

Early Effects of Castration and Replacement of Androgen on the Expression of Retrovirus-like Particles in Rat Ventral Prostate Epithelial Cells^{1,2} (41377)

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Abstract. Previous work has shown the presence of C-type retrovirus-like particles in normal prostate epithelial cells from Sprague-Dawley rats. We now report attempts to alter retrovirus-like particle expression by castration and replacement of androgen, i.e., testosterone propionate (TP). The frequency of C-type viral-like particle expression in the ventral prostates from the following groups of animals was estimated by a semiquantitative electron microscopic assay: (a) normal rats, (b) normal rats that received TP for 3 days, (c) rats 3 or 6 days after castration, and (d) rats that received TP for 3 days, beginning 3 or 6 days after castration, to induce cellular hypertrophy or hyperplasia, respectively. Three or six days following castration, the frequency of C-type particle expression in the regressed ventral prostates was markedly reduced. Typical C-type viral-like particle morphology was retained for those few particles detected. In contrast, prostates with hypertrophic epithelium from rats treated with TP starting 3 days after castration exhibited moderate to high frequencies of expression of both immature and mature, and aberrant C-type-like particles. Prostates with hyperplastic epithelium from rats treated with TP starting 6 days after castration had a wide range expression frequencies comparable to that of the normal, intact control group. Prostates from TP-treated normal intact rats exhibited low to moderate frequencies of C-type particle expression and showed no major change in the morphological pattern of virus-like particle expression. It appears that androgens are, at the very least, permissive for the expression of the retrovirus-like particles by rat ventral prostate epithelial cells, and that major *de novo* DNA synthesis and cellular replication are probably not essential for the increased release of the virus-like particles observed after short-term castration and replacement of androgen.

The apparently spontaneous release of morphologically typical C-type viral-like particles (retroviruses) from rat ventral prostate epithelial cells of young Sprague-Dawley or Long-Evans rats have been reported previously (1, 2). C-type particles have also been seen in the prostate from one of four aged CD rats (3). Although an extensive survey of many tissues from

normal Sprague-Dawley and Long-Evans rats did not reveal virus detectable by electron microscopy (4), C-type particles subsequently were observed in Sprague-Dawley rat placentas (5). Release of C-type particles from chemically induced rat hepatomas (6) after treatment with agents such as 5-bromodeoxyuridine (7) and "spontaneously" from cultured rat embryonic cells (8, 9) have been reported. An ecotropic virus (RaLV) (10, 11) is able to "rescue" fibroblast-transforming "sarcoma" viruses (RaSV) from several different rat tumor cell lines (12). RNA nucleotide sequences of the endogenous "sarcogene" and of the endogenous rat leukemia viral genome appear to differ (13-15).

That the content of retrovirus information in rat prostate and other cells probably

¹ Supported by the U.S. Department of Energy Contract W-31-109-ENG-38 and by Grant CA-22246 from the National Cancer Institute.

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is complex might be inferred from results with mouse cells. At least three biologically distinct viruses, one ecotropic and two xenotropic in host range, have been described in most of the 13 strains examined (16). These observations complement previous reports of the widespread presence of viral gp70 antigen in the mouse reproductive tract (17) and of C-type particles in the prostates of 10 strains examined (18). Thus, there is evidence that expression of several types of viral information may occur throughout much of the murine genitourinary tract.

Since the apparently spontaneous release of retroviral-like particles in the adult male rat has thus far been observed only in the prostate, it is reasonable to ask whether there is a direct or indirect relationship between the presence of intracellular receptor proteins for androgens and the expression of endogenous retroviral-like particles. Release of retroviral-like particles, whether of endogenous or exogenous origin, from prostate epithelial cells of young, apparently healthy, sexually mature rats suggests a role for testosterone or its metabolites in virus-like particle expression. Since ventral prostates enriched in growing (hypertrophic) or dividing (hyperplastic) epithelial cells can be obtained by suitable timing of castration and administration of androgens (19, 20), the effect of these manipulations on virus-like particle formation, as judged by electron microscopy of ventral prostates, was determined. Normal control prostate epithelium and prostates enriched in either hypertrophic or hyperplastic epithelial cells had comparable ranges of frequencies of viral particle expression. This is in contrast to the marked suppression of virus expressed in the dysplastic prostate epithelium of androgen-depleted, castrated rats. Together these observations suggest that the presence of androgen is, at the very least, permissive for the events related to virus-like particle expression and that these early phase events do not require the induction of extensive DNA synthesis and cellular replication.

Materials and Methods. Twelve 3-month-old Sprague-Dawley rats, from the

Sprague-Dawley Company, Madison, Wisconsin, were castrated via the scrotal route. After 3 or 6 days, half of these animals received 8 mg of testosterone propionate (TP) subcutaneously each day for 3 days. These animals were sacrificed the morning after the last injection (i.e., at 24 hr). It has been shown that prostates from rats that receive testosterone for 3 days, beginning 3 or 6 days after castration, contain large numbers of growing (hypertrophic) or dividing (hyperplastic or replicating) cells, respectively (19, 20). Control animals were also sacrificed and included six normal, intact (noncastrated) rats and three intact rats that received TP for 3 prior consecutive days.

After sacrifice by cervical dislocation, ventral prostates were quickly excised and immediately placed in glutaraldehyde fixative (1.5% glutaraldehyde in 0.05 M phosphate buffer, pH 7.4, containing 4% sucrose) at room temperature. While submerged in fixative, the tissues were further trimmed to 1-mm³ size blocks. The tissue blocks remained in fixative for 24 hr and were subsequently washed free of glutaraldehyde and postfixed with phosphate-buffered 1% osmium tetroxide for 1 hr. The fixed tissues were rinsed in 0.05 M phosphate buffer, stained overnight with 1% uranyl acetate in 50% ethanol, dehydrated first with increasing concentrations of ethanol, and finally with propylene oxide, and embedded in Epon (21).

The following approach was employed in attempting to quantify C-type virus-like particle expression within each of the prostate specimens. At least two blocks per specimen and three grids per block were processed for electron microscopic (EM) examination. Ultrathin sections were prepared by standard techniques and doubly stained with lead citrate and uranyl acetate. Each grid was systematically scanned for 30–60 min at 20,000× using a Siemens 101 electron microscope. During the examination period individual epithelial cells were closely studied and their numbers recorded, as were the number of clearly defined C-type particles. In general, 200–300 epithelial cells per tissue sample were evaluated. The

frequency of C-type particle expression within each of the processed tissues was computed by dividing the total number of C-type particles sighted by the total number of epithelial cells studied. This evaluation was carried out twice for each specimen. Either prior to or following the systematic scanning of the grid, representative areas were recorded photographically for the purpose of documenting viral-like particle-epithelial cell associations.

Results. Prostates from intact control rats. In this experiment as in our previous experience (1, 2) the majority (i.e., 83% in this experiment) of prostates from healthy adult Sprague-Dawley rats examined had moderate to high calculated frequencies of expression of C-type virus-like particles (Table I). Only one of the six (~17%) control prostates exhibited a low level of particle expression (i.e., frequencies ≤ 0.020 particles/cell). Although all of the normal control prostates were eventually shown to be positive after extended periods of observation (i.e., to contain at least a single extracellular C-type particle), the calculated frequencies of particle expression widely ranged from a low of 8×10^{-3} particles/cell to a high of 1.2 particles/cell (Table I).

Viral-like particles were generally observed in the small extracellular pockets in the basal regions of closely apposed epithelial cells (Figs. 1, 1b, and 2). Budding forms of the virus-like particles were seen as well, although at a much lower frequency. Extracellular particles were spherical in form with average outer diameters of ~110 nm. The inner cores (~90 nm) enveloped in unit membranes, typically consisted of flocculent nucleoids surrounded by dense concentric rings (Figs. 1b and 2). Aberrant morphologic forms (e.g., multinucleoids, nucleoid-deficient, etc.) were observed. C-type viral-like particles were never found in association with reticular elements of either the normal prostates or of the experimentally modified tissues described below.

Effect of testosterone treatment on prostates from intact rats. Similar to control groups, all of the prostates from intact, testosterone-treated rats were found to

contain C-type viral-like particles (Table I). Two of the three prostates examined had moderate frequencies of particle expression. Although the calculated mean particle frequency was low relative to the mean control value, the patterns of C-type particle expression and tissue distribution, as well as the ultrastructure of glandular epithelium, remained similar to those seen in control tissues.

Effects of castration. The estimated level of C-type particle expression appeared markedly reduced within rat prostates 3 or 6 days following castration (Table I). C-type particles were detected, and only in a very low number, in one of the six prostates studied. None of the tissues examined had moderate or high levels of particle expression, i.e., particle frequencies ≥ 0.020 .

The reduced level of virus-like particles was associated with pronounced ultrastructural alterations of the prostate glandular epithelium. In contrast to control tissues, substantial areas of epithelium were composed of cells that lacked the general characteristics of regional differentiation (i.e., apical pole, supranuclear region, and basal region type organelles) (22), and had reduced amounts of rough endoplasmic reticulum (ER) and secretion-filled cisternae (Fig. 3). The nuclei of these cells often appeared clefted, and the amount of condensed chromatin was greatly reduced.

Effects of castration and testosterone replacement. In contrast to the very low levels of C-type particle expression in the regressed prostate epithelium of castrated rats, moderate to high levels of particle expression were found in five of the six prostates from both groups of castrated, androgen-supplemented rats (Table I).

All of the prostates from rats supplemented with androgen beginning three days after castration had substantial levels of EM-detectable C-type particles. The virus-like particles were generally associated with the hypertrophic epithelial cells of lesser electron density (Fig. 4). These cells characteristically had large nuclei with prominent nucleoli and an open chromatin pattern. In contrast to fully mature, well-differentiated epithelial cells, the cytoplasm

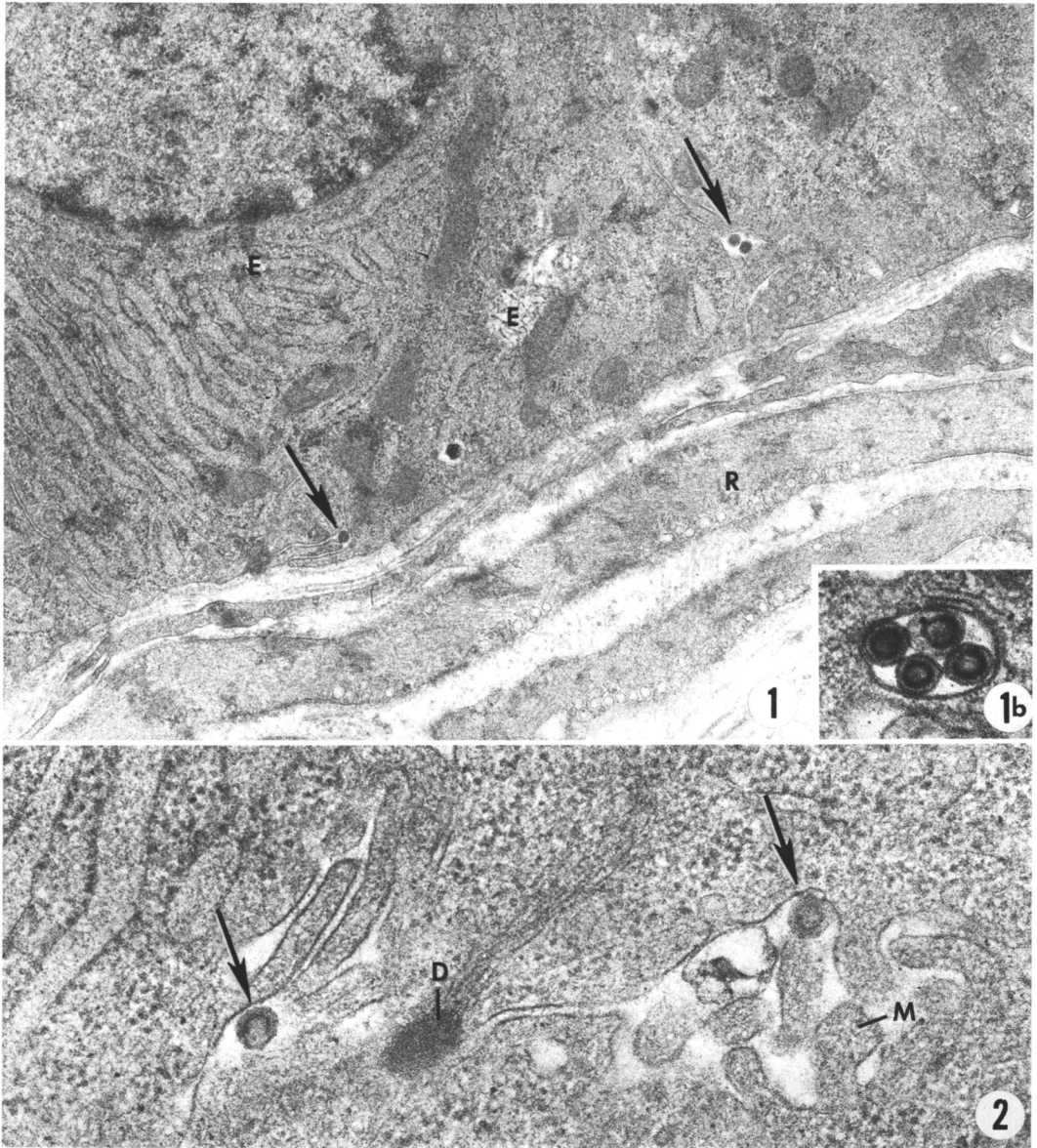
TABLE I. ESTIMATION OF THE RELATIVE NUMBERS OF C-TYPE VIRAL PARTICLES IN RAT VENTRAL PROSTATES BY ELECTRON MICROSCOPIC EVALUATION

Category	No. animals	Percentage positive cases ^a	Percentages frequency cases moderate-high ^b	Particle frequency per epithelial cell ^c	Mean frequency
I. Normal, intact	6	100	83	0.008	0.409
				1.204	
				0.797	
				0.047	
II. Intact, testosterone treated	3	100	67	0.035	0.026
				0.361	
				0.041	
				0.014	
III. Castrated	3	0	0	0.023	0.000
				0.000	
				0.000	
				0.000	
IV. Castrated, testosterone treated	3	33	0	0.000	0.004
				0.011	
				0.000	
				0.000	
3-day	3	100	100	0.175	0.271
				0.497	
				0.140	
6-day	3	100	67	0.019	0.266
				0.753	
				0.263	

^a Percentage of tissues examined for which at least one clearly defined extracellular or budding C-type-like particle was observed.

^b Percentage of tissues exhibiting C-type particle frequencies greater than 0.020.

^c Particle frequency = total number of extracellular C-type particles observed divided by the total number of epithelial cells examined.



FIGS. 1, 2. Normal rat ventral prostate and associated C-type viral-like particles. Fig. 1. Several C-type viral-like particles (arrows) are observed within the basal regions of the epithelium (E), but not within the supporting underlying reticular (R) elements. 20,000 \times . Fig. 1b (inset). The structural details of the prostate viral-like particles (~ 110 nm) are shown; thin unit membranes envelop dense, donut-shaped inner cores (~ 90 nm). 70,000 \times . Fig. 2. C-type viral-like particles (arrows) are shown in their typical location within the basally located extracellular spaces of closely apposed desmosome-joined (D) epithelial cells. Microvilli (M) are a common feature of these intercellular gaps. 61,500 \times .

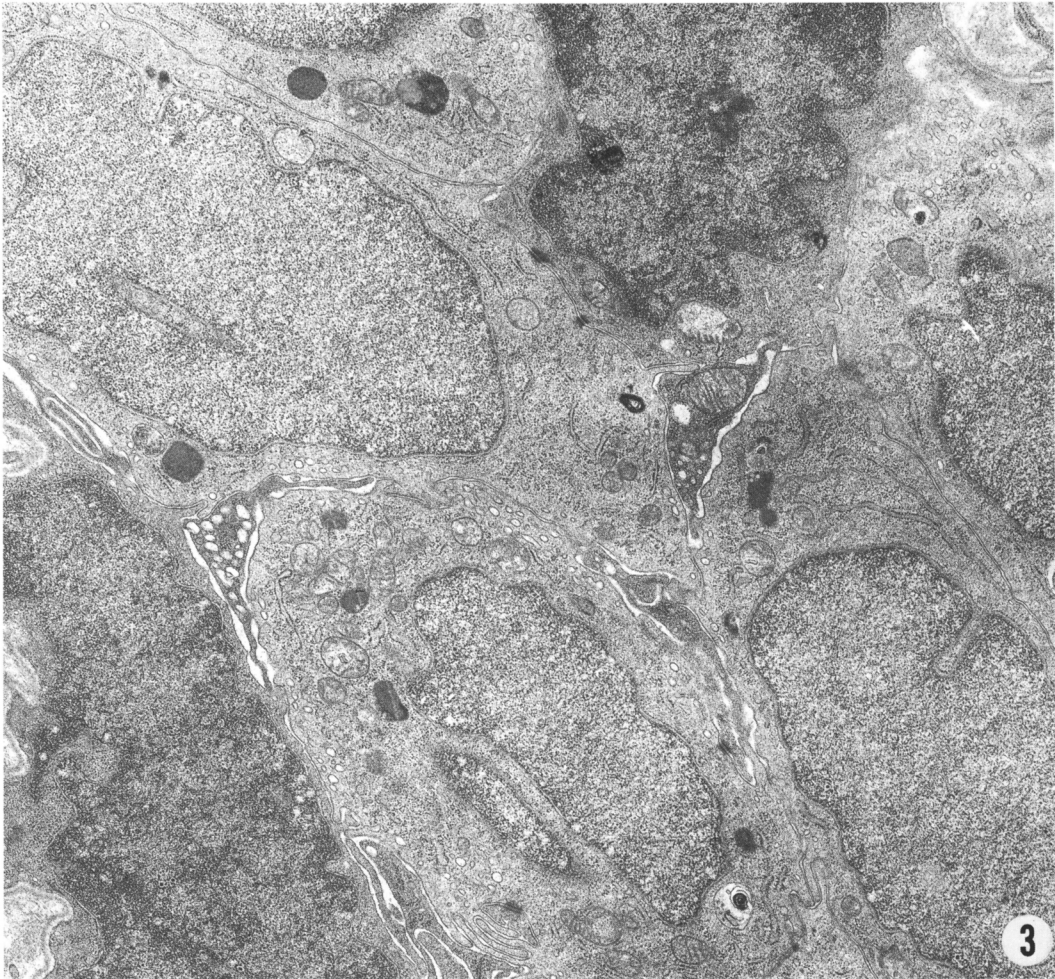


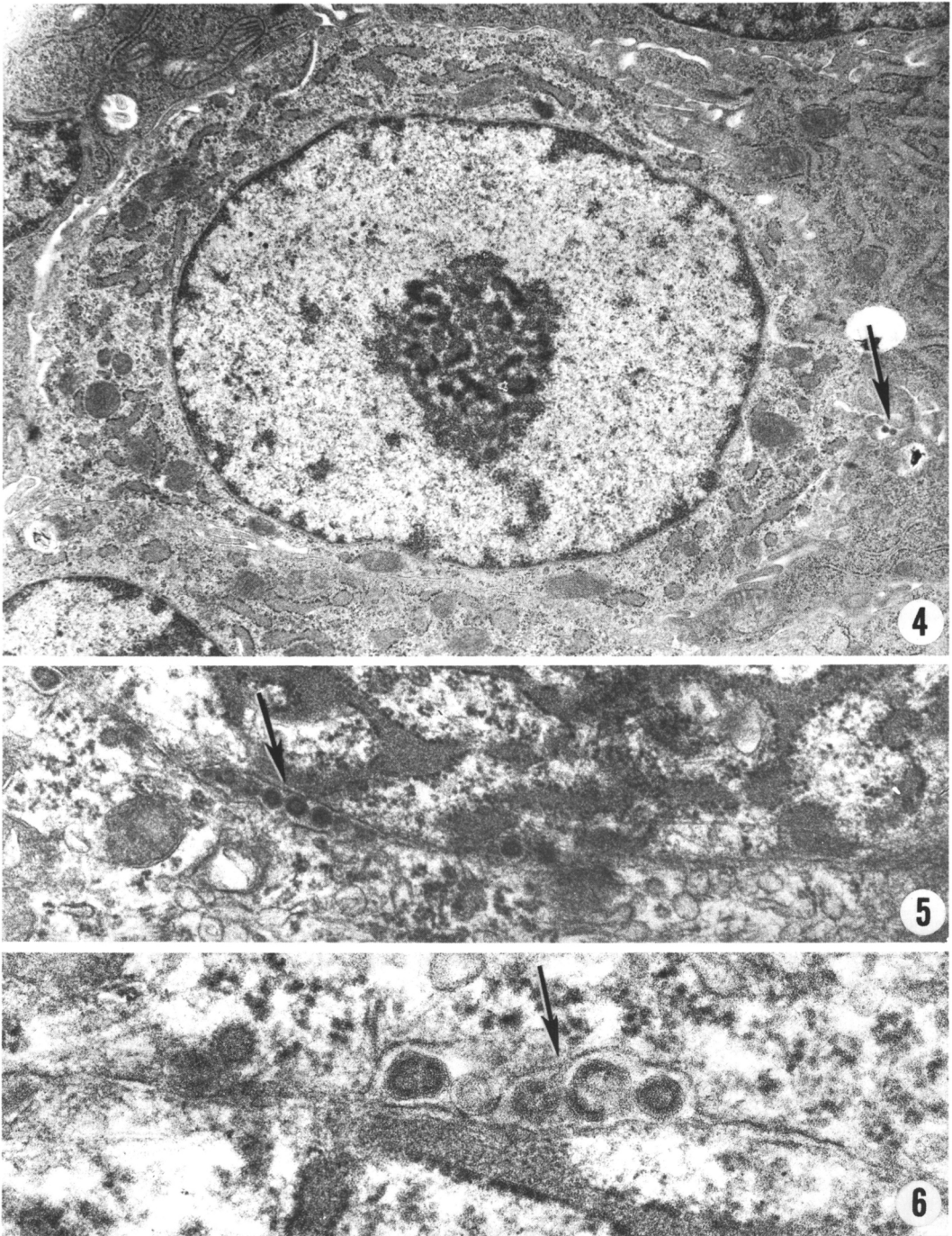
FIG. 3. Dysplastic prostate epithelial cells from a rat 6 days following castration. Marked nuclear and cytoplasmic changes are present in the epithelial cells; i.e., the nuclei are often clefted with an open chromatin pattern, while both length and width of rough ER cisternae are markedly reduced. C-type viral-like particles were not, except for a single exception, observed in prostates of these rats. 9000 \times .

had short segments of rough ER that formed small, secretion-filled cisternae. Figures 5 and 6 show a typical array of both normal appearing and aberrant C-type-like particles within the extracellular spaces.

The prostates of rats supplemented with androgen, beginning 6 days after castration, exhibited both low and high frequencies of C-type particle expression; i.e., a wide "control-like" range of particle frequencies was observed in this subgroup (Table I). These tissues had a preponderance of hyperplastic epithelial cells, characterized

by low electron density, a cytoplasm with reduced amounts of rough ER and associated secretion-filled cisternae, nuclei with open chromatin patterns undulate borders, and highly active cytoplasmic membrane borders.

Discussion. Several observations are compatible with the contention that the virus-like particles observed in the majority of prostates from normal intact Sprague-Dawley and Long-Evans rats represent spontaneously released endogenous retrovirus. Definitive proof of this, however,



FIGS. 4-6. Hypertrophic ventral prostate epithelium of a rat after 3 days of androgen injection beginning 3 days after castration. Fig. 4. Voluminous epithelial cells of low electron density characterize these prostates and are generally associated with increased numbers of C-type viral-like particles (arrow). These cells generally have large round nuclei with open chromatin patterns and prominent nucleoli. The cytoplasm is filled with short segments of rough ER cisternae filled with granular secretory material. 14,400 \times . Fig. 5. An array of retroviral-like particles (arrows) between hypertrophic epithelial cells. 36,000 \times . Fig. 6. A higher magnification of C-type viral-like particles (arrow) showing various ultrastructural forms (i.e., enlarged particles, particles with low-density cores). 78,000 \times .

will require additional biochemical⁴ and biological analyses. Regardless, these viral-like particles in question have the characteristic size, morphology, and budding pattern common to the C-type class of retroviruses (1–3). They do not appear to be artifacts of secretion (23). Several of the more prominent biological features of these presumptive rat prostate retroviruses include: (a) an organ and cell-type restricted expression, limited to epithelial rather than stromal cells of the prostate gland; (b) the frequency of expression being influenced by the functional status of the prostate's glandular epithelium, as mediated by androgen depletion or supplementation; and (c) the apparently benign nature of this retroviral-like species (i.e., in the Sprague–Dawley male rat). The latter is suggested because all of the animals studied appeared to be in good health and, upon sacrifice, lacked any gross signs of organ pathology. Prostatic tissues with either high or low levels of C-type particles appeared ultrastructurally similar and normal. The apparent lack of pathological potential might distinguish the rat prostate retroviruses from the ecotropic class of retroviruses that are established tumor inducers in certain species (24).

A small percentage of the prostates obtained from intact control rats or intact androgen-injected rats consistently exhibited very low levels of C-type retroviral-like particles. In contrast, a number of the prostates from the control group exhibited very high frequencies of C-type particle expression. The reason for such marked differences is not known. Multiple factors are undoubtedly involved, and include, no doubt, the genetic makeup of the animal,

and the functional status of the prostate. In different strains of inbred mice the degree of retrovirus expression has been reported as an autosomal dominant trait involving one or two genes, or even multiple genes, including the F_v locus (25).

The observation of a selective expression of C-type retroviral-like particles in the glandular epithelium of the prostate (and possibly in other regions of the androgen-dependent genitourinary system) is of interest. Feldman and Gross (4) made an extensive EM survey of various organs (i.e., thymus, spleen, lymph nodes, bone marrow, liver, lung, and kidney) of normal, healthy Sprague–Dawley male and female rats, but were unable to detect C-type particles. The prostate was one of the organs not examined, however. In a later paper, Gross *et al.* (5) reported finding C-type particles in the placentas of young, healthy, and pregnant female rats. In the study reported here, C-type particles with various morphological forms (i.e., both mature and immature particles, as well as aberrant forms) were shown clearly in the prostates of both normal and androgen-supplemented castrated rats. In other species different morphological classes of retroviruses have been identified. A, B, and C-type viruses have been observed simultaneously in both ventral and dorsal lobes of prostates from a number of different mouse strains (18). Two virus-like components distinct from known B, C, or H (hamster) virus particles have been observed in a poorly differentiated human adenocarcinoma of the prostate (26).

Previous studies have shown that replicative DNA synthesis in prostate epithelial cells of normal intact rats and androgen-treated intact rats is nil (20). Therefore, it would seem that DNA synthesis and cellular replication are not direct requirements to sustain retroviral-like particle synthesis and release. Similarly, prostates with hypertrophic epithelium of androgen-supplemented castrated rats contain proportionately few cells in S-phase (20), however, these hypertrophic tissues have relatively high frequencies of retroviral-like particle expression and are in contrast to the very suppressed levels found in the re-

⁴ In addition to detecting retroviral-like particles in rat prostate by electron microscopy, we have, with the kind help of Dr. Emerson W. Chan, attempted to detect retrovirus by carrying out exogenous template assays for retrovirus-specific reverse transcriptase (RT) in prostate extracts following fractionation in sucrose density gradients. Results, however, have been equivocal due to: (i) presence of strong inhibitors of RT in prostate cytosols; and (ii) atypical buoyant densities of isolated, RT-positive particles, consistent with partially degraded virus.

gressed prostates of the castrated rats. This might suggest that the retroviral-like particle expression may be an early, transient event linked to the androgen-mediated processes involved in the restitution and/or maintenance of cellular function. It has been demonstrated using several different experimental systems that steroid hormones stimulate expression of C-type viruses or presumptive viral information (24, 27–30). For example, these include enhanced expression of the mouse mammary tumor virus with 17β -estradiol (27), increased viral gs antigen, and reverse transcriptase in uteri of ovariectomized NIH-Swiss mice by estradiol (24), and release of virus from Chinese hamster ovary-K1 cells exposed to testosterone and dibutyryl cyclic AMP (28). Dexamethasone increases the yield of virus in other systems, apparently by a post-transcriptional mechanism (29). In primary avian fibroblasts, the amount of viral protein synthesized during active release of virus is from 1 to 3% of total cellular protein synthesis (30); this suggests an upper limit for viral protein synthesis in rat prostate epithelial cells.

Description of the early phase processes by which rat ventral prostate cells synthesize viral-like components and release intact retroviral-like particles, and elucidation of the direct or indirect role that dihydrotestosterone receptor proteins may play in mediating these events should provide additional insights into the biochemical actions of androgens.

We thank Dr. Emerson W. Chan for performing the reverse transcriptase and the gradient assays for prostate-associated retrovirus. The assistance of Ms. Lynn Purdy and Ms. DeLois Simmons is gratefully acknowledged.

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Received May 20, 1981. P.S.E.B.M. 1982, Vol. 169.