Mechanisms of Replenishment of Nuclear Androgen Receptor in Rat Ventral Prostate

By EDWARD VAN DOORN and NICHOLAS BRUCHOVSKY

Department of Medicine, University of Alberta, Edmonton, Alberta, Canada T6G 2G3

(Received 5 October 1977)

1. The concentration of androgen receptor in the nucleus of the prostatic cell is rapidly elevated by the administration *in vivo* of 2 µg of [³H]testosterone to 1-day-castrated rats. From a concentration of 2300 receptors/nucleus at 5 min after intravenous injection of hormone, there is an increase to 21 000 receptors/nucleus at 60 min. At the same time, the amount of binding of androgen in the cytoplasm remains constant at a relatively low value. 2. An identical dose of [³H]testosterone administered to 7-day-castrated rats produces a much smaller change in the concentration of nuclear receptor, from 700 receptors/nucleus at 5 min to only 4300 receptors/nucleus at 60 min. Thus the reservoir from which nuclear receptor is replenished is considerably smaller in regressed prostatic cells. Again, the amount of binding of androgen in the cytoplasm remains unchanged at a low value over the experimental time course of 60 min. 3. In contrast with the scant labelling of cytoplasmic receptor achieved by injecting animals with [³H]testosterone, labelling *in vitro*, by incubation of tissue slices with radioisotope, indicates that prostate of 1-day-castrated animals actually contains 21 400 receptors/cell in the cytoplasmic compartment, and prostate of 7-day-castrated animals 3000 receptors/cell. 4. Owing to the similarity between the concentration of nuclear receptor measured *in vivo* and the concentration of cytoplasmic receptor measured *in vitro*, the labelling techniques *in vivo* and *in vitro* were used in sequence to demonstrate the movement of most of the cytoplasmic receptor into the nucleus. In the 5–60 min interval after the administration of [³H]testosterone to 1-day-castrated rats, a decrease of 17 400 receptor molecules in the cytoplasm is exactly mirrored by an increase of 17 200 receptor molecules in the nucleus. 5. These results imply that, in prostate of 1-day-castrated rats, nuclear receptor is replenished exclusively by translocation of cytoplasmic receptor. However, in the regressed prostate of 7-day-castrated rats, only about 25% of the nuclear receptor is replenished through translocation of existing cytoplasmic receptor. The remainder is ultimately synthesized during new rounds of cell division induced by hormone.

The concentration of androgen receptor in the cytoplasm of the prostatic cell undergoes a rapid increase within 24 h after castration (Grover & Odell, 1975; Van Doorn *et al.*, 1976). This is matched by a decrease of similar velocity and magnitude in the concentration of nuclear receptor. Throughout the interval the net concentration of receptor in the cell remains constant. Owing to these observations and also the fact that no receptor-inactivating factors are detected in the nucleus, we inferred previously that the appearance of cytoplasmic receptor follows from the release of nuclear receptor into the cytoplasm (Van Doorn *et al.*, 1976; Rennie *et al.*, 1977). In such cells, nuclear receptor is replenished within minutes after a single intravenous injection of testosterone or dihydrotestosterone administered to castrated rats (Bruchovsky *et al.*, 1975). The rapidity of this effect suggests that receptor is simply recycled into the nucleus from an augmented cytoplasmic pool. How-

ever, if the period of androgen withdrawal extends to 7 days, when the pool of cytoplasmic receptor is diminished, the administration of dihydrotestosterone also stimulates the synthesis of nuclear receptor (Van Doorn *et al.*, 1976).

Although replenishment mechanisms have been the focus of several investigations on oestrogens (Capony & Rochefort, 1975; Mester & Baulieu, 1975; Sutherland & Baulieu, 1976; Clark *et al.*, 1977; Ferguson & Katzenellenbogen, 1977), progesterone (Mester & Baulieu, 1977) and glucocorticoids (Munck & Leung, 1976), less attention has been directed towards the manner in which androgen-receptor contents are adjusted in the prostatic cell [see reviews by Liao (1975) and Mainwaring (1977)]. In the present paper, we examine the relative importance of the recycling and synthesis mechanisms in contributing to the replenishment of nuclear receptor at different times after castration.

Materials and Methods

Experimental animals

Male Wistar rats, weighing 250–400g, were purchased from Woodlyn Laboratories (Guelph, Ont., Canada) and were maintained on a diet of Purina Laboratory Chow (Ralston Purina Co., St. Louis, MO, U.S.A.) and water *ad libitum*. Castration was performed under light ether anaesthesia through a scrotal incision. At appropriate times, the rats were killed by decapitation and the prostates were quickly removed, stripped free of connective tissue, placed on ice and weighed.

Preparation of subcellular fractions

All experimental procedures were performed at 4°C. Prostatic subcellular fractions were prepared by homogenizing fresh prostate in 5 vol. (v/w) of 10mM-Tes (2-[[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]-amino]ethanesulphonic acid) buffer, pH 7.0, containing 0.05mM-EDTA, 5mM-MgCl₂ and 0.5mM-mercaptoethanol (buffer A) in a Dounce apparatus. The homogenate was centrifuged at 800g for 10min to sediment the crude nuclei. The supernatant was centrifuged at 100000g for 60min to give the cytosol. The crude nuclei were purified and the nuclear extract was obtained as described by Bruchovsky *et al.* (1975).

Labelling of androgen receptors *in vivo*

Rats castrated 1 day and 7 days before the experiments were functionally hepatectomized, eviscerated and injected intravenously with 300μCi of [1,2-³H]-testosterone. At 5 and 60min after the injection, the rats were killed, and the prostatic tissue was separated into cytoplasmic and nuclear fractions. The content of receptor in each fraction was then measured by Sephadex G-25/G-200 dual-column chromatography.

Labelling of cytoplasmic receptor *in vitro*

Cytoplasmic receptor was labelled *in vitro* by using the mince-labelling technique described by Van Doorn *et al.* (1976). In brief, the prostatic tissue was minced with scissors and incubated in the presence of 750nM-[1,2-³H]dihydrotestosterone in a 37°C water bath for 2min. The reaction was terminated by cooling the incubation mixture rapidly to 4°C. The cytoplasmic fraction was obtained and the amount of receptor was determined by Sephadex G-25/G-200 dual-column chromatography.

Assay of translocation

This assay entailed the use of the labelling techniques *in vivo* and *in vitro* in sequence. Rats were

injected intravenously with 300μCi of [1,2-³H]-testosterone and were killed 5 and 60min later. The pooled prostates were divided into two fractions, and each was incubated in a 37°C water bath for 2min. One fraction contained 750nM-[1,2-³H]dihydrotestosterone, whereas the other fraction served as a control. The cytoplasmic and nuclear fractions were isolated and the receptor contents in each were measured as described below.

Assay of receptor concentration by Sephadex G-25/G-200 dual-column chromatography

The apparatus for this procedure consisted of a column (0.9cm×30cm) of Sephadex G-25 (Pharmacia, Montreal, Que., Canada) connected in series with a column (0.9cm×100cm) of Sephadex G-200 (Pharmacia). The sample (less than 2ml) was introduced into a sample loop and pumped into the short column. It was then eluted in an ascending direction with buffer A containing 0.6M-NaCl at a flow rate of 2–3ml/h. Fractions (1.4ml) were collected and assayed for radioactivity and u.v.-absorbing material.

Radioactive materials

[1,2-³H]Testosterone (40Ci/mmol) and [1,2-³H]-dihydrotestosterone (40Ci/mmol) were purchased from New England Nuclear (Boston, MA, U.S.A.). Solutions for intravenous injections and incubations *in vitro* were prepared as described by Van Doorn *et al.* (1976).

Liquid-scintillation counting

Aqueous samples were counted for radioactivity in a solution composed of 1 litre of toluene, 6g of 2,5-diphenyloxazole, 75ml of water and 232g of Bio-Solv (BBS-3, Beckman Instruments, Fullerton, CA, U.S.A.).

Other analytical procedures

Protein content of prostate tissue was assayed by using the differential u.v.-absorption method of Layne (1957) and confirmed by the method of Lowry *et al.* (1951), with bovine serum albumin as standard.

To express the results as molecules of receptor/cell, a steroid/receptor binding ratio of 1/1 was assumed, and the following constants were used: (1) A_{260} units/nucleus = $1.88 \times 10^{-7} \pm 0.11 \times 10^{-7}$ (mean ± S.E.M., $n = 43$) (light-path, 1cm), (2) nuclei/g of wet tissue = 11×10^7 (Bruchovsky *et al.*, 1975), (3) mg of cytosol protein/g of wet tissue = 31.6 ± 2.0 (mean ± S.E.M., $n = 28$) for 1-day-castrated animals, and 23.4 ± 2.4 (mean ± S.E.M., $n = 24$) for 7-day-castrated animals. The concentration of DNA in prostate is

1.4mg/g of wet tissue and 12.7pg/nucleus (Bruchovsky *et al.*, 1975).

Chemicals

Reagents used to prepare all solutions were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.) and Fisher Scientific Co. (Edmonton, Alberta, Canada) and were of the highest available purity. All steroids were obtained from Steraloids (Pawling, NY, U.S.A.). Deionized glass-distilled water was used in making up all solutions.

Results

Uptake of ^3H -labelled androgen into regressing prostate in vivo

To establish conditions for studying the replenishment of nuclear receptor, we first compared the relative distribution of androgen between cytoplasmic and nuclear compartments of prostate in 1-day- and 7-day-castrated animals. Animals were injected intravenously with 300 μCi (2 μg) of [1,2- ^3H]testosterone, a dose that rapidly saturates the nucleus with androgen (Van Doorn *et al.*, 1976). Prostate tissue was recovered at 5min and 60min after injection and assayed for radioactivity. The results in Table 1 indicate that there is no difference in the amount of androgen incorporated into prostatic cytoplasm of 1-day- and 7-day-castrated animals, at either 5min or 60min. However, the amount of androgen incorporated is about 3-fold greater at 60min than at 5min. More striking changes are observed in the nuclear uptake of androgen. Although the results at 5min are the same in 1-day- and 7-day-

castrated animals, by 60min a 3-fold difference is evident. Moreover, during the 60min time course of the experiment, the amount of androgen increases 6-fold in prostatic nuclei of 1-day-castrated animals, compared with only 2-fold in those of 7-day-castrated animals. Also, between 5 and 60min, the 2-fold increase in the amount of androgen in the prostatic cytoplasm of 7-day-castrated animals is equal to the 2-fold increase in the amount in the nucleus; by comparison, the corresponding increases in prostatic tissue of 1-day-castrated animals are 3-fold and 6-fold. These results indicate that the prostatic cell of the 7-day-castrated animal retains the capacity to transfer androgens into the nucleus, but at only 30% of that observed in the prostatic cell of the 1-day-castrated animal. They also indicate that the mechanism for incorporating androgens into the nucleus operates more efficiently in the latter case.

Translocation of receptor in regressing prostate

We next determined whether the restricted uptake of androgens into the prostatic nucleus of the 7-day-castrated animal is also paralleled by a lesser translocation of cytoplasmic receptor into the nucleus. To accomplish this, the androgen-binding capacities of cytoplasmic and nuclear fractions of prostate of 1-day- and 7-day-castrated animals were examined. Animals were injected with 300 μCi of [1,2- ^3H]testosterone and prostatic tissue was recovered 5 and 60min later. Analysis of cytoplasmic and nuclear binding of androgen by gel-exclusion chromatography yielded the results presented in Fig. 1. No major changes are observed between the profiles of cytoplasmic binding at 5min (●) and 60min (○), either 1 day (Fig. 1a) or 7 days (Fig. 1b) after castration. Although the amount of radioactivity recovered in the excluded volume (fractions 25–30) is greater in the latter experiment, and although the amount is slightly greater in almost all fractions at 60min than at 5min, it is unlikely that these changes are large enough to have significant meaning. On the other hand, a pronounced increase in the amount of nuclear binding of androgen is observed in the experiment 1 day after castration (Fig. 2a), and one of lesser magnitude in the experiment 7 days after castration. The peak of radioactivity corresponding to nuclear receptor is eluted in fractions 35–55, but skewing of the peak to the right suggests that the receptor is not homogeneous. Furthermore, the position of the principal peak of radioactivity in Fig. 2(b) relative to that in Fig. 2(a) indicates that the nuclear receptor in prostate of 7-day-castrated animals is smaller than its counterpart in prostate of 1-day-castrated animals.

The causes of the heterogeneity noted above and the alteration in size of receptor are not known. Since the nucleus apparently lacks any proteolytic factors capable of degrading receptor (Van Doorn *et al.*,

Table 1. *Uptake in vivo of ^3H -labelled androgens into regressing prostate*

Groups of 3–12 rats, castrated either 1 day or 7 days previously, were functionally hepatectomized and eviscerated. Immediately after surgery each rat was injected intravenously with 300 μCi of [1,2- ^3H]testosterone. Prostatic tissue was recovered 5 and 60min later, and separated into cytoplasmic and nuclear fractions. The fractions were analysed for radioactivity, and the results expressed as the means $\pm$ S.E.M. for the numbers of experiments shown in parentheses.

Time after injection (min)	Interval after castration (days)	Radioactivity recovered	
		Cytoplasm (10 ⁻⁵ $\times$ d.p.m./g)	Nucleus (10 ⁴ $\times$ d.p.m./nucleus)
5	1	10.3 $\pm$ 0.5 (5)	21.5 $\pm$ 1.6 (6)
	7	10.3 (1)	17.7 (1)
60	1	31.1 $\pm$ 3.5 (14)	125.0 $\pm$ 8.7 (16)
	7	24.5 $\pm$ 3.7 (4)	38.7 $\pm$ 10.6 (4)

Fig. 1. Binding of ³H-labelled androgens to cytoplasmic receptor *in vivo*

Groups of 3–12 rats, castrated either 1 day (a) or 7 days (b) previously were functionally hepatectomized and eviscerated. Immediately after surgery, each rat was injected with 300 μ Ci of [1,2-³H]testosterone. Prostatic tissue was recovered 5 and 60 min later, and the cytoplasmic fraction was analysed for binding by Sephadex G-25/G-200 dual-column chromatography. The column effluent was collected in 1.4 ml fractions. Radioactivity recovered: ●, 5 min after injection; ○, 60 min after injection.

1976), the observed variations may reflect conformational changes or multiplicity of forms (Katsumata & Goldman, 1974).

We assume that the peak of radioactivity recovered in the excluded volume (fractions 22–30) represents aggregated receptor or the binding of receptor to other nuclear components. With a 10-fold purification of nuclear receptor such formation of large androgen-binding complexes is no longer observed (N. Bruchovsky, unpublished work).

The actual amount of translocation that occurs in the 5–60 min interval after injection of [1,2-³H]-testosterone was estimated in several experiments

Fig. 2. Binding of ³H-labelled androgens to nuclear receptor *in vivo*

Nuclei were purified from the same tissue used in the experiments described in the legend to Fig. 1. Extracts of nuclei were analysed for binding by Sephadex G-25/G-200 dual-column chromatography. The column effluent was collected in 1.4 ml fractions. Experiment (a), 1 day after castration; experiment (b), 7 days after castration. Radioactivity recovered: ●, 5 min after injection; ○, 60 min after injection.

and the results are summarized in Table 2. In the prostatic nucleus of 1-day-castrated animals there is a net change of 18 700 receptors/nucleus, and in those of 7-day-castrated animals 3600 receptors/nucleus. This difference would be expected if the pool of cytoplasmic receptor were smaller in the prostate recovered from the latter group. According to the results in Table 2, although the capacity of cytoplasm to bind androgens is indeed lower in such tissue, in neither case is a significant decrease observed in total binding between 5 and 60 min. The lack of any time-dependent change in the binding of androgen to cytoplasmic protein is consistent with the possibility that most of the receptors are not detected by the labelling method *in vivo* owing to their very rapid

Table 2. Binding of ³H-labelled androgens to cytoplasmic and nuclear receptors *in vivo*

Prostatic tissue was recovered from castrated rats at intervals after injections of [1,2-³H]testosterone, and analysed for the presence of cytoplasmic and nuclear receptors as described in the legends to Figs. 1 and 2. The concentration of receptor is calculated in terms of the molecules of ³H-labelled androgen recovered in the fractions corresponding to the receptor peaks in Figs. 1 and 2. The results are expressed as the means ± S.E.M. for the numbers of experiments shown in parentheses.

Time after injection (min)	Interval after castration (days)	³ H-labelled androgen bound (molecules/cell)	
		Cytoplasm	Nucleus
5	1	10400 ± 2000 (5)	2300 ± 500 (7)
	7	3900 (1)	700 (1)
60	1	8500 ± 1400 (7)	21 000 ± 1800 (9)
	7	4600 ± 700 (3)	4300 ± 500 (4)

Table 3. Labelling *in vitro* of cytoplasmic receptor with ³H-labelled androgen

Prostatic tissue was obtained from rats castrated 1 day previously, minced and incubated for 2 min at 37°C in the presence of [1,2-³H]dihydrotestosterone at increasing concentrations. The cytosol fraction was treated with (NH₄)₂SO₄ at 80% saturation and the protein precipitate was analysed for binding by gel-exclusion chromatography. The results are expressed as the means ± S.E.M. for the numbers of experiments shown in parentheses.

[1,2- ³ H]Dihydro-testosterone (nM)	³ H-labelled androgen bound	
	(10 ⁻³ × d.p.m./mg of protein)	(10 ⁻⁵ × d.p.m./g wet wt. of tissue)
400	10.5 ± 1.2 (3)	1.68 ± 0.19 (3)
750	15.6 ± 1.7 (9)	2.54 ± 0.19 (9)
1000	14.6 ± 2.1 (3)	2.64 ± 0.18 (3)

translocation into the nucleus on contact with androgen. The background radioactivity that is observed may represent a combination of a small amount of residual androgen–receptor complex and some non-specific binding (Rennie & Bruchovsky, 1973), although other interpretations should not be overlooked (Shain *et al.*, 1975; Shain & Boesel, 1977).

Labelling *in vitro* of cytoplasmic receptor

To measure the true concentration of cytoplasmic receptor, we labelled prostate *in vitro*, using a method previously shown to be effective for this purpose (Bruchovsky *et al.*, 1975; Van Doorn *et al.*, 1976). The critical point is to limit the time of incubation at 37°C to only 2 min, so that no translocation of recep-

Fig. 3. Assay of cytoplasmic receptor *in vitro* by incubation of minced prostate with ³H-labelled androgen

Prostatic tissue was obtained from rats castrated 1 day (a) and 7 days (b) previously. The tissue was minced and incubated for 2 min at 37°C in the presence of [1,2-³H]dihydrotestosterone at 750 nM. Duplicate samples also contained non-labelled dihydrotestosterone at 75 μM. Protein recovered from the cytosol was analysed for binding by Sephadex G-25/G-200 dual-column chromatography. The column effluent was collected in 1.4 ml fractions. Radioactivity recovered: ○, without competition; ●, with competition.

tor takes place. For each measurement, minced prostate is divided into two samples. One sample is incubated with [1,2-³H]dihydrotestosterone, and the other with [1,2-³H]dihydrotestosterone plus a 100-fold excess of non-labelled dihydrotestosterone. The results shown in Table 3 indicate that saturation of specific binding sites is achieved at a steroid concentration of 750 nM. When the binding obtained at this concentration in prostate of 1-day- and 7-day-castrated animals is compared, the results in Fig. 3 show that the receptor molecules recovered differ in size. The principal peak of specific binding in Fig.

Table 4. *Concentration of cytoplasmic receptor*
Specific binding of [1,2-³H]dihydrotestosterone was measured by labelling of minced prostate *in vitro* as described in the legend to Fig. 3. The results were expressed as the means $\pm$ S.E.M. for the numbers of experiments shown in parentheses. The concentration of receptor is given by the molecules of ³H-labelled androgen specifically bound per cell.

Interval after castration (days)	³ H-labelled androgen bound (molecules/cell)		
	Total	Non-specific	Specific
1	31 100 $\pm$ 2500 (6)	9700 $\pm$ 800 (3)	21 400
7	12 700 $\pm$ 400 (4)	9700 $\pm$ 700 (3)	3000

3(a) (1-day-castrated animals) is eluted in fractions 35–55, whereas the one in Fig. 3(b) (7-day-castrated animals), eluted in fractions 45–55, represents a smaller molecule.

The results of several experiments in which specific binding was measured by labelling of tissue slices *in vitro* are summarized in Table 4. They indicate that there are 21 400 androgen receptors/cell in the prostatic cytoplasm of 1-day-castrated animals and 3000 in that of 7-day-castrated animals. Therefore the reservoirs of cytoplasmic receptor in prostate of such animals are indeed large enough to account for the changes in the concentrations of nuclear receptor recorded in Table 2.

Reciprocal relationship between the concentrations of cytoplasmic and nuclear receptor

To obtain more direct proof that nuclear receptor is replenished from a reservoir of cytoplasmic receptor, we attempted to define a time-dependent reciprocal relationship between the concentrations of cytoplasmic and nuclear receptor in prostate of 1-day-castrated rats. This was accomplished by combining the labelling technique *in vivo* described in Fig. 1 and Table 2 with the labelling technique *in vitro* described in Fig. 3 and Table 4. First, the rats were injected intravenously with 300 μ Ci of [1,2-³H]-testosterone; then prostatic tissue was recovered 5 and 60 min later, minced and incubated with 750 nM-[1,2-³H]dihydrotestosterone for 2 min at 37°C. Cytoplasmic and nuclear fractions were prepared and analysed for binding by gel-exclusion chromatography. The results presented in Fig. 4(a) show that the amount of cytoplasmic receptor labelled by the combined procedures is lower at 60 min ($\circ$) than at 5 min ($\bullet$). As expected, the amount of nuclear receptor increases with time (Fig. 4b).

In calculating the change in specific binding with time (Table 5), we corrected for the fixed pool of androgen binding *in vivo* (Fig. 1a) and the non-specific binding *in vitro* (Fig. 3a) by subtracting the respective

Fig. 4. *Assay of translocation*

Rats castrated 1 day before the experiment were injected intravenously with 300 μ Ci of [1,2-³H]testosterone and were killed 5 and 60 min later. The pooled prostates were divided into two fractions and each was incubated for 2 min at 37°C. One fraction contained 750 nM-[1,2-³H]dihydrotestosterone, and the other fraction served as a control. The cytoplasmic and nuclear fractions were isolated and analysed for binding by Sephadex G-25/G-200 dual-column chromatography. The column effluent was collected in 1.4 ml fractions. Experiment (a), cytoplasmic receptor; experiment (b), nuclear receptor. Radioactivity recovered: ●, 5 min after injection; ○, 60 min after injection.

values from the estimates of total cytoplasmic binding as furnished by data exemplified in Fig. 4(a). The results, as summarized in Table 5, indicate that, between 5 and 60 min after the injection of 300 μ Ci of [1,2-³H]testosterone, about 17 000 molecules of receptor/cell disappear from the cytoplasm and enter the nucleus. This evidence strongly implies that nuclear receptor is entirely replenished by translocation of cytoplasmic receptor in the 1-day-castrated animal.

Table 5. *Reciprocal relationship between the concentrations of cytoplasmic and nuclear receptor*

Sequential labelling *in vivo* and *in vitro* of prostate of 1-day-castrated rats was performed as described in the legend to Fig. 4. Cytoplasmic and nuclear fractions were prepared and analysed for binding by Sephadex G-25/G-200 dual-column chromatography. The total amount of ^3H -labelled androgen bound was measured from the chromatograms and corrected for non-specific binding as described in the text. The concentration of receptor is given in terms of the molecules of ^3H -labelled androgen bound in the cytoplasmic and nuclear compartments per cell, 5 and 60 min after the intravenous injection of $[1,2-^3\text{H}]$ testosterone. The change in the concentration is the amount of receptor translocated during the 5–60 min time course. The results are expressed as the means $\pm$ S.E.M. for the numbers of experiments shown in parentheses.

Fraction	^3H -labelled androgen bound (molecules/cell)		
	5 min	60 min	Change
Cytoplasm	20100 $\pm$ 2700 (3)	2700 $\pm$ 900 (3)	17400
Nucleus	2700 $\pm$ 900 (3)	19900 $\pm$ 2100 (4)	17200

Discussion

Our results indicate that the capacity to transport androgens into the nucleus, and the competence to form nuclear receptor, are reasonably well preserved in regressing prostate. However, the eventual decline in the concentration of cytoplasmic receptor after several days is paralleled by a decrease in the capacity of the cell to transfer both androgens and androgen receptors into the nucleus (Tables 1, 2 and 4). If the concentration of circulating androgen is restored to normal by parenteral administration of hormone, cytoplasmic receptor appears to migrate into the nucleus, but the molecules of androgen that enter the nucleus are more numerous than the molecules of translocated receptor (Tables 1 and 2). Another observation related to prostatic involution, and noted before by Bruchovsky & Craven (1975), is that cytoplasmic and nuclear receptors vary in size 1 day and 7 days after castration (Figs. 2 and 3).

Previously, we inferred that the cytoplasmic receptor recovered 1 day after castration originates from the nucleus (Van Doorn *et al.*, 1976; Rennie *et al.*, 1977). The results of our present work show that this receptor in turn probably accounts for the replenishment of nuclear receptor, when hormone is restored to the animal (Fig. 4 and Table 5). The fact that replenishment of nuclear receptor in prostate of 1-day-castrated animals is not inhibited by cycloheximide (Blondeau *et al.*, 1975) is further evidence that replenishment relies entirely on the recycling of receptor. Hence we conclude that nuclear receptor is replenished by a recycling mechanism

with no synthesis of protein involved, during the early stages of prostatic involution.

The process of replenishment is not as simple when the stage of prostatic involution is more advanced, since new synthesis of protein is also required to restore nuclear receptor contents to normal. Only 3000 cytoplasmic receptors/cell are present in the prostate of the 7-day-castrated animal (Table 4), and these appear to be rapidly translocated into the nucleus in the presence of androgen (Table 2). However, about 12000 molecules of nuclear receptor/cell are formed if regeneration of prostate is allowed to proceed (Van Doorn *et al.*, 1976). Regeneration is initiated by a single subcutaneous injection of dihydrotestosterone at a dose of 400 $\mu\text{g}/100\text{g}$ body wt. (Lesser & Bruchovsky, 1973). Within 6 h the nucleus accumulates 3000 receptor molecules (Van Doorn *et al.*, 1976); from the results presented in Tables 2 and 4 we infer that these arise by translocation of 3000 existing cytoplasmic receptors. Between 6 and 48 h the nucleus accumulates another 9000 receptor molecules. These are synthesized in early G1 phase during the first round of cell division induced by dihydrotestosterone (Van Doorn *et al.*, 1976). Thus, when prostatic regression is at a late stage, it is virtually certain that both recycling and new synthesis of protein are required for the replenishment of nuclear receptor.

The foregoing interpretation of our results partly hinges on the validity of the assumption that the cytoplasmic androgen-binding protein measured by the labelling procedure *in vitro* is a receptor. Indeed, our conclusions must be viewed in the light of the fact that the evidence for translocation remains circumstantial, and that it is not known whether the receptor plays any role in mediating androgen action. Nevertheless, the cytoplasmic receptor we describe is characterized by a number of properties commonly ascribed to a receptor molecule. It has a sedimentation coefficient of 4.4S in low salt and 7S in high salt. In competition experiments, the binding of radioactive dihydrotestosterone is inhibited by a 100-fold excess of dihydrotestosterone, testosterone, progesterone and oestradiol-17 β , but a similar excess of cortisol is less effective. High affinity of binding is suggested by the low rate of dissociation of the androgen–receptor complex at 0–4°C, and limited binding capacity is indicated by the results in Table 3. Finally, two lines of indirect evidence suggest that this receptor participates actively in androgen-stimulated reactions. Firstly, the concentration of receptor fluctuates in response to the acute withdrawal and replacement of androgens, and, secondly, the receptor binds extensively to DNA–cellulose. More complete information about these physical and biological properties is given in reports by Bruchovsky *et al.* (1975), Van Doorn *et al.* (1976) and Rennie *et al.* (1977).

Notwithstanding the uncertainty about the origin and fate of cytoplasmic receptor, the elucidation of several basic features of the replenishment process for nuclear receptor establishes the conditions for studying the actual synthesis of this protein.

We thank Sharon Craven for technical assistance and Glenda Dennis for typing the manuscript. These studies were supported by grants from the Medical Research Council of Canada and the National Cancer Institute of Canada. E. V. D. is a research scientist supported by the Provincial Cancer Hospitals Board and the Heritage Savings Trust Fund of the Province of Alberta.

References

- Blondeau, J. P., Corpechot, C., LeGoascogne, C., Baulieu, E. E. & Robel, P. (1975) *Vitam. Horm. (N. Y.)* **33**, 319-345
- Bruchovsky, N. & Craven, S. (1975) *Biochem. Biophys. Res. Commun.* **62**, 837-843
- Bruchovsky, N., Rennie, P. & Vanson, A. (1975) *Biochim. Biophys. Acta* **394**, 248-266
- Capony, F. & Rochefort, H. (1975) *Mol. Cell. Endocrinol.* **3**, 233-251
- Clark, J. H., Paszko, Z. & Peck, E. J. (1977) *Endocrinology* **100**, 91-96
- Ferguson, E. R. & Katzenellenbogen, B. S. (1977) *Endocrinology* **100**, 1242-1251
- Grover, P. K. & Odell, W. D. (1975) *J. Steroid Biochem.* **6**, 1373-1379
- Katsumata, M. & Goldman, A. S. (1974) *Biochim. Biophys. Acta* **359**, 112-129
- Layne, E. (1957) *Methods Enzymol.* **3**, 447-454
- Lesser, B. & Bruchovsky, N. (1973) *Biochim. Biophys. Acta* **308**, 426-437
- Liao, S. (1975) *Int. Rev. Cytol.* **41**, 84-172
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265-275
- Mainwaring, W. I. P. (1977) *The Mechanism of Action of Androgens*, pp. 72-73, Springer-Verlag, New York
- Mester, J. & Baulieu, E. E. (1975) *Biochem. J.* **146**, 617-623
- Mester, J. & Baulieu, E. E. (1977) *Eur. J. Biochem.* **72**, 405-414
- Munck, A. & Leung, K. (1976) in *Receptors and Mechanism of Action of Steroid Hormones* (Pasqualini, J. R., ed.), pp. 311-397, Marcel Dekker, New York
- Rennie, P. S. & Bruchovsky, N. (1973) *J. Biol. Chem.* **248**, 3288-3297
- Rennie, P. S., Van Doorn, E. & Bruchovsky, N. (1977) *Mol. Cell. Endocrinol.* **9**, 145-157
- Shain, S. A. & Boesel, R. W. (1977) *Mech. Ageing Dev.* **6**, 219-232
- Shain, S. A., Boesel, R. W. & Axelrod, L. R. (1975) *Arch. Biochem. Biophys.* **167**, 247-263
- Sutherland, R. L. & Baulieu, E. E. (1976) *Eur. J. Biochem.* **70**, 531-541
- Van Doorn, E., Craven, S. & Bruchovsky, N. (1976) *Biochem. J.* **160**, 11-21