The Effects of 5 α -Dihydrotestosterone on the Kinetics of Cell Proliferation in Rat Prostate

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The regenerating rat prostate was used as an experimental model to determine the effects of 5 α -dihydrotestosterone on certain parameters of cell proliferation, including the duration of the phases of the cell cycle and the size of the cellular growth fraction. Rats castrated 7 days previously were treated with daily subcutaneous injections of 5 α -dihydrotestosterone for 14 days; 48 h after the beginning of therapy, cells in the process of DNA synthesis were labelled with a single injection of radioactive thymidine and the progress of these cells through the division cycle was observed. Cell-cycle analysis was performed by fractionating prostatic nuclei according to their position in the cell cycle by using the technique of velocity sedimentation under unit gravity. The results indicate that during regeneration the cell population undergoes 1.8 doublings with a doubling time of 40 h, and that the process involves almost four rounds of cell division with a cell-generation time of 20 h. The growth fraction at any time is about 0.5, and about half the daughter cells produced do not re-enter the proliferative cycle. All cells present at the start of regeneration eventually undergo at least one division during the course of regeneration, although any given cell can divide from one to four times.

At least three experimental observations suggest that the function of 5 α -dihydrotestosterone (17 β -hydroxy-5 α -androstane-3-one) in the male accessory organs of reproduction is partly concerned with the control of DNA synthesis and cell division. First, it has been shown that a major effect of 5 α -dihydrotestosterone on rat prostate, both *in vivo* and *in vitro*, is to stimulate cell proliferation (Robel *et al.*, 1971; Lesser & Bruchovsky, 1973). Secondly, a significant increase in the intracellular concentration of 5 α -dihydrotestosterone has been detected in benign hyperplastic conditions of human and canine prostate (Siiteri & Wilson, 1970; Gloyne *et al.*, 1970). Thirdly, although the growth of rat prostate is induced by a number of natural androgens, circumstantial evidence has been obtained indicating that the potency of these may be related to the amount of 5 α -dihydrotestosterone formed from each androgen (Bruchovsky, 1971).

During initial experiments towards defining androgen-sensitive mechanisms in the cell that affect prostatic growth and function, we have characterized the growth response of prostate to 5 α -dihydrotestosterone. As reported previously (Lesser & Bruchovsky, 1973), the treatment of rats, after 7 days of castration, with daily doses of 5 α -dihydrotestosterone produces an increase in the rate of DNA synthesis and in the number of nuclei per prostate. There is a latent period of 24–36 h before these effects are observed, followed by a period of cell proliferation lasting 3 days during which the comple-

ment of cells is restored to normal. After this interval, despite continued administration of androgen, the rate of DNA synthesis declines to a negligible value and the rate of cell production falls abruptly.

This experimental model has been used in the present investigation to delineate the growth of prostate in terms of the duration of the phases of the cell cycle, the fraction of cells involved in the proliferative response, and the number of divisions that a given cell undergoes during the regenerative process.

It has been the practice in the past to study the kinetics of cell proliferation in reproductive organs by measuring the fraction of labelled mitoses as a function of time after exposure of cells to a radioactive nucleoside and by this means to follow the cohort of labelled cells through the division cycle (Tuohimaa & Niemi, 1968; Morley & Wright, 1972; Morley *et al.*, 1973). However, since nuclei of mammalian cells can be fractionated on the basis of size by the technique of velocity sedimentation (McBride & Peterson, 1970), we have utilized the latter method to separate prostatic nuclei according to their position in the cell cycle and to follow the progress of such nuclei through successive divisions.

Materials and Methods

Experimental animals

Adult male Wistar rats weighing 270–340 g were purchased from Woodlyn Laboratories, Guelph,

Ont., Canada, and maintained on a diet of rat chow and water *ad libitum*. Castration was performed via the scrotal route under ether anaesthesia. 5 α -Dihydrotestosterone (Steraloids, Pawling, N.Y., U.S.A.) in a dose of 800 μ g/100g body wt. was administered subcutaneously as a suspension in aqueous medium. The latter solution contained water-ethanol-polyoxyethylene sorbitan monopalmitate (8:1:1, by vol.), and about 0.6ml was injected with each dose of steroid. [Me - 3H]Thymidine or [Me - ^{14}C]thymidine at a dose of 5 μ Ci/100g body wt. was injected intraperitoneally in about 0.3ml of 0.9% NaCl. At the appropriate time, animals were killed by decapitation and prostates quickly removed, stripped of connective tissue, placed on ice, and weighed. Five to eight prostates were pooled for each experiment.

Isolation of nuclei

All operations were carried out at 0–4°C. The prostates were chopped with an automatic tissue sectioner and washed with phosphate-buffered saline (Dulbecco & Vogt, 1954). The tissue was resuspended in 20ml of Tris-HCl buffer, pH 7.5, (10mM) containing sucrose (0.25M), CaCl₂ (1.5mM), EDTA (0.05mM), MgCl₂ (5mM) and 2-mercaptoethanol (0.5mM) and then manually homogenized in a Dounce apparatus with 25 strokes of a loose-fitting plunger. The homogenate was filtered through three layers of gauze and the filtrate was homogenized with ten strokes of a tight-fitting plunger. Centrifugation of this suspension at 700 *g* for 10min in a Sorvall GLC-1 centrifuge (HL-4 rotor, r_{av} 12.5cm) yielded a crude nuclear pellet. This was resuspended in phosphate-buffered saline containing octylphenoxypolyethoxyethanol (Triton X-100; Sigma Chemical Co., St. Louis, Mo., U.S.A.; 0.05%, v/v) with five strokes of a loose-fitting plunger, and the resultant suspension was centrifuged at 700 *g* for 10min. The pellet was then resuspended by vortexing in 5ml of phosphate-buffered saline containing bovine serum albumin (fraction V powder; Sigma; 0.3%, w/v), and Triton X-100 (0.02%, v/v). This suspension was centrifuged at 40 *g* for 2min to remove debris and the supernatant was decanted as the nuclear fraction. A sample of the nuclear fraction was stained with Methyl Blue and nuclei were counted with a haemocytometer. The suspension was considered adequate for fractionation if less than 10% of the nuclei were present as small clumps. The yield of nuclei averaged 16×10^7 /g of prostate.

Velocity sedimentation of nuclei

The apparatus for velocity sedimentation under unit gravity was basically that described by Miller & Phillips (1969). It was purchased from O. H. Johns

Scientific Co. (Toronto, Ont., Canada) and modified by addition of a water jacket around the sedimentation chamber. All bovine serum albumin solutions were prepared in phosphate-buffered saline containing Triton X-100 (0.02%, v/v) and were filtered through a cellulose filter (pore size 0.22 μ m; Millipore Corp., Montreal, P.Q., Canada) before use. The two chambers containing bovine serum albumin at concentrations of 1 and 2% (w/v) respectively were filled with 600ml of solution. The remaining chamber was filled to the same level with bovine serum albumin (0.5%, w/v).

The nuclear suspension was diluted to a concentration of 3.0×10^6 nuclei/ml in phosphate-buffered saline containing bovine serum albumin (0.3%, w/v) and Triton X-100 (0.02%, v/v). Unless otherwise stated the volume introduced into the sedimentation chamber was 20ml. Next, the gradient was loaded at a rate of about 30ml/min, and then was left for 8h (measured from the time liquid reached the top of the cone on loading). After this interval, fractions (approx. 11ml) were collected at a flow rate of 15–20ml/min. The sedimentation velocity of each fraction was calculated by using the equation $V = [(N-F)D]/[S+C(F/N)]$ where the parameters are defined as follows: V , sedimentation velocity; N , total number of fractions; F , fraction number; D , depth/fraction, equal to the fraction volume/volume occupied by 1 vertical mm of the sedimentation chamber (22.4ml); S , standing time (from completion of loading to start of unloading); C , collection time (from start of unloading to descent of meniscus to top of cone.)

Counting of nuclei

A sample of each fraction was diluted with filtered phosphate-buffered saline and counted by using a Coulter counter model B (Coulter Electronics, Hialeah, Fla., U.S.A.) fitted with a 50 μ m aperture. Recovery of nuclei from the gradient was about 70%.

Measurement of radioactivity

After removal of a portion for counting of nuclei, the remainder of each fraction was deposited on a cellulose filter (pore size 0.45 μ m). The filters were washed with 5ml of phosphate-buffered saline and then with 2×5 ml of trichloroacetic acid (5%, w/v). After drying overnight at 37°C, the filters were analysed for radioactivity. Dried filters were deposited in glass vials containing 10ml of diphenyloxazole-toluene solution (4g of diphenyloxazole/litre of toluene), and the radioactivity was measured in an automatic liquid-scintillation system. The amount of radioactivity loaded per gradient was approx. 2000 c.p.m., and recovery of radioactivity from the gradient was greater than 80%.

DNA determinations

In experiments requiring DNA determinations, 2ml of HClO_4 (1.6M) was added to the dried filters and DNA was extracted by heating at 70°C for 20min. The filters were removed and a portion (1.5ml) of the extract was assayed for DNA by the diphenylamine procedure (Burton, 1956) with calf thymus DNA as standard. The remainder of the extract was added to 10ml of a diphenyloxazole-toluene solution that contained toluene (1 litre), diphenyloxazole (6g), water (75ml) and Bio-Solv (BBS-3; 116g; Beckman Instruments, Scientific Instruments Division, Fullerton, Calif., U.S.A.) and counted for radioactivity in an automatic liquid-scintillation system.

Radioactive materials

[$\text{Me-}^3\text{H}$]Thymidine (specific radioactivity 50–55 Ci/mmol) and [$\text{Me-}^{14}\text{C}$]thymidine (specific radioactivity 55mCi/mmol) were purchased from New England Nuclear Corp. (Boston, Mass., U.S.A.).

Radioautography

Cytological and radioautographic procedures were performed as described previously (Lesser & Bruchovsky, 1973).

Results

Separation of nuclei according to phases of the cell cycle

The technique of cell-cycle analysis by velocity sedimentation is based on the increase in size of nuclei as cells progress through the cell cycle, with G2-phase nuclei being about twice the size of G1-phase nuclei, and thus sedimenting 1.6 times as rapidly. In the present experiment, this analysis was performed with prostatic nuclei obtained from rats which had been castrated for 7 days and then treated with 5α -dihydrotestosterone for 3 days. As shown in Fig. 1, when a population of such nuclei is allowed to settle through a shallow bovine serum albumin gradient under unit gravity, two peaks of material are observed. Microscopic examination of the slowly sedimenting peak (about 0.4mm/h) shows it to consist of debris. It contains no DNA and no radioactivity, and for purposes of clarity will be omitted from subsequent Figures. Nuclei are distributed as a main peak centred at 2.0mm/h and skewed towards larger sedimentation velocities. After a 1 h pulse *in vivo* with [$\text{Me-}^3\text{H}$]thymidine just before killing, radioactivity is found in a peak heavier than that of most nuclei, with a maximum about 2.9mm/h. Nuclei sedimenting between 1.3 and 2.2mm/h contain the

Fig. 1. Fractionation of nuclei according to phases of the cell cycle

Rats castrated 7 days previously were injected subcutaneously with $800\mu\text{g}$ of 5α -dihydrotestosterone/100g body wt. daily for 3 days. At 1 h before death the rats were injected intraperitoneally with $5\mu\text{Ci}$ of [$\text{Me-}^3\text{H}$]thymidine (specific radioactivity 50–55 Ci/mmol)/100g body wt. Nuclei were isolated as described in the Materials and Methods section, and a total of 1.2×10^8 was applied to the gradient in a volume of 40ml. The distribution of particles after 8 h is shown for each fraction as a percentage of the value of the peak at 2mm/h. The distribution of radioactivity is expressed for each fraction as a percentage of the total radioactivity recovered, and DNA is shown as pg/nucleus. Fractions which contained a small number of nuclei were pooled to obtain sufficient DNA. Material recovered: Δ , nuclei; $\circ$, radioactivity; $\square$, DNA.

diploid amount of DNA and little radioactivity, and accordingly are considered to be in the G0 and G1 phases of the cell cycle. Nuclei sedimenting between 2.2 and 3.1 mm/h contain increasing amounts of DNA and most of the radioactivity, characteristic of nuclei in the process of DNA replication (S phase). Finally, nuclei sedimenting more rapidly than 3.1 mm/h contain twice the diploid amount of DNA and little radioactivity, and are considered to be in the G2 phase of the cell cycle.

Rate of prostatic growth: time required for doubling of cell population

As reported previously (Lesser & Bruchovsky, 1973) the rate of cell renewal in the rat prostate is estimated by measuring the increase in numbers of nuclei as a function of time during regeneration of the prostate induced by 5α -dihydrotestosterone. In 7-day castrate rats, the number of nuclei/prostate and the rate of DNA synthesis begin to increase 48 h after treatment is started. During the next 3 days rapid growth occurs and by day 5 the number of nuclei/prostate has returned to normal. At this time the rate of production of nuclei declines to 25% of the maximal rate and the rate of DNA synthesis falls to a negligible value. During the 3 days when the growth rate is at a maximum, the cell population undergoes about 1.8 doublings and the time required for one doubling is approx. 40h.

Rate of progression of cells through the cell cycle

The next group of experiments was designed to follow the progression of cells through phases of the cell cycle during a period of prostatic regeneration. Rats castrated 7 days previously were treated with daily injections of 5α -dihydrotestosterone. At 48 h after the start of therapy the animals received a single intraperitoneal injection of [Me^3H]thymidine (the time of this injection is equated to zero time in the following discussion). The fate of radioactively labelled nuclei was then followed for the subsequent 48 h by using the technique of velocity sedimentation. In all experiments prostatic weights and nuclear yields were monitored and compared with those of control animals to ascertain that the [Me^3H]thymidine had no deleterious effect on prostatic growth.

The results obtained during the first 12 h are shown in Fig. 2. After 1 h (Fig. 2b), radioactive nuclei are in the S-phase region of the gradient, and after 2 h the radioactivity has shifted to heavier regions. By 4 h (Fig. 2c) some radioactive label has appeared in the G1 region, representing nuclei that have completed their first division, and the major peak occurs in the late S and G2 regions. After 8 h (Fig. 2d) most of the radioactivity is in the G1 area, and by 12 h the

shift is almost complete and little radioactivity remains in the S and G2 regions. At 12 h the peak of radioactivity is actually slower than the peak of nuclei; this may indicate that nuclei that have just divided are smaller than most G1 nuclei.

The results for times later than 12 h are presented in Figs. 3 and 4. To demonstrate clearly enrichment or depletion of radioactivity in various phases of the cell cycle in this group of experiments, it was necessary to express the results as a ratio of the specific radioactivities of fractionated and unfractionated nuclei. Hence the specific radioactivity of each fraction, calculated as c.p.m./ 10^6 nuclei, has

Fig. 2. Fractionation of nuclei 1-12 h after the administration of [Me^3H]thymidine

Rats castrated 7 days previously were injected subcutaneously with $800\mu\text{g}$ of 5α -dihydrotestosterone/100 g body wt. at 24 h intervals. Along with the third injection at 48 h, the animals were injected intraperitoneally with $5\mu\text{Ci}$ of [Me^3H]thymidine/100 g body wt. At 1, 2, 4, 8 and 12 h after the injection of radioactive nucleoside, the rats were killed and prostatic nuclei were fractionated as described in the Materials and Methods section. The distribution of nuclei is indicated in terms of the number of nuclei recovered in each fraction calculated as a percentage of the peak value. The distribution of radioactivity is similarly expressed in terms of the amount recovered in each fraction as a percentage of the total amount recovered. Distribution of nuclei (a); distribution of radioactivity at 1 ($\circ$) or 2 ($\square$) h, (c) at 4 ($\circ$) h, (d) at 8 ($\circ$) or 12 ($\square$) h as shown.

Fig. 3. Fractionation of nuclei 12–24 h after the administration of $[Me-^3H]$ thymidine

The experimental method is the same as that described in the legend to Fig. 2 except that the rats were killed at 12, 16, 20 and 24 h after the injection of $[Me-^3H]$ thymidine. The distribution of nuclei is indicated in terms of the number of nuclei recovered in each fraction calculated as a percentage of the peak value. The radioactivity is shown as the specific radioactivity of the nuclei in each fraction relative to that of unfractionated nuclei set at 1.0. (a), Distribution of nuclei; (b), (c), (d), (e), distribution of radioactivity at 12, 16, 20, and 24 h respectively.

been divided by the specific radioactivity of an unfractionated population of nuclei, and the quotient is plotted as a function of the sedimentation velocity. Fig. 3(b) shows the data for 12 h replotted in this manner. Enrichment of radioactivity occurs in early G1 phase and depletion in late G1 phase. The situation is similar at 16 h (Fig. 3c), except that the enriched peak occurs slightly to the right. At this time the amount of radioactivity in the S and G2 regions of the gradient reaches a minimum of 12%, which may represent the degree of contamination of this region by G1 nuclei. At 20 h (Fig. 3d) the S and G2 regions are slightly enriched in radioactive label, and by 24 h (Fig. 3e) there has been a significant shift of label

Fig. 4. Fractionation of nuclei 30–48 h after the administration of $[Me-^3H]$ thymidine

The experimental method is the same as that described in the legend to Fig. 3 except that the rats were killed at 30, 36, 42 and 48 h after the injection of $[Me-^3H]$ thymidine. (a), Distribution of nuclei; (b), (c), (d), (e), distribution of radioactivity at 30, 36, 42 and 48 h respectively.

back into this region. At this time 32% of the radioactivity is present in the S and G2 areas of the gradient, representing an increase of about 20% over the amount present at 16 h. At 30 h (Fig. 4b) and 36 h (Fig. 4c) the S and G2 regions continue to be enriched in radioactivity and this trend is particularly noticeable at 42 h (Fig. 4d), but by 48 h (Fig. 4e) there is very little radioactive label left in these regions.

Maintenance of DNA synthesis

During the course of these experiments it was noted that after the first mitotic division most of the labelled cells at any given time were found in the G1 region of the gradient. To ascertain that this apparent G1 arrest was not due to a change in properties of S-phase nuclei or to a cessation of DNA synthesis after one round of division, a double-labelling experiment was performed. Castrated rats were

Fig. 5. Maintenance of DNA synthesis

Rats castrated 7 days previously were injected subcutaneously with $800\mu\text{g}$ of 5α -dihydrotestosterone/100g body wt. at 24h intervals. Along with the third injection at 48h, the rats were injected intraperitoneally with $5\mu\text{Ci}$ of $[\text{Me-}^3\text{H}]\text{thymidine}$ (specific radioactivity $50\text{--}55\text{Ci}/\text{mmol}$)/100g body wt. At 23h later the rats were injected intraperitoneally with $5\mu\text{Ci}$ of $[\text{Me-}^{14}\text{C}]\text{thymidine}$ (specific radioactivity $55\text{mCi}/\text{mmol}$)/100g body wt. Then 1h after the latter injection the rats were killed and prostatic nuclei were prepared and fractionated by velocity sedimentation under unit gravity. The distribution of nuclei is indicated in terms of the number of nuclei recovered in each fraction calculated as a percentage of the peak value. The distribution of ^3H and ^{14}C radioactivity was measured by double-isotope liquid-scintillation counting and the results are expressed in terms of the amount of radioactivity recovered in each fraction as a percentage of the total amount recovered. Material recovered: Δ , nuclei; $\bullet$, ^3H radioactivity; $\circ$, ^{14}C radioactivity.

treated with $[\text{Me-}^3\text{H}]\text{thymidine}$ as described above (48h after initiation of therapy with 5α -dihydrotestosterone) and were then treated again 24h later with an intraperitoneal injection of $[\text{Me-}^{14}\text{C}]\text{thymidine}$. As shown in Fig. 5, although the bulk of ^3H label is recovered in the G1 region of the gradient, the ^{14}C label occurs in the S-phase region. Thus DNA synthesis continues as expected, but some nuclei that synthesize DNA at later times during regeneration are probably different from those that synthesize DNA at early times.

Discussion

From an analysis of the preceding results it is possible to calculate certain kinetic parameters of the division cycle of prostatic cells during a period of active prostatic growth. By following the appearance of label in the G1-phase region of the gradient (Fig. 2), an estimate of the duration of the cell-cycle phases is obtained. The time required for radioactive nuclei to first appear in the G1-phase region corresponds to the minimum duration of G2-phase and mitosis. Since approx. 25% of the radioactivity is

present in the G1-phase region by 4h (Fig. 2c) a reasonable estimate for the length of G2-phase and mitosis is 2–3h. It will take an interval of time equal to the duration of S and G2 phases and mitosis for all the radioactive label to be transferred to the G1-phase region. Accordingly, after 8h (Fig. 2d), 80% of the label is in the G1-phase region and by 12h this value has reached 85%. Thus the length of S and G2 phases and mitosis is in the vicinity of 10h, leading to an estimate of 7–8h for the S-phase. The reappearance of label in the S-phase region (Fig. 3d) indicates that the minimum duration of the cell cycle is about 20h. Finally, from the values of 2–3h for the G2 and mitosis periods, 7–8h for the S-phase and 20h for the duration of the cell cycle, the length of G1-phase can be determined to be approx. 10h.

The fraction of cells involved in the prostatic growth process (growth fraction, GF) can be deduced from a further analysis of the above results. A population-doubling time of 40h with a cell-cycle time of 20h requires a minimal growth fraction of 0.41. A more precise estimate can be obtained by considering the kinetics of cell production in the tissue. In the regenerating prostate, these kinetics are certainly neither steady-state nor strictly exponential, but are probably described most accurately by considering them to be exponential with a growth fraction of less than 1.0. These kinetics can be described mathematically by modifying the derivation of Cleaver (1967) such that after one cell-cycle time the number of cells is equal to $(1+\text{GF})N_0$, where N_0 is the number originally present, rather than $2N_0$ as would be the case if the growth fraction were 1.0. The equation describing the kinetics of cell production then becomes

$$N_t = N_0 e[t/T \ln(1 + \text{GF})]$$

or

$$N_t = N_0(1 + \text{GF})^{t/T}$$

where N_t is the number of cells at time t , N_0 is the number of cells at time 0, and T is the cell-cycle time. Again, by using the analysis of Cleaver (1967), the mitotic index (MI) and labelling index (LI) can be described by:

$$\text{MI} = (1 + \text{GF})^{t_m/T} - 1$$

and

$$\text{LI} = (1 + \text{GF})^{t_s/T} [(1 + \text{GF})^{t_m/T} - 1]$$

where t_m , t_s and t_g are the durations of mitosis, G2-phase + 1/2 mitosis, and S phase respectively. At 48h after the initiation of therapy with 5α -dihydrotestosterone, the labelling and mitotic indices have been determined by radioautography to be 16 and 2.1% respectively (results not shown). By using the values for the duration of the various phases

determined above, the fraction of cells involved in the initial proliferative response to 5 α -dihydrotestosterone is calculated to be 0.51 if mitotic index is used or 0.50 if labelling-index data is used.*

It is noteworthy that after a cell has divided once, it does not necessarily proceed through the division cycle a second time. This conclusion can be inferred from the following observations. At 16h after a pulse of [3 H]thymidine, 12% of the radioactive label is found in the S- and G2-phase regions of the gradient (Fig. 3c). Between 20 and 42h, the amount of label in this region exceeds 12%. If the percentage of label in the S- and G2-phase regions at 16h is subtracted from the percentage in these areas at later times, it is possible to estimate the relative number of nuclei that enter the S phase for a second time. Assuming that the 6h interval between successive experiments is sufficiently long compared with the duration of the S phase (7h) such that each cell is only counted once during its passage through S phase, then the sum of the differences from the above calculation will correspond to the proportion of the radioactively labelled population of cells that undergoes a second round of division. This proportion is slightly greater than 50%, and is compatible with the idea that one daughter cell resulting from each division differentiates, while the other continues to divide.

Since 50% of the progeny cells from the first division do not continue to proliferate, other previously non-proliferating cells must be recruited if the growth fraction is to be maintained. As described above, cell production is supported by a growth fraction of 50%, and with a cell-cycle time of 20h, almost four rounds of division would be required to restore the number of cells in prostate to normal (1.8 doublings). Assuming a 50% loss at each round, it can be calculated that all of the cells originally present in the prostate after 7 days of castration must divide at some time during prostatic regeneration.

In addition to affording estimates of the duration of the phases of the cell cycle, and of the size of the growth fraction, the results of this work lend support

* If the method of Cleaver (1967) is used to calculate the growth fraction, then values of 0.60 and 0.54 for mitotic- and labelling-index data respectively are obtained. However, we feel our modification makes the analysis more applicable to the situation in the prostate.

to the following conclusions. First, the regeneration of prostate does not depend on the activation of a unique stem-cell population; rather, all cells that survive prostatic involution seem to participate in the growth response. Secondly, the mechanism that regulates prostatic size is independent of the number of cell divisions. In other words, it is unlikely that cell division is arrested because of the dilution of a regulatory molecule (for example, a steroid receptor) by successive equal partitionings of the cell. Thirdly, it is clear from both present results and earlier work (Lesser & Bruchovsky, 1973) that shutdown of proliferation does not involve a loss of sensitivity to 5 α -dihydrotestosterone, since prostatic weight continues to increase after proliferation is arrested. Finally, it seems likely that the proliferative potential of prostatic cells is regulated by a type of feedback mechanism that is dependent on total cell number.

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