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IN-VIVO EXPRESSION OF A C-TYPE RNA VIRUS IN RAT VENTRAL PROSTATE EPITHELIAL CELLS

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SUMMARY During evaluation of a procedure for separating rat ventral prostate epithelial from connective tissue cells, isolated fractions were examined by transmission electron microscopy. Characteristic C-type RNA viruses were seen budding from or in close proximity to the plasma membranes of isolated epithelial cells. Dissociation of rat prostate cells and concentration of the epithelial fraction facilitated detection of this virus.

INTRODUCTION Mechanical disruption of rat ventral prostate and centrifugation of cells through a Ficoll gradient (1) can be used to separate connective tissue from epithelial cells (2). Characteristically, 4 cellular fractions are obtained. Band 2 is enriched in connective tissue cells and band 4 in epithelial cells (1).

When epithelial cells from band 4, at the gradient-cushion interface were examined by TEM, we observed typical C-type particles. To our knowledge, observation of C-type viral particles in prostate cell fractions from Sprague-Dawley and Long-Evans rats has not been reported. Dissociation of prostate tissue, and sedimentation of cells through a Ficoll gradient facilitated their detection.

METHODS Sexually mature male Sprague-Dawley rats from 2½-5 months of age were purchased from ARS Sprague-Dawley, Madison, Wisconsin and Wistar and Long-Evans rats from Charles River, Willmington, Massachusetts. Prostates were removed aseptically and rinsed with 2 washes of alpha minimum essential medium (aMEM), finely minced in 10 ml. of aMEM and forced through an E.C. 10 mesh tissue sieve. Dispersed tissue was washed 3 times in aMEM, resuspended in 1 ml. of aMEM and layered over an isokinetic gradient prepared according to the method of Pretlow (2), with modification (1). Centrifugation was through a linear Ficoll gradient (2.7-5.5 percent) in aMEM using an I.E.C. 269 rotor for 8 minutes at 800 rpm (74 x g). One ml. samples were removed, starting from the top of the gradient. *In vivo* and *in vitro* labelling with ^{3}H-testosterone and its metabolites, determination of prostate-specific

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acid phosphatase, DNA, cell number, cell viability, and light microscopy will be presented elsewhere (ms. submitted).

Samples from intact glandular tissue as well as the cell fractions from the gradient were fixed and stained for TEM by standard procedures (3), and examined with a Siemens 101 electron microscope.

RESULTS While evaluating the ultrastructure of four prostate cell fractions from Sprague-Dawley rats, we observed C-type viral particles associated with a fraction #4 at the gradient-cushion interface enriched in epithelial cells (Fig. 1). Similar particles, present in fractions containing fewer epithelial cells, (i.e. fraction 1-3) and in intact prostatic tissue, were much less evident. C-type particles were not seen associated with cells of connective or vascular tissue origin.

Cells in band 4 that contained particles exhibited an extensive endoplasmic reticulum-cisternal system filled with a finely granular homogenous substance, and secretory granules and their related vacuoles (Fig. 1). The irregular surface of these cells appears to enhance particle entrapment. Viral particles were present intracisternally and extracellularly and more rarely, budding from plasma membranes of the epithelial cells. These morphologically typical C-type RNA viruses had an average outer diameter of 108 nm and an inner core of 90 nm. Typically, the core consisted of thick concentric rings (14-16 nm) with a dense flocculent nucleoid and an enveloping thin unit membrane (7-9 nm). Aberrant morphological forms of the virus-like particle were also commonly observed.

To date, 3 out of 5 individually processed rat prostates were positive for virus. Two of the 3 glands from Sprague-Dawley rats were positive as was the gland examined from a Long-Evans rat. The prostate from a Wistar rat appeared negative.

DISCUSSION We do not know of any other reports of replicating C-type RNA viruses observed in prostate epithelial cells isolated directly from young and seemingly healthy Sprague-Dawley or Long-Evans rats. C-type viruses were not detected in a variety of organs from Sprague-

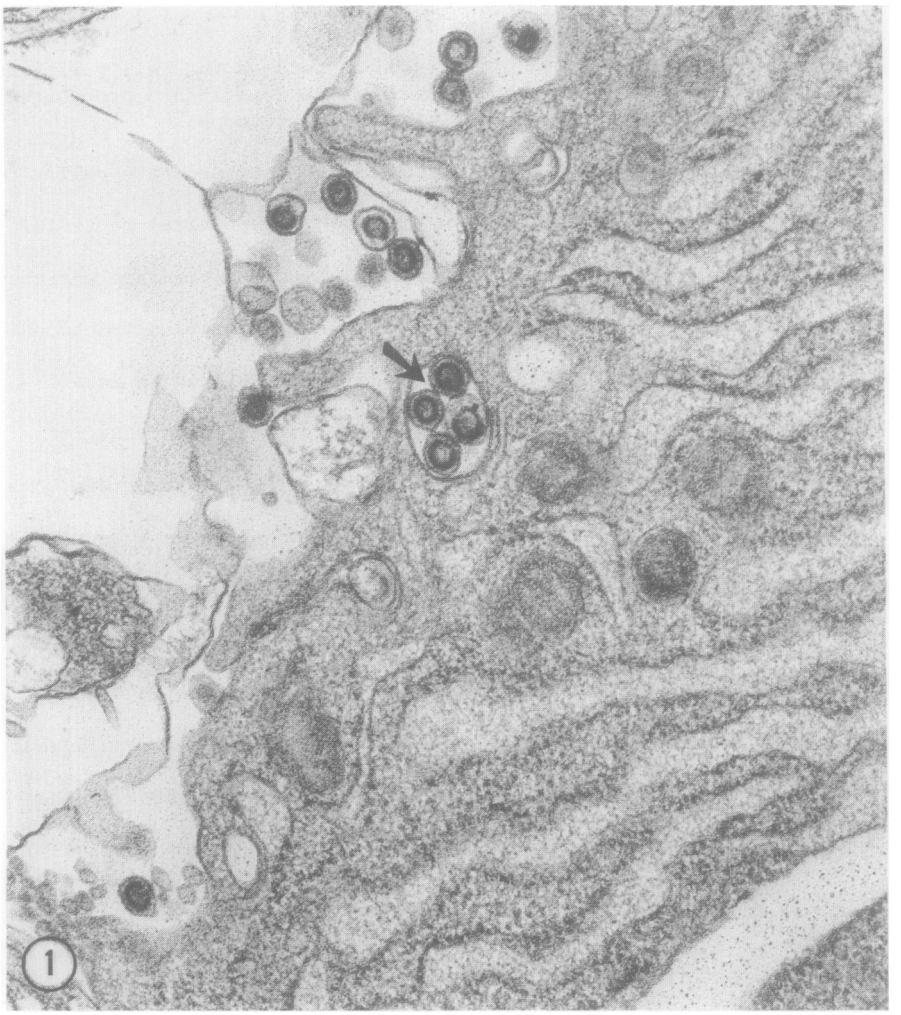

FIGURE 1. A representative electron photomicrograph illustrating the association of C-type viral particles (arrow) and rat prostate epithelial cells (70,000X).

Dawley or Long-Evans rats (4). These viruses were seen in early to mid-term placentas of Sprague-Dawley rats (5), and rats are known to harbor endogenous C-type viruses (6). An ecotropic C-type virus was isolated from Sprague-Dawley rat embryo cells in low passage, that subsequently transformed cells at higher passages (7,8). The Harvey and Kirsten strains of murine sarcoma virus were derived by passage in rats of a murine leukemia virus, thought to arise by recombination

between the exogenous virus and endogenous rat nucleotide sequences - the "sarc gene" (9).

Viral particles were much more readily observed associated with epithelial cells from band 4, than in the other fractions from the gradient, or in undissociated tissue remaining on the tissue sieve. Since virus replication is more frequent in epithelial cells compared to other prostate cell types, dissociation of prostatic tissue and isolation of epithelial cells on a Ficoll gradient increased the ease with which viral particles were detected. In view of the relative ease with which they were detected, the number of viruses shed from prostate epithelial cells must have been very large, perhaps greater than 10^6/ml as in tissue culture systems. It is possible that virus was lost in the supernatants during the tissue disruption, washing of cells, and centrifugation. High-speed pellets of these supernatants should be examined for viral particles.

Although Sprague-Dawley (and other) rats do not develop benign prostatic hypertrophy and prostatic cancer (10) , the former strain at least is subject to a high incidence of benign and malignant breast cancer, leukemias, lymphomas, and fibrosarcomas (5). The role, if any, for endogenous C-type RNA viruses or of superinfection by other viruses with "rescue" of potentially deleterious endogenous viral functions is unsettled. A contribution of viruses to human prostatic cancer has been sought (11), and there is evidence consistent with the presence of 70S viral RNA in benign and malignant human prostatic tissues (12-14). Further studies should help to clarify the generality of C-type viral replication in rat prostatic tissue and whether the virus originates from casual horizontal infection or represents expression of an endogenous virus. If this proves to be a consistent finding in prostatic tissue, biochemical evidence for the presence of reverse transcriptase, 70S viral RNA and its integration into the host genome, the effect

of castration and hormone replacement on the activation and development of the virus, and its' possible oncogenicity can be studied. Examination of specific cell types separated from normal tissue and from various solid human tumors may facilitate detection of viral particles, or the information necessary for their expression.

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