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IN VITRO METABOLISM OF TESTOSTERONE ON HEPATIC TISSUE OF CHICKEN (*GALLUS DOMESTICUS*)

YASURO SUGIMOTO, YOSHIHIRO OHTA, TETSUYA MORIKAWA, TATSUYA YAMASHITA, MASAMITSU YOSHIDA and BUN-ICHI TAMAOKI*

Department of Radiopharmacology, Faculty of Pharmaceutical Sciences, Nagasaki University, Bunkyo-machi, Nagasaki-shi 852, Japan

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Summary—Among the subcellular fractions of chicken liver homogenates, the microsomal and cytosol fractions were most active in metabolism of testosterone with mutually different enzymological features. On the other hand, the nuclear and mitochondrial fractions had far lower activity of metabolizing the steroid. Metabolism by *the cytosol fraction*: the following steroids were identified as the metabolites of testosterone. 5β-Dihydrotestosterone (17β-hydroxy-5β-androstan-3-one), 5β-androstane-3α,17β-diol and its 3β-epimer, 3α-hydroxy-5β-androstan-17-one and its 3β-epimer and 5β-androstanedione. Metabolism by *the microsomal fraction*: from testosterone under aerobic condition, androstenedione was obtained as the major metabolite, besides the minor polar metabolites, production of which diminished when incubated in the atmosphere of carbon monoxide.

From the results, testosterone was accepted to be firstly converted by the cytosol fraction into 5β-dihydrotestosterone which was then reduced to 5β-androstane-3α,17β-diol and its 3β-epimer. These diols were further converted partially to 3α-and 3β-hydroxy-5β-androstan-17-ones. These pathways were supported by the results of our incubation study with 5β-dihydrotestosterone and 5β-androstanedione as substrates. By the microsomes, testosterone was aerobically and anaerobically transformed to androstenedione as the major metabolite. Throughout our incubation experiments, no 5α-reduction of a Δ^4-3-oxo-steroid was detected in the chicken liver.

INTRODUCTION

In chicken organs such as testes [1], ovaries [2, 3], embryonic gonads [4], adrenal [5], epididymis [6, 7], oviduct [8], kidney [9] and brain [10], 5β-reduction of Δ^4-3-oxosteroids were observed. Even in chicken comb, one of the target organs of androgen, testosterone was reduced to 5β-steroids more than 5α-steroids [11]. Also in plasma of chicken, 5β-reduced steroids were detected [12]. These results suggested to us that one of the characteristic features of steroid hormone metabolism in chicken would be very intensive 5β-reduction.

As *in vitro* metabolism of steroid hormones in chicken hepatic tissue has not been precisely examined up to the present, we fractionated hepatic homogenates of chicken into organella fractions together with cytosol fraction, and incubated testosterone respectively with these subcellular fractions of the liver. Among them, the steroid hormone was most actively metabolized by the supernatant fluid at 10,000 ***g*** or a mixture of the microsomal and cytosol fractions. Thereafter we found that the two subcellular fractions retained activity of mutually different enzymes related to the steroid metabolism. On the other hand, the nuclear and mitochondrial fractions were found to have less enzyme activity of metabolizing the steroid and no enzyme activity specific to these organellae, either.

In this paper, we report of *in vitro* metabolism of testosterone in hepatic tissue preparations with special reference to intracellular distribution of 5β-reductase activity and also attempt to establish metabolic pathways of testosterone in the hepatic tissue.

MATERIALS AND METHODS

Chemicals

[4-^{14}C]Testosterone (Sp. act 1.9 GBq/mmol) was purchased from New England Nuclear (Boston, U.S.A.). [^{14}C]Androstenedione was prepared from [4-^{14}C]testosterone by oxidation with 0.5% CrO_3 in 90% aqueous acetic acid solution. 5β-Androstanedione was chemically derived from ^{14}C-labeled 5β-reduced steroids by the chemical oxidation. [^{14}C]5β-Dihydrotestosterone was enzymatically prepared from ^{14}C-labeled 5β-androstanedione. These products were purified by TLC.

$NADP^+$ and NADPH were purchased from Kohjin Co. (Tokyo, Japan). Before incubation, the radioactive steroids were diluted with the respective

*Author to whom all correspondence should be addressed.

unlabeled steroids (gifts from Dr H. Inano, National Institute of Radiological Sciences, Chiba-shi, Japan). All chemicals and solvents were of analytical grade.

Preparation of respective enzyme solutions

Chicken livers (male of the Broiler strain, purchased from Nagasaki Keikyo Foods Co., Nagasaki-shi, Japan) of approx. 60 days of age were used. The livers were homogenized by Ultra-Turrax TP (Ika Werk, Germany) with 3 vol of ice-cold 10 mM phosphate buffer (pH 7.4). After the homogenates were centrifuged at 800 *g* for 20 min, the resulting supernatant fluid was centrifuged at 10,000 *g* for 20 min. The supernatant fluid at 10,000 *g* was centrifuged at 105,000 *g* for 60 min to precipitate the microsomal fraction, while the supernatant fluid was used as cytosol fraction. The precipitate at 800 *g* contained mostly nuclei and cell debris, and the precipitate at 800–10,000 *g* contained largely mitochondria.

Protein concentration were measured by Bradford's method [13], using bovine gamma globulin as the standard.

Incubation

The radioactive steroid (approx. 100,000 dpm, 300 nmol per incubation flask) dissolved in a benzene–ethanol (9:1, by vol) mixture was transferred into an incubation flask, to which 2 drops of propylene glycol were added. The volatile solvent were evaporated under reduced pressure immediately before incubation. To the flask, a mixture which consisted of 4.5 ml of chicken hepatic preparation (approx. 10 mg protein each per incubation flask) and 0.5 ml of cofactor solution (990 nmol per incubation flask) was added. The mixture was incubated for 60 min with constant shaking at 38°C in an atmosphere of air, or when necessary, of 100% carbon monoxide.

Separation and purification of the metabolites

Incubation was terminated by transferring the incubation flasks into an ice-water bath and by addition of CH_2Cl_2 (5 ml per flask) to the mixture. The extraction was repeated to more times. The CH_2Cl_2 layer was pooled and the solvent was evaporated, after being dried with anhydrous Na_2SO_4. An aliquot of the extract was taken for measurement of recovery of total radioactivity and the rest was spotted on a silica-gel precoated thin-layer plate ($60F_{254}$, E. Merck A.G., Germany), together with Δ^4-3-oxosteroids (progesterone, 17α-hydroxyprogesterone, androstenedione, testosterone and 11-deoxycortisol) as markers. The steroid metabolites were separated by TLC, primarily developed in a benzene–acetone (4:1, by vol) system. For further separation of mixture of some steroids, a solvent system (cyclohexane–ethyl acetate, 1:1, by vol) was employed. Radioactive spots were detected by autoradiography, by exposing the thin-layer plate to a sheet of medical X-ray film (HR-S, Fuji Film Co., Tokyo, Japan) for 4–5 days.

Identification

For identification of the purified metabolites, the following criteria were employed.

(1) Identical mobilities of the metabolites on thin-layer chromatograms with those of the authentic preparations. (2) Identical behavior of the metabolites to those of the authentic preparations, through acetylation by acetic anhydride and pyridine (1:1, by vol), and oxidation by 0.5% CrO_3 in 90% aqueous acetic acid solution, and (3) Similarity between the retention times and mass-spectra obtained by gas-chromatography–mass-spectrometric analyses of a metabolite and its corresponding authentic preparation [14, 15]. The gas-chromatography–mass-spectrometric analyses were carried out by the instrument (JMS-DX 303, Ms-GCG 06, JEOL Co. Tokyo, Japan), using a glass column 3 mm in inner diameter and 2 m in length packed with 2% silicon OV-1 coated on acid-washed and silanise on chromosorb W 80–100 mesh. The temperatures at the injection site and the separator were controlled at 250°C, and the column was gradually heated from 120 to 250°C after injection.

Quantitation of the metabolites

Radioactivities were measured by a liquid scintillation spectrometer (LSC-703, Aloka Co., Tokyo, Japan), and were expressed in dpm. From the radioactivity of a metabolite, its amount in nmol was estimated by dividing by the specific activity (dpm/nmol).

RESULTS

Metabolism of testosterone in the supernatant fluid at 10,000 g

After an aerobic incubation of radioactive testosterone with supernatant fluid at 10,000 *g* in the presence of NADPH, the metabolites were separated by TLC. As the result, seven radioactive steroids were obtained as the metabolites. The least polar metabolite was named as 'A', and the most polar metabolite was named as 'F'. The metabolites localized between them were designated as B, C, D and E, successively, as shown in Fig. 1. Each metabolite was respectively isolated from the spot on the thin-layer chromatogram. Exceptionally the steroid fraction at spot E on the first TLC (Fig. 1, left) was further subjected to another TLC, developed in cyclohexane–ethyl acetate (1:1, by vol) system, by which three different steroids were separable. As shown in the second TLC (Fig. 1, right), the three steroids were named as E1, E2 and E3 in the order of polarity.

Metabolite A. Its radioactivity was detected at a less polar position than that of androstenedione on the initial TLC. After the oxidation or acetylation of A, no change in polarity on TLC was observed. The mobility of this metabolite was in agreement with that of authentic 5β-androstanedione. According to

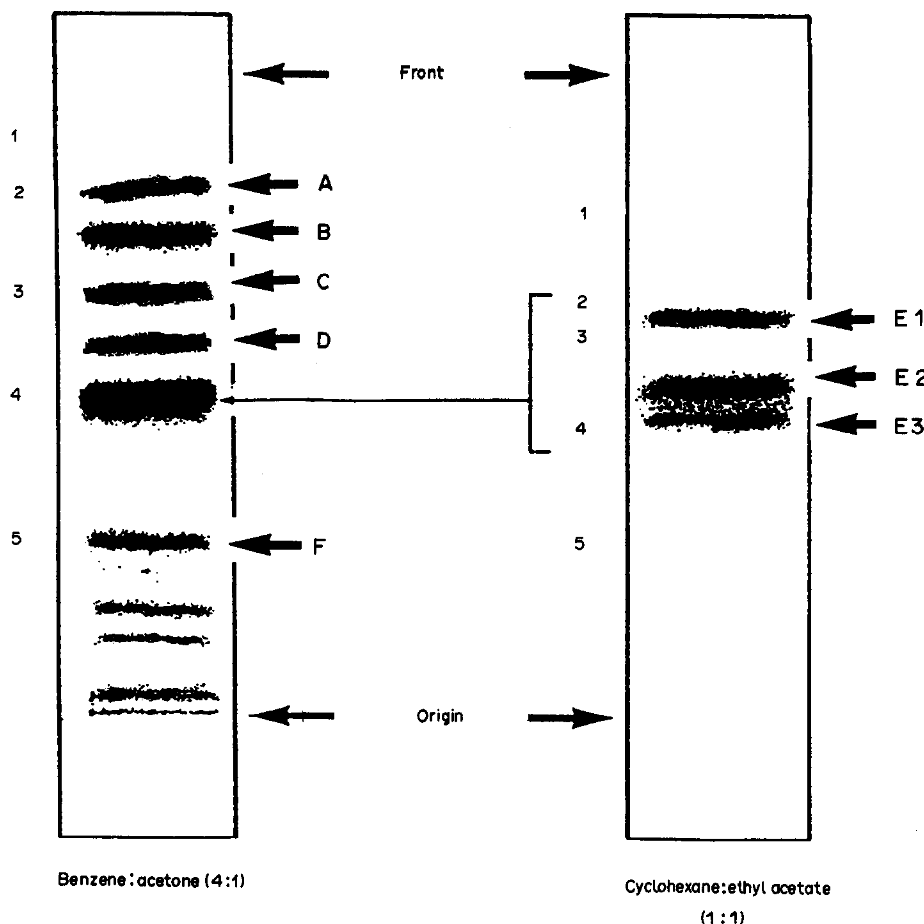


Fig. 1. Autoradiograms of the thin layer chromatograms on which metabolites of [4-^{14}C]testosterone were separated. An aliquot of the extract was spotted on a thin-layer plate, together with the following Δ^4-3-oxosteroids as markers. 1: progesterone, 2: androstenedione, 3: 17α-hydroxyprogesterone, 4: testosterone and 5: 11-deoxycortisol.

the gas-chromatography–mass-spectrometric analysis, the molecular ion peak of this metabolite was found at 288 *m/e*. The pattern of fragmentation was identical with that of authentic 5β-androstanedione. The retention time of this metabolite in gas-chromatography was agreeable with that of the standard preparation.

Metabolite B. The radioactivity of B was found in the same location of androstenedione on the initial TLC. The polarity of metabolite B was not influenced by either the chemical oxidation or acetylation. When this metabolite was subjected to the mass-spectrometric analysis, its molecular ion peak was found at 286 *m/e*, being agreeable with that of the authentic androstenedione. The pattern of the fragments such as the fragments at 244 *m/e* and 124 *m/e* was identical with that of authentic androstenedione (Fig. 2). The retention time of this metabolite in the gas-chromatography was in agreement with that of the standard preparation.

Metabolite C. The oxidized product of metabolite C was thin-layer chromatographically identical with 5β-androstanedione. Acetate of this metabolite showed the same mobility as the acetate of authentic 3β-hydroxy-5β-androstan-17-one on TLC. When this metabolite was mass-spectrometrically analyzed, the molecular ion peak of C was found as 290 *m/e*. The pattern of the fragment at 272 *m/e* and other fragments in the mass spectrum was identical with that of authentic 3β-hydroxy-5β-androstan-17-one. The retention time of this metabolite in the gas-chromatography was agreeable with that of the standard preparation.

Metabolite D. After oxidation of metabolite D, the product was found as identical to TLC with 5β-androstanedione. Acetate of this metabolite showed the same chromatographic mobility as that of acetate of the authentic 5β-dihydrotestosterone by TLC. When this metabolite was mass-spectrometrically analyzed, the molecular ion peak of D was found at 290 *m/e*. The pattern of the fragment at 272 *m/e* and other fragments in the mass spectrum was identical with that of authentic 5β-dihydrotestosterone (Fig. 3). The retention time of metabolite D in the gas-chromatography was identical with that of the authentic preparation.

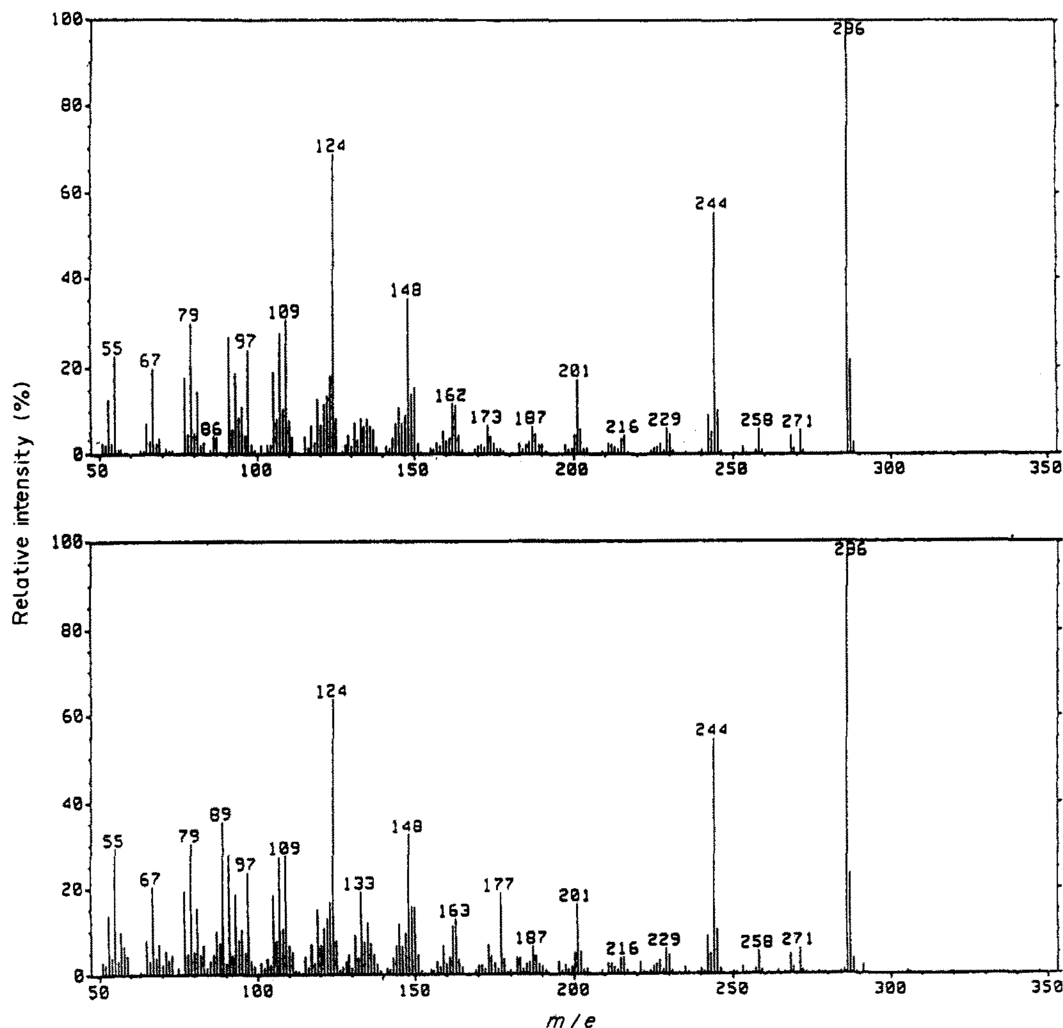


Fig. 2. Mass spectrum of metabolite B (Lower chart) in comparison with that of authentic preparation of 4-androstenedione (Upper chart).

Metabolite E1. On the initial chromatography, this metabolite migrated together with standard testosterone. In the second TLC developed in cyclohexane–ethyl acetate (1:1, by vol) system, however, it migrated together with standard androstenedione. After CrO_3 oxidation of this metabolite, the product was thin layer chromatographically identical with 5β-androstanedione. When this metabolite was subjected to acetylation, the product was identical with the acetate of authentic 5β-androstane-3β,17β-diol. When this metabolite was subjected to a gas-chromatography–mass-spectrometric analysis, the molecular ion was found at 292 *m/e*. The pattern of the fragments found at 274 *m/e*, 256 *m/e* and other fragments in the mass spectrum was identical with that of authentic 5β-androstane-3β,17β-diol. Retention time of this metabolite in gas chromatography was in agreement with that of the authentic preparation of 5β-androstane-3β,17β-diol.

Metabolite E2. In the initial chromatography, metabolite E2 migrated together with standard testosterone. In the second TLC, however, radioactivity of E2 was found between the spots of testosterone and 17α-hydroxyprogesterone. After oxidation of this metabolite, the product was identical by TLC with 5β-androstanedione. When this metabolite was subjected to the mass-spectrometric analysis, the molecular ion peak was found at 290 *m/e*. The pattern of the fragment at 272 *m/e* and other fragments in the mass spectrum was identical with that of authentic 3α-hydroxy-5β-androstan-17-one. Retention time of this metabolite in the gas-chromatography was similar to that of the standard preparation.

Metabolite E3. In the first TLC, this steroid migrated with the standard testosterone. E3 showed a characteristic absorption around 240 nm. This was finally identified as unconsumed testosterone.

Metabolite F. Its radioactivity was detected close to the spot of the standard 11-deoxycortisol by the initial TLC. After oxidation of metabolite F, the product was identical by TLC with 5β-androstane-

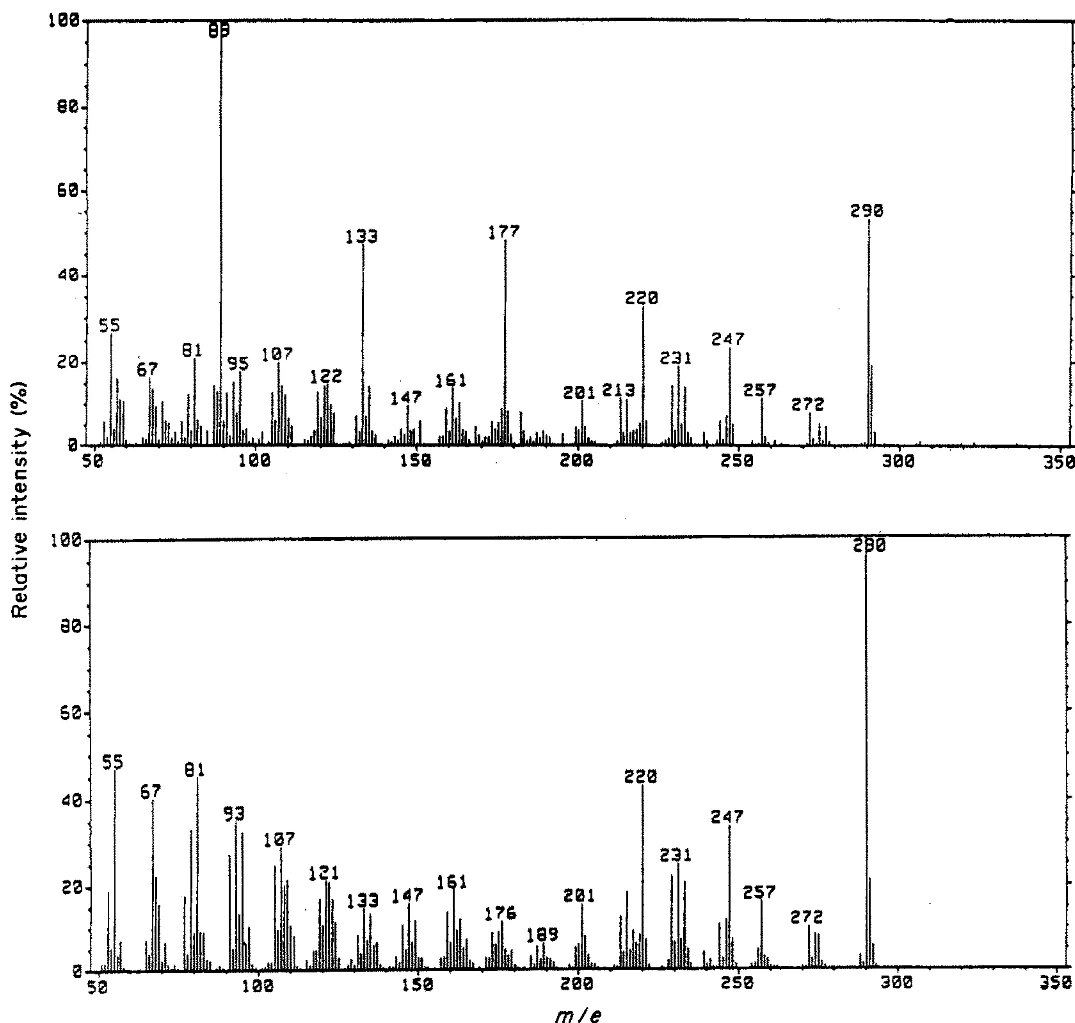


Fig. 3. Mass spectrum of metabolite D (Lower chart) in comparison with that of authentic preparation of 17β-hydroxy-5β-androstan-3-one (Upper chart).

dione. Acetate of metabolite F showed the same mobility by TLC as that of acetate of authentic 5β-androstane-3α,17β-diol. When its metabolite was analyzed by a gas-chromatography–mass-spectrometry, its molecular ion peak was found at 292 *m/e*. The pattern of the fragments found at 274 *m/e* and 256 *m/e* and others in the mass spectrum was identical with that of authentic 5β-androstane-3α,17β-diol as shown in Fig. 4. The retention time of this metabolite in the gas-chromatogram was agreeable with that of the standard preparation.

Besides the above seven metabolites, a few minor metabolites were detected in more polar regions than 11-deoxycortisol on the initial TLC, but their radioactivities were not sufficient to allow further identification of them.

Testosterone metabolism in the microsomal fraction

(a) Aerobic condition: after incubation of testosterone with the microsomal fraction under an aerobic condition in the presence of $NADP^+$, testosterone was converted to androstenedione as the major metabolite, while limited amount of the substrate was converted to more polar metabolites than 11-deoxycortisol on TLC. The production of these metabolites was summarized in Table 1.

(b) Anaerobic condition: when incubation of testosterone was performed under a carbon monoxide-saturated condition, testosterone was exclusively converted to androstenedione. Production of the more polar metabolites which had appeared under the aerobic condition almost completely diminished.

(c) Androstenedione metabolism in the presence of NADPH: after an aerobic incubation of androstenedione in the presence of NADPH, it was converted to testosterone as the major metabolite.

(d) Metabolism of 5β-androstanedione in the presence of NADPH: when an aerobic incubation was performed in the presence of NADPH, 5β-androstanedione was converted into the following metabolites: 3β-hydroxy-5β-androstan-17-one, 5β-dihydrotestosterone and 5β-androstane-3α,17β-diol. The production rates of the metabolites were shown in Table 2.

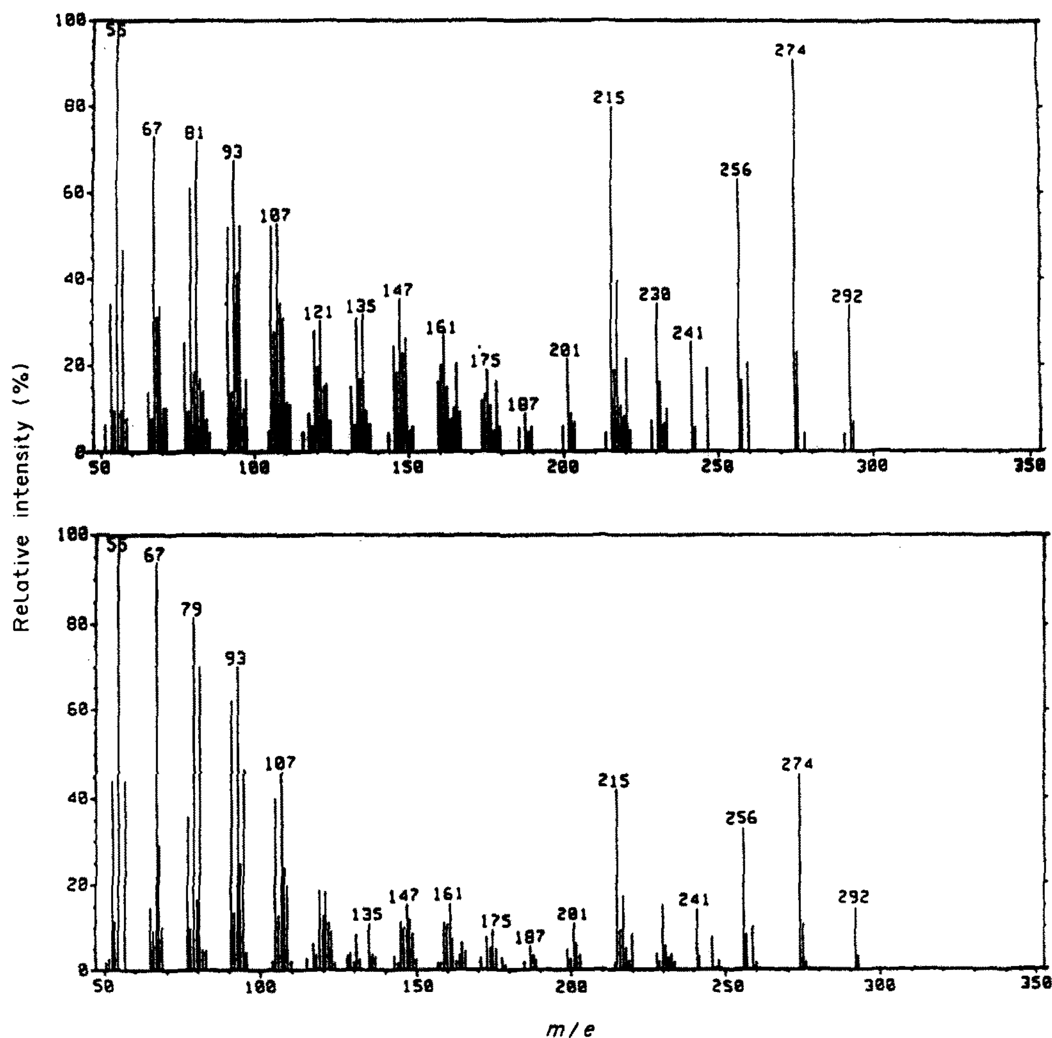


Fig. 4. Mass spectrum of metabolite F (Lower chart) in comparison with that of authentic preparation of 5β-androstane-3α,17β-diol (Upper chart).

Testosterone metabolism in the cytosol fraction

(a) When testosterone was aerobically incubated with the cytosol fraction in the presence of NADPH, it was converted to the following metabolites: 5β-androstanedione, 5β-dihydrotestosterone, 5β-androstane-3α,17β-diol, 5β-androstane-3β,17β-diol, 3α-hydroxy-5β-androstan-17-one and 3β-hydroxy-5β-androstan-17-one. Production of the metabolites from testosterone was quantitatively expressed, as shown in Table 1.

Table 1. Production rates of metabolites of [4-^{14}C]testosterone, produced by the microsomal and cytosol fractions of chicken liver

Steroid	Fraction Microsomes	Cytosol
Testosterone (recovered)	67.71[a]	54.36[a]
4-Androstenedione	14.97	4.88
5β-Dihydrotestosterone	ND	3.31
5β-Androstane-3α,17β-diol	2.41	24.24
5β-Androstane-3β,17β-diol	1.46	6.93
3α-Hydroxy-5β-androstan-17-one	ND	—[b]
3β-Hydroxy-5β-androstan-17-one	ND	5.52
Unidentified metabolites	13.45[c]	0.83[d]

Radioactive testosterone (100,000 dpm, 300 nmol) was aerobically incubated with hepatic microsomal and cytosol fractions (*ca.* 10 mg protein, each) respectively, in the presence of NADPH (990 nmol) in 5 ml of phosphate buffer (pH 7.4) for 60 min.
[a]Ratio (percentage) of radioactivity of a metabolite to the total radioactivity; [b]tentatively identified; [c]four metabolites were separated; [d]one metabolite at least was detected; ND, not detected.

(b) After an aerobic incubation of 5β-dihydrotestosterone in the presence of NADPH, the major metabolites were identified as 5β-androstane-3α,17β-diol and 5β-androstane-3β,17β-diol.

Table 2. Production rates of metabolites of 5β-androstanedione produced by the microsomal and cytosol fraction of chicken liver

Steroid	Fraction Microsomes	Cytosol
5β-Androstanedione (recovered)	1.55[a]	8.40
3α-Hydroxy-5β-androstan-17-one	ND	6.30
3β-Hydroxy-5β-androstan-17-one	15.71	47.60
5β-Dihydrotestosterone	79.38	1.00
5β-Androstane-3α,17β-diol	ND	33.30
5β-Androstane-3β,17β-diol	3.36	3.50

[a]Ratio (percentage) of radioactivity of a metabolite to the total radioactivity; ND, not detected.

(c) After an aerobic incubation of 5β-androstanedione in presence of NADPH, the metabolites were identified as 5β-dihydrotestosterone, 5β-androstane-3α,17β-diol, 5β-androstane-3β,17β-diol, 3α-hydroxy-5β-androstan-17-one and 3β-hydroxy-5β-androstan-17-one. Production of these metabolites was shown in Table 2.

Metabolism of testosterone in organellae fractions other than the microsomal fraction

Testosterone was aerobically incubated with the mitochondrial and nuclear fractions in the presence of NADPH, respectively. However, a limited amount of the substrate was consumed, accordingly with diminished production of 5β-reduced steroids. Production of no steroid other than those obtained by the supernatant fluid at 10,000 *g* was confirmed. In addition, the more polar metabolites than 11-deoxycortisol which were produced by the microsomes were not detected among the metabolites formed by either the mitochondrial or the nuclear fraction.

DISCUSSION

On the first TLC of the metabolites, we noticed that there was discrepancy between the u.v.-absorbing spot due to authentic testosterone and the radioactive spot on the autoradiogram of the TLC. Therefore we carried out further separation by the second TLC developed in a different solvent system, and then detected two more metabolites besides testosterone. Each spot other than the above on the chromatogram was confirmed as a single steroid.

In this study, we employed gas-chromatography–mass-spectrometric analysis for final identification of the steroidal metabolites which were derived from non-radioactive substrates. Assignment of several fragments in mass spectra was attempted to elucidate structure of each metabolite as follows:

Metabolite A as 5β-androstanedione. The mass spectrum of metabolite A was not only agreeable with that of authentic preparation of 5β-androstanedione, but also essentially in accord with the mass spectrum of 5β-androstanedione obtained previously reported [16].

Metabolite B as androstenedione. Among the fragments of authentic androstenedione in the mass spectrum, the fragments specific to Δ^4-3-oxosteroids were found at (M^+–42) or 244 *m/e* which was assigned as formed by removal of (—CH_2—CO—) from the A-ring of androstenedione, and also at 124 *m/e* which was formed by cleavage of the bonds between C_6 and C_7, and between C_9 and C_{10} [17]. In the spectrum of metabolite B, these peaks were observed with the similar intensities to those of the corresponding peaks of the authentic preparation (Fig. 2), supporting that this metabolite was a Δ^4-3-oxosteroid.

Metabolites C, D and E2 respectively as 3β-hydroxy-5β-androstan-17-one, 17β-hydroxy-5β-androstan-3-one and 3α-hydroxy-5β-androstan-17-one. According to our mass spectrometric analyses, the metabolites C, D and E2 showed the same molecular ion peak at 290 *m/e* with the dehydrated fragment (M^+–18) at 272 *m/e*. However, these three metabolites were mutually distinguishable by (1) the retention time in gas-chromatographic analyses and also (2) the intensity of the dehydrated fragments relative to those of the respective molecular ion peak on the mass spectrum.

Metabolite F as 5β-androstane-3α,17β-diol and metabolite E1 as 5β-androstane-3β,17β-diol. In the mass-spectra, the both molecular ion peaks of these two steroids were found at 292 *m/e*. The pattern of the dehydrated fragments at (M^+–18) or 274 *m/e* and at (M^+–36) or 256 *m/e* and other fragments of those two metabolites (F and E) in the mass spectra were identical respectively with those of the authentic prepartions of 5β-androstane-3α,17β-diol and 5β-androstane-3β,17β-diol. The intensity of the fragment (M^+–36) or 256 *m/e* of 5β-androstane-3α,17β-diol in relative to the molecular ion peak (292 *m/e*) was 73.3, whereas that of 5β-androstane-3β,17β-diol was 18.1.

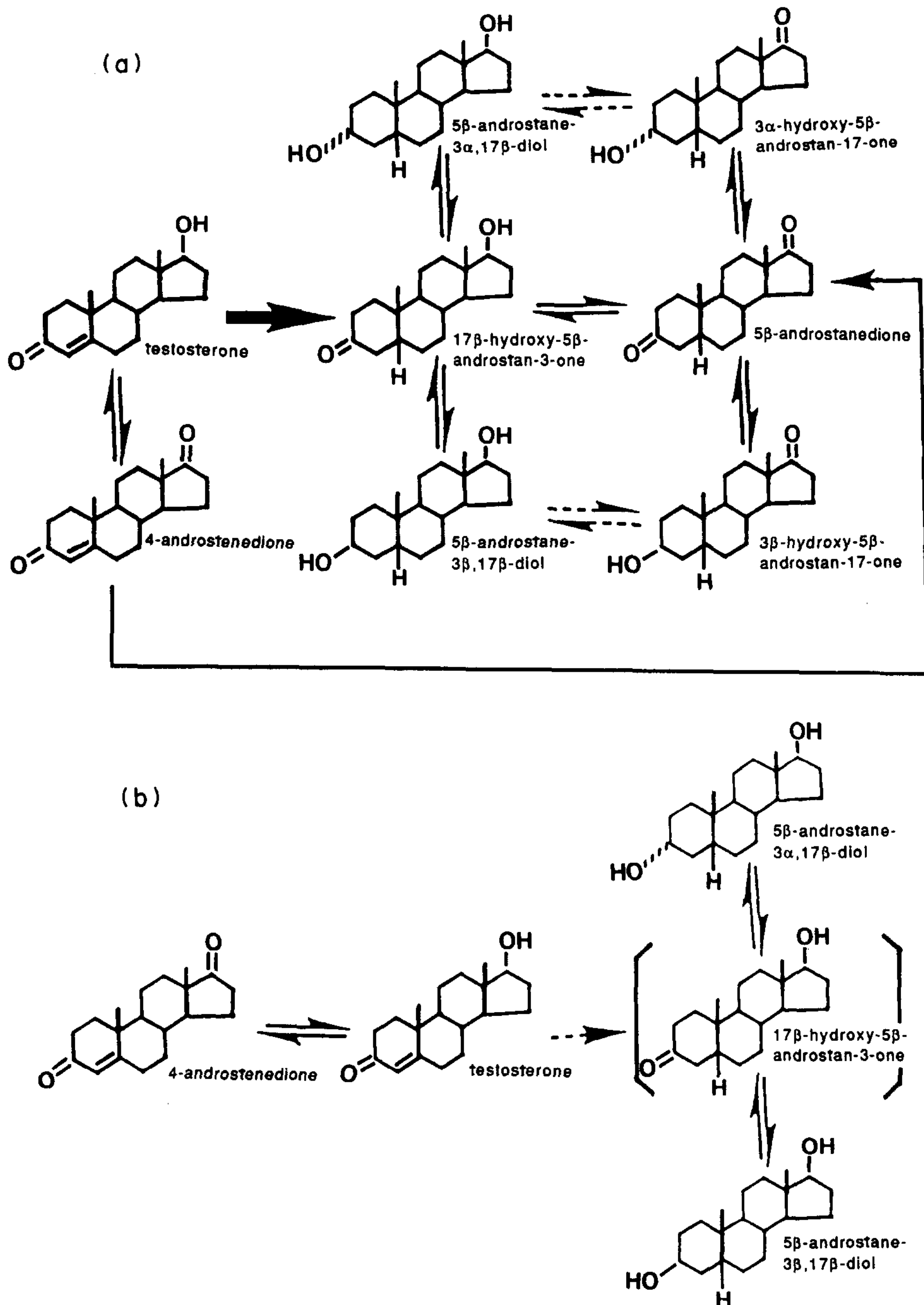
As our preliminary study on the hepatic metabolism of testosterone, we employed the supernatant fluid at 10,000 *g* as the enzyme source. This supernatant fluid was separated by centrifugation at 105,000 *g* into the microsomal fraction as the precipitate and cytosol fraction as the supernatant fluid. All the metabolites produced by the supernatant fluid at 10,000 *g* were detected among the metabolites obtained by individual incubation with the cytosol or the microsomes.

By the microsomes, testosterone was almost predominantly oxidized to androstenedione in the presence of $NADP^+$ and NADPH, in addition to limited amount of 5β-androstane-3α,17β-diol and its 3β-epimer (Table 1). We observed that the minor metabolites with more polarities than 11-deoxycortisol were produced by aerobic incubation of testosterone with the microsomal fraction. These metabolites were suggested to be metabolites of testosterone to which hydroxy groups were introduced by cytochrome *P*450-involving enzyme systems [18], because production of these metabolites were severely inhibited by carbon monoxide. From 5β-androstanedione as substrate, reduction of 17-oxo and 3-oxo groups occurred in the incubation with the microsomes (Table 2).

By the cytosol fraction, however, testosterone was selectively reduced in 5β-dihydrotestosterone, followed by reduction of its 3-oxo-group into 3α- and 3β-hydroxy groups. These products or 5β-androstane-3α,17β-diol and its 3β-epimer remained partly as the end products and partly their 17β-hydroxy groups were oxidized into 3α-hydroxy-5β-androstan-17-one or its 3β-epimer, besides oxidation

of testosterone to androstenedione (Table 1). From 5β-androstanedione, formation of 3α-hydroxy-5β-androstan-17-one and its 17β-reduced product was noticeable in the case of the cytosol. When testosterone and 5β-androstanedione were respectively employed as substrate, production rate of 17β-hydroxy-5β-androstan-3-one was significantly lower than that of 5β-androstane-3β,17β-diol in the cytosol fraction.

Therefore, it was suggested that in the cytosol fraction, testosterone was exclusively reduced by 5β-dihydrotestosterone, followed by efficient conversion of the 3-oxo group of 5β-dihydrotestosterone into the 3α- or 3β-hydroxysteroids by the respective hydroxysteroid dehydrogenases, as these dehydrogenases were probably more active than 5β-reductase. On the other hand, testosterone was transformed by the microsomes into androstenedione which was reduced to 5β-androstanedione. Thereafter, it was further converted into 17β,3α(β)-dihydroxysteroids. From these results, the major metabolic pathways of testosterone in the chicken liver were proposed as Scheme 1. In Scheme 1a,



Scheme 1. Tentative metabolic pathways of testosterone in the microsomal and cytosol fractions of chicken liver. Solid arrows indicated the established and broken arrows showed the tentative pathways of testosterone metabolism. (a) Cytosol fraction and (b) microsomal fraction.

testosterone was 5β-reduced in the first place by the cytosol from which 5β-reductase was recently purified in this laboratory to an apparent homogeneity by SDS–polyacrylamide gel electrophoresis [19].

In the present experiment, we could detect no 5α-reduced steroid formed by the chicken hepatic preparations, even though we paid our particular attention to detection of 5α-reduced metabolites. As mentioned above, we identified almost all the radioactive metabolites derived from ^{14}C-labeled testosterone, but no 5α-reduced steroid among the metabolites. Furthermore, after incubation of ^{14}C-labeled testosterone with the supernatant fluid at 10,000 *g*, all the steroids were extracted. Immediately thereafter, the pooled steroidal extract was subjected to chemical oxidation with CrO_3 in the acetic acid solution. The products were then separated by TLC. On the chromatogram, androstenedione and 5β-androstanedione were detected, but no detectable amount of 5α-androstanedione was observed.

From the standpoint of comparative endocrinology, it is interesting to find that central nervous system of other aves, such as the European stalling [20], dove [21] and quail [22] contained appreciable activity of 5β-reductase. Also in testes of Japanese quail, 5β-reductase activity was reported in addition to the steroidogenic enzymes [23]. However, the same authors reported that no 5β-reduced product was formed by pituitary glands of laying hen [24]. Physiological role of 5β-reduced steroids other than hematopoietic activity has not been well investigated of aves [25].

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