Journal of Steroid Biochemistry, 1973, Vol. 4, pp. 139–151. Pergamon Press. Printed in Great Britain

IN VIVO AND *IN VITRO* TESTOSTERONE METABOLISM BY THE ANDROGEN INSENSITIVE RAT*

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(*Received* 9 *October* 1972)

SUMMARY

The androgen insensitive (tfm) rat is a male pseudohermaphrodite with inherited tissue insensitivity to androgens. To understand the nature of this genetic defect better, *in vivo* and *in vitro* testosterone metabolism was studied in preputial glands from tfm as well as normal male and female rats. *In vivo* studies in normal rats indicated that testosterone metabolism in cytoplasm and nuclei of preputial glands was similar to that of normal prostate. In the tfm rat, testosterone uptake by the preputial gland was normal, but dihydrotestosterone (DHT) was not retained in cytoplasm or nuclei. Reduction of testosterone to DHT by 5α-reductase in tissue slices of eight organs as well as preputial gland cytoplasm and nuclei from tfm rats was indistinguishable from that in normal animals.

We conclude that the inability of the tfm rat to retain intracellular DHT is due to a defect of a cytoplasmic binding component which normally concentrates DHT in the cell and facilitates its transfer to the nucleus. Since DHT is believed to be one of the intranuclear effectors of testosterone action, the androgen insensitivity of the tfm rat may be explained by the inability to concentrate this steroid at its presumed site of action.

STUDIES of androgen metabolism in prostate have suggested that the mechanism of testosterone† action may be mediated via its metabolite, dihydrotestosterone (DHT)[1–3]. DHT is formed in cytoplasm and transferred to the nucleus of the cell by a specific cytosol receptor protein. This binding is associated with intracellular androgen retention by target organs after the hormone has been cleared from blood. Within the nucleus, the DHT-protein complex is bound to chromatin where androgen dependent stimulation of RNA synthesis is presumably initiated [4–6].

To further document the essential features of androgen action, we have employed a male pseudohermaphroditic rat (tfm)‡[7–12]. This animal has an inherited end organ insensitivity to testosterone and other androgens and has been examined with the assumption that better definition of its gene defect would establish at least one of the steps required for the initiation of androgen action in

*A preliminary report of this study was presented at the Annual Meeting of the American Federation for Clinical Research, May, 1971. These studies were initiated while Dr. Bullock was a special fellow, Endocrine Branch, National Cancer Institute.

†The abbreviations and trivial names used are: testosterone: 17β-hydroxy-4-androsten-3-one; dihydrotestosterone: 17β-hydroxy-5α-androstan-3-one; androstanediol(s): a mixture of: 5α-androstane-3α,17β-diol and 5α-androstane-3β,17β-diol; androsterone: 3α-hydroxy-5α-androstan-17-one; 5α-reductase-NADPH: 4-ene-3-ketosteroid 5α-oxidoreductase; 3-hydroxysteroid dehydrogenase-3α-hydroxy steroid: NAD(P) oxidoreductase, EC 1.1.1.50 and 3-β-hydroxy steroid: NAD(P) oxidoreductase (EC 1.1.1.51).

‡In previous publications from this laboratory the androgen insensitive male pseudohermaphrodite was called the Ps rat.

the cell. Because the tfm rat lacks a prostate, we focused our studies of testosterone metabolism on the preputial gland, an androgen responsive tissue in both normal male and female rats[13]. The present study demonstrates that testosterone metabolism to DHT and androstanediols (DIOL) in tfm rats is similar to that in normal animals and that the androgen insensitivity of these animals correlates with the inability to incorporate DHT into the nucleus of the cell.

EXPERIMENTAL PROCEDURE

Animals. The origin of the tfm rat has been described previously[14]. The defect is transmitted by phenotypically normal females to half of their male offspring. The affected animals have a female phenotype, inguinal testes, and absence of Mullerian and Wolffian derivatives. Mullerian regression is consistent with the known effect of testes on these structures while the lack of androgen dependent differentiation of the Wolffian system is due to the end organ insensitivity to androgens. Normal and tfm male and female rats weighing 250–300 g were obtained from the University of Oklahoma colony established by Stanley and Gumbreck. Holtzman rats were also used in some experiments. Castration was performed under ether anesthesia 3 days prior to study. Animals were killed by cervical dislocation; tissues were removed immediately and placed in ice cold saline. Further processing was performed at 4°C.

Materials. All solvents were glass distilled (Burdick & Jackson Laboratories, Inc., Muskegon, Michigan). Reagents were obtained from the following suppliers: non-radioactive steroids, Mann Research Labs, New York, N.Y.; yeast glucose-6-phosphate dehydrogenase in 3·2M ammonium sulfate, Calbiochem, Los Angeles, California; glucose-6-phosphate and NADP, Sigma Chemical Co., St. Louis, Missouri.

The following solutions were used: *Buffer*-1: 0·32 M sucrose, 0·03 M Tris, 3 mM $MgCl_2$, pH 7·6; *Buffer*-2: 2·2 M sucrose, 0·03 M Tris, 1 mM $MgCl_2$, pH 7·6; *Buffer*-3: 0·14 M NaCl, 8·8 mM $Na_2HPO_4 \cdot 7\ H_2O$, 2·7 mM KCl, 1·5 mM KH_2PO_4, 0·7 mM $CaCl_2 \cdot 2H_2O$, 0·5 mM $MgCl_2 \cdot 6\ H_2O$, 6·7 mM glucose, pH 7·6; *Buffer*-4: 1 unit glucose-6-PO_4 dehydrogenase per ml of 0·01 M Tris, 0·05 M NaCl, 5·0 mM $MgCl_2$, 0·5 mM mercaptoethanol, 5×10^{-5} M EDTA, 2·5 mM glucose-6-PO_4, 0·2 mM NADP, pH 7·0.

The following radioactive steroids were purchased from New England Nuclear Corp.: [1-2-^{3}H]-testosterone (45 Ci/mmol), [1,2-^{3}H]-dihydrotestosterone (46 Ci/mmol), and [4-^{14}C]-testosterone (56 m Ci/mmol). A small aliquot of radioactive steroid was added to the appropriate unlabeled steroid and purified by thin layer chromatography (t.l.c.) using solvent system benzene: ethyl acetate 3 : 2 for testosterone and chloroform : methanol 49 : 1 for dihydrotestosterone. In all cases at least 92% of the radioactivity was eluted with the carrier steroid. The bulk of the labeled steroid was then chromatographed in the appropriate t.l.c. system and eluted relative to a dye marker. Other [^{14}C]-steroids were synthesized by incubating 300 mg of minced female rat preputial gland with 5 μCi of [4-^{14}C]-testosterone. The contents of the incubation flask were homogenized and extracted with 8 volumes of methylene chloride. [^{14}C]-DHT and [^{14}C]-DIOLS were purified as described under isolation of radioactive steroids (below). The steroid acetates were saponified under nitrogen in 0·15 N NaOH in 80% methanol. The steroid alcohols were developed in system t.l.c.-III and 5α-androstane-3α,17β-diol and 5α-androstane-3β, 17β-diol separated by paper chromatography in a

modified Bush A_2 system. The radiochemical purity of the isolated steroids was established by crystallizing a portion of each sample with authentic standards.

Chromatography. Thin layer chromatography was performed on 20 × 20 cm glass plates coated with silica gel GF (type D: Analtech Inc., Wilmington, Delaware) in the following solvent systems: t.l.c.-*I*-benzene, t.l.c.-*II*-chloroform: methanol 49:1 v/v, t.l.c. III-benzene: ethyl acetate 4:1 v/v, t.l.c. IV-benzene: ethyl acetate 9:1 v/v. Appropriate marker steroids were included in each plate. Steroids were located on t.l.c. plates by Rhodamine-6-G spray, iodine vapor or U.V. absorption. Paper chromatography was performed on Whatman No. 1 paper in a modified Bush A_2 system (Ligroin: methanol: water 10:7:3 by vol). Steroids were visualized on paper by U.V. absorption, Zimmerman reaction or alcoholic sulfuric acid and radiographic peaks were located with a strip scanner (Actigraph III-Nuclear Chicago). Samples were eluted with acetone and ethanol.

Isolation of cytoplasm and nuclei. From 0·8 to 1·0 g of preputial gland or prostate from 6 to 10 rats was used in each experiment. In some studies 1·0 g of liver from 1 to 2 animals was also used. Tissues were minced and homogenized in 4 ml of buffer-1. Homogenates were filtered through flannel and centrifuged at 800 *g* for 10 min. The supernatant was decanted and designated cytoplasm. The crude nuclear pellet was suspended in 1 ml buffer-1, layered on 4 ml of buffer-2 and centrifuged at 50,000 *g* (ave) for 60 min (Spinco Model L-SW 39) at 2°C. The nuclear pellet was suspended in buffer-1 or 4 and dispersed with several strokes in a Dounce homogenizer. An aliquot of nuclei was stained with methylene blue, and counted in a Spencer counting chamber.

Nuclei from all tissues were grossly free of cytoplasmic tags by light microscopy, although preputial gland preparations did contain some cellular debris. Electron microscopy revealed that preputial gland nuclei had small cytoplasmic tags which were not evident under phase contrast microscopy. When β-glucuronidase was used as a marker enzyme, the purified nuclear preparation contained less than 0·02% of original cytoplasmic protein. While nuclei from prostate and liver were relatively uniform in size, preputial gland nuclei were variable. Some were similar in size to those of prostate and contained both eu- and heterochromatin. Others, however, were small and heterochromatic and in various stages of degeneration. Only the larger uniform nuclei containing some euchromatin were counted since radioautographic studies indicated that only these nuclei concentrated radioactivity following an IV dose of [^{3}H]-testosterone [10]. Based on the number of viable nuclei counted, the DNA content per nucleus was 8×10^{-12} g in liver and 32×10^{-12} g in preputial gland. The high DNA content calculated for preputial gland nuclei is due to the wholocrine nature of the gland which results in the presence of a significant number of cells undergoing lysis. The degenerating nuclei of these cells contribute DNA yet are nonviable and were not counted.

In vivo *uptake.* 175 μCi of [^{3}H]-testosterone in 1 ml 5% ethanol was injected into the jugular vein of 6 castrated, ether anesthetized male or tfm rats. The animals recovered from anesthesia and were killed 30, 60 and 90 min after treatment. Tissues were removed, pooled and minced. Samples of 900–1000 mg were taken for isolation of cytoplasm and nuclei, necessitating, in the case of preputial glands, the addition of several hundred mg of tissue from uninjected rats. Corrections were made in the final calculations so that radioactivity was expressed per unit amount of nuclei or protein from animals that received [^{3}H]-testosterone. Cytoplasm and nuclei were divided into aliquots for determination of total methylene

chloride extractable radioactivity and for isolation of individual radioactive metabolites. Total radioactivity was determined in cytoplasmic samples with average protein concentrations of 3·9, 4·8, 12·5 mg and on nuclear samples of 0·52, 6·6, $4{\cdot}0 \times 10^6$ nuclei for preputial gland, prostate and liver respectively. Fractions analyzed for steroid metabolites averaged 27, 32, 84 mg cytoplasmic protein and 3·6, 73, 54×10^6 nuclei for preputial gland, prostate and liver, respectively.

In vitro *metabolism*. Tissue minces and cell fractions were incubated at 37°C with constant shaking (Dubnoff incubator) under 95% O_2: 5% CO_2. Samples were pre-incubated for 5 min prior to the addition of substrate. Reactions were stopped by freezing in dry ice-acetone and samples were held at −16°C until analysis.

In mince incubations tissues from 3 to 5 animals were pooled and aliquoted (80–100 mg) into 2 ml buffer-3. [1,2-^{3}H]-testosterone (140,000 c.p.m., 5×10^{-7} M) was added in 10λ ethanol and incubated for 15–240 min. Samples were homogenized with a teflon pestle before extraction.

In incubations of cell fractions, purified nuclei ($1{\cdot}1$–$1{\cdot}6 \times 10^7$) were added to 2·0 ml of buffer-4 and 1·5 ml cytoplasm (8–10 mg protein) were added to 0·5 ml of a 4-fold concentration of buffer-4. [1,2-^{3}H]-testosterone (140,000 c.p.m., 5×10^{-6} M) was added in 10λ ethanol and incubated for 7·5–60 min in cytoplasm and 60–120 min with nuclei.

Isolation of radioactive steroids from tissue. Testosterone, DHT and DIOLS (200–900 c.p.m.-^{14}C) were added to each sample to correct for procedural losses. In addition, 50–70 μg of each of these steroids were added as cold carrier. Samples were extracted with 6–8 volumes methylene chloride and extracts were washed successively with 0·1 N NaOH, 6% acetic acid and water. Samples for determination of total radioactivity were transferred directly to counting vials, while samples for isolation of individual metabolites were spotted on t.l.c. plates, developed in t.l.c. I to remove non-polar lipids, then compressed into a fine line at the origin with acetone and developed in t.l.c. II.

This chromatography resolved each sample into three major fractions (testosterone, DIOL and DHT plus androsterone) which were acetylated. Testosterone acetate and androstanediacetate were developed in t.l.c. III and IV respectively. Androsterone and DHT acetates were resolved in t.l.c. IV. Recovery of [^{14}C]-steroid markers through extraction and 2 chromatographies was 71, 73 and 76% for testosterone, DHT and DIOL respectively. Purity of the testosterone and DHT acetates was confirmed by constant specific activity of crystals in the initial two ethanol–water crystallizations.

Measurement of radioactivity. Dried samples were dissolved in 5 ml phosphor [42 ml Liquifluor (New England Nuclear) and 1000 ml toluene] and radioactivity measured in a Packard liquid scintillation counter (Model 4322). The [^{14}C]-runover into the [^{3}H]-channel was 24% and the [^{3}H]-runover into the [^{14}C]-channel was less than 0·1%.

Assays. Protein was determined by the method of Lowry *et al.*, using bovine serum albumin as standard[15]. DNA was determined by the method of Burton using calf thymus DNA as standard[16]. β-glucuronidase activity was measured by the method of Fishman using phenolphthalein as standard[17].

RESULTS

In vivo *metabolism of* [^{3}H]-*testosterone*. In a typical androgen target organ

such as prostate, DHT accumulates in cytoplasm and nuclei as a product of testosterone metabolism. In the liver, however, testosterone undergoes extensive oxidative and reductive metabolism so that very little DHT accumulates[1]. In order to characterize *in vivo* preputial gland testosterone metabolism, it was thus pertinent to compare this organ with prostate and liver. Following IV administration of [^{3}H]-testosterone to normal rats, the total cytoplasmic radioactivity in preputial gland and prostate was less than that of liver; in addition, nuclear radioactivity in the latter tissues was at least 10-fold greater than in liver (Fig. 1). Further similarities of androgen metabolism in preputial gland and prostate were evident when individual metabolites were measured (Fig. 2). DHT was the major [^{3}H]-androgen isolated from cytoplasm and nuclei of preputial gland and prostate. By contrast, DIOLS were the predominant metabolites isolated from

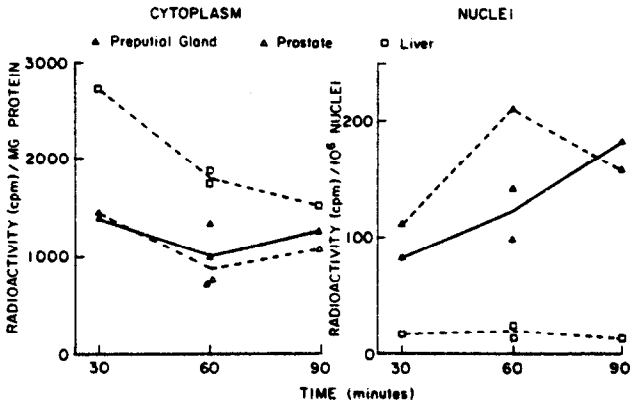

Fig. 1. Total methylene chloride extractable radioactivity in cytoplasm and nuclei of tissues from castrated male rats following intravenous [1,2-^{3}H]-testosterone (175 μCi). Each experiment of 6 animals is shown as a single point. Tissue preparation and steroid extraction were as described in the text.

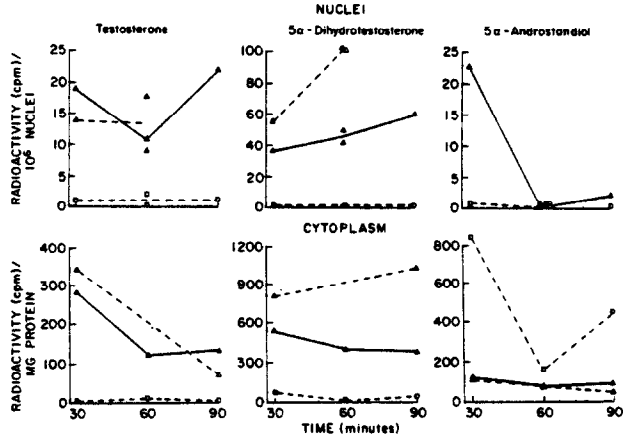

Fig. 2. Radioactive steroids isolated from cytoplasmic and nuclear fractions of tissues from castrated male rats following intravenous [1,2-^{3}H]-testosterone (175 μCi). These data and those in Fig. 1 were from the same groups of animals. Tissue preparation and steroid isolation were as described in the text. Preputial gland (▲———▲), prostate (△----△). liver (◙----□).

liver cytoplasm, and nuclei contained only a very low androgen concentration. These observations suggest that the uptake and intracellular distribution of testosterone and its metabolites in preputial gland are similar to that of prostate.

In vivo testosterone metabolism by preputial glands and liver of normal male and tfm rats was then compared. The distribution of total radioactivity between nuclei and cytoplasm following intravenous administration of [^{3}H]-testosterone is shown in Fig. 3. Total radioactivity in cytoplasm and nuclei from preputial glands of normal and tfm rats was similar during the 1st h of study. This observation was confirmed by dry-mount autoradiography[10]. After 90 min, however, preputial gland cytoplasmic and nuclear radioactivity in tfm rats had markedly decreased compared to that in normal androgen sensitive animals. There was a similar decrease in total radioactivity in hepatic cytoplasm from tfm rats compared to normal animals. There was no difference in the tritium content of hepatic nuclei from the two groups.

Testosterone and its 5α-metabolites were then isolated from the methylene chloride extracts of cytoplasm and nuclei. Two to 3 times more testosterone and 1·5–9 times more DHT were recovered from preputial gland cytoplasm of normal males than from that of tfm males (Fig. 4). Similarly, liver cytoplasm from normal rats contained more DHT and DIOL than cytoplasm from pseudohermaphrodites. An even greater difference between normal and tfm rats was evident when nuclear androgen content was examined. DHT concentration in preputial gland nuclei from normal rats was up to 12 times that from tfm rats (Fig. 5). Although the DHT content of liver nuclei was several orders of magnitude lower than in preputial gland, a similar difference between normal and tfm rats was observed (Fig. 5). From these observations we conclude that the most striking abnormality of androgen metabolism in tfm rats is the inability to concentrate DHT in the cell. This observation is particularly pertinent since DHT is thought to be the intranuclear effector of testosterone action in some tissues[2, 18].

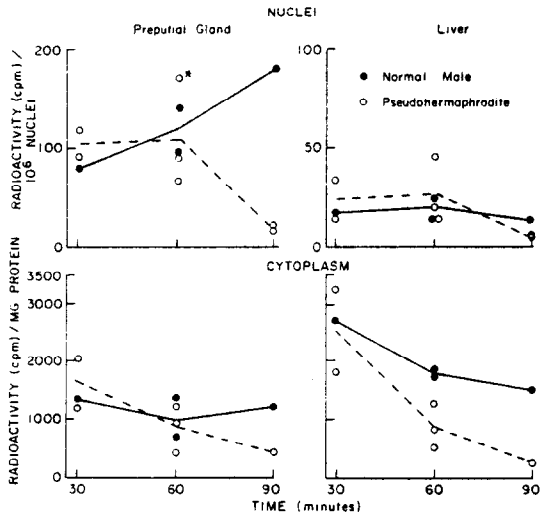

Fig. 3. Total methylene chloride extractable radioactivity in cytoplasm and nuclei of tissues from castrated male and tfm (pseudohermaphrodite) rats following intravenous [1,2-^{3}H]-testosterone (175 μCi) administration. Each point represents a single experiment in which the tissues from 6 rats were pooled. The experiment designated with the asterisk (*) is also shown in Fig. 5.

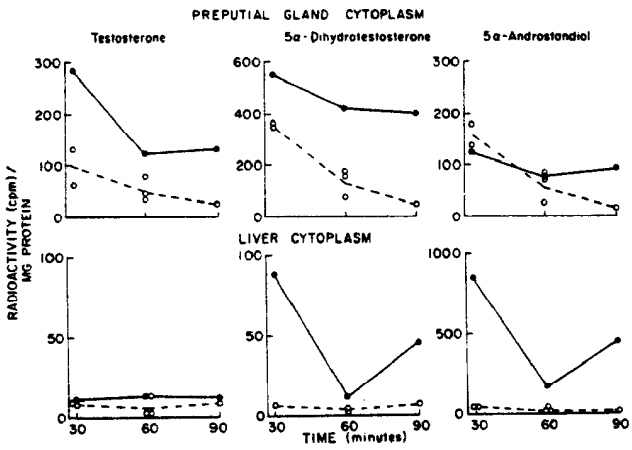

Fig. 4. Radioactive steroids isolated from cytoplasm from castrated male and tfm rats following intravenous administration of [1,2-^{3}H]-testosterone (175 μCi). These observations and those of Fig. 3 were from the same groups of rats. Each point represents a single experiment in which the tissues from 6 rats were pooled. Steroids were fractionated in 2 thin layer systems (see text) and corrected for procedural losses by recovery of [^{14}C]-markers. Male (●——●), tfm (○----○).

In vitro *testosterone metabolism by tissue minces*. As low tissue levels of DHT could result from a decreased rate of testosterone metabolism, it was important to examine 5α-reductase activity in tissues of the androgen insensitive rat. The following *in vitro* studies were of added interest since defective DHT formation, secondary to reduced 5α-reductase activity, has been postulated as a cause for androgen insensitivity in man[19–21].

Studies were first performed to ascertain optimal conditions for testosterone metabolism by preputial gland minces. Following 1 h of incubation in buffer-3,

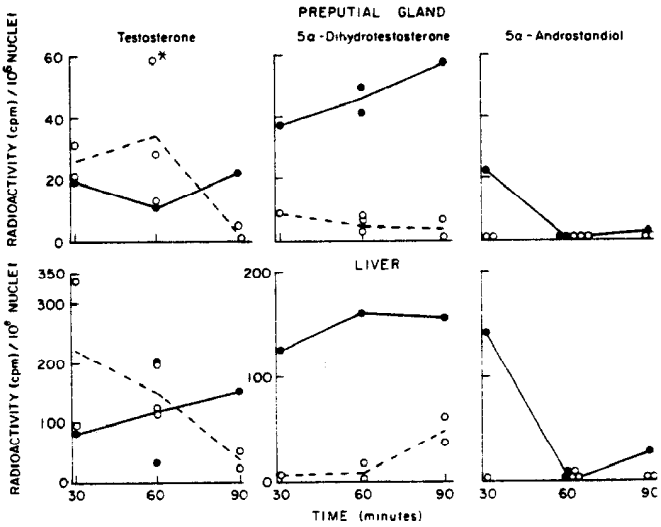

Fig. 5. Radioactive steroids isolated from nuclei of castrated male and tfm rats following intravenous administration of [1,2-^{3}H]-testosterone (175 μCi). These observations were made on the same group of rats as in Figs. 3 and 4. Male (●——●), tfm (○----○).

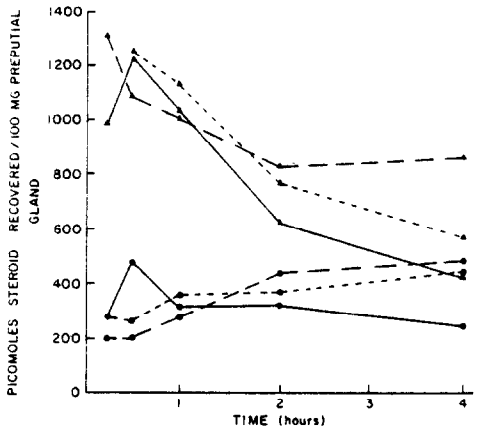

Fig. 6. Dihydrotestosterone metabolism by preputial gland. Tissues (80–100 mg) from male, female and tfm rats were minced and incubated with [1,2-^{3}H]-dihydrotestosterone (146,000 c.p.m., 5×10^{-7} M) as described in the text: Male (————), female (----), tfm (– –). Dihydrotestosterone remaining (▲) and androstanediol formed (●) were isolated by thin layer chromatography.

testosterone conversion to DHT, like that by prostate[1], increased linearly with substrate concentrations between 5×10^{-8} and 5×10^{-6} M. A substrate concentration of 5×10^{-7} M was used in subsequent incubations. DHT formation from testosterone (5×10^{-7} M) was linear during 1 h of incubation. The addition of an NADPH generating system or 50 units of penicillin G plus 5 mg of streptomycin was without effect upon DHT accumulation. DHT (5×10^{-7} M) was degraded at a similar rate by preputial gland minces from male, female and tfm rats during 2 h of incubation (Fig. 6).

Since all organs of the tfm rat examined to date are relatively androgen insensitive[10], 5α-reductase activity was studied in several tissues and the results are

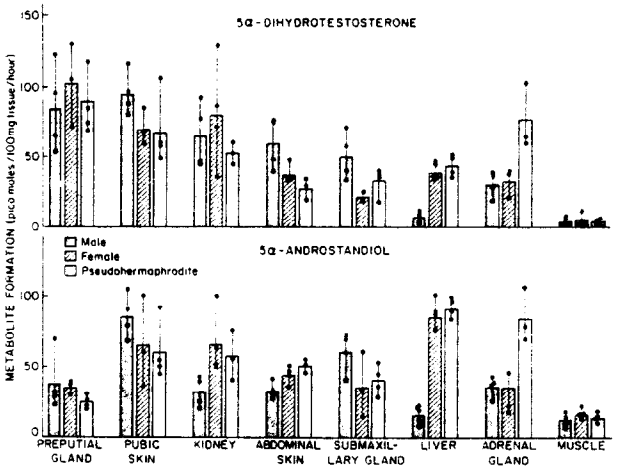

Fig. 7. Testosterone metabolism to dihydrotestosterone and androstanediols by tissue minces from normal and tfm (pseudohermaphrodite) rats. Tissues (80–100 mg) were incubated with [1.2-^{3}H]-testosterone (140.000 c.p.m., 5×10^{-7} M) for 60 min and the metabolites isolated as described in the text. Bars give mean results from individual incubations.

shown in Fig. 7. Enzyme activity in tissues from male animals was similar to that reported by other investigators[18]. When organs from male, female and tfm rats were compared, three patterns of metabolism were observed. In the first group (preputial gland, kidney and muscle) 5α-reductase activity was the same in tissues from all three types of animals. In the second group (liver, submaxillary gland and skin) there was a sex difference in enzyme activity and the activity in tfm rats was similar to that of females. Since the sex related pattern of enzyme activity in liver is androgen dependent[22], 5α-reductase may also be androgen responsive in submaxillary gland and skin. In addition, this enzyme is androgen dependent in prostate[23]. In the third group (adrenal) 5α-reductase activity in the tfm rat was increased over that of both male and female rats. The increased adrenal enzyme activity is possibly related to elevated LH levels in tfm animals[11]. This latter postulate is consistent with the adrenal hyperplasia and neoplasia associated with castration induced hypergonadotrophism in mice[24]. It is particularly significant that neither DHT nor DIOL formation in the tfm rat was lower than normal in any of the 8 tissues examined. While these experiments suggest that total 5α-reductase activity in tfm animals is normal, it is possible that an abnormality of this enzyme in the nucleus could have been obscured in the mince studies. This consideration led to the following investigations.

In vitro *testosterone metabolism by cytoplasm and nuclei.* Initial studies were done to determine the effects of various incubation conditions on testosterone metabolism by cytoplasmic and nuclear fractions from preputial glands of normal rats. Increasing final cofactor concentration in cytoplasm up to 4 times that shown for buffer-4 had no effect on testosterone (5×10^{-6} M) utilization or DHT accumulation during 15 min of incubation. In subsequent studies, cofactor was added to cytoplasm such that the final concentration was that given for buffer-4. DHT and DIOL accumulation in cytoplasm was a function of testosterone concentration up to 5×10^{-6} M (Fig. 8). At higher substrate concentrations DHT and DIOL formation plateaued. It was of interest to note that at all substrate concentrations of 5×10^{-6} or below the ratio of DIOL to DHT was approximately unity (Figs. 8, 9). When the DIOL isomers were resolved by paper chromatography, 85–90% was in the form of 5α-androstane-3β, 17β-diol.

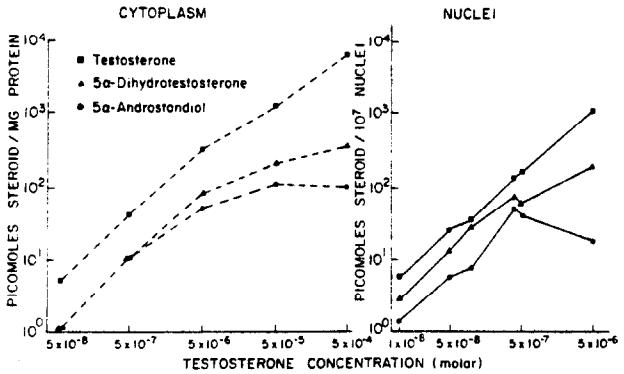

Fig. 8. Testosterone metabolism by cytoplasmic and nuclear fractions of preputial glands from normal rats. Increasing concentrations of testosterone (140,000 c.p.m.-^{3}H) were added to samples containing a NADPH generating system (buffer-4) and incubated in cytoplasm for 15 min and nuclei for 60 min. Sample preparation and steroid isolation were described in the next.

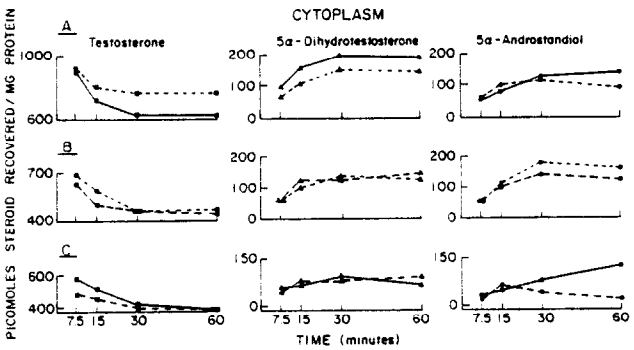

Fig. 9. Testosterone metabolism by preputial gland cytoplasm from male, female and tfm rats. Cytoplasm was incubated with [1,2-^{3}H]-testosterone (140,000 c.p.m., 5×10^{-6} M) and a NADPH generating system as described in the text. Three separate experiments (A, B, C) were performed pairing the animals as shown. Male (———), female (----), tfm (– – –). Testosterone (■) and its metabolites, dihydrotestosterone (▲) and androstandiol (●) were isolated by thin layer chromatography.

Nuclear DHT formation increased linearly with number of nuclei (1×10^6 to 1×10^7), time (15–120 min), and substrate concentration (1×10^{-8} to 5×10^{-6} M testosterone) (Fig. 8). The fact that "nuclear" DIOL formation was also dependent upon testosterone concentration up to at least 5×10^{-7} M was of some concern since in prostate little, if any, [18, 25, 26] 3-hydroxy steroid dehydrogenase is thought to be in the nucleus. Even though electron microscopy and β-glucuronidase determination indicated that cytoplasmic contamination was small, one could still conclude that DIOL and possibly DHT isolated from nuclear preparations were formed by preputial gland cytoplasmic contamination. The possibility, however, that a major fraction of nuclear DHT was of cytoplasmic origin was precluded by comparing the DHT: DIOL ratio obtained from cytoplasmic and nuclear incubations. In cytoplasm the ratio was near unity; whereas, in nuclei the ratio was greater than one. In similar studies of preputial gland nuclei by Richardson and Axelrod [27] the DHT : DIOL ratio was 3. Thus, even if one assumed that all DIOL recovered from nuclear incubations resulted from cytoplasmic contamination relative nuclear 5α-reductase activity could still be estimated by the difference in DHT and DIOL concentrations.

Testosterone metabolism by preputial gland cytoplasmic and nuclear fractions from male, female or tfm animals were compared in 3 separate studies and the results are summarized in Figs. 9 and 10. These experiments show that there is no significant difference in androgen metabolism in preputial gland cytoplasm or nuclei from the three groups. These studies, as well as those on tissue minces, indicate that decreased 5α-reductase activity cannot explain the decreased retention of DHT in the cytoplasm and nucleus of the tfm rat.

DISCUSSION

Inherited end organ insensitivity to androgens has been described in rat [7], mouse [28], cattle [29] and man [30]. In each species, the defect is transmitted by the female to ½ of her male offspring in a pattern consistent with either a sex limited autosomal dominant or an x-linked recessive gene. Linkage studies have

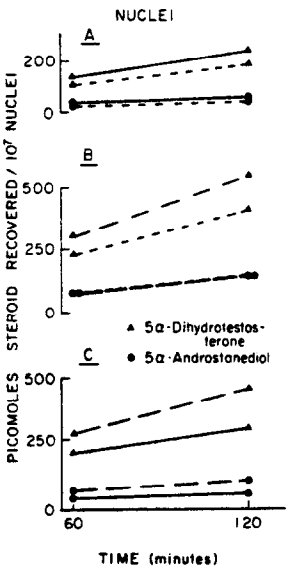

Fig. 10. Testosterone metabolism by isolated preputial gland nuclei from male, female and tfm rats. Nuclei ($1{\cdot}2 \times 10^7$–$1{\cdot}6 \times 10^7$) from the groups (A, B, C) shown in Fig. 9 were incubated with [1,2-^{3}H]-testosterone (140,000 c.p.m., 5×10^{-6} M) and a NADPH generating system. Male (———), female (----), tfm (— —).

shown the latter mode of inheritance to exist in the androgen insensitive tfm/y mouse[31]. The tfm defect results in androgen-insensitivity of all end organs. As a consequence, tissues such as prostate, Wolffian duct derivatives and external genitalia, which require androgens for growth during fetal developement, do not differentiate and the tfm animal develops as a male pseudohermaphrodite.

Androgen metabolism has been extensively described for prostate, but the absence of this organ in tfm rats necessitated the selection of another organ for study. The preputial gland does not require testosterone for fetal organogenesis although it is androgen responsive in the normal adult rat. The present investigation, along with others in the literature, indicates that the preputial gland and prostate share certain features characteristic of androgen target organs: (a) Both tissues respond to androgen administration with a several fold increase in protein, RNA and DNA; (b) Preputial gland and prostate accumulate testosterone and its metabolites at greater concentrations than are found in blood; (c) The cytoplasmic and nuclear distribution of testosterone metabolites are similar in both organs. These similarities imply that androgen action may be initiated in both the preputial gland and prostate by a common series of metabolic events.

In prostatic cytosol, testosterone is metabolized to DHT which is then bound to a 7S protein receptor[1–6, 32]. The DHT-protein complex is converted to a 4–3.5S complex which binds to nuclear chromatin[2, 5]. The specificity of DHT binding in prostatic nuclei resides in both the 7S cytosol receptor and in the acidic proteins of chromatin[5]. Testosterone stimulation of prostatic RNA and protein synthesis may be initiated by the chromatin bound DHT-receptor complex [2, 33]. A similar mechanism has also been proposed for the action of estradiol and progesterone on their respective target tissues[34, 35]. It was thus pertinent

to compare intracellular androgen metabolism and retention in normal and insensitive tfm animals.

Androgen retention by preputial glands of tfm rats was less than normal following a single IV injection of [^{3}H]-testosterone. The greatest difference was in intranuclear DHT accumulation where concentrations in normal males were up to 12 times that of tfm animals. A similar decrease in intranuclear androgen retention has been noted in androgen insensitive tfm/y mice[36]. Since DHT is thought to be the intranuclear mediator of androgen action in several tissues, the inability of the tfm rat to concentrate this steroid at its active site could explain the androgen insensitivity. Low DHT levels in tfm tissue could be due to decreased 5α-reduction of testosterone. This explanation was excluded, however, as 5α-reductase activity was normal in studies of testosterone metabolism by tissue minces, cytoplasm and nuclei from tfm animals.

It is of interest that reduced 5α-reductase activity has been observed *in vivo* and *in vitro* in patients with "testicular feminization" and has been suggested as the basis for androgen-insensitivity in these subjects[19–21]. It should be noted, however, that patients with testicular feminization, prepubertal boys and women all excrete less 5α-metabolites than normal men, pubertal boys and virilized women[19], suggesting that 5α-reductase activity in man is androgen dependent. If this interpretation is correct, the low 5α-reductase activity in patients with testicular feminization is the result, rather than the cause of the androgen insensitivity. The demonstration that men[37, 38] and rodents[28, 39] with testicular feminization are unresponsive to DHT further supports the concept that reduced formation of this steroid is not etiologic to the end organ insensitivity.

An alternative explanation for low DHT levels in preputial glands of the androgen insensitive rat is that the tfm mutation results in reduced binding activity of the cytosol receptor for DHT. This abnormality would not only account for decreased retention of DHT in cytoplasm but would also explain the inefficient transfer of this steroid into the nucleus. This postulate was recently proven for both the tfm rat and tfm/y mouse in this laboratory[40], and by Gehring *et al.*[41] for the tfm/y mouse. The requirement of cytosol receptor for nuclear androgen transfer and action is supported by studies with anti-androgens such as cyproterone acetate which exert their biologic effect by competition with DHT for the 7S binder[2, 42]. From the available observations, it is not possible to ascertain whether reduced DHT retention by the tfm cell is due to a reduction in total cytosol binding sites or to altered affinity of androgens for the receptor. It is unlikely that a defect in nuclear binding *per se* could explain all the observations in the present study since, if the abnormality were in the nucleus, normal DHT uptake and retention would be expected in cytoplasm.

By custom, studies of androgen action have compared "sensitive" tissue such as prostate with "resistant" tissues such as liver. In fact, however, androgens exert some biological effect on almost all organs of the rat. The occurrence of a genetic defect in the tfm rat which produces a generalized end organ insensitivity implies that androgen action may be implemented by a common mechanism in many tissues. The decreased retention of DHT by liver as well as preputial gland nuclei from tfm rats is consistent with this hypothesis. A logical extension of this consideration is that the two step mechanism of steroid induced gene activation, involving cytoplasmic binding followed by nuclear binding of a steroid-protein complex, may be generally applicable to all androgen responsive tissues.

ACKNOWLEDGEMENTS

This research was supported in part by USPHS Grant HD 05276 and the Lalor Foundation. We wish to thank Drs. A. J. Stanley, L. G. Gumbreck and J. E. Allison for supplying the normal and tfm rats.

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