Specific Protein Synthesis in Isolated Epithelium of Guinea-Pig Seminal Vesicle

EFFECTS OF CASTRATION AND ANDROGEN REPLACEMENT

By CARLO M. VENEZIALE, JOHN M. BURNS,* JON C. LEWIS
and KASPAR A. BÜCHI†

Mayo Medical School, Rochester, MN 55901, U.S.A.

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Four intrinsic soluble secretory proteins are synthesized *in vitro* by isolated seminal-vesicle mucosa from sexually mature guinea pigs. Newly synthesized specific proteins labelled with [^{14}C]glycine and [^{14}C]lysine were precipitated by using double-antibody immunoprecipitation techniques and their radioactivity was assessed. Rates of synthesis were determined on each of 5 days after castration. By 5 days after castration the wet weight of the epithelium decreased to 42% of intact control values; the absolute amount of specific protein synthesized *in vitro* after 60 min incubation decreased to 28% and the 27500g cytoplasmic protein content decreased to 31%. Thus androgen deprivation leads to a decrease in general protein synthesis *in vivo*, as well as to a decrease in specific protein synthesis *in vitro*. Specific protein synthesis comprised 76% of the total protein formed in isolated tissue from animals 5 days after castration as compared with 99–100% in tissue from intact animals. At 72h after an injection of testosterone or dihydrotestosterone, seminal-vesicle epithelium wet weight, cytoplasmic protein content and capability for synthesizing specific proteins *in vitro* were restored to approx. 70% of normal values. At 72h after onset of therapy with 3 α -androstenediol, both epithelium wet weight and cytoplasmic protein content had increased significantly, but without a corresponding increase in the capability of the isolated tissue to synthesize specific proteins. The soluble labelled proteins synthesized *in vitro* by isolated epithelium from intact animals during 60 or 120 min incubation were essentially entirely immunoprecipitable, i.e. specific. In contrast, approx. 29% of all soluble protein newly synthesized by isolated epithelium from animals 5 days after castration was acid-precipitable, but not immunoprecipitable, i.e. 'non-specific'. The injection of testosterone into castrated animals inhibited the synthesis of the non-specific fraction by isolated tissue. The effects of castration on the ultrastructure of guinea-pig seminal-vesicle epithelium are also presented.

The availability of an androgen-dependent tissue comprised only of homogeneous columnar epithelial cells, which can synthesize and secrete relatively large amounts of multiple cell-specific soluble proteins *in vitro*, provides a unique opportunity to study the nature of androgen-dependence. The isolated seminal-vesicle mucosa of mature guinea pig is such a tissue (Veneziale, 1977). Our use of this preparation as a model tissue preparation for studies on androgen action has been helped by the elucidation of certain of its general biochemical features

which have been summarized elsewhere (Veneziale *et al.*, 1977). In the present paper we elaborate on the influence of castration on fine structure and on synthesis of multiple specific proteins *in vitro*. We report on the efficacy of various androgens when injected into castrated animals to restore to isolated mucosa the capability to synthesize specific proteins. In addition we demonstrate a unique effect of injected 3 α -androstenediol in castrated animals, which was to augment the cytoplasmic protein content of seminal-vesicle mucosa *in vivo*, and an effect of injected testosterone and dihydrotestosterone, which was to inhibit the synthesis *in vitro* of an unidentified protein fraction.

* Present address: Department of Biological Sciences, Texas Tech University, Lubbock, TX 79409, U.S.A.

† Present address: Kantonsspital Zürich, Frauenklinik, Zürich, Switzerland.

Materials and Methods

Adult male guinea pigs, weighing 700–800 g, from the inbred Mayo Colony were the source of seminal-vesicle mucosa. For electron microscopy seminal vesicles were fixed overnight in 2.5% (v/v) glutaraldehyde buffered to pH 7.2 with 0.1 M-potassium phosphate buffer. Post-fixation in 1% (w/v) OsO_4 was followed by dehydration through a graded ethanol series and embedding in Epon 812. Thin sections, obtained with a diamond knife in an LKB Ultratome, were double stained with uranyl acetate and lead citrate before being examined in a Philips EM 201C electron microscope.

Excision of the vesicles, isolation of the mucosa and the incubation conditions for the protein-synthesis experiments *in vitro* have been described (Prendergast & Venezia, 1975; Venezia, 1977). Wet weight (mg) in the Results section refers to wet weight of the isolated mucosa at the start of incubation. The labelled amino acid precursors added to the incubation flasks were 0.05 ml of $[\text{U-}^{14}\text{C}]$ glycine (9 nmol; 2.25×10^6 d.p.m.) and 0.05 ml of L- $[\text{U-}^{14}\text{C}]$ lysine hydrochloride (3 nmol; 2.10×10^6 d.p.m.). Both were obtained from Amersham-Searle, Arlington Heights, IL, U.S.A.

After 60 or 120 min incubation the tissue was homogenized in its incubation medium at 4°C by using 15 strokes. The homogenate was centrifuged at 27 500 g for 50 min, ensuring precipitation of plasma membranes, nuclei and mitochondria. The supernatant was 2.0 ml in volume and its protein composition comprised, not only cell cytoplasmic protein of the incubated mucosa, but also all of its newly formed soluble secretory cell-specific proteins. The supernatant was analysed for total protein concentration by a modification (Harfree, 1972) of the Lowry *et al.* (1951) method with bovine serum albumin as standard; mg of protein given in the Results section is for that of the 27 500 g supernatant fraction. On the basis of previous calculations (Veneziale, 1977) that only microgram quantities of specific soluble proteins are formed *in vitro*, the protein measured in the supernatant fraction after incubation reflected largely intracellular protein that had been present *in vivo*. The supernatant was assayed for specific proteins by immunoprecipitation methods (Veneziale, 1977) and the results reported as mean d.p.m. $\pm$ S.E.M.

Supernatant (0.5 ml) was acidified with 0.5 ml of 20% (w/v) HClO_4 . The protein precipitate was collected by centrifugation (1200 g, 30 min), washed with 2×1.0 ml of 6% HClO_4 and dissolved in 0.5 ml of 0.5 M-NaOH. This protein solution was quantitatively transferred to a scintillation vial containing 15 ml of Aquasol (New England Nuclear, Boston, MA, U.S.A.), which was neutralized with acetic acid and counted for radioactivity in a Nuclear-Chicago

scintillation counter. The results are quoted as d.p.m. in total newly formed protein.

Testosterone propionate was from the Schering Co. (Kenilworth, NJ, U.S.A.) under the trade name Oreton; dihydrotestosterone and dehydroepiandrosterone were from Schwarz/Mann (New York, NY, U.S.A.), and 5α -androstane- $3\alpha,17\beta$ -diol (3α -androstenediol) and 5α -androstane- $3\beta,17\beta$ -diol (3β -androstenediol) were from the Sigma Chemical Co. (St. Louis, MO, U.S.A.). Testosterone propionate and dehydroepiandrosterone were dissolved in sesame oil, and dihydrotestosterone was dissolved in propane-1,2-diol. The 3α -androstenediol and 3β -androstenediol were suspended in warmed sesame oil before injection. All injections were intramuscular.

Results

Fine structure of seminal-vesicle epithelium can be seen in Plates 1–4. Epithelial cells from intact animals were elongated, with basally located nuclei, numerous secretory granules and an orderly network of rough endoplasmic reticulum. Additionally, the apical surfaces of these cells were characterized by multiple elongated microvilli projecting into the lumen (Plate 2). Several alterations in ultrastructure were apparent after castration (Plates 3 and 4). Cells from animals 4 days after castration were less elongated and had flattened apical regions with a concomitant decrease in the numbers of microvilli and secretory granules. Increases in the number of lipid inclusions

Fig. 1. Effect of castration on isolated guinea-pig seminal-vesicle mucosal wet weight and soluble protein content. Protein concentration multiplied by 2 reflects cytoplasmic protein (27 500 g fraction) of mucosa from one gland after incubation for 60 min. See the Materials and Methods section for details. Values are means of the numbers of determinations indicated in parentheses and bars denote $\pm$ S.E.M.

EXPLANATION OF PLATE I

Epithelial cells from seminal vesicles of non-castrated animals

Elongate cells have basally located nuclei (N) and many secretory granules (arrow). Magnification $\times 4800$.

EXPLANATION OF PLATE 2

Apical portion of epithelial cells from non-castrated animals showing secretory granules (G) and numerous microvilli (Mv)
Magnification $\times 11000$.

EXPLANATION OF PLATE 3

Epithelium 4 days after castration

Cells are less elongated than in control animals and contain lipid inclusions (Li) and lysosomes (Ly) (lower arrows). Apically flattened cells have few microvilli (upper arrow). Magnification $\times 7000$.

EXPLANATION OF PLATE 4

Epithelium 8 days after castration

Note extensive dilation of endoplasmic reticulum (ER) and numerous lysosomes (Ly). Magnification $\times 3400$.

Fig. 2. *Specific protein synthesis*

Numbers in parentheses are the numbers of isolated-mucosa incubation experiments. The mucosa from one gland was used for each incubation. See the Materials and Methods section for details. Values are means and bars denote $\pm$ S.E.M. Incubation was for (a) 60 min and (b) 120 min. Radioactivity in specific proteins: $\circ$, protein 1; $\bullet$, protein 2; $\square$, proteins 3 and 4. Relative radioactivity in specific proteins: $\blacksquare$, protein 1; ∇ , protein 2; $\blacktriangledown$, proteins 3 and 4. Δ , Percentage of immunoprecipitable protein relative to acid precipitable protein. 'Ratio' is the percentage of immunoprecipitable protein to acid-precipitable protein.

and lysosomes (Plates 3 and 4) and a dilatation in cisternae of endoplasmic reticulum were most prominent at 8 days after castration (Plate 4). A tendency towards more electron-dense cytoplasm, which accompanied dilatations of endoplasmic reticulum, appeared to be the result of ribosome dissociation from membranes. Only minimal structural changes could be detected in the nuclei. These changes were mainly visible in animals 8 days after castration, especially in cells with maximally dilated endoplasmic reticulum (Plate 4) where the nuclei had a rounded appearance and the nucleoplasm seemed to be disrupted.

The effects of castration on mucosa wet weight and cytoplasmic protein content are shown in Fig. 1. At 5 days after castration wet weight of isolated mucosa decreased to 42% of control values and protein concentration to 31%.

The absolute rates of specific protein synthesis decreased rapidly after castration (Fig. 2a). After the 5-day interval after castration isolated vesicle mucosa could synthesize proteins 1, 3 and 4 at only 22% of the rate of control tissue. The mucosa could synthesize protein 2 at 43% of the rate of control tissue. The decrease in the rate of protein synthesis of the four proteins taken together after 120 and 60 min of incubation is shown in Fig. 3.

Specific proteins (immunoprecipitated) comprised 99% of the total protein synthesized by tissue from the intact animal (Fig. 2a) and 76% by tissue from animals that had been castrated 5 days previously.

Radioactivity in newly formed specific protein per

Fig. 3. *Radioactivity in four immunoprecipitable proteins*

The data represent the means (with the numbers of determinations indicated in parentheses) of the sum of radioactivity in proteins 1, 2, 3 and 4 after 60 ($\bullet$) and 120 ($\circ$) min incubation. See the Materials and Methods section for details.

Table 1. *Effects of androgen replacement on protein synthesis in isolated seminal-vesicle epithelium*

Treatment with hormone was given as a single intramuscular injection 5 days after castration. See the Materials and Methods section for details. Values are means $\pm$ S.E. for the numbers of preparations shown (*n*).

Treatment	Time between treatment and death (h)	<i>n</i>	$10^{-3} \times$ Total radioactivity in specific protein per 60 min incubation (d.p.m.)	% of intact control	Protein (mg)	Tissue wet wt. (mg)
None (intact animals)	—	8	836 $\pm$ 49	100	12.2 $\pm$ 0.4	436 $\pm$ 41
Testosterone propionate (5 mg)	24	1	238	28	—	—
	48	2	508	61	8.5	385
	72	6	719 $\pm$ 22	86	8.2 $\pm$ 0.3	310 $\pm$ 18
Testosterone propionate (50 mg)	24	4	266 $\pm$ 42	32	3.4 $\pm$ 0.5	165 $\pm$ 15
	48	2	514	61	8.1	300 $\pm$ 10
	72*	6	668 $\pm$ 34	80	7.8 $\pm$ 0.3	313 $\pm$ 16
Dihydrotestosterone (5 mg)	24	2	177 $\pm$ 70	21	5.1	275
	48	6	449 $\pm$ 61	54	6.4 $\pm$ 0.7	278 $\pm$ 26
	72	6	400 $\pm$ 40	48	6.6 $\pm$ 0.9	267 $\pm$ 28
Dihydrotestosterone (20 mg)	24	2	144	17	4.9	180
	48	6	448 $\pm$ 41	54	5.0 $\pm$ 0.6	234 $\pm$ 30
	72	6	654 $\pm$ 27	78	9.5 $\pm$ 0.4	305 $\pm$ 20
None (5 days after castration)	0	7	235 $\pm$ 20	28	3.8 $\pm$ 0.2	180 $\pm$ 9
Sesame oil (8 days after castration)	72	4	133 $\pm$ 20	16	3.8 $\pm$ 0.2	155 $\pm$ 30

* Four of the six seminal-vesicle mucosa preparations were from animals treated with 20 mg of testosterone propionate. Data from all six were combined because results were not significantly different (unpaired *t* test < 0.001).

Table 2. *Differential effects of testosterone and 3 α -androstanediol on protein synthesis by isolated epithelium*

Treatment was started 5 days after castration and the seminal vesicles were excised 72 h later. See the Materials and Methods section for details. Data for the testosterone-treated group were combined because results were not significantly different. Values are means $\pm$ S.E. for the numbers of preparations given (*n*).

Treatment	<i>n</i>	$10^{-3} \times$ Radioactivity in specific proteins per 60 min incubation (d.p.m.)	% of intact control	Protein (mg)	Mucosa (mg wet wt.)
Testosterone propionate* (5, 20 or 50 mg)	12	693 $\pm$ 20	83	8.0 $\pm$ 0.2	312 $\pm$ 11
3 α -Androstanediol* (25 mg)	8	313 $\pm$ 75	37	6.7 $\pm$ 0.6	266 $\pm$ 18
3 α -Androstanediol† (10 mg)	5	293 $\pm$ 75	35	7.7 $\pm$ 0.6	272 $\pm$ 14
3 β -Androstanediol* (25 mg)	6	240 $\pm$ 45	29	4.3 $\pm$ 0.3	173 $\pm$ 10
Sesame oil	4	133 $\pm$ 20	16	3.8 $\pm$ 0.2	155 $\pm$ 30

* Single intramuscular injection.

† Daily intramuscular injection for 3 days.

Table 3. *Effects of castration on non-specific protein synthesis*
See the Materials and Methods section for details. Values are means $\pm$ S.E.M.

Incubation (min) ...	$10^{-3} \times$ Radioactivity (d.p.m.)			
	60		120	
	Intact	5 days after castration	Intact	5 days after castration
Immunoprecipitable (specific proteins)	836 $\pm$ 49 (8)	236 $\pm$ 20 (7)	1310 $\pm$ 59 (8)	462 $\pm$ 46 (7)
Acid-precipitable	847 $\pm$ 48 (8)	335 $\pm$ 20 (5)	1240 $\pm$ 55 (8)	652 $\pm$ 59 (5)
Difference (non-specific proteins)	11	99		190

mg of total protein present in the 27 500g fraction remained almost unchanged (Fig. 2a). This is consistent with the demonstrated decrease in total cytoplasmic protein content of the mucosa (Fig. 1) and the decrease in the rate of specific protein synthesis *in vitro* (Fig. 2a). Patterns of specific protein synthesis after 120 min incubation were essentially as after 60 min incubation (Fig. 2b).

The effects of androgen replacement in pharmacological doses on radioactivity in specific protein, mg of protein and mg wet weight are given in Table 1. Treatment with testosterone or dihydrotestosterone had little or no effect on specific protein synthesis at 24 h, but had a marked effect at 72 h. However, tissue capacity for specific protein synthesis was still not yet restored to normal values at that time. A 5 mg dose of testosterone propionate was just as effective as 50 mg of testosterone propionate and more effective than 5 mg of dihydrotestosterone.

3 α -Androstenediol had much less influence on specific protein synthesis compared with testosterone and dihydrotestosterone (Table 2). Testosterone and dihydrotestosterone stimulated specific protein synthesis and also caused nearly comparable increases in total cytoplasmic content and wet weight. However, 3 α -androstenediol influenced total protein content and wet weight much more than specific protein synthesis. In 13 separate observations of seminal-vesicle mucosa incubation experiments with tissue from 3 α -androstenediol-treated animals, cytoplasmic protein content was 7.1 ± 0.4 mg and wet weight was 268 ± 12 mg. These values represent 88 and 86% respectively of those from the testosterone-treated group. However, specific protein synthesis was only 44% of that in tissue derived from testosterone-treated animals.

At 72 h after the injection of 25 mg of dehydroepiandrosterone into animals 5 days after castration, isolated incubated seminal-vesicle mucosa incorporated only 247000 ± 15600 d.p.m. ($n = 4$) into specific proteins, which was only 30% of that incorporated by mucosa from intact control animals.

The radioactivity in acid-precipitable protein synthesized *in vitro* by mucosa from animals 5 days after castration decreased to 40% of control values after 60 min incubation and to 53% of controls after 120 min incubation (Table 3).

The effects of castration on 'non-specific' protein synthesis by isolated mucosa are also shown in Table 3. Non-specific protein is that fraction which is acid-precipitable, but not immunoprecipitable. Mucosa from the intact animal synthesized specific protein almost exclusively (99–100%). By 5 days after castration the mucosa was able to synthesize a non-specific fraction which constituted as much as 30% of the total protein formed. We do not know the nature of the non-specific fraction at the present time. It is possible that it represents aborted fragments of

proteins 1, 2, 3 and 4 that are not bound by antibody, but this is speculation only. Alternatively, and more likely, the non-specific fraction is other protein.

The effects of a single injection of testosterone (5 or 50 mg) or of dihydrotestosterone (20 mg) in suppressing non-specific protein synthesis within 72 h was evaluated. In 18 separate 60 min incubation experiments there were 680000 ± 16700 d.p.m. in the immunoprecipitable fraction and 698000 ± 39400 d.p.m. in the acid-precipitable fraction. Thus 18000 d.p.m. or approx. 2.6% of all newly formed protein was non-specific. These data indicate that an androgen(s) inhibited, perhaps repressed, non-specific protein synthesis.

Discussion

The functional and structural integrity of the seminal vesicle of the adult male guinea pig is dependent on the presence of an appropriate concentration of circulating androgen(s). The weight of the organ decreases after bilateral orchidectomy (Sayles, 1942), as does the amount of nucleic acids, especially RNA (Kochakian *et al.*, 1964), and the quantity of fructose and citric acid (Ortiz *et al.*, 1956). Castration decreases the activities of succinate dehydrogenase and alkaline and acid phosphatases (Chinoy *et al.*, 1973); it also lowers the RNA polymerase activities of nuclei prepared from the seminal-vesicle mucosa (Büchi & Veneziale, 1977) and decreases the amount of amino acids incorporated into protein by a seminal-vesicle homogenate (Kochakian, 1964). The histological changes that follow castration have been described. They include a decrease in the height of epithelial cells, weight of the entire gland and size of the Golgi body (Sayles, 1942; Ortiz *et al.*, 1956). However, we have not found any reports on the influence of castration on the ultrastructure of seminal-vesicle mucosa in the guinea pig.

Prominent dilatation of the endoplasmic reticulum was among the most distinguishing features of cells from castrated animals. Such dilatations have not been observed in the rat prostate (Helminen & Ericsson, 1971; Dahl & Kjaerheim, 1973; Brandes, 1974) nor in the rat seminal vesicle (Szirmai & van der Linde, 1965; Dahl & Tveter, 1973).

In other regards the structural changes induced by castration in the epithelium of the guinea-pig seminal vesicle followed the pattern described in the male accessory sex organs of the rat. An increase in lipid inclusions and the appearance of giant vacuoles in rat seminal vesicles after castration have been reported (Helminen & Ericsson, 1971; Dahl & Tveter, 1973). These observations are consistent with our findings. It must be noted, however, that the lysosomes we observed in guinea-pig columnar cells were considerably smaller and less heterogeneous

than the autophagic vacuoles described in the same organ of the rat. The dissociation of ribosomes from endoplasmic reticulum after castration has also been observed in the seminal vesicle of the rat (Szirmai & van der Linde, 1965). Nuclei showed *in situ* only minimal ultrastructural changes after castration, a finding that is consistent with our observation that castration within this interval of time has no influence on gross chemical composition of isolated nuclei (Büchi & Veneziale, 1977).

There have been many excellent reviews and discussions on the effects of androgens on nucleic acid metabolism (Williams-Ashman & Reddi, 1972; Coffey, 1974; King & Mainwaring, 1974; Higgins *et al.*, 1976a,b), on cell renewal and mitotic activity (Tuohimaa & Niemi, 1974; Higgins *et al.*, 1976b) and on receptor mechanisms by which these effects are believed to be mediated (Liao *et al.*, 1974; Liao, 1976). Additional discussion of androgen mechanisms is not indicated here.

Our androgen-replacement experiments were of the acute variety, i.e. the effects of castration were followed for only 5 days and androgen replacement was studied only between the 5 and 8 days after castration. Deliberately we chose an early time after castration to study androgen-replacement responses, because we wanted to deal with only a partially involuted cell population, i.e. one in which the predominant response would be normalization of existing cells rather than proliferation of new cells. Higgins *et al.* (1976b) found by actual cell count only a small decrease in the number of epithelial cells in rat seminal vesicle 5 days after castration. The effect of androgens in restoring specific protein synthesis was therefore probably mostly due to the restoration of partially involuted cells to a more normal state rather than to the proliferation of new cells. In this regard our experimental system paralleled that of Higgins *et al.* (1976b), for which it was convincingly demonstrated that the induction of specific protein synthesis by testosterone preceded cell proliferation.

It has been suggested that in the oestrogen- and progesterone-sensitive chick oviduct system the hormones modulate synthesis of specific secretory proteins, but not of intrinsic cellular protein (Schimke *et al.*, 1975). At 5 days after castration the guinea-pig seminal-vesicle mucosa undergoes a marked decrease in wet weight, cytoplasmic protein content and ability to synthesize the specific soluble secretory proteins. Thus both general protein synthesis *in vivo* after castration and specific protein synthesis *in vitro* were dependent on androgens, and this also agrees with the conclusions of Higgins *et al.* (1976a). However, isolated mucosa from the intact animal synthesized an amount of specific protein that was 99–100% of the total soluble protein synthesized during incubations of 60 and 120 min, whereas after incubation of tissue from animals 5 days after castration approx.

71% of the total was specific protein and 29% was unidentified protein that could not be precipitated by antibodies (Table 3). Thus specific protein synthesis had been impaired and 'non-specific' protein synthesis apparently increased by castration. Subsequently, it was found that androgen replacement restored specific protein synthesis and inhibited synthesis of the unidentified so-called 'non-specific' fraction. Elucidation of the apparent inhibition phenomenon will depend in large part on identification of the unknown fraction.

3 α -Androstanediol was a major product in the interconversion of multiple radioactively labelled steroids that had been incubated with isolated seminal-vesicle mucosa (Steer & Veneziale, 1977; Veneziale *et al.*, 1977). Testosterone, dihydrotestosterone, dihydroepiandrosterone and androsterone all gave rise to 3 α -androstanediol in amounts that constituted a significant percentage of all the labelled steroid recovered. In contrast, 3 α -androstanediol remained largely unmetabolized. This led us to investigate possible effects of the steroid in this study. We found that 3 α -androstanediol injected *in vivo* had a positive effect on nitrogen balance within the mucosal cells of the seminal vesicle *in vivo* without also influencing to a comparable extent the tissue's capacity to synthesize the four soluble secreted specific proteins *in vitro*. Thus 3 α -androstanediol could mimic testosterone and dihydrotestosterone with respect to mucosa wet weight and cytoplasmic protein content, but not with respect to specific protein synthesis. The findings suggest that 3 α -androstanediol could have a unique role in mediating one kind of androgen action and testosterone and/or dihydrotestosterone mediate other actions. The androgenic effects of testosterone in seminal-vesicle mucosa might not then be attributable only to testosterone or dihydrotestosterone. Although a special role for 3 α -androstanediol has not been demonstrated for seminal-vesicle mucosa previously, research has suggested that this steroid might induce prostatic growth (Moore & Wilson, 1973; Walsh & Wilson, 1976; Jacobi & Wilson, 1976) and protein synthesis in tissue culture (Vilée, 1975), and that it might maintain fertilizing capacity of epididymal spermatozoa (Lubicz-Nawrocki, 1973). Investigations of 3 α -androstanediol must be extended before the significance of these findings can be ascertained.

The isolated seminal-vesicle mucosa can synthesize abundant cell-specific soluble secretory protein and might be an ideal model tissue for certain studies on the mechanism(s) of action of various androgens. Because it is cellularly homogeneous, such measurements as wet weight, protein and nucleic acid content, rates of specific protein synthesis and other parameters of cell function are totally unambiguous inasmuch as the measurements reflect absolutely the cells of interest (Veneziale *et al.*, 1977; Veneziale,

1977). We have come to appreciate the advantage of the cellular homogeneity, especially during the course of current studies on the nature of cytosolic androgen receptors. Although preliminary, the results described in the present paper indicate that the isolated guinea-pig seminal-vesicle mucosa preparation will be very useful in elucidating the nature of androgen-dependence.

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