Journal of Steroid Biochemistry 1975 Vol. 6 pp. 1363 1370. Pergamon Press. Printed in Great Britain

METABOLISM OF TESTOSTERONE AND DIHYDROTESTOSTERONE IN CULTURED RAT HEPATOMA CELLS

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(*Received* 1 *July* 1974)

SUMMARY

The metabolism of [^{3}H]-testosterone and [^{3}H]-dihydrotestosterone (17β-hydroxy-5α-androstan-3-one) has been studied in cultured male rat hepatoma (HTC) cells. In contrast to glucocorticoids androgens were extensively transformed by this cell line. The main reactions observed were: 5α-reduction, 3α-, 3β- and 17β-hydroxysteroid dehydrogenation. The pattern of metabolism was very reproducible and varied with time, substrate concentration and the number of cells incubated.

All attempts to provoke in this system inductions or repression of steroid metabolizing enzymes failed. A number of steroids interfered directly with androgen metabolism, however. 17β-Estradiol, 17-epitestosterone and progesterone competed for the 17β-hydroxysteroid dehydrogenase. The latter two steroids also inhibited the formation of 5α-reduced metabolites. Competition experiments with 3-epiandrosterone suggest that in HTC cells the same enzyme catalyzes both 17β-hydroxy- and 3β-hydroxysteroid dehydrogenation.

INTRODUCTION

Some hepatomas induced by feeding rats a diet containing 0·04% N,N'-2,7-fluorenylene bis-2,2,2-trifluoroacetamide have been cultured to yield cell lines with stable characteristics [1]. Although these cells cannot be considered to be normal hepatocytes [2], they have been used widely in recent years to investigate various problems. Examples of this approach are studies by Thompson *et al.*[3] on the induction of tyrosine α-ketoglutarate transaminase by steroid hormones and studies of the same group on glucocorticoid activity [4] and glucocorticoid receptors [5, 6].

Steroid metabolism in cell cultures derived from organs other than the liver, has been investigated by several authors [7, 8]. Data on steroid metabolism in hepatoma tissue culture cells are rather scarce, however. Baxter and Tomkins[9] did show that, while dexamethasone was not metabolized by hepatoma tissue culture (HTC) cells, cortisol was to a limited extent. These authors, however, did not identify with certainty the metabolites produced. Androgen metabolism in HTC cells has not yet been investigated.

Sexual differentiation of steroid metabolism in the rat liver has been extensively studied during the last years (for review i.a. [10]). The mechanism of the delayed masculinisation observed in this organ remains unknown and could perhaps be approached more easily in a cell culture system. Rat liver cells, however, are very difficult to culture in a reproducible way. In this paper the authors investigated steroid metabolism in HTC cells derived from a male rat according to the scheme shown in Fig. 1.

EXPERIMENTAL

Cell cultures. The hepatoma tissue culture cells (HTC cells) used are cells derived from the rat hepatoma 7288c established by H. P. Morris. Subcultures of this stabilized cell line, classified as minimal deviation HTC [2], were provided to us by J. Desmyter. The cells were grown at 37°C in loosely stoppered glass tubes kept nearly horizontal in the incubator. About 1×10^6 trypsinized cells were transferred to each of these tubes in 1 ml of Eagle's medium containing Earle's salts and lactalbumine [11], to which were added 1% $NaHCO_3$ and 10% fetal bovine serum (fbs). After 24 h in this medium the trypsinized cells had regained their full metabolic capacity and adhered to the glace surface. At that time each tube contained between 0·8 and $1·0 \times 10^6$ living cells. From then on the culture remained stable as far as number of cells was concerned and remained viable for at least 3 more days as shown in the next paragraph. Before starting metabolism studies the culture medium containing 10% fbs was replaced by medium containing only 2% fbs in order to minimalize extracellular binding of steroid substrate. Steroids were added in ethanol and incubated with the cells for various lengths of time. The volume of ethanol employed to dissolve the steroids never exceeded 1% of the incubation volume; control tubes contained the same amount of ethanol.

Viability of HTC cells in the stationary phase. HTC-cells were grown in a 10% fbs medium for 24, 48, 72 and 96 h. Afterwards they were counted and incubated with testosterone for another 24 h in a 2% fbs medium. It appeared that after 24 h of growth the cells formed a monolayer and that the cell number did not increase any more. The metabolite patterns

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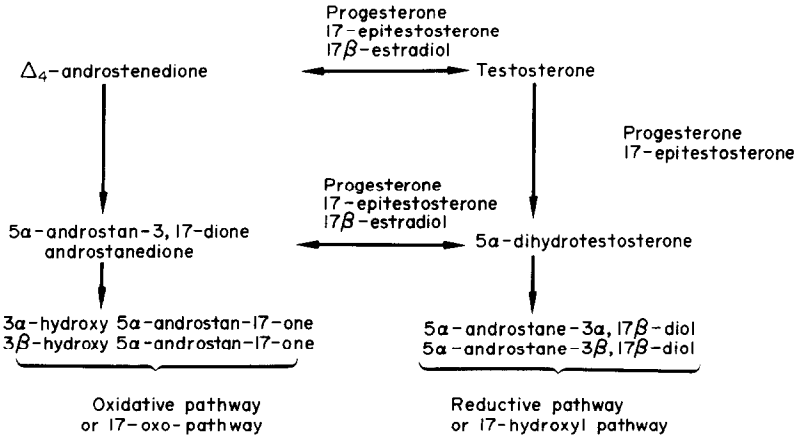

Fig. 1. Schematic view of testosterone metabolism and of the competitive influence of various steroids on androgen metabolism in HTC cells.

obtained after 24 h of preincubation did not differ significantly from those obtained after either 48, 72 or 96 h of preincubation. This, in our opinion, is a good argument for the viability of full grown HTC cells over these periods of time.

Chemicals. [1,2,^{3}H]-testosterone (56 Ci/mmol) and [1,2,^{3}H]-dihydrotestosterone (47 Ci/mmol) were obtained from CEN, Mol, Belgium. The tritiated steroids were used without further purification. Reference steroids were obtained from Sigma. All further reagents were analytical grade.

Chromatography. For thin-layer chromatography, precoated Silicagel F_{254} plates (Merck A. G.) were developed twice in dichloromethane–ether 85:15, v/v). Paper chromatography was performed on Schleicher & Schüll 2040 B paper using either the Bush A′ system (methanol–water–isooctane, 40:10:50; by vol.) or the Bush B_3 system (petroleum benzene–benzene–methanol–water, 33:17:40:10, by vol.).

Incubation with tritiated steroids. Monolayers of HTC cells (0·8–1·2 × 10^6 per tube), adhering to the bottom and side wall of glass tubes were used. The incubation period was started by replacing the 10% fbs containing medium in which the cells had grown by an analogous medium containing 2% fbs. Tritiated (1 μCi) and cold steroids (100 ng per tube except when stated otherwise) were added in 10 μl of ethanol. The incubation was stopped after varying lengths of time by adding a five-fold volume of chloroform–methanol (2:1, v/v). After vigorous shaking and centrifugation for 10 min at 3000 rev./min, the water phase was discarded and replaced by an equal volume of chloroform–methanol–water (3:48:47 by vol). Preliminary experiments showed that conjugated derivatives were virtually absent in this system. The radioactivity remaining in the cells or adhering to the tube was less than 1%. Thereafter the extract was evaporated to dryness after addition of 50 μg of the following reference steroids: testosterone, 5α-dihydrotestosterone (DHT), 5α-androstan-3α, 17β-diol (THT), 3α-hydroxy-5α-androstan-17-one (androsterone), 4-androstene-3,17-dione and 5α-androstane-3,17-dione. First thin layer chromatography was performed. 4-en-4-3-ketosteroids were visualized under U.V. at 254 nM. The other reference steroids were localized by a non-destructive colour method [12]. It was confirmed by radiochromatogram scanning that only the zones containing the six mentioned reference steroids and the start line of the chromatograms contained significant amounts of radioactivity. Five of these zones could be shown to be homogeneous on further paper chromatography. These zones were immediately scraped off and transferred to counting vials. Zones II and IV, however, could be shown to contain two metabolites each. After elution, the latter components were separated by chromatography on Schleicher and Schüll 2040 B paper respectively in a Bush B_3 and Bush A′ system. 5α-Androstan-3α/β,17β-diol (from zone II) and 3α/β-hydroxy-5α-androstan-17-one (from zone IV) were recognized by their R_T-values. After elution the latter 3α and 3β metabolites were quantitated in a liquid scintillation counter. The eight mentioned metabolites (*i.e.* the six reference steroids *plus* 5α-androstan-3β,17β-diol and 3β-hydroxy-5α-androstan-17-one) accounted for 92·2 ± 8·6% (mean ± S.D.) of the radioactivity after incubation of testosterone. Zone I containing the hydroxylated metabolites remaining on the start line averaged 4·1 ± 2·4%. The radioactivity that could be accounted for by elution of the chromatogram averaged 67·9 ± 11·3% of the radioactivity added to the extract. The total radioactivity on the chromatogram was calculated by adding the radioactivity of the 7 individual zones and the results of a particular zone were expressed as the percentage of this total without correction for recovery. The metabolites localized by their mobility in the chromatography systems were identified by crystallization with authentic reference steroids to constant specific activities (Table 1).

Repeated experiments at separate times gave reproducible results, provided the same number of cells were used. In each series of incubations, two tubes

Table 1. Crystallization to constant specific activity of the eight major metabolites resulting from the incubation of [^{3}H]-testosterone (results given in c.p.m./mg)

	Testosterone	DHT	3α-5α-17β THT	3β-5α-17β THT	Androsterone	Epiandrosterone	Androstanedione	Androstenedione
Starting material	4057	7116	2465	10,283	1857	4259	2003	3276
1st Crystallization	3655	7070	2117	8806	1589	4401	1878	2287
2nd Crystallization	3567	7428	2037	10,323	1477	4379	1774	3259
3rd Crystallization	3584	7047	2033	9834	1420	4059	1723	3149
4th Crystallization	3725	7394	1990	9179	1498	3933	1576	3229
5th Crystallization	3820	7049	2016	9531	1476	3912	1751	3152
% Purity	91·4	100	81·6	87·9	78·8	93·2	87·4	96·7

DHT = 5α-dihydrotestosterone; THT = tetrahydrotestosterone.

without cells were included. The results of these incubations were used as blank.

Preincubation studies. Since full grown HTC cells proved to be viable for at least 4 days preincubation studies were planned as follows. After 24 h of growth the medium was changed and replaced by a similar 10% fbs medium to which various non-labelled steroid hormones were added either singly or in combination in the hopes of altering the subsequent enzymatic activity. After various length of time (preincubation) the medium was again drained off and replaced by the 2% fbs incubation medium.

RESULTS

I. Metabolism of testosterone and dihydrotestosterone as a function of time

Incubations with testosterone ($3{\cdot}5 \times 10^{-7}$ M) were performed as described in Experimental. Testosterone disappeared exponentially (Fig. 2). During the first 6 h of incubation this disappearance could largely be accounted for by the production of DHT. After 6 h the production of 17-oxo derivatives became more and more important and the production of DHT levelled off. An increasing fraction of the 17-oxo and 17-hydroxy dihydro derivatives was transformed into tetrahydro compounds.

Small amounts of 5α-dihydrotestosterone (DHT) did not disappear as fast as did similar amounts of testosterone (Fig. 3). The substrate is metabolized progressively, but after 12 h still 50% of the original DHT remains unchanged. At that moment, THT production is maximal, and further DHT metabolism yields only 17-oxo derivatives: production of androstanedione as well as of 17-oxo-tetrahydro derivatives increases.

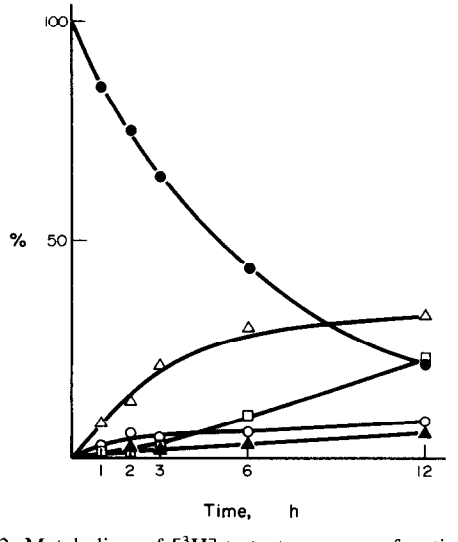

Fig. 2. Metabolism of [^{3}H]-testosterone as a function of time. HTC cells (900,000 cells/ml) were incubated with [^{3}H]-testosterone ($3{\cdot}5 \times 10^{-7}$ M) for various periods of time. The disappearance of testosterone (●——●) and the production of DHT (△——△), androstanedione (□——□), 3α- and 3β-androstanediol (○——○), androsterone and epiandrosterone (▲——▲) was measured as described in the experimental section.

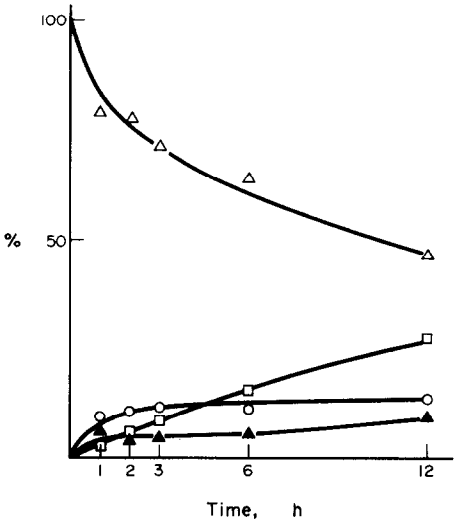

Fig. 3. Metabolism of [^{3}H]-dihydrotestosterone as a function of time. HTC cells (900,000 cells/ml) were incubated with [^{3}H]-dihydrotestosterone ($3{\cdot}6 \times 10^{-7}$ M) for various periods of time. The disappearance of DHT (△——△) and the production of androstanedione (□——□), 3α- and 3β-androstanediol (○——○), androsterone and epiandrosterone (▲——▲) was measured as described in the experimental section.

II. Influence of the number of cells on the metabolism of androgens

The same concentration of substrate was incubated with various numbers of cells for a fixed period of time (Table 2). Using more cells increased the 5α-reduction: a 24 h incubation of $1{\cdot}2 \times 10^6$ cells/ml left no testosterone ($3{\cdot}5 \times 10^{-7}$ M) unmetabolized. The equilibrium in the resulting interconvertible reactions clearly favoured production of tetrahydro metabolites of either the 17-oxo or 17-hydroxysteroid pathway. With DHT as substrate an increased cell number incubated for 6 h yielded an increased DHT metabolism along the 17-hydroxy pathway, without influence on the 17-oxo-steroid pathway. But when incubations were done for 24 h an increase in the number of cells favoured the 17-oxo pathway (Table 3). Although these results are consistent, one could argue that the number of cells does not intervene directly but influences the pH of the medium and so cofactor availability or enzyme equilibria.

III. Influence of substrate concentration on the metabolism of androgens

HTC cells (800,000 cells/ml) were incubated for 24 h with 1 μCi of testosterone or dihydrotestosterone. Non tritiated androgens were added in concentrations ranging from $2{\cdot}1 \times 10^{-8}$ M to $3{\cdot}7 \times 10^{-5}$ M (Table 4). The results show that the production of 5α-reduced metabolites clearly levels off at a substrate concentration of $3{\cdot}7 \times 10^{-6}$ M. Similar experiments were performed with 5α-dihydrotestosterone. In the range

Table 2. Influcnece of number of cells on the metabolism of testosterone (percentage of total amount of radioactive steroids recovered from the chromatogram)

Number of cells per ml	Time (h)	Residual testosterone	DHT production	THT production	Aa-dione production*	a + e Production†
650,000	24	63·8	15·8	11·2	3·2	2·1
800,000	24	25·0	13·2	38·0	2·2	7·0
1,200,000	24	1·8	20·0	43·0	9·5	20·0

* Aa = androstanedione.
† a + e = Androsterone + epiandrosterone.
DHT = 5α-dihydrotestosterone; THT = tetrahydrotestosterone either 3α or 3β.
Incubation of $3{\cdot}5 \times 10^{-7}$ M testosterone with various numbers of cells for a fixed period of time. Conditions of extraction, separation and quantitation are the same as described in the experimental section.

Table 3. Influence of number of cells on the metabolism of 5α-dihydrotestosterone (percentage of radioactivity recovered on the chromatogram)

Number of cells per ml	Time (h)	Residual DHT	THT production	Aa-dione production	a + e Production
870,000	6	65·9	18·3	10·5	2·5
900,000	6	64·0	14·0	11·0	4·5
950,000	6	64·0	11·2	16·5	5·2
950,000	6	29·3	44·8	8·4	11·5
1,200,000	6	36·0	50·0	3·1	10·0
1,520,000	6	40·0	45·0	5·0	—
870,000	12	27·8	26·3	20·0	21·7
950,000	12	46·0	13·5	26·5	9·1
630,000	24	44·0	26·0	8·7	10·0
700,000	24	46·9	32·9	11·2	5·6
800,000	24	26·0	53·5	3·5	12·9
850,000	24	25·3	12·0	37·6	18·2
870,000	24	23·6	13·8	34·0	19·4

DHT = 5α dihydrotestosterone; THT = tetrahydrotestosterone either 3α or 3β; a + e = androsterone + epiandrosterone.
Incubation of $3{\cdot}5 \times 10^{-7}$ M DHT with various numbers of cells for a given period of time. Conditions of extraction, separation and quantitation are the same as described in the experimental section.

of concentrations tested, disappearance of 5α-dihydrotestosterone decreased only moderately. A constant fraction was transformed into THT. As already mentioned, the capacity for 3α- or 3β-hydroxysteroid dehydrogenation seemed to be much higher than the capacity for 5α-reduction and the capacity for 17β-dehydrogenation (Table 5).

IV. Production of tetrahydro metabolites with either a 3α- or a 3β-configuration

The tetrahydro derivatives both of the 17-hydroxyl and the 17-oxo pathway were separated into their 3β- and 3α-components. Between 65 and 75% of total tetrahydro derivatives had a 3β-configuration; in a

Table 4. Influence of substrate concentration on the metabolism of testosterone (percentage of radioactivity recovered on the chromatogram)

Non tritiated substrate (M)	Residual testosterone	DHT production	THT production	a + e Production	Total 5α-reduced metabolites
$2{\cdot}1 \times 10^{-8}$	42·0	13·8	33·4	6·3	53·5
$3{\cdot}7 \times 10^{-7}$	33·7	12·6	41·0	7·6	61·2
$1{\cdot}85 \times 10^{-6}$	54·8	14·6	24·8	2·4	41·8
$3{\cdot}7 \times 10^{-6}$	41·3	14·4	35·1	4·8	54·3
$1{\cdot}85 \times 10^{-5}$	62·6	6·7	17·8	1·2	25·7
$3{\cdot}7 \times 10^{-5}$	80·0	4·1	13·6	0·4	18·1

T = testosterone; DHT = 5α-dihydrotestosterone; THT = tetrahydrotestosterone either 3α or 3β; a + e = androsterone + epiandrosterone.
HTC-cells (800,000 cells/ml) were incubated with 1 μCi T for 24 h. Non tritiated testosterone was added in various concentrations. Conditions of extraction, separation and quantitation were the same as described in the experimental section.

Table 5. Influence of substrate concentration on the metabolism of dihydrotestosterone (percentage of radioactivity recovered on the chromatogram)

Non tritiated substrate (M)	Residual DHT	THT production	a + e Production
$2{\cdot}1 \times 10^{-8}$	26·3	53·4	12·4
$3{\cdot}7 \times 10^{-7}$	26·2	54·1	13·4
$1{\cdot}85 \times 10^{-6}$	24·8	59·2	10·3
$3{\cdot}7 \times 10^{-6}$	36·0	53·2	6·03
$3{\cdot}7 \times 10^{-5}$	45·5	50·8	1·6

DHT = 5α-dihydrotestosterone; THT = tetrahydrotestosterone either 3α or 3β; a + e = androsterone + epiandrosterone.

HTC cells (800,000 cells/ml) were incubated with 1 μCi DHT for 24 h. Non tritiated DHT was added in various concentrations. Conditions of extraction, separation and quantitation were the same as described in the experimental section.

given experiment the coefficient of variation for the latter percentage is around 5%. Contrary to what one observes *in vivo* (*i.e.* [17]), neither preincubation nor incubation with 17β-estradiol, testosterone or cortisol influences this ratio. Under normal basic conditions apparently somewhat more 3β-derivatives were formed by the 17-oxo pathway as compared with the 17-hydroxyl pathway. But this difference is probably artificial due to the small amounts of tetrahydro derivatives formed in the 17-oxo pathway.

V. Inhibition of androgen metabolism with various steroids

Full-grown cultures were incubated for 24 h with labelled testosterone (± 106 ng/ml) or dihydrotestosterone (± 106 ng/ml) as described previously. Various non-labelled steroids were added at the beginning of the incubation in concentrations ranging from 200 to 10,000 ng/ml (Fig. 3). The latter concentrations were used as screening procedure. Some of the results obtained are shown in Tables 6 and 7.

Cortisol and dihydrocortisol had no influence on testosterone or dihydrotestosterone metabolism. Estradiol, in the concentrations used, inhibited the 17β-hydroxysteroid dehydrogenase without influencing the 5α-reductase or the 3α- and 3β-hydroxysteroid dehydrogenase activity. The blockade of the 17β-hydroxysteroid dehydrogenase diminished, of course, the production of 17-oxo derivatives and so increased the production of 17-hydroxy derivatives.

Addition of *large* (10,000 ng/ml) doses of progesterone, 17-epitestosterone and 3-epiandrosterone inhibited completely the metabolism of small (± 106 ng/ml) doses of testosterone. As far as DHT metabolism

Table 6. Inhibition of androgen metabolism by various steroids (percentage radioactivity recovered from the chromatogram)

Incubation of testosterone	Residual T	DHT	THT	a + e	Aa
Experiment 1					
Control	4·32	23·28	30·37	20·28	14·72
$6{\cdot}8 \times 10^{-7}$ M epiandrosterone	30·71	13·59	42·49	6·12	1·59
$1{\cdot}3 \times 10^{-6}$ M epiandrosterone	39·56	12·41	37·47	4·52	1·24
$2{\cdot}1 \times 10^{-6}$ M epiandrosterone	33·15	12·10	42·66	5·22	1·29
Experiment 2					
Control	17·15	41·48	17·68	6·39	12·67
$3{\cdot}4 \times 10^{-6}$ M epiandrosterone	76·40	10·49	8·71	0·91	0·45
$1{\cdot}7 \times 10^{-5}$ M epiandrosterone	84·86	4·64	6·82	0·94	0·15
$6{\cdot}7 \times 10^{-6}$ M epitestosterone	80·30	7·69	6·06	0·89	0·45
$1{\cdot}7 \times 10^{-5}$ M epitestosterone	90·13	1·21	4·34	0·42	0·16
Experiment 3					
Control	25·74	33·01	17·30	6·19	12·08
$3{\cdot}4 \times 10^{-6}$ M epitestosterone	59·24	9·63	20·90	3·07	1·15
$3{\cdot}4 \times 10^{-5}$ M epitestosterone	89·75	0·88	4·88	0·56	1·19
$3{\cdot}4 \times 10^{-5}$ M epiandrosterone	87·81	2·22	5·62	0·59	0·14
Experiment 4					
Control	10·70	24·80	17·70	21·60	19·30
$3{\cdot}1 \times 10^{-5}$ M progesterone	96·60	0·00	0·00	0·00	0·00
$3{\cdot}6 \times 10^{-5}$ M 17β-estradiol	39·30	27·70	23·10	2·80	3·60
$2{\cdot}7 \times 10^{-5}$ M cortisol	15·10	34·00	17·60	14·20	11·10

T = testosterone; DHT = 5α-dihydrotestosterone; THT = tetrahydrotestosterone either 3α or 3β; a + e = androsterone + epiandrosterone; Aa = androstanedione.

Full-grown cultures of HTC cells (approximately 800,000 cells/ml) were incubated with $3{\cdot}5 \times 10^{-7}$ M testosterone. Non-labelled steroids were added in various concentrations at the beginning of the incubation. Conditions of extraction, separation and quantitation were the same as described in the experimental section.

Table 7. Inhibition of androgen metabolism by various steroids (percentage radioactivity recovered from the chromatogram)

Incubation of 5α-DHT	Residual T	THT	a + e	Aa
Experiment 1				
Control	31·54	42·99	12·80	8·96
$3{\cdot}4 \times 10^{-6}$ M epitestosterone	30·49	64·09	2·62	0·65
$6{\cdot}7 \times 10^{-6}$ M epitestosterone	24·63	68·46	3·43	0·80
$3{\cdot}4 \times 10^{-5}$ M epitestosterone	27·93	65·47	3·37	0·77
Experiment 2				
Control	34·50	24·70	13·00	23·20
$3{\cdot}1 \times 10^{-6}$ M progesterone	35·40	37·00	5·60	8·80
$3{\cdot}1 \times 10^{-5}$ M progesterone	31·60	62·80	0·40	0·90
$3{\cdot}6 \times 10^{-6}$ M 17β-estradiol	27·50	35·00	16·50	12·10
$3{\cdot}6 \times 10^{-5}$ M 17β-estradiol	39·80	53·20	1·40	2·50
$2{\cdot}7 \times 10^{-6}$ M cortisol	33·00	32·00	15·00	16·50
$2{\cdot}7 \times 10^{-5}$ M cortisol	32·00	26·00	15·50	22·00

T = testosterone; THT = tetrahydrotestosterone either 3α or 3β; a + e = androsterone + epiandrosterone; Aa = androstanedione.

Full-grown cultures of HTC-cells (approximately 800,000 cells/ml) were incubated with $3{\cdot}5 \times 10^{-7}$ M DHT. Non labelled steroids were added in various concentrations at the beginning of the incubation. Conditions of extraction, separation and quantitation were the same as described in the experimental section.

was concerned, only the production of 17-oxo derivatives was affected. *Small* doses of the latter steroids, *e.g.* 200–600 ng/ml of 3-epiandrosterone, or 1000 ng/ml of 17-epitestosterone, stimulated the production of 3α,5α- and 3β,5α-derivatives of the 17-hydroxyl series and diminished the production of 17-oxo derivatives.

It could be shown by thin-layer chromatography that the 17-epitestosterone added to the HTC cells was metabolized by the latter cells.

DISCUSSION

The metabolism of androgens has been studied in an HTC cell line derived from a hepatoma developed in a *male* rat. It could be demonstrated that testosterone as well as dihydrotestosterone is extensively transformed. Moreover, the pattern of metabolism was reproducible after more than 9 months in at least 25 subcultures. The main enzyme activities detected were 5α-reduction and 3α-, 3β- and 17β-hydroxysteroid dehydrogenation.

The high capacity of the HTC cell line to metabolize androgens contrasts sharply with its relative inability to transform glucocorticoids [4]. This might indicate that in the rat liver a distinct 5α-reductase and distinct 3α/3β-hydroxysteroid dehydrogenases handle androgens and progesterone, while another set of enzymes (also?) handles glucocorticoids *and* that only the former pathway has been retained in HTC cells. Several findings derived from our competition experiments support the latter contention. Firstly, the 5α-reductase in HTC cells is strongly competed for by progesterone and 17-epitestosterone but not by glucocorticoids. Nevertheless, in intact liver cells the latter steroids are reduced equally well. With respect to its substrate specificity the 5α-reductase in HTC cells thus resembles the 5α-reductase described in rat prostate [13], rat kidney [14], human skin microsomes [15] and recently also in rat liver nuclei [16]. Secondly, dihydrocortisol also does not compete for 3α- or 3β-hydroxysteroid dehydrogenases able to transform dihydrotestosterone.

It has been extensively documented that the administration of sex hormones to intact rats during critical periods of life causes temporary or even permanent changes in the activity of steroid metabolizing enzymes [17]. To explore further the molecular base of this phenomenon we attempted to provoke similar inductions in HTC cells by the additions of sex hormones or sex hormone derivatives to the pre- and/or the incubation medium. As 3β-hydroxysteroid dehydrogenase activity has been shown to be a sensitive marker of the influence of androgens on steroid metabolizing enzymes in the liver, the production of 5α-androstan-3β,17β-diol was used as an endpoint in these experiments. Unfortunately we were not able to provoke inductions or repressions of steroid metabolizing enzymes in HTC cells, all the effects observed could be explained by direct competiton of the added steroids for enzyme sites involved in the metabolism of testosterone or dihydrotestosterone. This may indicate either that the ability of HTC cells to respond to sex hormones has been lost during the dedifferentiation process or that other non-steroidal factors intervene in the expression of the male or female pattern of metabolism in liver cells. Arguments for the latter contention have been advanced recently by Denef [18–20]. Consequently, several hypophyseal hormones were added to the preincubation medium but due probably to the absence of an adenylcyclase system in the HTC cells no effects were observed (unpublished observations).

Finally our experiments have put some doubt on the usefulness of the 3β-hydroxysteroid dehydrogenase activity in HTC cells as a parameter of masculinisation. In fact, further exploration of the paradoxical inhibition of the 17β-hydroxysteroid dehydro-

genase activity in experiments with 17-*epi*testosterone showed that, on the one hand, 17-epitestosterone is intensively metabolized to tetrahydro derivatives and, on the other hand, that 3β-derivatives of C_{19}-steroids (for instance 3-epiandrosterone) and possibly also of C_{21}-steroids compete for the active site of the 17β-hydroxysteroid dehydrogenase. This led us to conclude that 3β- and 17β-reduction in HTC cells may be catalyzed by the same enzyme. This enzyme might very well be a 3β,17β-hydroxysteroid dehydrogenase comparable to the one described in rat blood cells [21]. It might differ considerably from the 3β-hydroxysteroid dehydrogenase assayed in intact liver cells in studies concerning the sexual differentiation of androgen and corticoid metabolism. A hepatoma derived from a *female* rate (unpublished observations) also produced sizeable amounts of 3β-derivatives of testosterone.

Acknowledgements—Dr. J. Desmyter made it possible for us to start this work by providing the cell line and the facilities for cell culture. Mrs. M. Schoofs-Franssens typed the manuscript with her customary precision.

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