism in man has not yet been definitely established.

Pathways of Metabolism of the Steroids:

The liver can metabolize the steroid hormones and their products along two general pathways:-

(i) Complete oxidation of the molecule to, say, water

and Carbon Dioxide; or degradation to a small molecule, for example, Acetic Acid.

(ii) Partial modification of the molecule, so that a steroid of lesser physiological activity is produced, and then excretion of this compound in the urine in a conjugated form.

The chemical transformations which will result in the latter form of metabolism of Testosterone will be discussed later. (See page 67) Heller (73) has observed in vitro using rat and rabbit liver slices that Estrone and \( \alpha \)-Estradiol are interconvertible, and has, therefore, concluded that the second
pathway is the one normally associated with Estrogen metabolism. However, there is at present a dearth of information on the pathways of metabolism of the steroids in general and Testosterone in particular, and it is not possible to evaluate the relative importance of the pathways listed above. Both are presumably in use in man, since only small amounts of steroid metabolites of the sex hormones have been isolated from normal human urine, and since the yield of urinary steroids has been well below 50% following exogenous hormone administration. In the case of Progesterone, for example, it is estimated that only 10–20% of endogenous Progesterone is recovered as Pregnanediol.

Rakoff et al (129) reported that a fraction of the Estrogens in human pregnancy blood was present in combination with proteins. Szego and Roberts (153) have recently published a report concerning the nature of circulating Estrogen in the blood and sera of normal, pregnant and gonadotrophin injected animals and pregnant women. They have confirmed Rakoff et al’s findings and also observed that the Estrogens in blood are present in two forms -- protein-bound Estrogens and a hydrophilic conjugated form. The ratio is approximately 2:1 respectively. It is suggested that the protein-bound Estrogens are available for use by tissues, since they can dialyze through a collodion membrane. This theory has yet to be substantiated, but the results do indicate that more than one pathway for transport and possibly for metabolism of Estrogens exists in both man and experimental animals. It is noteworthy that the amount of Estrogens present in the blood was very small, since values
approximating 0.5 micrograms of \( \alpha \)-estradiol equivalents per 100 cc. of blood were found. Similar studies on the transport of Androgens have not yet been reported.

**Role of Enzyme Systems in Steroid Metabolism:**

Studies in carbohydrate, fat and protein metabolism have demonstrated that the metabolic activities of the various organs and tissues of the body are dependent upon the presence of functional enzyme systems. The physiological actions of the liver in particular have been closely related to the enzymes believed to occur therein.

It is probable, therefore, that enzyme systems are involved in the metabolism of the steroid hormones in the liver and other as yet undetermined tissues. It is probable also that enzyme systems are responsible for the biochemical reactions involving steroid compounds as carried out by micro-organisms. (See page 22) As yet, unfortunately, information about the enzyme systems associated with the metabolism of Testosterone and the other steroid hormones is limited.

A large part of the experimental results thus far reported on the enzyme systems deals with those associated with Estrogen metabolism. Thus, Heller (73) has shown that Sodium Cyanide will inhibit the enzymes -- presumably oxidizing enzymes -- in rat's liver slices and thereby prevent the inactivation of \( \alpha \)-Estradiol, Estrone and Estriol. This enzyme system is heat labile at 100°C. Engel and Rosenberg (56) have obtained an aqueous extract of beef liver which will inactivate Estrone, and, to a lesser degree, Stilbestrol; but the nature and properties of the active components of this extract are not fully known. The Estrogen inactivating principle is extractable at pH 4, pH 5.5.
pH 7 and pH 8, and can be precipitated with alcohol.

Singer and his associates (147) have demonstrated that the inactivation of \( \alpha \)-estradiol in liver slices of rats is dependent upon the concentration of Thiamine and Riboflavin in the liver, possibly through the role of these vitamins as part of an oxidative enzyme system. Whether this oxidative system is similar to that involved in the oxidation of carbohydrates has not been experimentally determined. Incidentally, Koreff and Engel (92) have reported that Folic Acid will also inhibit the estrogenic activity of Estrone, but only to a moderate degree.

It is of interest to note that Pesterfeld (153) has presented evidence for the inactivation of Estrone by Tyrosinase. This is an oxidative process exhibited by potato Tyrosinase (127). Similar enzyme preparations obtained from plant sources are described by Pincus and Pearlman (127).

Meyer et al (115) have reported the presence of the succinic dehydrogenase system in the corpora lutea of the rat and have found a direct relation between succinic dehydrogenase activity and corpus luteum function. However, the role of this enzyme system in corpus luteum activity is not known, and the possible function of succinic dehydrogenase in the metabolism of Progesterone (and the Estrogens) has not yet been determined.

As for Testosterone, limited advances have been made in determining the nature and role of enzyme systems in its metabolism. It is possible that the enzymes involved are similar to those necessary for the oxidation of the Estrogens. This is
indicated by the work of Samuels (121) who incubated Testosterone with liver minces and minces of other tissues, and who has shown that Sodium Cyanide, Sodium Fluoride, or an atmosphere of Nitrogen will inhibit the metabolism of Testosterone by the liver tissue. He thereby correlated the work of Heller (73) on Estrozones and confirmed the above possibility. Samuels suggests that oxidative--possibly phosphorulating--enzymes are involved. This enzymatic reaction incidentally also occurs in the presence of tissue juices containing no organized cells.

One might point out that the work of Singer et al., Heller and Samuels was done in vitro with liver preparations, but the significance of their results and its applicability to in vivo mechanisms must nevertheless not be minimized.

At any rate, it is apparent that the nature and role of enzyme systems in steroid metabolism has still to be determined, and that a study of the role of the liver (or any other tissue) in steroid metabolism must be closely bound up with the function of enzyme systems in such metabolism.

During the discussion of the urinary hydroxylonon-ketonic steroids, it was pointed out that little is known but much is suspected about the urinary cebelinol derivatives of Testosterone in man. In an attempt to further elucidate this problem and to re-examine the ketonic fraction of the urine, 5 gm. of crystalline Testosterone were administered orally to a normal adult man. The procedure and experimental results are accordingly presented.
II

Experimental Section

A. Acid Hydrolysis of the Urine.

One gm. of crystalline Testosterone was administered orally per day for five days to a normal male, twenty years old. The urine was collected for four days prior to administration, and for the five days of administration and the subsequent three days (the administration period urine). The post-administration control urine was collected for two days. The urine was preserved in the cold after acidification to pH 4. Twenty-four-hour collections of urine were hydrolyzed and processed daily in the following manner.

The administration period urine totalled 12.08 litres. The acidified urine was adjusted to pH 7. The urine was then transferred to a round-bottom distilling flask, and brought to the boiling point. 15 cc. of concentrated HCl per 100 cc. of urine were added and the acidified urine was then boiled under reflux for exactly fifteen minutes. The urine was then cooled and saturated with NaCl. The urine was then extracted four times with one-third its volume of redistilled Ether. The Ether extracts were combined and extracted with 1 N NaOH until the NaOH washings were alkaline. The NaOH washings were collected and kept in the refrigerator. The Ether extracts were then washed with small volumes of distilled H2O until the water washings were neutral. The Ether extracts were dried over Na2SO4 and then distilled and the residue dried under Nitrogen.
The daily neutral steroid residues thus obtained were combined, the two control periods and the administration period being kept separate.

The NaOH washings obtained above were combined, acidified with HCl until acid to Congo Red, and extracted with Ether. The Ether extracts were washed with 10% Na₂CO₃ to remove acids. The Ether phase was then discarded. The Na₂CO₃ phase was acidified and re-extracted with Ether. The Ether extracts were washed free of acid with H₂O, and then distilled and the residue was taken to dryness under Nitrogen. The Acid Fraction of the urine was thus obtained. Again, the two control periods and the administration period fractions were kept separate.

B. Fractionation of the Total Neutral Steroid Residue.

The administration period total neutral steroid residue, which weighed 3.3262 gm., was then fractioned as outlined in Figure 5.

Separation of Ketonic and Non-Ketonic Steroid Fractions:

To the total neutral steroid residue of the administra-
tion period was added 104 cc. of Absolute Ethanol, 10.4 cc. of Girard's Reagent T, and 10 cc. of Glacial Acetic Acid. The solution was refluxed for one hour on a steam-bath. The refluxed solution was poured into 405 cc. of H₂O, containing 725 cc. of ice and 16.7 cc. of 6N NaOH. The pH of the solution was determined and adjusted to pH 7.

The aqueous solution was extracted four times with approximately one-third its volume of Ether. The Ether extracts
Fractionation Procedure of the Administration Period Urine

Total Neutral Steroid Residue
3.3282 gm.

Girard's Reagent T

Non-Ketonic Steroids
561.7 mg.

Chromatographed

Column A1
Acetylation
Etiocolandiol-3(α)17(α)
Column A5
Unidentified Crystals

Column A2
Cholesterol

Etiocolandiol-3(α)17(α)
Column A6
Oil

Ketonic Steroids
2.3472 gm.

(4-hr. Hydrolysis = 2.3448 gm.)
(24-hr. " = -0.0024 gm.)

2.3472 gm.

204.2 mg. of crude crystals isolated from the emulsion arising during the Ether extraction of the ketonic steroid phase. Added to Androsterone of Column A3.

Digitonin Separation

Digitonin-Precipitable Ketonic Steroids
1.9102 gm.
(1753.6 mg. 17-K3, using the Zimmerman method of Holtorff and Koch)

Digitonin-Precipitable Ketonic Steroids
109 mg.

Chromatographed

Column A4

Δ2 Androstenone-17
Androsterone
Etiocolanol-3(α) one-17

Column A3

17-K3 = 2.2600 gm. using the Zimmerman method of Holtorff and Koch (86)

17-K3 = 2.0125 gm. using the Antimony Trichloride method of Pincus (128)
containing the non-ketonic steroids were combined and processed as described below, after sufficient concentrated HCl (1/11 volume of the aqueous phase) was added to the aqueous phase to form a 1N solution. The acidified aqueous phase containing the ketonic steroid complex was allowed to stand at room temperature for four hours.

(a) Non-Ketonic Steroids. The ether phase containing the non-ketonic steroids was washed four times with one-quarter its volume of distilled H₂O. The H₂O washings were collected and added to the acidified aqueous phase. The ether extracts were then dried over Na₂SO₄ for twenty-five minutes, distilled, and the residue dried under nitrogen. The non-ketonic residue thus obtained was subjected to a second ketonic separation procedure using Girard's Reagent T. The final non-ketonic steroid residue weighed 561.7 mg.

(b) Ketonic Steroids. The acidified aqueous phase, after standing for four hours at room temperature, was extracted four times with one-third its volume of Ether. The Ether extracts were combined. (To the residual aqueous phase was added a small volume of Ether, and the solution was allowed to stand overnight in the refrigerator.) The emulsion obtained with the Ether extraction was centrifuged and the Ether phase treated in the same manner as the initial Ether extracts. The residues were not combined, however, because crystals (of impure Androsterone) were obtained from the emulsion.

The Ether extracts were washed with one-third their
volume of 1N NaOH and washed to pH 7 with H₂O; dried over Na₂SO₄ and distilled to dryness. The overnight acid-hydrolyzed aqueous phase was also extracted with ether and subjected to the same procedure. The ketonic fractions thus obtained were combined with those obtained from the second fractionation of the non-ketonic residue. The total ketonic residue weighed 2.5472 gm.

Crystalline material weighing 204.2 mg. was obtained from the aforementioned emulsion. After three recrystallizations from aqueous Ethanol, the crystals melted at 175-83°C Uncorrected, and gave no depression in melting point (180-84°C) upon admixture with authentic Androstenedione (M. Pt. = 179-83°C). These crystals, together with those obtained from the mother liquors, weighed 148.6 mg.

(c) Estimation of 17-Ketosteroids. The 17-ketosteroid content of the total ketonic steroid fraction was estimated using the Zimmerman method outlined by Holtorff and Koch (86), and the Antimony Trichloride method of Fincus (128). With the m-Dinitrobenzene reagent, the 17-ketosteroid value was 2.2600 gm.; with the Antimony Trichloride reagent it was 2.0125 gm.

The 17-ketosteroid content of the digitonin-non-precipitable ketonic fraction was estimated using the m-Dinitrobenzene reagent and the value obtained was 1.7563 gm.

Digitonin Separation of the Total Ketonic Steroid Residue of the Administration Period:

The total ketonic steroid residue weighing 2.3472 gm. was taken up in 50 cc. of 94%; Ethanol. To the Ethanol solution
was added 2 gm. of Digitonin in 200 cc. of 80% Ethanol. The solution was then allowed to stand in the refrigerator for forty-eight hours. The solution containing the precipitated digitonides was centrifuged and the supernatant liquid containing the excess Digitonin and the digitonin-non-precipitable-steroids was decanted off. The precipitate of digitonides was washed three times with 25 cc. of cold Absolute Ethanol. The Ethanol washings were combined with the supernatant fluid. The precipitate of digitonides was dried thoroughly first under Nitrogen and then by allowing to stand for two weeks at room temperature.

(a) Digitonin Non-Precipitable Ketonic Steroids.
The total Ethanol solution of 3-(α)-hydroxy-ketonic steroids containing the excess Digitonin was concentrated in vacuo to approximately one-tenth its volume. The solution was then poured into sufficient Ether to form a 10% solution. The Digitonin which precipitated out was recovered by centrifugation. The Ether solution was washed three times with one-quarter its volume of H₂O. The Ether solution was then dried over Na₂SO₄, distilled, and the residue dried under Nitrogen. The residue, weighing 1.9102 gm., was the digitonin non-precipitable ketonic steroid fraction of the administration period urine.

(b) Digitonin Precipitable Ketonic Steroids.
To the dried, powdery digitonide precipitate were added 20 cc. of dry Pyridine. The solution was then heated for forty-five minutes on a Water-bath, to dissolve the digitonide and to decompose the complex. The volume of the solution was then reduced to one-tenth
under reduced pressure. Sufficient dry Ether was added to make a
10% solution, and the Digitonin precipitated out. The solution was
then centrifuged, and the supernatant liquid decanted. The precip-
itate of Digitonin was washed three times with 10 cc. Ether. The
Ether washings were combined with the above supernatant liquid. The
Digitonin was then dried at room temperature.

The Ether phase was then washed five times with one-third
its volume of 25 HCl to remove the Pyridine. The Ether extracts
were then washed with 1 volume of ether, dried over NaSO₄ for twenty-
five minutes, distilled, and the residue of Digitonin-precipitable
steroids was dried under nitrogen.

The precipitate of Digitonin was then redissolved in
Pyridine and the above procedure was repeated. The combined
Digitonin-precipitable ketonic steroid residues weighed 109 mg.

2. Chromatographic Fractionation of the Non-Ketonic and Ketonic
Steroid Residues.

All melting points were done on the Fischer-J hus Apparatus
and are reported uncorrected.

Non-Ketonic Steroid Residue:

The non-ketonic fractions obtained from the administration
period urine were chromatographed using Anhydrus Al₂O₃ columns
(Tables 4 and 5). The Alumina used was that prepared by the Merck
Co., after the method of Brockmann.

(a) COLUMN Al. The results of this column are summarized
in Table 4. A cylindrical column of Alumina, length 8.5 cm. and
diameter 1.77 cm. was used.
TABLE 4

Column A1: 561 mg. of the Non-Ketonic Steroid Fraction of the Administration Period Urine.

<table>
<thead>
<tr>
<th>Fraction No.</th>
<th>Eluant</th>
<th>Eluate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nature</td>
<td>Vol. in cc</td>
</tr>
<tr>
<td>1 - 2</td>
<td>Benzene-Pentane Solution of the Non-Ketonic Fraction</td>
<td>287.0</td>
</tr>
<tr>
<td></td>
<td>1:1 Benzene: Ether</td>
<td>16.4</td>
</tr>
<tr>
<td>3 - 16</td>
<td>1:3 Benzene: Pentane</td>
<td>20 cc.</td>
</tr>
<tr>
<td></td>
<td>100% Benzene</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3:1 Benzene: Ether</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1:1 Benzene: Ether</td>
<td>1:1 Ether: Abs. Ethanol</td>
</tr>
<tr>
<td>17</td>
<td>1:1 Benzene: Ether</td>
<td>8.8</td>
</tr>
<tr>
<td>18</td>
<td>1:1 Benzene: Ether</td>
<td>19.6</td>
</tr>
<tr>
<td>19 - 24</td>
<td>1:1 Benzene: Ether</td>
<td>166.0</td>
</tr>
<tr>
<td>25 - 27</td>
<td>100% Ether</td>
<td>2.4</td>
</tr>
<tr>
<td>28</td>
<td>1:1 Ether: Abs. Ethanol</td>
<td>36.8</td>
</tr>
<tr>
<td>29</td>
<td>1:1 Ether: Abs. Ethanol</td>
<td>12.6</td>
</tr>
<tr>
<td>30 - 34</td>
<td>1:1 Ether: Abs. Ethanol</td>
<td>16.8</td>
</tr>
<tr>
<td></td>
<td>100% Absolute Ethanol</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100% Glacial Acetic Acid</td>
<td></td>
</tr>
</tbody>
</table>
Fractions 1 and 2 were chromatographed as outlined in Column A2.

After three recrystallizations of fraction 19 from aqueous Ethanol, crystals (narrow, rectangular plates) were obtained which melted at 238-40°C, and which did not give any depression in melting point (235-40°C) upon admixture with a sample of authentic Etiocolandiol-3(\(\alpha\)) 17(\(\alpha\)) (M. P. = 235-27°C). Fraction 21, whose melting point was 236-39°C, likewise gave no depression in melting point (275-27°C) in a mixed melt with authentic Etiocolandiol-3(\(\alpha\)) 17(\(\alpha\)). It was concluded, therefore, that fractions 1-21, inclusive, were Etiocolandiol-3(\(\alpha\)) 17(\(\alpha\)).

Fractions 17 and 18 were combined and after two recrystallizations, crystals which melted at 230-37°C were obtained. These crystals were added to the Etiocolandiol fractions 19-21.

Fractions 20 and 21 were used for the synthesis of Etiocolandiol-diacetate as outlined below. 6g. of the diacetate -- equivalent to 52.6 mg. of the free diol -- were obtained.

The total yield of Etiocolandiol-3(\(\alpha\)) 17(\(\alpha\)) from Column A1 was 125 mg.

Fractions 20 and 21 were combined and attempts to recrystallize these fractions from aqueous Ethanol and aqueous Ethanol cooled. The fractions were, therefore, acetylated, as noted below. and then chromatographed, the results being summarized in Column A5.

(b) Column A2. The results are summarized in Table 5.
A cylindrical column of Alumira, 8.5 cm. in length with a diameter of 1.77 cm. was used.
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Benzene-Pentane Solution of the Total Fraction</td>
<td>20 cc.</td>
<td>36.4</td>
<td>Yellow Oil</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>100% Pentane</td>
<td>20 cc.</td>
<td>2.2</td>
<td>Oil</td>
<td></td>
</tr>
<tr>
<td>3 - 8</td>
<td>100% Pentane</td>
<td>20 cc.</td>
<td>0</td>
<td>Oil</td>
<td></td>
</tr>
<tr>
<td>9 - 14</td>
<td>3:7 Benzene:Pentane</td>
<td>20 cc.</td>
<td>7.2</td>
<td>Oil</td>
<td></td>
</tr>
<tr>
<td>15 - 16</td>
<td>3:1 Benzene:Pentane</td>
<td>19.6</td>
<td>Oil</td>
<td>Acetylated-Chromatographed-Column</td>
<td></td>
</tr>
<tr>
<td>17 - 20</td>
<td>3:1 Benzene:Pentane</td>
<td>29.0</td>
<td>Oil and Crystals</td>
<td>5.4 mg. Cholesterol</td>
<td></td>
</tr>
<tr>
<td>21 - 23</td>
<td>1:1 Benzene:Ether</td>
<td>7.8</td>
<td>Oil</td>
<td>Oil</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>1:1 Benzene:Ether</td>
<td>23.0</td>
<td>Oil</td>
<td>Oil</td>
<td></td>
</tr>
<tr>
<td>25 - 26</td>
<td>1:1 Benzene:Ether</td>
<td>37.8</td>
<td>Oil</td>
<td>Minute amount of Crystals</td>
<td></td>
</tr>
<tr>
<td>27 - 28</td>
<td>100% Ether</td>
<td>34.8</td>
<td>Crystals</td>
<td>31.5 mg. Atiocholanol-3(α) 17(α)</td>
<td></td>
</tr>
<tr>
<td>29 - 34</td>
<td>100% Ether</td>
<td>29.4</td>
<td>Oil</td>
<td>Oil</td>
<td></td>
</tr>
</tbody>
</table>
After three recrystallizations from aqueous Ethanol, the crystals in fraction 27 melted at 235-37°C, with preliminary sublimation. Upon admixture with authentic Etiocholandiol-3(α') 17(α)(H. Pt. = 232-34°C), there was no depression in the melting point (234-35°C). Similarly, after two recrystallizations of fraction 28, the crystals obtained melted at 235-36°C with preliminary sublimation, and gave no depression in melting point (235-37°C) in a mixed melt with authentic Etiocholandiol (239-41°C). The yield of crystals of diol obtained from these fractions was 31.5 mg. The total yield of diol from Columns A1 and A2 was 156.5 mg.

10 mg. of Etiocholandiol-3(α') 17(α) crystals were acetylated as outlined on Page 49, and the crystals obtained melted at 117-21°C after three recrystallizations from aqueous Ethanol, thereby confirming the observation that the crystals were the diol.

One crystallization of fraction 26 yielded a minute amount of crystals which melted at 225-30°C with preliminary sublimation. The nature of this material could not be ascertained because further attempts to recrystallize it from aqueous Ethanol and aqueous Methanol failed. Likewise attempts to recrystallize fraction 25 did not meet with success.

Fractions 15 and 16 were combined, but could not be recrystallized from aqueous Ethanol or aqueous Methanol. The fractions were, therefore, acetylated. The oil, weighing 17 mg., was then chromatographed and the results described in Column A6 - Table 7.

Attempts to crystallize fractions 1 and 2 of Column A2 likewise failed.

Fractions 18-20 were combined and after three recrystallizations
from aqueous Ethanol, crystals were obtained which melted at 142-45°C with preliminary softening. No depression in the melting point (142-47°C) was observed upon admixture with authentic Cholesterol (M. Pt. = 146-48°C). Fraction 17 likewise yielded Cholesterol. The total yield of Cholesterol from Column A2 was 5.4 mg.

(c) Acetylation Procedures.

1. Preparation of Etiocholanolone-3α,17α-Diacetate. Fractions 20 and 21 of Column A1 were combined and taken into solution in 5 cc. of Acetic Anhydride, to which was added 2 cc. anhydrous Pyridine. The solution was allowed to stand overnight at room temperature, and was then diluted to a 10% solution with H2O. The aqueous solution was then extracted four times with one-third its volume of Ether. The Ether extracts were washed five times with 2N HCl, and then washed with 1M NaOH, H2O, and dried over MgSO4; distilled and the residue dried under Nitrogen. After two recrystallizations from aqueous Ethanol, crystals were obtained which melted at 192-20°C. With authentic Etiocholanolone-3α,17α-diacetate (M. Pt. = 191-23°C), a mixed melt showed no depression in melting point (119-23°C). 68 mg. of the diacetate (equivalent to 52.6 mg. of free diol) were obtained.

Etiocholanolone-3α,17α-Diacetate was prepared in the same way with crystals obtained from the other liquids of fraction 27 of Column A2. In this case 1 cc. Acetic Anhydride, 0.5 cc. Pyridine and 10 mg. of crystals were used. The melting point of the acetylated product was 117-91°C, with prelim-
inary softening, thereby confirming the presence of dihydroxy-
diacetate.

2. Other Acylations. Attempts to crystallize
fractions 28 and 29 of Column 1 and fractions 15 and 16 of Column 2 from
aqueous Ethanol and aqueous Methanol did not meet with success. These
fractions were therefore acetylated as above. The fractions were then
chromatographed and are Column A5 and Column A6 respectively.

Fractions 28 and 29, weighing 53.8 mg., were
dissolved in 5 cc. Acetic Anhydride. Fractions 15 and 16, weighing
17 mg., were dissolved in 2 cc. Acetic Anhydride. The subsequent
procedure followed was that outlined above.

(d) Column A5. The results are summarized in Table 6. A
cylindrical column of alumina, 4 cm. in length and 1.77 cm. in
diameter, was used.

Fractions 28 and 29 of Column 1 were acetylated and
chromatographed.

Fractions 16 and 17 and fraction 19 of Column A5 yielded
crystals.

After two recrystallizations from aqueous Ethanol, the
crystals of fraction 17 melted at 237-40°C. These crystals gave a
positive Tetra-nitromethane test, thereby indicating the presence of
unsaturation in the molecule. The crystals of fraction 17 did not
give a precipitate with Digitonin, but it was noted that \( \Delta^5 \)
androstenediol-3(\( \beta \)) 17(\( \beta \))-diacetate and Dehydroisoandrosterone-acetate
gave no precipitate with Digitonin, hence it is possible that the
acetyl radical at the C9-hydroxy-position masks the presence of a
<table>
<thead>
<tr>
<th>Fraction No.</th>
<th>Eluant</th>
<th>Vol. in cc</th>
<th>Wt. in mg</th>
<th>Eluate</th>
<th>Nature</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 5</td>
<td>Benzene-Pentane Solution of the Total Residue</td>
<td>10 cc.</td>
<td>0.6 Oil</td>
<td>0 Oil</td>
<td>100% Pentane</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>1:3 Benzene:Pentane</td>
<td>0 Oil</td>
<td>0 Oil</td>
<td>0 Oil</td>
<td>1:3 Benzene:Pentane</td>
<td></td>
</tr>
<tr>
<td>7 - 11</td>
<td>1:1 Benzene:Pentane</td>
<td>0 Oil</td>
<td>0 Oil</td>
<td>0 Oil</td>
<td>3:1 Benzene:Pentane</td>
<td></td>
</tr>
<tr>
<td>12 - 15</td>
<td>3:1 Benzene:Pentane</td>
<td>2.8 Oil</td>
<td>2.8 Oil</td>
<td>0 Oil</td>
<td>100% Benzene</td>
<td></td>
</tr>
<tr>
<td>16 - 17</td>
<td>1:3 Ether:Benzene</td>
<td>15.8 Oil</td>
<td>15.8 Oil</td>
<td>15.8 Oil</td>
<td>Per</td>
<td>18.4 mg. crystals, M.T. = 224-400°C, are unsaturated and may have a C₃ &quot;Cis&quot; hydroxyl group</td>
</tr>
<tr>
<td>18</td>
<td>1:3 Ether:Benzene</td>
<td>1.8 Oil</td>
<td>1.8 Oil</td>
<td>1.8 Oil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>3:1 Ether:Benzene</td>
<td>11.8 Oil</td>
<td>11.8 Oil</td>
<td>11.8 Oil</td>
<td></td>
<td>8.0 mg. crystals, M.T. = 207-160°C; upon resolidification crystals melt at 204-00°C; are unsaturated.</td>
</tr>
<tr>
<td>20 - 32</td>
<td>3:1 Ether:Benzene</td>
<td>20.2 Oil</td>
<td>20.2 Oil</td>
<td>20.2 Oil</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 6**

*Column A5: The Acetylated Fractions 28 and 29 of Column A1 - 54.6 mg.*
"cis" hydroxyl group. Hence in view of the possible presence of a C3 "cis" hydroxyl group, of the unsaturation, and of the high melting point (237-40°C), it was within the realm of possibility that the crystalline was Δ5 Androstenediol-3(β) 16,17-3-monoacetate. (Melting point of the authentic 3-monoacetate is 240-41°C.) After a third recrystallization, the admixture of these crystals (M. Pt. = 238-37°C) with authentic Δ5 Androstenediol-3(β) 16,17-3-monoacetate (M. Pt. = 240-41°C) resulted in a mixed melting point of 210-29°C, with decomposition and continued melting terminating at 235°C. Some depression in the melting point had, therefore, occurred in the mixed melt. A fourth recrystallization of fraction 17 yielded crystals melting at 224-35°C with preliminary softening. It is apparent, therefore, that fraction 17 consists of a mixture of what may be the mono-, di- and triacetates of Δ5 Androstenediol-3(β) 16,17.

Fraction 16, after one crystallization from aqueous ethanol, yielded crystals melting at 220-36°C.

It was decided, therefore, to saponify fraction 16 and the mother liquors of fraction 17. The combined material weighed 11.2 mg. before saponification and 8.2 mg. after saponification. Saponification was carried out by dissolving the material in 3 cc. of ethanol, adding 5 cc. of 4% NaOH, and letting the solution stand overnight at room temperature. The solution was then diluted with H2O and extracted with Ether. The Ether solution was then washed with small amounts of H2O till the pH was equal to 7, dried over Na2SO4, and redistilled. Attempts to crystallize the residue from aqueous ethanol failed, and it was, therefore, not possible to determine if the saponified residue was free Δ5 Androstenediol-3(β) 16,17.
After four recrystallizations of fraction 19 from aqueous ethanol, crystals were obtained which melted at 207-16°C with preliminary softening and sublimation. The melted crystals, upon resolidification, melted at 204-06°C. The crystals gave a positive tetranitromethane test, indicating the presence of unsaturation in the molecule. Further identification of the crystals was not carried out. The relation of these crystals to the isomer of Hirschmann's \( \Delta^5 \) androstenediol-3(\( \beta \)) 16, 17 synthesized by Butenandt et al (24) and Stodola et al (151) could not be ascertained. This isomer melts at 205-06°C, while the triacetate melts at 221-230°C. Whether fraction 19 contains a mixture of these acetates is not known.

(a) Column A6. Fractions 15 and 16 of Column A2 were acetylated and chromatographed as outlined in Table 7. A cylindrical column of Alumina, 3 cm. in length and 1.77 cm. in diameter, was used.

Unfortunately, no crystals were obtained in any of the fractions obtained from the chromatogram. Hence, the nature of the compounds in the acetylated fractions 15 and 16 of Column A2 remain unknown.

Ketonic Steroid Residue:

(a) Column A3: Digitonin-non-precipitable. The results of the chromatogram are summarized in Table 8. A cylindrical column of Alumina, 12.7 cm. in length and 2.54 cm. in diameter, was used.

The digitonin-non-precipitable ketonic steroid fraction was eluted with CCl\(_4\), containing varying amounts of Absolute Ethanol. The CCl\(_4\) was redistilled and dried over CaCl\(_2\) before use.
**TABLE 7**

**Column A6: The Acetylated Fractions 15 and 16 of Column A2 - 17 mg.**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Benzene:Pentane Solution of the Total Residue</td>
<td>10 cc.</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>2 - 3</td>
<td>100% Pentane</td>
<td></td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>4 - 6</td>
<td>3:1 Benzene:Pentane</td>
<td>Per</td>
<td>3.4</td>
<td>Oil</td>
<td></td>
</tr>
<tr>
<td>7 - 8</td>
<td>100% Benzene</td>
<td></td>
<td>1.4</td>
<td>Oil</td>
<td></td>
</tr>
<tr>
<td>9 - 11</td>
<td>3:1 Ether:Benzene</td>
<td>Fraction</td>
<td>8.8</td>
<td>Oil</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>100% Ether</td>
<td></td>
<td>0.6</td>
<td>Oil</td>
<td></td>
</tr>
<tr>
<td>13 - 14</td>
<td>100% Abs. Ethanol</td>
<td></td>
<td>1.2</td>
<td>Oil</td>
<td></td>
</tr>
</tbody>
</table>
Fractions 1 and 2 were crystallized from aqueous Ethanol. After two recrystallizations, fraction 1 gave rise to crystals which melted at 103.02°C-05°C with preliminary softening. The crystals gave no depression in melting point (102-04°C) in a mixed melt with authentic \( \Delta^2 \) Androstenone-17 (M. Pt. = 103-04°C). Fraction 2 melted at 97-100°C with preliminary softening after one crystallization, and was added to fraction 1. The combined fractions were then recrystallized from aqueous Ethanol and 204.8 mg. of \( \Delta^2 \) Androstenone-17 was obtained.

Fractions 6-29 were recrystallized from aqueous Ethanol. After one crystallization, fraction 6 yielded crystals which melted at 182-85°C, with preliminary sublimation at 145°C. These crystals gave no depression in melting point (178-81°C) in a mixed melt with authentic Androsterone (M. Pt. = 178-81°C). After two recrystallizations, fraction 29 melted at 181-84°C, and gave no depression in the melting point (181-84°C) upon admixture with authentic Androsterone (M. Pt. = 185-86°C). Fraction 18, after one crystallization from aqueous Ethanol, yielded crystals which melted at 182-85°C with preliminary sublimation. There was no depression in the melting point (183-86°C) upon admixture with authentic Androsterone (M. Pt. = 185-86°C). Fractions 6-29 inclusive were, therefore, combined and upon recrystallization, 546.2 mg. of Androsterone were obtained. The Androsterone crystals obtained from the emulsion arising during the Ether extraction of the ketonic steroid fraction of the urine (see page 42) weighed 148.6 mg. The total yield of Androsterone was, therefore, 694.8 mg.

Fraction 30 was recrystallized once from aqueous Ethanol and crystals showing the characteristic double melt of Etiocholanolone...
### TABLE 3

**Column A3: The Digitonin Non-Precipitable Ketonic Steroid Fraction of the Urine - 1.9102 gm.**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 2</td>
<td>100% CCl₄</td>
<td>25 cc</td>
<td>216.2 Crystals</td>
<td>204.8 mg. Δ²-Androstenone-17</td>
<td></td>
</tr>
<tr>
<td>3 - 5</td>
<td>100% CCl₄</td>
<td></td>
<td>102.4 Oil</td>
<td>10 mg. 17-KS. Could not be crystallized</td>
<td></td>
</tr>
<tr>
<td>6 - 29</td>
<td>0.1% Abs. Ethanol &amp; CCl₄</td>
<td>Per</td>
<td>554.3 Crystals</td>
<td>546.2 mg. Androsterone</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.2% &quot; &quot; &quot;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.3% &quot; &quot; &quot;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.5% &quot; &quot; &quot;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1% &quot; &quot; &quot;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2% &quot; &quot; &quot;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 - 31</td>
<td>2% Abs. Ethanol &amp; CCl₄</td>
<td></td>
<td>845.8 Crystals</td>
<td>784.1 mg. Etocholanolone</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5% &quot; &quot; &quot;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>32 - 39</td>
<td>5% Abs. Ethanol &amp; CCl₄</td>
<td></td>
<td>74.5 Oil</td>
<td>25.8 mg. 17-KS. Could not be crystallized</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10% &quot; &quot; &quot;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>20% &quot; &quot; &quot;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>100% Abs. Ethanol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>100% Glacial Acetic Acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
were obtained. The melting points were 127-35°C and 145-46°C, with preliminary softening at 110°C. Upon admixture with authentic Etiocholanolone (M. Pt. = 134-36°C, 145-48°C), there was no depression in the melting points (129-35°C, 145-48°C), with preliminary softening at 115°C. After two recrystallizations from aqueous methanol and two from aqueous Ethanol, fraction 31 yielded impure Etiocholanolone which melted first at 124-32°C (with preliminary softening). The resolidified crystals then melted again at 125-44°C. Fractions 30 and 31 were, therefore, combined and 784.1 mg. of Etiocholanolone were obtained.

17-ketosteroid determinations were carried out on the combined fractions 3-5 and fractions 32-39, using the m-Dinitrobenzene reagent. The former set of fractions contained 10 mg. of 17-ketosteroids, while the latter set contained 26.8 mg. of 17-ketosteroids. Neither set of fractions could be crystallized from aqueous Methanol or aqueous Ethanol.

(b) Column A4: Digitonin-precipitable. The results are summarized in Table 9. The cylindrical column of Alumina in this case was 5 cm. in length and 1.77 cm. in diameter.

After two recrystallizations from aqueous Ethanol, crystals from fraction 5 melted at 101±0.04°C with preliminary softening. The crystals gave no depression (M. Pt. = 101-05°C) upon admixture with authentic Δ² Androstenone-17 (M. Pt. = 104-05°C). Fractions 1-5 were combined and after two recrystallizations from aqueous Ethanol, crystals were obtained which melted at 95-105°C. Fractions 1-5 were, therefore, combined and the total yield of Δ² Androstenone-17 was 17.6 mg.

Fractions 14-24 were combined and after four recrystallizat-
TABLE 9

Column A4: The Digitonin Precipitable Ketonic Steroid
Fraction of the Urine - 109 mg.

<table>
<thead>
<tr>
<th>Fraction No.</th>
<th>Eluant</th>
<th>Vol. in cc.</th>
<th>St. in mg.</th>
<th>Nature</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 5</td>
<td>100% Pentane</td>
<td></td>
<td>18.2</td>
<td>Crystals</td>
<td>17.5 mg. Δ²-Androstenone-17</td>
</tr>
<tr>
<td></td>
<td>1:4 Benzene:Pentane</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 - 13</td>
<td>1:4 Benzene:Pentane</td>
<td>10 cc.</td>
<td>13.6</td>
<td>Oil</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2:3 &quot; &quot;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3:2 &quot; &quot;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14 - 24</td>
<td>4:1 Benzene:Pentane</td>
<td></td>
<td>46.2</td>
<td>Crystals</td>
<td>36.6 mg. Isoandrosterone</td>
</tr>
<tr>
<td></td>
<td>100% Benzene</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1:3 Ether:Benzene</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>1:3 Ether:Benzene</td>
<td></td>
<td>12.0</td>
<td>Oil and Crystals</td>
<td>Isoandrosterone and Dehydroisoandrosterone (?)</td>
</tr>
<tr>
<td>26 - 35</td>
<td>1:3 Ether:Benzene</td>
<td></td>
<td>8.2</td>
<td>Oil</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1:1 &quot; &quot;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3:1 &quot; &quot;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>100% Ether</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1:1 Abs. Ethanol:Ether</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>36</td>
<td>100% Abs. Ethanol</td>
<td></td>
<td>0.6</td>
<td>Oil and Crystals</td>
<td>M.Pt. = 251-260°C, with decomposition and preliminary sublimation.</td>
</tr>
<tr>
<td>37 - 39</td>
<td>100% Abs. Ethanol</td>
<td></td>
<td>3.8</td>
<td>Oil</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100% Glacial Acetic Acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
ions from aqueous Ethanol, crystals were obtained, which melted at 167-72°C with preliminary softening. The crystals gave no depression in melting point (173-75°C) upon admixture with authentic Isoandrosterone (M. Pt. = 174-77°C). The total yield of Isoandrosterone was 36.6 mg.

Fraction 25 was recrystallized three times from aqueous Ethanol. Two sets of crystals were obtained repeatedly, as indicated by the melting points observed. The first melting point was 152-56°C and the second was 163-74°C. These melting points were distinct. This mixture of crystals could not be separated and, consequently, their identity could not be definitely established. It is probable that the crystals melting at 163-74°C were Isoandrosterone, since the preceding fractions in the column were Isoandrosterone. Selvy's Encyclopedia lists melting points for Dehydroisoandrosterone ranging from 144-53°C. It is, therefore, within the realm of possibility that the crystals melting at 152-56°C were Dehydroisoandrosterone, or a mixture of Dehydroisoandrosterone and Isoandrosterone. Since it was not possible to isolate a satisfactory separation of the crystals, one can only state that the presence of Dehydroisoandrosterone in the 3,4/3)-hydroxy-ketonic column is indicated.

Pearlman (123, 124) has also reported the isolation of crystals melting at 156.1-57°C, which he suspects to be a mixture of Dehydroisoandrosterone and Isoandrosterone.

Fraction 36 yielded a minute amount of crystals melting at 251-60°C with decomposition and preliminary sublimation. The nature of these crystals is unknown.
III Discussion

A. Summary of Experimental Results.

The results of the experiment are summarized in Tables 16 and 11.

17-Ketosteroid Metabolites of Testosterone:

It is evident from these results that Androsterone and Etioccholanolone are urinary metabolites of exogenous Testosterone in man. As mentioned in the introduction, $\Delta^2$ Androstenone-17 is assumed to be an artifact of Androsterone arising during acid hydrolysis of the urine. The isolation of these three steroids from the urine, therefore, confirms the work of earlier workers (29, 43, 45, 49, 136). The individual yields of each of these three steroids obtained in this experiment was, however, much higher than those previously reported (Table 3), and the total yield of 17-ketosteroids was, therefore, also much higher.

For example, Schiller et al (136) reported that the total conversion of 1.2 gm. of Testosterone-propionate (administered intramuscularly to a normal woman) to 17-ketosteroids was 25%, and this was the highest rate of conversion reported up to that time. In the present experiment, the total conversion to urinary 17-ketosteroids was 34.75%. The latter yield may be due to the fact that the Testosterone was administered orally in the present experiment.

It will be noticed from Table 3 that Isoandrosterone was never isolated from urine at the same time as Etioccholanolone. Androsterone, and $\Delta^2$ Androstenone-17 after administration of Testosterone (regardless of the mode of administration). In this experiment, however, 36.6 mg. of Isoandrosterone was isolated. This is an excretion
TABLE 10

Summary of the yields of substances isolated from the urine in crystalline form after the oral administration of 5 gm. of Testosterone to a normal adult man.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Total mg. of Substance</th>
<th>mg./l.</th>
<th>% Conversion of 5 gm. Testosterone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Etiocholandin-3(β) 17(α)</td>
<td>156.5</td>
<td>12.95</td>
<td>3.13</td>
</tr>
<tr>
<td>Etiocholanolone</td>
<td>784.1</td>
<td>64.90</td>
<td>15.88</td>
</tr>
<tr>
<td>Δ^2 Androstenone-17</td>
<td>222.4</td>
<td>18.41</td>
<td>4.44</td>
</tr>
<tr>
<td>Androsterone</td>
<td>694.8</td>
<td>57.51</td>
<td>13.90</td>
</tr>
<tr>
<td>Δ^2 Androstenone-17 &amp; Androsterone</td>
<td>917.2</td>
<td>75.92</td>
<td>18.34</td>
</tr>
<tr>
<td>Etiocholandin &amp; Etiocholanolone</td>
<td>940.6</td>
<td>77.85</td>
<td>18.01</td>
</tr>
<tr>
<td>Isoandrosterone</td>
<td>36.6</td>
<td>3.03</td>
<td>30.73</td>
</tr>
<tr>
<td>Dehydroisoandrosterone</td>
<td>Trace</td>
<td>Trace</td>
<td>----</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>5.4</td>
<td>0.45</td>
<td>----</td>
</tr>
<tr>
<td>3(β)-OH-Ketonic Steroid (⁺ Pt. = 251-60°C)</td>
<td>&lt;0.6</td>
<td>----</td>
<td>----</td>
</tr>
</tbody>
</table>

TABLE 11

<table>
<thead>
<tr>
<th>Total Yield</th>
<th>Etiocholandin-3(β) 17(α)</th>
<th>Grand Total of all Metabolites of Testosterone</th>
</tr>
</thead>
<tbody>
<tr>
<td>17-E5.</td>
<td>1737.9 mg.</td>
<td>156.5 mg.</td>
</tr>
<tr>
<td>Total Yield</td>
<td>1737.9 mg.</td>
<td>156.5 mg.</td>
</tr>
<tr>
<td>Total % Conversion of Testosterone</td>
<td>34.75%</td>
<td>3.13%</td>
</tr>
<tr>
<td></td>
<td>37.88%</td>
<td></td>
</tr>
</tbody>
</table>
of 3.03 mg. per litre. The excretion of Isoandrosterone in human urine is reported as 0.2 mg. per litre (127), hence it is apparent that in the present experiment, Isoandrosterone -- as well as the other 17-ketosteroids -- are metabolites of Testosterone.

Pincus and Pearlman (127) have reported that Androsterone and Etiocholanolone are excreted in approximately equal amounts (1 mg./l.) in normal human urine. The results of the present experiment (Table 10) indicate that exogenous Testosterone is likewise converted in almost equal amounts to Androsterone and Etiocholanolone. Continuing the $\Delta^2$-Androstenone-17 and Androsterone excretion values, it is seen that the Androsterone to Etiocholanolone ratio is approximately 1 to 1 (53% to 47%), the Androsterone fraction being somewhat larger.

Since it is probable that the Etiocholandiol obtained is closely related to Etiocholanolone, both chemically and metabolically (see page 73), let us add the yield of diol to that of Etiocholanolone. Now it is observed (Table 10) that the Androsterone to Etiocholanolone ratio is indeed 1 to 1.

From the data in Table 3, it is seen, however, that more Androsterone than Etiocholanolone was obtained by Schiller et al (136), Callow (28), and Fish and Dorfman (61). This difference in the amounts excreted may perhaps be explained in part by the inability of these investigators to isolate Etiocholandiol. The Androsterone to Etiocholanolone ratio in Schiller et al's (136) experiment was approximately 2 to 1, and the same is true for Fish and Dorfman's experiment. Only in Callow's experiment was there a ratio approximately 1 to 1. At any rate, the present experiment indicates that Androsterone and Etiocholanolone are formed in almost equal amounts during the metabolism of Testosterone in man. This, incidentally, is indirect evidence to
support the theory that Testosterone is the male sex hormone.

**Carbinol Metabolites of Testosterone:**

156.5 mg. of Etiocholandiol-3(α) 17(α) was isolated from the urine -- a conversion of 3.13% of the administered Testosterone. This diol has never before been reported as a metabolite of Testosterone, and its presence in human urine has been indicated with certainty only by Butenandt et al (22). In the latter case, however, it was probably an artifact arising during the fractionation of the urine using amalgamated Aluminum.

In the present experiment, none of the procedures used in the isolation of the diol necessitated the use of reducing agents; also, the rapidity with which the urine was worked up after its collection makes unlikely the possibility of bacterial hydrogenation of any of the urinary steroids; hence the Etiocholandiol-3(α) 17(α) did not arise from reduction of urinary Etiocholanolone or any other Etiocholane derivative. One may assume, therefore, that the Etiocholandiol isolated was the result of in vivo metabolism. The presence of the diol has been indicated only qualitatively in normal human urine (127), but that yield -- 12.95 mg./l. -- indicates that the orally administered Testosterone was converted to the diol. The excretion of Androstane and Etiocholane and other 17-ketosteroids in normal human urine (127) further emphasizes this fact -- for the excretion has never exceeded 2 mg./l. (ranging from 0.07-1.7 mg./l.).

To summarize (Table 11) the total yield of all steroids which may be considered urinary metabolites of Testosterone in R.R. has amounted to 37.88%.
(a) **Comparison of Results in the Present Experiment with Those of a Similar Experiment in the Rabbit.** It is of interest at this point to compare the results of the present experiment with the unpublished results of the experiment conducted by Hoffman, Desbarats and Knowles at the McGill University Clinic. Hoffman et al administered orally 5.640 gm. of Testosterone-propionate (equivalent to 4.709 gm. of Testosterone) to two male rabbits and collected a total of 7.165 litres of urine. The yields of the urinary metabolites excreted was different from that observed in the human urine, since the bulk of the neutral steroids excreted was non-ketonic.

For example, in man, the ratio of ketonic to non-ketonic steroids was approximately 5 to 1, whereas in the rabbit this ratio was 1 to 3. This difference is more strikingly illustrated by the observation that the amount of Etiocholandiol-3(α) 17(α) actually isolated by Hoffman et al exceeded by approximately 40% the total amount of the non-ketonic steroid fraction of the human urine. Furthermore, Hoffman et al have isolated a new diol -- Androstanediol-3(β) 17(α) -- from the rabbit urine, but the presence of this compound could not be detected in the human urine.

Only Etiocholanolone and Isoandrosterone have been isolated from the ketonic fraction of the rabbit urine. The former compound was excreted in approximately 30% of the amount observed in man, while the latter compound was excreted in slightly twice the amount. The presence of Androsterone is indicated, but has not been conclusively illustrated. It is not possible to make a quantitative comparison of the excretion of the aforementioned steroids in human and rabbit urine, but qualitatively, it is apparent that there is a species difference in the metabolism of
Testosterone. This is manifested by excretion of the metabolites in significantly different amounts, indicating that excretion of these metabolites occurs in different phases of the metabolic pathways. The sequence of events in these pathways in the rabbit is not known, and in man can only be inferred.

**Urinary Steroids Not Considered to be Metabolites of Testosterone:**

Also isolated from the urine in the present experiment was 5.4 mg. of Cholesterol -- equivalent to 0.45 mg./l. The excretion of Cholesterol in normal human urine has been reported as ranging from 0.01-0.51 mg./l. by various investigators (23, 54, 78, 136). It is probable, therefore, that the Cholesterol isolated in the present experiment was the result of endogenous metabolism and that it is not a metabolite of Testosterone. It is of interest to note that Neustadt et al (120) have determined the Cholesterol content of normal human urine using a photoelectric-colorimetric method and they have reported normal values ranging from 2.5-4.0 mg. Cholesterol per day. The latter values are much larger than those obtained by isolation of crystalline Cholesterol. The urine hydrolysis techniques employed by Neustadt et al was less drastic than the one employed in the present experiment. For example, Neustadt et al added 60-65 cc. concentrated HCl to 500 cc. urine, and the solution was then layered with 200 cc. CHCl₃. The solution was then refluxed gently for thirty minutes. Furthermore, the photoelectric method of measurement is a more sensitive one than the gravimetric method employed in this experiment. This, therefore, may account for the higher yield of Cholesterol obtained by Neustadt et al. There is, at any rate, no evidence in the present experiment to clarify the metabolic relation between Cholesterol and
Testosterone.

The nature of the substance obtained from the 3α-hydroxy-ketonic fraction of the urine (Column A4) which melted at 251-260°C could not be determined because of the minute amount of material obtained.

The possible presence of Dehydroisoandrosterone was indicated in fraction 25 (M. Pt. = 152-56°C) of Column A4 (Table 9). Since it was not possible to isolate this steroid in a free form, conclusive evidence for its presence cannot be claimed. However, Pearlman (123, 124) has also reported the isolation of crystals melting at 156-57°C (the crystals of Column A4 melted at 152-56°C), and he has suggested that it was a mixture of Dehydroisoandrosterone and Isoandrosterone. Pearlman (124) has actually recrystallized a mixture of equal amounts of these two steroids, and the melting point observed was 157.5-58°C. Furthermore, this mixture gave no depression in a mixed melt with the urinary product. This, therefore, is circumstantial evidence for the presence of Dehydroisoandrosterone in the present experiment.

Since the weight of fraction 25 did not exceed 12 mg., the amount of Dehydroisoandrosterone present was small. In terms of mg. per litre, the amount present was much less than 1 mg./l. The excretion of Dehydroisoandrosterone in normal urine has been reported (127) as ranging from 0.1-2.0 mg./l., hence the Dehydroisoandrosterone in the urine of the present experiment was most probably a result of endogenous secretion and is not a metabolite of Testosterone. The isolation of Dehydroisoandrosterone in amounts greater than normal from urine has never been reported following Testosterone administration.
and the evidence quoted above confirms the theory that Dehydroiso-androsterone is not a normal urinary metabolite of Testosterone (and the male sex hormone), and hence is probably a product of adrenal cortical secretion.

Marrian (106) isolated $\Delta^5$-androstenetriol-3(16,17) from the urine of normal men and women, in yields of 0.1 mg. per litre. This triol was identical with Hirschmann's triol (80). In the present experiment crystals were isolated which may be $\Delta^5$-androstenetriol-3(16,17), but conclusive evidence for their presence was not obtained. The evidence consists of a high melting point (224-350C), the presence of unsaturation, and the possible presence of a C3 "Cis" hydroxyl group. There was some indication that an isomer of this triol was also present, but again evidence in support of this suggestion is slight, being simply the melting point (207-160C) and the presence of unsaturation. This isomer may be identical with the isomer of Hirschmann's triol that was synthesized by Butenandt et al (24) and Stodola et al (151).

The crystals of fraction 16 and 17 were excreted in amounts equivalent to 0.67 mg. per litre, while the crystals of fraction 19 were excreted in amounts of 0.2 mg. per litre. It is apparent, therefore, that these sets of crystals, regardless of their identity, are probably the product of endogenous steroid metabolism rather than metabolites of the administered Testosterone.

B. Metabolic Significance of the Urinary Steroids.

In the Intermediary Metabolism of Testosterone:

One of the primary goals of a study of the metabolism of Testosterone is the discovery of the nature and sequence of the
intermediates arising during the course of this metabolism. As yet, there is no clear-cut knowledge of the metabolic pathways along which Testosterone passes in man or any experimental animal. The present experiment has confirmed the fact that Isoandrosterone, Etiocholanolone, and Androsterone are normal metabolites of Testosterone, and has shown that the latter two 17-ketosteroids are excreted in approximately equal amounts in the urine in man. It has indicated further that Etiocholandiol-3(α') 17(α') is also derived from Testosterone. The possible intermediates arising during this conversion and their sequence is indicated in Figure 6. It must be pointed out that this scheme of events is necessarily incomplete for the following reasons:

1. More than half of the administered Testosterone in this and other experiments could not be accounted for in the urine, indicating that metabolism of the hormone to products as yet undetermined may have occurred with this fraction of the total.

2. It is not yet possible to make accurate, quantitative in vivo studies of the reactions listed in Figure 6, or of similar reactions.

(a) Role of the Urinary 17-ketosteroids. With regard to Figure 6, the work of Dorfman and Hamilton (47) has indicated that $\Delta^4$ Etiocholenedione-3,17 (i.e. $\Delta^4$ Androstenedione-3,17) and Androstenedione-3,17 do indeed give rise to urinary Androsterone after oral administration to a eunuchoid. The yields of Androsterone was 2.5% in the case of the former compound, and was unspecified for the latter compound. Furthermore, the work with yeast and bacteria previously quoted (see page 22) has shown that every one of the
A POSSIBLE PATHWAY OF TESTOSTERONE METABOLISM

Testosterone

Δ^4-Androstenedione - 3,17

Androstenedione - 3,17

Etiocholanedione - 3,17

Androstane

Isoandrostene

Etiocholanol - 3α,17α

Etiocholanol - 3β,17β

Androstane

Androstenediol - 3α,17α
and isomer

Androstenediol - 3β,17β
and isomer

Etiocholandiol - 3α,17α
and isomer

Etiocholandiol - 3β,17β
and isomer

The Δ^4-Androstenedione - 3,17 leads to the excretion of a greater amount of androgenically active substances in the urine than...
reactions listed in Figure 6 (with the exception of the conversion of Androsterone and Etiocholanolone to saturated diols) can be carried out in yields varying from 11-47%, and in the direction indicated. One objection to the work done with yeast and bacteria is, namely, that it has not been carried out in the animal organism.

The results obtained in these studies with micro-organisms are highly suggestive, however, and are, therefore, of value in determining the pathway of metabolism of Testosterone. The scheme outlined in Figure 6 is one that draws strong support from these studies with micro-organisms.

Until the results of further enzyme studies have been reported and can be correlated with the above information, one can only say that Figure 6 represents a highly possible pathway of metabolism of Testosterone.

Gallagher (66) states that $\Delta^1$-Androstenedione-3,17 may be derived from Testosterone, but he feels that reduction of the dione will result in the formation of greater amounts of Dehydroisoandrosterone than of Androsterone. Barker et al (103) have shown that reduction of an $(\alpha,\beta)$-unsaturated ketone in Ring A of a sterol will result in the formation of a $\Delta^5$-3($\beta$)-hydroxy compound, but this observation does not by any means suggest that reduction of a Ring A $(\alpha,\beta)$-unsaturated ketonic-sterol must follow this route. The fact that Dorfman and Hamilton (47) were able to isolate Androsterone after administration of $\Delta^4$-Androstenedione-3,17 but did not report the isolation of Dehydroisoandrosterone further supports this suggestion.

The $\Delta^4$ Androstenedione-3,17 led to the excretion of a greater amount of androgenically active substances in the urine than
did Testosterone or Androstanediol-3(α) 17(α) or Androstanedione-3,17. (Only the administration of Androsterone itself resulted in a higher excretion of androgenic substances.) Gallagher (26) suggests that this high androgenic activity is due to conversion of the ionone to Dehydroisoandrosterone. However, it is not clear what fraction of the androgenic substances are accounted for by the amount of Androsterone isolated (2.4%); and the experiment as a whole cannot be interpreted in a quantitative manner. Hence, the position of $\Delta^4$ Androstenedione-3,17 in the scheme outlined in Figure 6 is not contra-indicated.

With the present state of knowledge regarding the metabolism of Testosterone, any attempt to state dogmatically that a given scheme represents the true picture of the intermediary metabolism of this hormone will meet with failure, since there is no unequivocal evidence in favour of any such pathway. Koch (91) has presented several alternative schemes -- for all of which there is some evidence, but for none of which the evidence is conclusive. Figure 7 is such a pathway. One objection to this scheme is that Triphcholanol-17-one-3 has never been administered as a possible precursor of Triphcholanolone (or of urinary androgens). At any rate, Figure 7 differs from Figure 6 only in the nature of the first intermediates arising from Testosterone.

(b) Role of Dehydroisoandrosterone. Koch (91) has also suggested that $\Delta^4$ Androstenedione-3,17 is converted to Dehydroisoandrosterone, which is then converted to Androsterone, etc. Theoretically, this is indeed possible and, as Kason and Kepler (108) have reported, Dehydroisoandrosterone has been converted to Androsterone and Triphchol-
Figure 7

A possible pathway of testosterone metabolism

Testosterone

Androstanediol-3(17)

Etiocolanediol-17-one-3.

Androstanediol-3,17

Etiocolanediol-3,17

Androsterone

Isandrosterone

Etiocolanol-3(17)

Androstenediol-3(17)

Etiocolanol-3(17)

Etiocolanol-3(17)

Etiocolanol-3(17)
anolone by an anterior pituitary deficient man. However, as was noted in the Introduction (see page 9), Dehydroisoandrosterone is probably a product of adrenocortical secretions, so that its role as an intermediary in the metabolism of Testosterone is not entirely likely. The conversion of Testosterone to \( \Delta^5 \)androsterediols and the subsequent conversion of these diols to Dehydroisoandrosterone and to 17-ketosteroids (91) is, for the above reason, not a likely pathway of Testosterone metabolism.

(c) Role of Etiocholanolone-3(\( \alpha \)) 17(\( \alpha \)). It will be noticed in Figure 6 that Androsterone, Etiocholanolone, and Isoandrosterone are regarded as the precursors of the saturated diols, Etiocholandiol-3(\( \alpha \)) 17(\( \alpha \)), Androstanediol-3(\( \beta \)) 17(\( \alpha \)) etc. This is the logical place for the diols in the scheme as it is outlined. It is, of course, within the realm of possibility that the diols may be the precursors of the urinary 17-ketosteroids, but neither the results of the present experiment nor the unpublished results of the rabbit experiment of Hoffman et al (see page 64) -- nor the experiment of Ranish and Hamilton (47) -- provide a clue to the answer to this problem.

Etiocholanolone and Etiocholandiol-3(\( \alpha \)) 17(\( \alpha \)) are closely related chemically, and theoretically one might expect to isolate urinary Etiocholandiol-3(\( \alpha \)) 17(\( \alpha \)) following the administration of Etiocholanolone to an experimental animal. The reverse reaction may also be expected following Etiocholandiol administration. Such experiments have, however, not been carried out as yet, and the metabolic relation of Etiocholandiol-3(\( \alpha \)) 17(\( \alpha \)) to Etiocholanolone has still to be established. The cis configuration of the \( C_5 \) hydrogen
atom in the steroid nucleus of Etiocholaniol precludes the possibility that Androsterone is a closely related precursor of the diol metabolically.

As has been stated previously (see page 64), androstanediol-3(β) 17(α) has been isolated by Hoffman et al from rabbit urine following Testosterone administration, but the relation of this diol to Isoandrosterone and the other urinary 17-ketosteroids (and the other steroids listed in Figures 6 and 7) is also not known.

A study of the enzymatic processes and the thermodynamics involved may help to elucidate the metabolic relation of the aforementioned saturated diols to their ketonic homologues.

(d) Conclusion. On the whole, the in vivo information available at present -- supplemented by the studies with yeast and bacteria -- tend to favour the pathway outlined in Figure 6 as the likely route of Testosterone metabolism, but the evidence in favour of this intermediary pathway is not conclusive.

Ratio of the 17-Ketosteroids in the Urine:

Etiocholanol-3(β)-one-17, the isomer of Etiocholanolone, has never been isolated from urine, despite repeated attempts by many investigators to isolate it. It will be noticed that Androsterone and Etiocholanolone -- the digitonin-non-precipitable 17-ketosteroids -- have been isolated in approximately equal amounts from urine after Testosterone administration, but that the digitonin-precipitable 17-ketosteroids have been isolated in the case of one compound (Isoandrosterone) in much smaller amounts, and in the case of the second compound (Etiocholanol-3(β)-one-17) has not yet been
isolated. A similar state of affairs has been noted for normal human urine.

This preference for the biological reduction of Testosterone (and the male sex hormone) to digitonin-non-precipitable (that is, to steroids with a trans hydroxyl at the C3 position in Ring A) is as yet inexplicable. It is probably not associated with biological activity, since Androsterone is androgenically active, whereas Etiocholanolone is not. A possible explanation is that it is thermodynamically simpler to carry out the reduction in this fashion, but this theory has yet to be proven. The nature of the enzymes associated with the biological reduction of Testosterone may also modify the direction of the reactions.

It is of interest to note that the energy relationships of the reactions outlined in either Figure 6 or Figure 7 have yet to be evaluated. A study of the energy levels involved in these reactions may throw some light upon the sequence of events in the metabolism of Testosterone.

**Reversibility of Intermediary Metabolic Reactions:**

The present methods of studying the metabolism of the androgenic steroids in animals has brought to light one important fact, namely, that the reactions encountered are reversible and these compounds, therefore, are closely related. Typical experiments which have made this clear are those of Mason and Kepler (109) and Hoffman et al (83) who have isolated Etiocholanolone and Androsterone (digitonin-non-precipitable compounds) after the administration of Dehydroisoandrosterone (a digitonin-precipitable compound) to a man.
and the rabbit, respectively. Dorfman et al. (50) have demonstrated the converse reaction in guinea pigs, who converted Androsterone \( C_3 (\alpha)-\text{hydroxy} \) to Isoandrosterone \( C_3 (\beta)-\text{hydroxy} \). The studies with micro-organisms (see page 22) have confirmed these animal experiments. It is obvious on the basis of these observations that no one metabolic pathway will ever account for all the experimental results reported. There are probably a series of closely related paths (the metabolism of Testosterone representing but one spoke of the metabolic wheel) with one compound as the hub of the wheel. The nature of this compound is as yet unknown.

A possible compound of such importance is \( \Delta^4 \)-Androstenedione-3,17. This compound can be regarded theoretically as an intermediate in the metabolism of both Testosterone and Dehydroisoandrosterone, and as has already been noted (47, 97) it can be converted to compounds (e.g. Androsterone) which are acknowledged metabolites of both parent molecules. However, much more evidence than is available at present must be provided before \( \Delta^4 \)-Androstenedione-3,17 can be regarded conclusively as a crossroad in the metabolism of the steroid hormones. This dione may be the biological precursor of Testosterone (95), but as yet this is still a matter of conjecture. Isotopic studies may be useful in clarifying these issues.

C. Pathways of Metabolism of Testosterone to Compounds of Smaller Molecular Size.

In discussing the role of the liver in the metabolism of Testosterone, it was pointed out that there were two general pathways along which Testosterone and the other steroid hormones travel. Th-
first pathway deals with the excretion in the urine of conjugated metabolites which are either less active or inactive biologically. The chemical transformations which result in the excretion of such metabolites of Testosterone have been discussed above. The second pathway deals with the transformation of the hormone to compounds which are smaller in size, e.g., degradation to acetic acid or to CO₂ and H₂O, and to other as yet unrecognized products. It is not yet possible to evaluate accurately the relative importance of these two types of metabolic paths in normal physiological processes. Isolation experiments such as the present one tend to indicate that the latter pathway is used more extensively, since the isolation of compounds from the urine has not exceeded 50% of the amount of steroid administered -- regardless of the mode of administration. At any rate, the application of isotopes to the study of the steroid hormones will throw considerably more light upon this phase of the subject.

Degradation of the Steroid Nucleus:

(a) Theory of Oxidative Scission. The nature of the chemical changes which must take place in the structure of Testosterone during the formation of the as yet unknown degradation products is not clear. Several theories have been proposed as to the initial changes (127) and these deal with oxidative scission of Ring D.

The isolation of Δ⁵ Androstenetriol-3β(16,17) by Hirschmann (80) suggests the following series of reactions in the intermediary metabolism of the neutral 17-ketosteroids:

\[
\begin{align*}
\text{I} & \xrightarrow{+ H₂O} \text{II} & \text{II} & \xrightarrow{C_\text{OOH}} \text{III} \\
\end{align*}
\]
The observations that Estrone is converted in vivo to Estriol (127) confirms steps I to III. Step III and the subsequent reactions represent the unknown. Marrian (107) has suggested that \( \Delta^5 \) Androstenetriol-3(\( \beta \)) 16, 17 has the same metabolic relation to Dehydroisoandrosterone as Estriol has to Estrone, but as yet there is no support for this hypothesis.

(b) Metabolic Role of the Saturated Etiocholane and Androstanene Cortinols. The conversion of Estrone to \( \alpha \)-Estradiol has been demonstrated by several investigators (127). It is possible that Etiocholandiol-3(\( \alpha \)) 17(\( \alpha \)) is a metabolite of Etiocholanolone, and that both \( \alpha \)-Estradiol and Etiocholandiol-3(\( \alpha \)) 17(\( \alpha \)) and other saturated diols are intermediates in the formation of compounds (e.g., Estriol) having two hydroxyl groups in Ring D of the steroid nucleus. These trihydroxy compounds would then be metabolized as indicated above. This theory extends that of Marrian's regarding \( \Delta^5 \) Androstenetriol-3(\( \beta \)) 16,17 and Dehydroisoandrosterone. Thus, for example, Androstanediol-3(\( \beta \)) 17(\( \alpha \)) would be an intermediate in the conversion of Dehydroisoandrosterone to \( \Delta^5 \) Androstenetriol-3(\( \beta \)) 16, 17.

The absence of Androstanediols from the urine of the present experiment has not yet been explained. Nor is there any knowledge of the fate of approximately 62% of the administered Testosterone which could not be recovered as urinary metabolites. The above theory may provide a possible explanation for these observations (and for the low yield of Etiocholandiol-3(\( \alpha \)) 17(\( \alpha \)) -- 3.13%), since one might assume that the diols were converted to smaller molecules in the
manner outlined above. This theory is an attractive one, but evidence in its support has yet to be reported.

(c) Further Theories of Oxidative Scission. Another theory of oxidative scission (127) is based on the similarity between reactions carried out by the body and those effected through oxidation with HgO₂. Westerfeld (164) has shown that Estrone and cyclopentanone may be converted into lactones by HgO₂ in an alkaline medium (see below) and the Smiths (149, 150) have reported that the Estrone Lactone is of great biological importance, since it can duplicate the changes in the anterior pituitary which are observed after administration of Estrozens:

\[
\begin{align*}
\text{H}_2\text{O}_2 &\rightarrow \begin{array}{c} \text{CH}_2\text{OH} \\
\text{D} &\rightarrow \text{D} \end{array} \\
\text{OH}^- &\rightarrow \begin{array}{c} \text{CH}_2\text{O} \\
\text{D} &\rightarrow \text{D} \end{array} \\
\text{Acid} &\rightarrow \begin{array}{c} \text{CH}_2\text{O} \\
\text{D} &\rightarrow \text{D} \end{array} \\
\text{CO} &\rightarrow \begin{array}{c} \text{CH}_2\text{O} \\
\text{D} &\rightarrow \text{D} \end{array}
\end{align*}
\]

The subsequent reactions undergone by the lactone are not known. Heard and Hoffman (127) have isolated a substance, C₁₈H₂₂O₃, from the neutral steroid fraction of mare's pregnancy urine which they have identified as a keto-lactone, but this substance is not identical with Westerfeld's Lactone. The conversion of neutral 17-ketosteroids to such lactones is, therefore, a distinct possibility, but reports to this effect have not yet been published.

In discussing the degradation of the steroid molecule, it must be remembered that the point of attack may be not only Ring D, but also Rings A and B, and possibly Ring C. For example, a possible starting point in the degradation of Ring A may be the formation of a diketone (see below) with a subsequent splitting of the ring:
Such a reaction can be carried out in vitro, using Chromic acid.
The side-chains thus formed can be split off in the same way that
the side-chains of the Bile Acids are broken down. Thus, stripping
of the steroid molecule can be accomplished with the formation of
the hypothetical "smaller molecules".

Ring B may be split and a molecule having a configuration
similar to the nucleus of Vitamin D2 may be formed. Again, opportu-
nities for degradation of the molecule are manifold.

\[
\text{17-Ketosteroids} \rightarrow \begin{array}{c}
\text{CH}_3 \\
\text{OH}
\end{array} \quad (\text{Vitamin D}_2)
\]

The formation of Cholesterol from Deuteronic Acid and
Acetic Acid containing isotopic Carbon is well known; hence the
converse reaction is within the realm of possibility. Accurate
information about the above reactions and about the possible forma-
tion of Acetic Acid, CO\textsubscript{2} and H\textsubscript{2}O from Testosterone will probably
be obtained with the use of isotopes.

D. Biosynthesis of Testosterone.

The problem of the metabolism of Testosterone is related
to the biosynthesis of Testosterone, since Testosterone is believed
to be the male sex hormone. Hence, comment upon the biosynthesis of
Testosterone is in order. Briefly, there is little definite informa-
available at present about the latter problem. Bloch et al (11, 12) have shown that Deuterocholesterol is converted to Deuterocholic Acid in the bile of dogs. Bloch has also (13) presented strong evidence to indicate the conversion of Deuterocholesterol to Deuteropregnadiol in a pregnant woman, (thereby suggesting that the Deuterocholesterol was converted to Deuteroprogesterone), but there is no similar evidence to indicate that Cholesterol can be converted to Testosterone in vivo. Severs et al (139-34) have shown that the administration of Adrenotrophic hormone to rats will result in a decrease of the Cholesterol and Ascorbic Acid content of the adrenal gland. One possible interpretation of this is that the Cholesterol and Ascorbic Acid have been converted to adrenal steroid hormones. Indeed, Zweren and Lowenstein (167) have reported the isolation of a compound which appears to be a biologically active complex of Ascorbic Acid and an adrenal steroid. In line with this information and the isotopic evidence presented by Bloch et al, it is possible that Cholesterol is a precursor of Testosterone in vivo. Attempts to solve this problem histochemically are still in the preliminary stages (36). It is of interest to note that commercially (129), Testosterone is actually prepared by oxidative degradation of Cholesterol, with Dehydroisandrosterone, for example, as the intermediate.

E. Summary of the Discussion.

It is apparent from the data presented in the Introduction and in the Discussion of the results of the present experiment that studies on the metabolism of Testosterone are far from complete, and that extensive work has yet to be done on this subject. Future studies should, perhaps, consider the following phases:
1. Improved urine hydrolysis techniques.
2. Study of the enzyme systems associated with steroid metabolism.
3. Application of physical chemistry to steroid metabolism.
4. Application of isotopes to steroid metabolism.

1. The isolation of urinary steroids which are considered to be artifacts (e.g. $\Delta^2$ androstene-17, $\Delta^3:5$ androstadienone-17 and 3-Chlorodehydroisoandrosterone) indicates that the present urine hydrolysis techniques are too drastic, and until milder but equally effective techniques are developed, the matter of artifacts will always be a spectre hovering over isolation experiments such as the present one. The possible direction in which these new developments should proceed is indicated by the work of Talbot et al (154) -- who hydrolysed Dehydroisoandrosterone sulfate with BaCl$_2$ at pH 5 -- and the isolation of an enzyme system in aceton dried rat liver which can hydrolyze urinary Sodium-pregnanediol glucuronide (185) and Sodium-pregnane triol glucuronide (109). The isolation of urinary adrenal steroids -- which have proven to be very labile to the present hydrolysis techniques -- will especially be facilitated by such developments. Furthermore, comparison of yields of urinary steroid metabolites -- at present a grossly quantitative procedure at best -- will become a more exact mathematical process, and will, for example, enable investigators to evaluate in a quantitative fashion the relative importance of the digitonin-precipitable and the digitonin-non-precipitable steroids. Urinary isolation studies in general will become more quantitative.
2. The desirability of further studies on the enzymatic systems associated with the intermediary metabolism of the steroids -- and in particular the intermediary metabolism of Testosterone -- has been indicated repeatedly, and consequently, does not bear repetition. Suffice it to say that accurate knowledge of the intermediary metabolism of Testosterone will probably not be achieved without investigation of the related enzymatic processes. The importance of dietary factors (e.g. Thiamine, Riboflavin, adequate dietary protein) in the metabolism of the steroids has already been pointed out, but the specific role of these factors remains to be elucidated.

3. The physical chemical approach to steroid metabolism should include the thermodynamic aspects of the problem. This approach to the subject, however, received scant attention in the past. The value of such studies in elucidating the intermediary metabolism of a compound is well illustrated by the classical studies on the intermediary metabolism of Glucose. One reason why the information available at present about the metabolism of Testosterone cannot be interpreted in an accurate and universally satisfactory manner is probably the fact that the energy levels and the oxidation-reduction potentials of the reactions observed have not been determined. This defect was especially apparent when an attempt was made to draw up a scheme (Figures 6 and 7) representing the initial stages of the metabolism of Testosterone. It is difficult to predict the precise nature of the thermodynamic changes which will be encountered, but it is reasonable to presume that such changes will be closely related to the enzyme systems associated with Testo-
sterone metabolism.

Another mode of application of physical chemical techniques has been suggested by the work of Schueler (138), who has reported that compounds having the highest estrogenic activity are all molecules 8.55 Å in length. The applicability of such observations to the intermediary metabolism of the estrogens (and androgens) may, however, be limited.

4. The need for the application of isotopes to steroid metabolism studies is especially great. Instances for the use of isotopes have already been cited during the course of the discussion. Among the isotopes which can be used are \( \text{D}^2 \), \( \text{C}^{11} \), \( \text{C}^{13} \), and \( \text{C}^{14} \). The latter isotope, \( \text{C}^{14} \), may prove to be the most useful, because of its radioactivity and its long half-life (approximately 1,000).

In conclusion, it is apparent that knowledge of the metabolism of Testosterone is in its infancy.
IV

Summary

1. The oral administration of 5 gm. of crystalline Testosterone to a normal adult man resulted in the isolation for the first time of a saturated hydroxy non-ketonic urinary metabolite of the Etiocholane Series -- Etiocholandiol-3(α) 17(α). The yield of this carbinol was 156.5 mg. (12.95 mg./l.), representing a conversion of 3.13% of the administered Testosterone.

2. 694.8 mg. (13.9%) of Androsterone; 784.1 mg. (15.68%) of Etiocholanol-3(α)-one-17; as well as 36.6 mg. (0.73%) of Isoandrosterone were also isolated. 222.4 mg. (4.44%) of Δ² Androstenone-17, an artifact of Androsterone was also isolated. This experiment, therefore, confirms the work of previous investigators, with the following modifications: the yields were higher than those previously reported, and Isoandrosterone had never before been isolated at the same time as Etiocholanolone and Androsterone after the exogenous administration of Testosterone.

3. This experiment has shown that oral Testosterone is converted in approximately equal amounts to Androsterone and Etiocholanolone. Since these two steroids are found in approximately equal amounts in the urine of normal men, the former observation may be construed as indirect evidence to support the theory that Testosterone is the male sex hormone.

4. The role of Etiocholandiol-3(α) 17(α) and the other urinary metabolites in the intermediary metabolism of Testosterone
was discussed.

5. 5.4 mg. of Cholesterol (0.25 mg./l.) was also isolated. The Cholesterol most probably arise from normal endogenous metabolism, and the metabolic relation between this steryl and Testosterone could, therefore, not be clarified.

6. The possible presence of Dehydroisoandrosterone was indicated, but the amount present was very small (<1 mg./l.). This experiment, therefore, indicates that urinary Dehydroisoandrosterone does not normally arise from Testosterone and supports the theory that it is a product of adrenal cortical secretion.

7. 0.6 mg. of crystals melting at 251-60°C with decomposition were isolated. The nature of these crystals could not be ascertained.

8. 12.4 mg. of crystals which may be identical with the Δ5 Androstenetriol-3(β)16,17 isolated by Hirschmann were isolated from the urine.

9. Crystals (8.0 mg.) which melted at 307-12°C and are unsaturated were also obtained from the urine. The nature of these crystals could not be determined.
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