Selective Retention and Formation of a \( \Delta^5 \)-Androstenediol-Receptor Complex in Cell Nuclei of the Rat Vagina*

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Tsang-Cheng Shao,† Evangelina Castañeda, Robert L. Rosenfield, and Shutsung Liao

From the Ben May Laboratory for Cancer Research, and the Departments of Biochemistry and Pediatrics, The University of Chicago, Chicago, Illinois 60637

SUMMARY

Cellular protein binding of a number of androsterone and androstane derivatives that promote the growth of the vagina in rats has been studied. It was found that cell nuclei of the rat vagina contain a tissue-specific protein that binds \( \Delta^5,17\beta \)-dihydroxy-androst-5-ene (\( \Delta^5 \)-androstenediol), a unique steroid causing growth and keratinization of the vaginal epithelium. The formation of the steroid-protein complex can be demonstrated by the administration of \( \Delta^5,17\beta \)-[\( ^3\H \)]androstenediol to ovariec-tomized rats or by the incubation of minced vagina with the radioactive steroid. The steroid can interact with purified vaginal cell nuclei even in the absence of a cytosol preparation, forming the same steroid-protein complex. The formation of the complex is temperature-dependent; it occurs much more readily at 37° than at 0°.

The \( \Delta^5,17\beta \)-androstenediol-protein complex migrated as about 4 S in a sucrose gradient medium containing 0.4 M KCl. A similar complex can be detected when nuclei of vaginal cells are incubated with \( \Delta^5,17\beta \)-di- or \( \Delta^5,17\beta \)-mono-hydroxy-5a-androstan-17-one \( \Delta^5 \)-androstenediol derivatives that promote the growth of the vagina in rats has been studied. It was found that cell nuclei of the rat vagina contain a tissue-specific protein that binds \( \Delta^5,17\beta \)-dihydroxy-androst-5-ene (\( \Delta^5 \)-androstenediol), a unique steroid causing growth and keratinization of the vaginal epithelium. The formation of the steroid-protein complex can be demonstrated by the administration of \( \Delta^5,17\beta \)-[\( ^3\H \)]androstenediol to ovariec-tomized rats or by the incubation of minced vagina with the radioactive steroid. The steroid can interact with purified vaginal cell nuclei even in the absence of a cytosol preparation, forming the same steroid-protein complex. The formation of the complex is temperature-dependent; it occurs much more readily at 37° than at 0°.

The \( \Delta^5,17\beta \)-androstenediol-protein complex migrated as about 4 S in a sucrose gradient medium containing 0.4 M KCl. A similar complex can be detected when nuclei of vaginal cells are incubated with \( \Delta^5,17\beta \)-di- or \( \Delta^5,17\beta \)-mono-hydroxy-5a-androstan-17-one which also have the capability of stimulating vaginal epithelium, although in somewhat different ways. These steroids may bind to different groups of chromatin-bound receptor proteins in various layers of vaginal epithelium.

The \( \Delta^5 \)-androstenediol binding protein is not found in the vaginal cytosol fraction that contains receptor proteins for estrogens and progestins, nor in the cytosol or nuclei of rat ventral prostate that contains a receptor protein for androgens. The protein has been found to be present in the nuclei of rat uterus cells, but not in muscle, brain, kidney, or liver. Testosterone and 5\( \alpha \)-dihydrotestosterone bind weakly to the protein, whereas cortisol, androstenedione, 17\( \beta \)-estradiol, and progestosterone do not bind to the same protein to any significant extent.

In mammals, \( \Delta^5 \)-androstenediol* exerts moderate androgenic activity on peripheral target tissues such as the prostate (1, 2). The steroid also induces comb growth in the capon (1, 3). The action of \( \Delta^5 \)-androstenediol in androgen-sensitive tissues may be explained in part by its conversion to major androgens such as testosterone and \( \Delta^5,17\beta \)-dihydrotestosterone (4, 5), which apparently function by binding to a specific receptor protein in the target cells (6). In immature female rats, \( \Delta^5 \)-androstenediol also promotes the growth of the uterus and the keratinization of vaginal epithelium (3, 7) and the related steroid, dehydroepiandrosterone sulfate, has estrogenic activity in man (8). Since these latter actions are not mimicked by the major androgens, \( \Delta^5 \)-androstenediol appears to function in the vagina by another mechanism.

We report here that, in immature female rats, \( \Delta^5 \)-androstenediol can be retained by the cell nuclei of the vagina without a metabolic conversion. Such nuclear retention appears to be due to a specific nuclear protein that may be considered as a \( \Delta^5 \)-androstenediol receptor.

**EXPERIMENTAL PROCEDURE**

**Materials**—Nonradioactive steroids were purchased from Steraloid, Inc. The tritiated steroids (44 Ci per mmol for \( \Delta^5,17\beta \)-dihydrotestosterone, [1,2-\( ^3\H \)]testosterone, [1,2-\( ^3\H \)]cortisol, [6,7-\( ^3\H \)]estradiol, [1,2-\( ^3\H \)]androstenedione, [1,2-\( ^3\H \)]progesterone, and \( \Delta^5,17\beta \)-di- or \( \Delta^5,17\beta \)-mono-hydroxy-5a-androstan-17-one; 21 Ci per mmol for [\( \Delta^5,17\beta \)-dihydroepiandrosterone] were products of New England Nuclear Corp. Radioactive \( \Delta^5 \)-androstenediol was prepared by reduction of [\( \Delta^5 \)-\( \Delta^5 \)-dihydroepiandrosterone] in the presence of 17\( \beta \)-hydroxysteroid dehydrogenase and NADH (9). The purity of the radioactive steroids was checked and, if necessary, they were repurified by thin layer or paper chromatographic methods. Purified enzymes were supplied by Sigma and Worthington. Immature female rats of the Sprague-Dawley strain, 3 to 5 weeks of age, were used for the experiments. In some experiments, 11-week-old adult male rats were used. All rats were castrated under ether anesthesia 18 to 20 hours prior to the experiment.

**Subcellular Fractionation**—Animals were killed by cervical dislocation. The vagina and other tissues were dissected and chilled in an ice-cold medium containing 0.32 M sucrose, 1 mm MgCl\(_2\), and 20 mm Tris-HCl, pH 7.6 (Medium A). Subcellular fractionation was performed in the manner described in previous reports (6, 11) from this laboratory. Briefly, the chilled tissue was minced and homogenized in 3 volumes of Medium A. The homogenate was centrifuged at 600 x g for 10 min, and then at 100,000 x g for 1

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hour, to yield the cytosol fraction. Cell nuclei were purified from the sediment obtained after the first centrifugation. The procedure included the sedimentation of the cell nuclei in a hypertonic medium containing 2.2 M sucrose, 1 mM MgCl₂, and 20 mM Tris HCl buffer, pH 7.0 (6).

**Incubation of Cellular Preparations with **₁⁻°**⁻**-Steroids**—For the in vivo study on the retention of ₃⁻°⁻**⁻**-androstenediol by the vagina, 0.1 ml of 0.9% NaCl (saline) containing 0.14 μCi of ₃⁻°⁻**⁻**-androstenediol was pipetted into the vaginal opening of the anesthetized rat. The opening was then closed with a hemostat for 40 min after which the animal was killed and various tissues were dissected for further analysis.

For the in vitro incubation of tissue minces, the freshly obtained vagina was minced into pieces of about 1 mm³. These were suspended in Medium A, so that 1 ml of the medium contained about 0.1 g of minced tissue. The minced tissues were then allocated by a wide mouth pipette into glass bottles (diameter 2.6 cm, height 6 cm). The radioactive steroid dissolved in Medium A was added to the tissue suspension in a final volume of 3 ml that contained about 0.5 to 0.4 g of vagina (usually from three to four rats). Incubation was carried out in a Dubnoff shaker (50 oscillations per min). After incubation, the bottle was chilled on ice and the minced tissue was transferred to a centrifuge tube.

Tissues labeled during in vivo and in vitro experiments were washed three times, each with 10 ml of Medium A, by resuspension and centrifugation in a Sorvall RC2-B centrifuge with a SS-34 rotor. The washed tissue was then homogenized and the cell nuclei were isolated.

**Incubation of cell nuclei with  ᵃ⁻°⁻**⁻**-steroid was carried out in a final volume of 1 ml of Medium A at 37° for 40 min. Each experimental tube usually contained cell nuclei isolated from 0.4 g of tissue. Vaginas from four rats were used for each tube.

All cell nuclei labeled with  ᵃ⁻°⁻**⁻**-steroids (in vivo or in vitro) were washed (by centrifugation at 500 × g for 10 min) once with 10 ml of Medium A containing 0.4% Triton X-100, and twice with 10 ml of Medium A containing no Triton. The washed nuclei were then suspended in 10 ml of 20 mM Tris-HCl buffer, pH 7.5, containing 1·5 mM EDTA and centrifuged at 10,000 × g for 10 min.

**Extraction of **₁⁻°**⁻**-Steroids Bound to Cell Nuclei**—In many experiments the washed nuclei were mixed with an equal volume of 0.8 M KCl containing 20 mM Tris-HCl buffer, pH 7.5, and 1·5 mM EDTA (final concentration of KCl, 0·4 M) and extracted for 30 min at 0°. The extract was separated from the residue by centrifugation at 15,000 × g for 10 min. About 50 to 70% of total  ᵃ⁻°⁻**⁻**-steroid was normally solubilized. Extraction of the residue with media containing higher concentrations of KCl can result in the extraction of more radioactive steroids, but considerable amounts of DNA and other chromat materials are then also solubilized. When the radioactivity and the DNA content of cell nuclei were to be analyzed, the nuclear preparations were heated at 90° for 10 min in 2 to 3 ml of 10% trichloroacetic acid. The hydrolysate obtained after centrifugation of 3,000 × g for 10 min was used for the measurements.

**Identification of **⁻°⁻**⁻**-steroids**—  ᵃ⁻°⁻**⁻**-steroids bound to cellular preparations were extracted 5 times with methylene chloride saturated with H₂O. Unlabeled steroids were added as carriers. The extracts were pooled and dried with a stream of N₂. The residue was dissolved in a small volume of methanol, and  ᵃ⁻°⁻**⁻**-steroids were identified by chromatography. Paper chromatography was performed on Whatman chromatographic paper, either with a mixture of ligroin and 2,4-dihydroxypropane (1:1, v/v) or with a solution containing ligroin-benzene-methanol-water (33.3: 16.6: 40: 10, v/v). In these systems,  ᵃ⁻°⁻**⁻**-androstenediol can be separated clearly from 3α, 17β-dihydroxy-androst-4-ene. The identity of  ᵃ⁻°⁻**⁻**-androstenediol was also confirmed by recrystallization to constant specific radioactivity.

**Gradient Centrifugation**—The sample (0.2 ml) was layered on the top of a sucrose gradient (10 to 30% linear in Spinco SW 50 rotor tube) containing 0.4 M KCl, 1.5 mM EDTA, and 20 mM Tris HCl buffer, pH 7.5. The tube was centrifuged at 54,000 rpm for 18 hours at 0 to 2°. Fractions (0.2 ml each) were collected and measured starting from the bottom of the tube, and the radioactivity was measured. Bovine albumin ([ρ₀ₐ₉ = 4.6 S) and other proteins were used as standards (10, 11).

**Other Methods**—DNA was measured by the diphenylamine test, with calf thymus DNA as the standard (12). Protein was determined by the method of Lowry et al. (13), with bovine serum albumin as standard. Radioactivity was measured in a Packard scintillation counter. The scintillation fluid was a mixture of 1.5 liters of toluene, 500 ml of Triton X-100, 8 g of 2,5-diphenyloxazole, and 100 mg of 1,4-bis[2-(5-phenyloxazolyl)]benzene (10). The counting efficiency was about 60%.

**RESULTS**

**Tissue Uptake and Nuclear Retention of  ᵃ⁻°⁻**⁻**-Androstenediol in vivo**—When 10 to 20 μCi of  ᵃ⁻°⁻**⁻**-androstenediol were injected intraperitoneally into an immature female rat, a large quantity of radioactive metabolites of the injected steroid was found in the liver. Very little radioactivity was found in the vagina, however. Because of the limited supply of the radioactive steroid, we were not able to make a systematic study of the steroid uptake and retention by intraperitoneal injection of higher doses of  ᵃ⁻°⁻**⁻**-androstenediol. Subsequently, we found that a high vaginal uptake and retention of the radioactive steroid could be obtained when the  ᴢ⁻°⁻**⁻**-steroid was placed in the vaginal opening (Table I). By this method, significant quantities of  ᵃ⁻°⁻**⁻**-steroids were also found in the liver and uterus, but not in the kidney.

When cell nuclei were isolated from those tissues and analyzed, nuclear retention of  ᵃ⁻°⁻**⁻**-steroid was clearly seen in the vagina and uterus, but not in liver and kidney. Kinetic studies showed that maximal labeling of the tissues and cell nuclei was reached within 1 hour after the application of  ᵃ⁻°⁻**⁻**-androstenediol. The nuclear radioactivity in the vagina remained higher than that in other tissues studied for more than 3 hours. A comparison (Table 1) of the ratio of the amounts of radioactive steroids in the isolated nuclei to the amount in the whole homogenates suggested that the tissue-specific difference in nuclear retention was not directly related to the difference in the amounts of  ᵃ⁻°⁻**⁻**-steroids taken up by these tissues. The results, therefore, indicated that the vagina and uterus, but not the liver or kidney, might have a tissue-specific mechanism for retaining  ᵃ⁻°⁻**⁻**-androstenediol (or some of its metabolites) in their cell nuclei.

When the vaginal nuclei, previously labeled in vivo with  ᵃ⁻°⁻**⁻**-androstenediol, were extracted with a 0·4 M KCl medium, about 50 to 70% of radioactivity was solubilized. When the extract was analyzed by gradient centrifugation with a sucrose solution containing 0·4 M KCl, the solubilized  ᵃ⁻°⁻**⁻**-steroid was found to sediment as a 4·3 S complex (Fig. 1). A small, but distinct
Nuclear Retention of $\Delta^4$-$[\text{H}]$Androstenediol during Incubation of Whole Tissue Homogenates

- Freshly dissected tissue was minced and incubated with 1.33 µCi of $\Delta^4$-$[\text{H}]$androstenediol at 37°C for 1 hour. Cell nuclei were isolated and extracted by a 0.4 M KCl solution. The nuclear extract was analyzed by gradient centrifugation in the way described in Fig. 1, except that centrifugation was performed for 16.5 hours.

Table II

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Radioactivity (cpm/mg DNA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vagina</td>
<td>12,750</td>
</tr>
<tr>
<td>Uterus</td>
<td>2,260</td>
</tr>
<tr>
<td>Liver</td>
<td>431</td>
</tr>
<tr>
<td>Kidney</td>
<td>924</td>
</tr>
<tr>
<td>Diaphragm</td>
<td>670</td>
</tr>
<tr>
<td>Thymus</td>
<td>220</td>
</tr>
<tr>
<td>Ventral prostate</td>
<td>560</td>
</tr>
</tbody>
</table>

- From ovariectomized female rats.
- From orchiectomized male rats.

Cytosol Steroid-Binding Proteins—Despite various efforts under in vitro and in vivo conditions, we have been unable to detect any $\Delta^4$-$[\text{H}]$androstenediol-binding macromolecule in the cytosol preparations of various rat tissues such as vagina, uterus, ventral prostate, and liver. Since we were able, under our experimental conditions, to demonstrate the existence of the receptor binding of $[\text{H}]$estradiol (14), $[\text{H}]$progesterone (16), and $[\text{H}]$dihydrotestosterone (10) in the cytosol preparations of their target tissues, our results suggest that a $\Delta^4$-androstenediol-binding macromolecule is not present or is totally inactivated during the preparation of the cytosol fractions.

Cytosol-independent Formation of $\Delta^4$-$[\text{H}]$Androstenediol-Macromolecular Complex—We found, however, that the $\Delta^4$-$[\text{H}]$androstenediol-macromolecular complex could be formed readily by incubation of either the crude nuclear preparation (600 × g pellet) or the purified nuclei of vaginal cells with $\Delta^4$-$[\text{H}]$androstenediol in the absence of a cytoplasmic preparation (Fig. 3). The ability of the nuclear preparations to form the radioactive complex remains the same after the nuclei are treated with Triton X-100 to remove the outer nuclear membranes (6, 10), or ruptured and washed extensively with a hypotonic medium (0.02 M Tris-HCl buffer, pH 7.5). The cytosol independence appears to be in keeping with the view that, in vaginal cells, the steroid-binding macromolecule is in the cell nuclei and binds tightly to the nuclear chromatin.

An experiment, the result of which is shown in Fig. 4, also indicated that the cell nuclei alone are responsible for the tissue-specific formation of the steroid-macromolecular complex. Thus, the formation of the complex was observed with cell nuclei of the vagina and uterus, but not with cell nuclei of skeletal muscle, brain, or kidney. Other experiments also showed no significant formation of a $\Delta^4$-androstenediol-macromolecular complex with cell nuclei of the liver or ventral prostate, either in the presence or in the absence of the cytosol fractions of the same tissues.

The formation of the vaginal cell nuclear complex was temperature-dependent. Under our incubation conditions, a maximum level (equivalent to that attained by incubation of vaginal minces) was reached within 1 hour. The amount of complex formed was only about 10% of this maximum level if incubation was carried out at 0°C.

Properties of Nuclear $\Delta^4$-$[\text{H}]$Androstenediol-Protein Complex of Vagina—We found that the radioactive steroid associated with the nuclear KCl extract and the 4 S complex obtained by gradient centrifugation could be extracted quantitatively by treatment with Triton X-100 or by paper or thin layer chromatographic methods (Fig. 5) and by recrystallization to constant specific radioactivity. The nuclear macromolecule that bound the radioactive steroid was apparently a protein, since treatment with pronase, but not with pancreatic DNase-I or a mixture of pancreatic RNase and T1 RNase, diminished steroid binding to the 4 S complex. Heating of the complex as many as 1500 and 250 molecules of the steroid per diploid nucleus (assuming 6 pg of DNA per nucleus), respectively, under the conditions of the experiment. The extent of $\Delta^4$-$[\text{H}]$androstenediol retention by the cell nuclei of kidney, liver, diaphragm, thymus, and prostate was less than 10% of that by vaginal cell nuclei.

When the labeled nuclei were extracted with the 0.4 M KCl medium and analyzed by gradient centrifugation, the steroid macromolecular complex was found to sediment at about 4 S. The formation of the 4 S complex during the incubation of vaginal minces or homogenates occurred readily at 30 to 40°C, but only very slowly at 0°C (Fig. 2).
Fig. 3. (left) Cytosol-independent formation of Δ^4-[\text{H}]androstenediol-macromolecular complex during the incubation of vaginal cell nuclei. Cell nuclei isolated from 0.8 g of vagina obtained from 3 ovariectomized rats were incubated in a final volume of 1 ml in Medium A containing 1 nCi of Δ^4-[\text{H}]androstenediol. Incubation was carried out at 37° for 40 min. Nuclei were reisolated, washed, and extracted with 0.4 M KCl solution. The nuclear extract was analyzed by gradient centrifugation in the same way as in Fig. 2. In separate tubes, a whole homogenate or a crude nuclear preparation (600 X g pellet) was used instead of the purified nuclei. In the fourth tube, a nuclear extract obtained from purified nuclei not previously exposed to the H-steroid was used. Before gradient centrifugation, the nuclear extract was mixed with an amount of Δ^4-[\text{H}]androstenediol equivalent to that present in other nuclear extracts (6.7 nCi). Other conditions are described under "Experimental Procedure."

Fig. 4. (right) Tissue-specific formation of Δ^4-[\text{H}]androstenediol-macromolecular complex during the incubation of isolated cell nuclei. The experiment was carried out in the way described in Fig. 3, except that cell nuclei were prepared from the various tissues shown. The labeled nuclear extracts were analyzed by gradient centrifugation in a way described in Fig. 1. The presence of the complex in the uterus has been confirmed in separate experiments using larger quantities of uterine cell nuclei.

at 50° or above for 10 min also resulted in complete destruction of the complex.

Fig. 6 shows the steroid specificity for the formation of the 4 S complex during incubation of vaginal cell nuclei and H-steroids. Of all the steroids tested so far, Δ^4-[\text{H}]androstenediol appeared to bind most efficiently. Considerable protein binding also occurred with 3α,17β-dihydroxy-Δ^5-[\text{H}]androstane, 3α,17β-dihydroxy-Δ^5-[\text{H}]androstane, and [\text{H}]dehydroepiandrosterone. Radioactive testosterone and 5α-dihydrotestosterone bind to a much lesser extent. It is not known whether all of these steroids bind to the same protein or to the same steroid binding site. No significant binding was observed with androstenedione, cortisol, progesterone, and 17β-estradiol.

Further study on the chemical and physical properties of the steroid binding protein has been hampered by our failure to extract the nuclear protein and to show its binding of Δ^4-[\text{H}]androstenediol in a nucleus-free system (Fig. 3). It is possible that, in the absence of the steroid, the protein released from the nuclear chromatin is unstable and loses its steroid binding ability rapidly even at 0°. Alternatively, the release of the protein from the chromatin may be facilitated by steroid binding. Since vaginal cell nuclei can be frozen for several weeks without losing their steroid binding property, the nuclear protein appears to be stabilized by associating to the nuclear chromatin. The Δ^4-[\text{H}]androstenediol-protein complex solubilized from the incubated vaginal cell nuclei is also stable at 0° for 20 hours, and −20° for 2 weeks.

As has been observed for other steroid-receptor complexes (10, 14–20), very little (<10%) exchange of the [\text{H}]steroid bound to the protein and the nonradioactive free steroid added to the preparation occurred at 0° over 18 hours. Preliminary studies based on equilibrium dialysis (21) suggested that the affinity constant (K_a) for the nuclear Δ^4-[\text{H}]androstenediol-protein complex of the rat vagina may be higher than 10^6 M⁻¹. This value is similar to that reported recently for the 5α-dihydro-
As-androstenediol in preference to many other steroids which we
classic androgen receptor, for it was not detected in the cell
various blood proteins failed to affect As-androstenediol binding
tains a large quantity of a receptor protein for Sa-dihydrotestos-
nuclei or in the cytosol fraction of rat ventral prostate that con-
extract was divided into two equal portions. A preparation of
vaginal cell nuclei incubated with the radioactive steroid. The
incubated at 2° for 2 hours. After incubation, they were analyzed
The high affinity of the As-[^H]androstenediol-protein complex
was also indicated by the inability of an antibody to the radio-
receptor complexes.

The vaginal cell nuclear protein or proteins appear to bind
As-androstenediol protein complex of the vagina. (15). The high affinity of the As-[^H]androstenediol-protein complex was also indicated by the inability of an antibody to the radio-
active steroid to compete with the nuclear protein for binding
As-[^H]androstenediol (Fig. 7). As described elsewhere (22), it
demonstrated that As-[^H]androstenediol at high concentrations did not
compete with these female hormones for binding to their receptor,
the nuclear protein is clearly not the female hormone receptor
available. For example, steroid-protein complexes have been
formed migrate into the cell nuclei. Other experiments showing the
direct interaction of steroid hormones with cell nuclei are
formulated. In these cases, however, steroids also bind to the
cytosol receptors and the cytosol complexes are retained by the
The presumed nuclear estrogen-protein complex
is not extractable by a 0.4 M KCl solution as is the nuclear As-
androstenediol protein complex of the vagina.

Fig. 7. Steroid antibody-resistant binding of As-[^H]androstenediol to the nuclear protein of vaginal protein of vaginal cells. The As-[^H]androstenediol-protein complex was extracted from vaginal cell nuclei incubated with the radioactive steroid. The extract was divided into two equal portions. A preparation of testosterone antibody (20 µg of protein) that could bind As-[^H]androstenediol was added to one portion, and both portions were incubated at 3° for 2 hours. After incubation, they were analyzed by gradient centrifugation in the way described under "Experimental Procedure." It will be noted that the steroid antibody binds free[^H]steroid, but not the steroid bound to the 4 S nuclear protein. The[^H]steroid-antibody complex migrated at about 8 S, as has been described elsewhere (22).

discussion

The present work indicates that there is a high affinity, tissue-
specific nuclear protein in vaginal cells which binds As-[^H]androstenediol preferentially. This protein is not found in the cytosol fraction of the vagina that contains receptor proteins for estrogens and progesterones. Since our experiments (data not shown) also showed that As-[^H]androstenediol at high concentrations did not compete with these female hormones for binding to their receptor, the nuclear protein is clearly not the female hormone receptor
described previously by others (14, 15). The protein is not a
classic androgen receptor, for it was not detected in the cell
nuclei or in the cytosol fraction of rat ventral prostate that contains a large quantity of a receptor protein for 5a-dihydrotestosterone (10). As we reported earlier, the prostatic androgen receptor
does not bind any natural androstanes or androstanes which have hydroxy groups at both the C-3 and C-17 positions (9). The protein is not a blood protein; it was not found in blood, and the steroid antibody that can eliminate steroid binding to various blood proteins failed to affect As-androstenediol binding by the vaginal protein (Fig. 7).

The vaginal cell nuclear protein or proteins appear to bind
As-androstenediol in preference to many other steroids which we
have tested. However, 3β-(or 3α),17β-dihydroxy-5α-androstane
and dehydroepiandrosterone also bind to a significant extent. Huggins et al. (7) showed that although these steriods stimulate the growth of uterus they also induce different responses in different layers of vaginal epithelium cells. Munification of the vaginal epithelium was seen following the injection of 3α,17β-
dihydroxy-5α-androstane, whereas stratification of the epithelium was induced by 3β,17β-dihydroxy-5α-androstane and dehydroepiandrosterone. As-Androstenediol was unique among the 26 related 19-carbon steroids tested in that it alone shared the property of the phenolic estrogens of causing keratin formation, in significant amounts, by the deeper layers of the vaginal epithelium. It may be, therefore, that the dihydroxylated steroids
bind to different groups of receptor proteins in different types of
cells in the vaginal epithelium.

If the As-androstenediol binding protein is indeed a function-
related receptor, our finding may indicate that As-androstenediol acts directly on vaginal cells and that it functions without metab-
olic conversion. If this is in fact the case, our observation sug-
gests that the steroid acts on vaginal cell nuclei by interacting
with and binding directly to a nuclear chromatin protein by a
temperature-dependent process. This is in contrast to the re-
ceptor mechanism proposed for the action of estrogen in the
uterus (14), of androgen in the ventral prostate (10), progesterone in the chick oviduct (16), and glucocorticoids in liver cells (17-
19). In these systems (cf. Ref. 15), the steroid receptor interaction is believed to occur in the cytoplasm and the complexes formed migrate into the cell nuclei. Other experiments showing the direct interaction of steroid hormones with cell nuclei are available. For example, steroid-protein complexes have been extracted from purified rat thymus nuclei incubated with[^H]cortisol (23) and purified kidney nuclei incubated with[^H]aldosterone (24). In these cases, however, steroids also bind to the
cytosol receptors and the cytosol complexes are retained by the
nuclear protein is clearly not the female hormone receptor
available. For example, steroid-protein complexes have been
extracted from purified rat thymus nuclei incubated with[^H]cortisol (23) and purified kidney nuclei incubated with[^H]aldosterone (24). In these cases, however, steroids also bind to the
cytosol receptors and the cytosol complexes are retained by the
cell nuclei (17, 20). It has been suggested further that estrogen
may interact directly with the nuclear receptor of chick liver
(25). However, the presumed nuclear estrogen-protein complex
is not extractable by a 0.4 M KCl solution as is the nuclear As-
androstenediol protein complex of the vagina.

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