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ANDROGEN METABOLISM BY AND BINDING TO RABBIT EPIDIDYMAL TISSUE: STUDIES ON NUCLEI

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SUMMARY

When minces of epididymides from 3-day castrated rabbits are incubated with [^{3}H]-testosterone at 23°C, macromolecular bound radioactivity enters nuclei. The increase in radioactivity associated with the nuclear fraction is accompanied by a decrease in radioactivity bound to the cytoplasmic receptor indicating that the classically described transfer of the hormone–receptor complex has occurred. Radioactivity is associated with at least two distinct compartments within nuclei. Macromolecular bound hormone can be released from one of these compartments by extracting the nuclei with 1.0 M KCl. The other compartment, which is associated with the post KCl treated nuclear debris, is resistant to salt extraction. Radioactivity can be released from this compartment by treatment of the debris with ethanol. The kinetics of association of radioactivity appears to be more rapid in the salt-solubilized than in the salt resistant fraction. On an absolute basis more radioactivity is associated with the salt resistant than with the salt solubilized fraction of nuclei. [^{3}H]-testosterone and [^{3}H]-5α-dihydrotestosterone (5αDHT) can be detected in both compartments. No other metabolite of testosterone could be isolated from nuclei. When the minces were incubated for 1 hr with increasing concentrations of [^{3}H]-testosterone at 23°C, [^{3}H]-testosterone was the predominant androgen present in both nuclear compartments. When the incubation at 23°C was extended for 2 h [^{3}H]-5αDHT was the predominant androgen in the salt-solubilized compartment, but [^{3}H]-testosterone remained the major androgen in the salt resistant compartment. The [^{3}H]-testosterone and the [^{3}H]-5αDHT binding sites are saturable in both compartments.

INTRODUCTION

The epididymis is an organ that is dependent on the presence of androgen for the maintenance of its structural and functional integrity [1–2]. Since the current hypothesis regarding the mechanism of steroid hormone action posits that these hormones interact with cytoplasmic receptors and that the hormone–receptor complex be transferred to nuclear sites, these events have been extensively investigated in the rat [3–6] and the rabbit [2, 7–8] epididymis. Studies on the mechanism of androgen action must also take into consideration the additional phenomenon of its metabolism by target tissues [9–11]. In previous communications, we have demonstrated the presence of an epididymal androgen cytoplasmic receptor [7], the nuclear transfer of the androgen–receptor complex [8], the metabolism of testosterone by the rabbit epididymis and the binding of the metabolites to the cytoplasmic receptor [8, 12].

We have previously shown [8] that at least two androgen binding compartments exist within epididymal nuclei. Bound androgens can be solubilized from one of these compartments by using solutions of increasing ionic-strength. The other compartment is present in the debris sedimented from nuclei following KCl extraction. The androgens present in this fraction are resistant to extraction with 1.0 M KCl, but can be solubilized with organic solvents [8].

The studies to be reported here were designed: (a) to examine nuclear transfer of the androgen–receptor complex under conditions that would permit varying amounts of androgen metabolism, (b) to determine what androgen metabolites were present in the nuclear compartments following various incubation conditions, and (c) to ascertain whether quantitative and/or qualitative differences might exist between the androgens bound to the KCl solubilized fraction and those present in the nuclear debris.

MATERIALS AND METHODS

Chemicals. [1,2,6,7-^{3}H]-testosterone (85 Ci/mmol) was purchased from New England Nuclear Corporation and purified by thin layer chromatography (TLC) prior to use. Unlabeled steroids were purchased from Steraloids. Ultra pure sucrose and buffer grade Tris (base) were purchased from Schwarz-Mann. Spectrafluor was purchased from Amersham-Searle. Pre-coated silica gel thin layer chromatography plates were purchased from EM Laboratories, Inc. and Sephadex was purchased from Pharmacia Fine Chemicals. All other chemicals used were reagent or analytical grade.

Animals and surgical procedures. Sexually mature (at least six months old) New Zealand white rabbits were used in all the experiments reported here. Animals to be castrated were anesthetized with sodium

nembutal (30 mg/kg). The scrotum was incised in the midline and a small incision was made in the tunica vaginalis testis through which the testis and epididymis were withdrawn. The epididymis was dissected free of the testis and the testicular blood vessels were ligated. The testis was excised, the epididymis was returned to the scrotum, and the incisions were closed.

Preparation and incubation of tissue. Three days following castration, the rabbits were killed by administering a lethal dose of nembutal. The epididymides were removed from 2 animals, trimmed of fat and connective tissue, rinsed in TES buffer [10 mM Tris–HCl (ph 7.5), 1.0 mM EDTA, 0.25 M sucrose], and weighed. The weighed tissues were pooled, thoroughly minced with iridectomy scissors, and divided into aliquots of approximately 800 mg. The aliquots were then mixed with [^{3}H]-testosterone alone or in combination with unlabeled testosterone, and TES in a final vol. of 2 ml. The samples were then incubated for various periods of time at 0°C and/or at 23°C. All procedures following the incubations were conducted at 0–4°C.

Preparation and analysis of cytosol. At the conclusion of the incubation period, the minces were homogenized using a Polytron Pt-10. The homogenates were centrifuged for 15 min at 1,020 *g*, the supernatants were decanted and further centrifuged for 30 min at 229,000 *g* to obtain the cytosol fraction. Unlabeled testosterone was added to the cytosol after incubation at a concentration of approximately 8.6×10^{-5} M to dissociate hormone bound to the rapidly dissociating binding moieties [6, 13–16], androgen binding protein (ABP) and testosterone binding globulin (TeBG) which might be present in the cytosol. The remaining bound hormone should represent androgens bound to the slowly dissociating epididymal androgen receptor [6–7, 12–13, 5]. After the cytosols had been incubated with unlabeled hormone for at least 30 min, a 1 ml aliquot of each cytosol was applied to 6 mm × 17 cm columns of Sephadex G-25 and eluted with TE buffer [10 mM Tris–HCl (pH 7.5) 1.0 mM EDTA]; 45 fractions (≅0.25 ml each) were collected. The flow rate of the columns was approximately 30 ml/h. The columns were used to separate macromolecular bound radioactivity from that which was not bound. Column fractions known to correspond to the regions of bound and free radioactivity were pooled separately and extracted with ether.

Preparation and analysis of nuclei. The 1,020 *g* pellets obtained from the homogenates of epididymal tissue were resuspended in 10 ml of TES using a glass Teflon homogenizer. The resuspended pellets were poured over cheese cloth and the cloth was rinsed with 30 ml of TES. The filtrates were centrifuged at 4,080 *g* for 15 min. The supernatants were discarded, the pellets were resuspended in 40 ml of TES and the wash step was repeated twice. The washed nuclear pellets were resuspended in 1.0 ml of 1.0 M KCl using a glass rod with a rubber tip. The samples were then incubated on ice for one hour to extract nuclear bound receptor. At the conclusion of the incubation period, the samples were centrifuged at 16,000 *g* to sediment the nuclear debris. The supernatant fluid was chromatographed on G-25 columns as described above for cytosol. The nuclear debris was resuspended in deionized water and transferred to conical tubes and extracted with ether as described below for the column fractions.

Extraction and characterization of radioactive steroids. The pooled column fractions were extracted three times with anhydrous diethyl ether using 10 ml of ether for each extraction. The organic phases were combined and 50 μg each of testosterone; 5α-androstan-17β-hydroxy-3-one (5αDHT); 5α-androstane-3α,17β-diol; and 5α-androstane-3β, 17β-diol were added. The ether was then evaporated to dryness. The walls of each tube were washed with 2 ml of ether. The ether was evaporated and 100 μl of absolute ethanol was added to each tube. Fifty μl of each sample were then spotted on silica gel plates as were unlabeled standards and labeled testosterone and 5α-DHT. The plates were chromatographed in a solvent system containing chloroform–acetone (93:7, v/v). Testosterone was visualized using U.V. light and the other standards were detected after spraying the plates with a 10% solution of phosphomolybdic acid in acetone and heating at approximately 100°C for 5 min. The plates were divided into columns perpendicular to the origin, and then subdivided into 1/2 cm sections horizontal to, and beginning 1/2 cm below, the origin. Testosterone, 5α-DHT, and the diols were well separated under these conditions. However, the 3α and 3β diols were not usually clearly separated from each other. The areas were then scraped and counted in 5 ml of a toluene-spectrafluor scintillation fluid.

We have monitored the losses of label at each step in the procedure. The 1.0 ml aliquots of cytosol added to the Sephadex columns usually contained about 26% of the total label in the incubation mixture. Loss on the column was 10–15% of the amount applied. Appropriately 40% of the radioactivity associated with the KCl extract of nuclei was lost during column chromatography. Recovery from the ether extraction was generally greater than 90%. Recovery from the thin layer plate was about 80%.

The identity of the steroids recovered from the plates was determined by recrystallization to constant specific activity. Recrystallization was performed by separately pooling extracts of the bound fractions and the free fractions from several experiments by washing of the extraction tubes with ether. The ether was then evaporated to dryness and 150 μl of ethanol were added to each tube. Aliquots (50 μl) of the ethanol solubilized steroids, in duplicate, were applied to t.l.c. plates, chromatographed as described above, and the steroids were localized using one of the duplicates. Areas on the other duplicate, corresponding to the

migration of the standards, were scraped into soxhlet extraction thimbles and refluxed over boiling chloroform for two hours. The chloroform was allowed to cool and then was transferred quantitatively to a conical centrifuge tube and evaporated to dryness under a stream of nitrogen over a steam bath. Chloroform was then added to the conical tube to solubilize the steroid. The chloroform soluble steroid was then transferred to a preweighed 1/2 dram vial. The conical tube was washed twice with chloroform and the washes were transferred to the vial. The chloroform was evaporated using nitrogen and the sample was further dried under vacuum. The weight of the sample in the vial was determined and a measured amount of the appropriate unlabeled steroid was added to it. The samples were recrystallized using the solvent systems methanol–water and acetone–water.

RESULTS

The effect of temperature and duration of incubation on androgen metabolism by and binding to cytosol

Epididymal minces from 3-day castrated rabbits were incubated with [^{3}H]-testosterone on ice for 0.5 to 4 h. At the end of each time period cytosol was prepared, unlabeled testosterone was added to the cytosols to dissociate loosely bound label, the samples were chromatographed on Sephadex G-25 to separate macromolecular-bound label from unbound label, and the types of steroids present in the bound fraction were determined by t.l.c. Following these procedures,

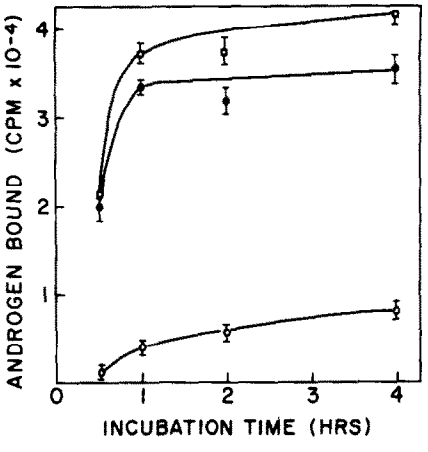

Fig. 1. The effect of incubation at 0–4°C on androgen metabolism by and binding to the rabbit epididymal cytoplasmic androgen receptor. Minced epididymal tissue pooled from two 3-day castrated adult rabbits was incubated with approximately 8×10^{-9} M [^{3}H]-testosterone on ice for 0.5 to 4 h. At the end of each time period cytosol was prepared, unlabeled testosterone ($\cong 8.6 \times 10^{-5}$ M) was added to the cytosol (at 0°C) to dissociate loosely bound label, the samples were chromatographed on Sephadex G-25 to separate macromolecular bound label from unbound label, and the types of steroids present in the bound fraction were determined by t.l.c. The points plotted represent the mean ± S.E.M. of 4 separate experiments. Testosterone (●——●), 5αDHT (○——○), total (□——□).

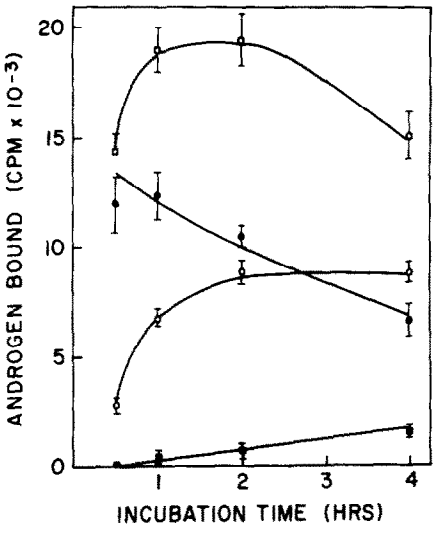

Fig. 2. The effect of incubation at 23°C on androgen metabolism by and binding to the rabbit epididymal cytoplasmic androgen receptor. The epididymal minces were treated in a manner identical to that described in the legend to Fig. 1 except that the incubations were conducted for 0.5–4 h at 23°C. Total binding (□——□), testosterone (●——●), 5αDHT (○——○). 5α-androstan-3α,17β-diol (■——■). The points plotted represent the mean ± S.E.M. of 4 separate experiments.

we noted (Fig. 1) that the binding reaction was essentially completed after 1 h of incubation at 0–4°C and that the predominant ligand bound to the receptor was testosterone. As the length of incubation increased, there was an increase in the amount of 5αDHT bound to the receptor, but even after 4 h of incubation this metabolite constituted only 20% of the receptor bound label.

When the incubations were conducted at 23°C. (Fig. 2), there was a decline in the amount of total binding activity present in cytosol following 2 h of incubation time. This decline was not due to degradation of the receptor since little decrease in binding was observed when labeled cytosol alone was incubated for this time period (data not shown). The decline in receptor concentration in the cytosol was taken as suggestive evidence for nuclear translocation of the hormone–receptor complex. During the 4 h incubation period, there was an increase in the amount of [^{3}H]-5α-DHT bound to the receptor and a concomitant decrease in amount of bound [^{3}H]-testosterone indicating the greater affinity of the receptor for 5αDHT. Binding of adrostandiols to the receptor could be detected after 2 hours of incubation at 23°C (Fig. 2).

Andogen binding to nuclei

To examine androgen binding to nuclei, we isolated nuclei from epididymal minces that had been incubated with [^{3}H]-testosterone alone or with [^{3}H]-testosterone plus unlabeled testosterone. The nuclei were then extensively washed, extracted with 1.0 M KCl and the nuclear extracts were chromatographed on

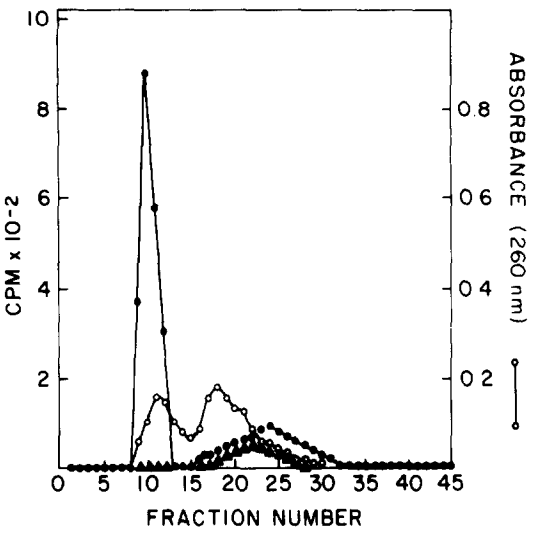

Fig. 3. Sephadex G-25 chromatography of epididymal nuclear extracts from 3-day castrated rabbits. Epididymal minces were preincubated on ice for 2 h with 8×10^{-9} M [^{3}H]-testosterone alone (●——●) or in combination with 8.6×10^{-5} M unlabeled testosterone (▲——▲). The samples were then further incubated for 1 h at 23°C. Nuclei were prepared, extracted with 1.0 M KCl, and binding was analyzed on Sephadex G-25 columns as described in Materials and Methods. Absorbance of the column fractions was monitored at 260 nm (○——○).

Sephadex G-25 as described in Materials and Methods. Elution profiles that were obtained are illustrated in Fig. 3. When the tissues were incubated with [^{3}H]-testosterone alone, a symmetrical peak of binding activity was eluted at the void volume of the column followed by a low broad peak of unbound label. When unlabeled testosterone was included in the incubation medium, the peak of macromolecular bound radioactivity was eliminated (Fig. 3). These studies indicated that Sephadex chromatography provided a suitable means for separating bound from unbound label in nuclear extracts and indicated that androgen binding was to a limited number of sites. These data also provided evidence that relatively little unbound label is associated with the KCl soluble fraction of nuclei.

We further evaluated androgen binding to epididymal nuclei by studying the effects of duration of incubation at 23°C on the absolute uptake of androgens by nuclei and on the types of androgens present in nuclei. The effects of the concentration of [^{3}H]-testosterone in the incubation medium on the binding of testosterone and its metabolites to nuclei was also studied.

Effects of duration of incubation on androgen binding to nuclei

Figure 4 illustrates data obtained when nuclei prepared from epididymal minces which had been incubated for 60 minutes at 0°C and for 0.5 to 4 h at 23°C were analyzed to determine the total amount of binding and relative amount of testosterone and its metabolites that were present in them. Panel A shows that during the first hour of incubation at 23°C there was rapid increase in the percentage of 5αDHT that was bound to the 1.0 M KCl extract of nuclei and a concomitant decrease in the percentage of bound testosterone. During the later time periods there was a slower increase in the percentage of 5αDHT bound. At the 4 h time period 5αDHT represented 60–70% of the bound steroids. The percentage of free 5αDHT in the KCl extract increased linearly with time and attained a maximum value of 20 ± 3% of the total free androgens at 4 h of incubation (data not shown). When the steroids present in the nuclear debris were examined (Fig. 4B), we observed that there was also a time dependent increase in the percentage of 5αDHT in this fraction. However, the accumulation of 5αDHT appeared to be slower than in the KCl soluble compartment.

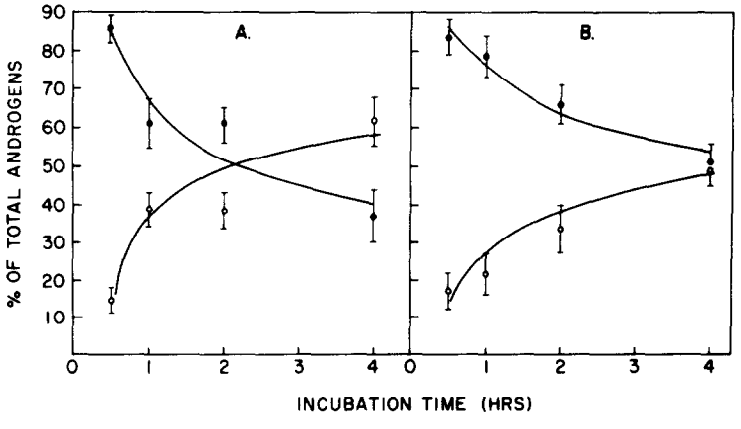

Fig. 4. The effect of duration of incubation on androgen binding to epididymal nuclei from 3-day castrated rabbits. Epididymal minces were preincubated on ice for 1 hour with 8×10^{-9} M [^{3}H]-testosterone and further incubated at 23°C for 0.5–4 h. Nuclear extract (Panel A) and nuclear debris (Panel B) were prepared and the types of androgens present in these fractions were determined. Testosterone (●——●), 5αDHT (○——○). The points plotted represent the mean ± S.E.M. of 4 separate experiments.

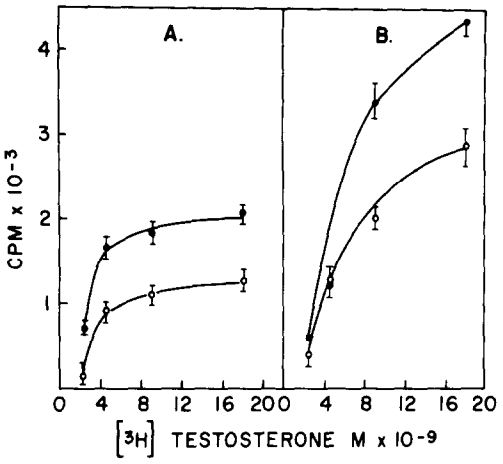

Fig. 5. The effect of [^{3}H]-testosterone concentration and incubation at 23°C for 1 h on binding of androgens to epididymal nuclei from 3-day castrated rabbits. Rabbit epididymal minces were preincubated on ice for 1 h with various concentrations [^{3}H]-testosterone (2.06–16.50 × 10^{-9} M) and further incubated for 1 h at 23°C. Nuclear extract (Panel A) and nuclear debris (Panel B) were prepared and the types of androgens present in them were determined. Testosterone (●——●), 5αDHT (○——○). The values plotted represent the mean ± S.E.M. of 4 separate experiments.

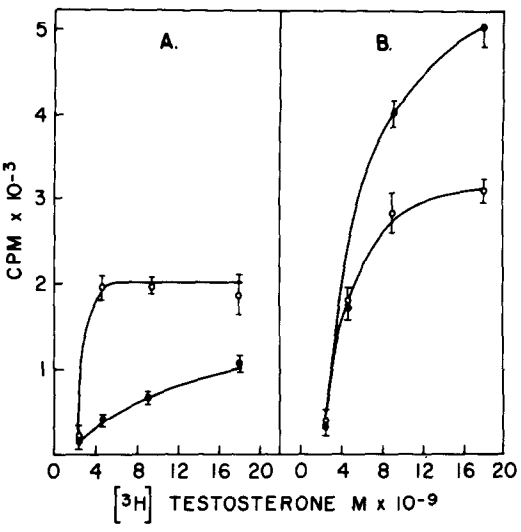

Fig. 6. The effect of [^{3}H]-testosterone concentration and incubation at 23°C for 2 h on androgen binding to epididymal nuclei from 3-day castrated rabbits. The procedures were conducted as described in Fig. 5 except that the incubation at 23°C was conducted for 2 h. Nuclear extract (Panel A), nuclear debris (Panel B) Testosterone (●——●), 5αDHT (○——○). The values plotted represent the mean ± S.E.M. of 4 separate experiments.

The effect of [^{3}H]-testosterone concentration on nuclear androgens

The effect of the concentration of [^{3}H]-testosterone in the incubation medium on the accumulation of radioactivity in nuclei was studied after a one h incubation at 0°C followed by incubation at 23°C for either 1 h or 2 h. The results of the one h incubation at 23°C are presented in Fig. 5. Following this incubation, testosterone was the major steroid present in nuclei at every concentration of [^{3}H]-testosterone used. The saturation of a limited number of sites for testosterone and 5αDHT was observed in the KCl extract of nuclei (Fig. 5A). The amount of steroids present in the free fraction from the KCl solubilized material increased linearly with increasing [^{3}H]-testosterone concentration as would be anticipated if the radioactivity associated with this fraction represented contamination from unbound hormone in the incubation medium.

When steroids present in the nuclear debris were evaluated (Fig. 5B), it was noted that a greater absolute amount of testosterone and 5αDHT were present in this fraction than in the KCl soluble fraction. Although the change in slope of the binding curves suggests the presence of a limited number of binding sites, saturation was not achieved.

When the incubations were performed for 2 h at 23°C, the results presented in Fig. 6 were obtained. In this case 5αDHT was the predominant steroid present in the KCl extract of nuclei (Fig. 6A) and the saturation of a limited number of 5αDHT and testosterone binding sites was obtained at a [^{3}H]-testosterone concentration of $\cong 4.0 \times 10^{-9}$ M. When the steroids present in the nuclear debris were examined (Fig. 6B), it was noted that saturation of binding sites for 5αDHT had occurred at a [^{3}H]-testosterone concentration of $\cong 8 \times 10^{-9}$ M. However, the amount of testosterone in this compartment continued to rise suggesting that there are sites in excess of the 5αDHT sites to which hormone is binding and/or that an increasing amount of contaminating unbound hormone might be present in this fraction. We were not able to detect any metabolite of testosterone other than 5αDHT in nuclei at any incubation time or at any [^{3}H]-testosterone concentration.

DISCUSSION

In the studies reported here, we have examined the effect of various incubation conditions on the types of testosterone metabolites bound to the epididymal androgen receptor and have given special emphasis to the types of androgen metabolites accumulated within the salt soluble and salt resistant fractions of nuclei.

We have demonstrated that androgen binding to unoccupied cytoplasmic receptors is independent of testosterone metabolism, since saturation of the sites can be achieved by one hour of incubation at either 0–4°C or at 23°C. However, the types of metabolites bound to the receptor are markedly influenced by incubation temperature. When the accumulation of radioactivity within nuclei was examined, we noted that this was a time-dependent phenomenon that favored the accumulation of 5αDHT. Although the same general patterns were observed in both the salt

extractable bound radioactivity and in the residual radioactivity, i.e., a time-dependent increase in the proportion of androgens represented by 5αDHT and attainment of saturation, differences were also noted. The primary differences between the two compartments were (a) the higher relative proportion of 5αDHT in the salt soluble compartment than in the residual compartment (b) the apparent faster rate at which binding equilibrium occurred in the salt soluble versus the residual compartment and (c) the apparently greater number of binding sites in the residual compartment as compared to the salt-extractable compartment. The studies further suggest that the residual fraction may have fewer acceptor sites for the 5αDHT–receptor complex than for the testosterone–receptor complex or that the acceptor sites may have a greater affinity for the testosterone–receptor complex than for the 5αDHT–receptor complex.

These alternatives were partially resolved when studies on the combined effects of duration of incubation at 23°C and the concentration of [^{3}H]-testosterone in the incubation medium were examined. We observed that whether incubations were conducted at 23°C for one h or 2 h saturation of a limited number of binding sites in the salt-soluble fraction occurred. The primary difference being, that when the incubations were conducted for 1 h at 23°C, testosterone was the major androgen present in this fraction, whereas, 5αDHT was the major androgen present after 2 h of incubation.

When androgens present in the residual fractions were examined, we noted that at low [^{3}H]-testosterone concentrations (at or below $\cong 4 \times 10^{-9}$ M) in the incubation medium, the amount of testosterone and 5αDHT present in the residual fraction were similar. However, at the higher concentrations of [^{3}H]-testosterone used, the amount of radioactivity present as testosterone clearly exceeded that present as 5αDHT whether the incubation at 23°C was conducted for one h or 2 h. The slopes of the binding curves indicated that the saturation of a limited number of binding sites was being approached in the one h incubation and saturation of the 5αDHT sites was reached after 2 h of incubation. Saturation of these sites in the residual fraction occurred at a [^{3}H]-testosterone concentration in the incubation medium twice that necessary for the saturation of 5αDHT sites in the salt-soluble fraction. The saturation of the 5αDHT sites does not appear to be an artifact resulting from saturation of the enzyme 4-ene-3-ketosteroid 5α-oxidoreductase that is necessary for the conversion of testosterone to 5αDHT, since we have shown [12] that there is a linear increase in the formation of 5αDHT from testosterone over the full range of [^{3}H]-testosterone concentrations used in these studies. It would appear, therefore, that the salt resistant fraction possesses testosterone binding sites in addition to those for 5αDHT.

The possibility exists that the residual fraction contains radioactivity non-specifically associated with nuclear membranes and other contaminating materials and/or unbound radioactivity. However, since the recovery of radioactivity from this fraction is greatly decreased by incubation in the presence of [^{3}H]-testosterone and unlabeled testosterone [8], it would appear that a limited number of binding sites are present in this fraction for androgens and/or the androgen–receptor complex. From the data thus far obtained we cannot distinguish between these alternatives. Further studies remain to be conducted to determine if prolonged incubation with high ionic-strength solutions, and/or enzymatic treatment will significantly influence the proportion of radioactivity extractable from nuclei.

The observation that a portion of the radioactivity present in the nuclear fraction following incubation of various tissues with various tritiated steroids is resistant to salt extraction has been made previously by several investigators [8, 17–21]. These observations in conjunction with those reported here may add an additional dimension to the theory of steroid hormone action since it becomes of interest to determine which type of binding represents the physiologically meaningful interaction of the hormone–receptor complex with target tissue nuclei. It is possible that the two (or more) orders of nuclear binding sites may be involved in different aspects of hormone action.

The non-physiological nature of the model system used in these studies was necessary for the experimental manipulations required. However, it gives rise to the possibility that events analogous to those described here do not occur under *in vivo* conditions in intact animals. This is particularly so regarding the observed translocation of testosterone–receptor complexes into nuclei. In castrated rabbits, the concentration of unoccupied cytoplasmic receptor sites would be higher and the endogenous steroid concentration would be lower than in non-castrated animals. In intact animals the epididymal concentration of 5αDHT greatly exceeds that of testosterone [22]. These considerations, taken in conjunction with the greater affinity of the receptor for 5αDHT than for testosterone, suggests that little testosterone would complex with the cytoplasmic receptor and be translocated to nuclei in intact animals. However, under physiological conditions one cannot exclude the possibility of testosterone–receptor complexes interacting with the same or different sites in the genome as do 5αDHT receptor complexes.

Several other studies have been conducted on the uptake of androgens by rat epididymal nuclei[4–5, 23]. Those of Tindall *et al.* [4] showed that one hour following an i.v. injection of [^{3}H]-testosterone approximately 70% of the radioactivity recovered from nuclei was dihydrotestosterone. From the data that they present, it would appear that "other metabolites" of testosterone, which were not specified, constitute the remainder of the radioactivity isolated during intermediate periods (1–3 h) following injection. Blaquier and Calandra [5], using minces of rat epididymides

incubated with [^{3}H]-testosterone, showed that the amount of bound radioactivity extracted from nuclei that corresponded to DHT varied according to the concentration of [^{3}H]-testosterone in the incubation medium from 92–55%. The remaining radioactivity corresponded to testosterone. These investigators have also reported [24] that 5α-androstane-3α-17β-diol could be isolated from epididymal nuclei following its injection into or its incubation with minced epididymal tissue. The studies of Djøseland *et al.* [23] were unable to detect nuclear 5α-androstane-3α-17β-diol. In our previous studies [8, 12] and in those reported here we have shown that 5α-androstane-3α-17β-diol is produced by the rabbit epididymis from testosterone and binds to the cytoplasmic androgen receptor. However, we have been unable to isolate this metabolite from epididymal nuclei. Whether alteration of the incubation conditions used would permit the 5α-androstane-3α-17β-diol–receptor complex to enter nuclei remains to be elucidated.

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