

STIMULATION OF NUCLEIC ACID AND PROTEIN SYNTHESIS IN THE EPIDIDYMIS AND ACCESSORY ORGANS OF THE RAT BY TESTOSTERONE

M. RAJALAKSHMI AND M. R. N. PRASAD

Department of Zoology, University of Delhi, Delhi-110007, India

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SUMMARY

The effects of a single dose of testosterone on the content of DNA, RNA and protein and the incorporation of [³H]phenylalanine into protein in the epididymis, vas deferens and ventral prostate of the rat were studied. A single dose of testosterone did not increase the weights of the accessory organs but restored the incorporation of [³H]phenylalanine into proteins in the caput, corpus and cauda epididymides, vas deferens and ventral prostate to the normal level. Within 1 h of hormone administration, significant increases in the content of DNA, RNA and protein were noticed in the cauda epididymidis and ventral prostate. The caput and corpus epididymides and vas deferens showed decreasing order of responsiveness to testosterone. These data are discussed with respect to the relative responsiveness of these organs to reinitiation of their function by administration of a single dose of testosterone. The new protein(s) synthesized in response to hormonal stimulation associated with growth and secretory activity of the cells may be different from the new protein macromolecules synthesized after hormone withdrawal or inhibition of hormone action which are involved in autolytic processes.

INTRODUCTION

The structural and functional integrity of the epididymis is regulated by androgens; the threshold of androgens required to maintain the functions of the epididymis is much higher than that needed by the accessory glands (Prasad, Rajalakshmi, Gupta & Karkun, 1973; Gupta, Rajalakshmi & Prasad, 1974). Androgens stimulate the synthesis of nucleic acids and specific proteins which mediate the manifestation of the physiological effects of the hormone in the accessory glands (Williams-Ashman & Reddi, 1971). Chronic exposure to androgens *in vivo* or in organ culture for 3-7 days stimulates the synthesis of DNA or protein by the epididymis (Niemi & Tuohimaa, 1971; Blaquier, 1973). However, little or no attention has been paid to the time sequence of action of androgens on nucleic acids and protein synthesis in the epididymis after a single administration of the hormone. Such studies are necessary to understand the physiological basis of the differential androgen threshold hypothesis (Prasad *et al.* 1973) and the differences in the response of the epididymis and accessory glands to the administration of a single dose of androgen.

MATERIALS AND METHODS

Adult male rats of the Holtzman strain, 3-3.5 months old, and weighing between 200 and 250 g were used. The rats were castrated and used in the experiments 30 days after surgery. One group of sham-operated rats of the same age served as intact controls. Castrated rats received a single s.c. injection of 400 µg testosterone in 0.2 ml olive oil and were

autopsied 1, 3, 6, 12, 24, 48, 72, 96 or 144 h after administration of the hormone. A group of castrated rats receiving the vehicle alone served as castrated controls. Three hours before autopsy, the rats were injected with 50 μ Ci [3 H]phenylalanine (sp. act. 6.15 Ci/mmol; New England Nuclear Corporation) into the jugular vein while under light ether anaesthesia. Three animals were used in each group.

At autopsy, the epididymis, vas deferens and ventral prostate were removed; the epididymis was divided into the caput, corpus and cauda epididymides and weighed on a torsion balance to the nearest 0.2 mg. The tissues were homogenized in a glass homogenizer fitted with a Teflon pestle in 5.0 ml physiological saline; 3 ml of the homogenate were used for counting radioactivity and the remaining 2 ml were used for the estimation of RNA, DNA and protein. Both the portions were acidified with enough 30% perchloric acid to give a final acid concentration of 0.5 mol/l. The tubes were shaken and allowed to stand overnight.

For counting radioactivity, the homogenate was processed according to the method of Reel & Gorski (1968). The samples were centrifuged at 800 g for 10 min to obtain the acid-insoluble and -soluble fractions. The acid-insoluble protein pellet was washed twice with 5.0 ml cold 5% perchloric acid, once with 5.0 ml 95% ethanol, once with 5.0 ml ethanol-ether (2:1, v/v) and twice with 5.0 ml ether. The dried protein pellet was dissolved in 1 ml Soluene (Packard Solubilizer); aliquots of this were taken in counting vials and 5 ml scintillation fluid [5 g 2,5-diphenyl oxazole (PPO) and 50 mg 1,4-bis-2(5-phenyl oxazolyl) (POPOP) in 1 l scintillation grade toluene] were added. To an aliquot of the acid-soluble fraction (0.2 ml), 2 ml ethanol and 5 ml scintillation fluid containing Triton X100 were added. The radioactivity was counted in a Packard Tri-Carb Liquid Scintillation Spectrometer with an efficiency of 45% for tritium. The counts were corrected for background activity and quenching. The amount of radioactivity was expressed in terms of specific activity of protein incorporated.

The samples for estimation of nucleic acids and protein were processed according to the method of Rendina (1971). RNA, DNA and protein were determined by the procedures of Schneider (1957), Burton (1956) and Lowry, Rosebrough, Farr & Randall (1951) respectively. The data were analysed using Student's *t*-test.

RESULTS

Castration for 30 days decreased significantly the weights of the epididymis, vas deferens and ventral prostate, their contents of DNA, RNA and protein as well as the incorporation of [3 H]phenylalanine into proteins in these tissues (Table 1).

Administration of a single dose of testosterone evoked no significant increase in the weights of the epididymis (Table 2), vas deferens or ventral prostate at any of the time intervals studied.

Changes in the content of DNA, RNA and protein

Caput epididymides

The content of DNA increased, but not significantly ($P > 0.05$) 1 h after testosterone administration; the numerical variations observed at later intervals were also not statistically significant with respect to the castrated controls ($P > 0.05$; Fig. 1*a*).

The content of RNA increased significantly within 1 h after a single dose of testosterone ($P < 0.001$) and increased progressively to reach a peak at 48 h but decreased at 72 h; the content of RNA by 144 h was not statistically different from the castrated control values due to the high standard error ($P > 0.05$; Fig. 1*a*).

The content of protein showed a gradual increase to reach a maximum at 24 h ($P > 0.05$)

Table 1. Changes in the weight, content of DNA, RNA and protein, and the incorporation of [³H]phenylalanine into the epididymis, vas deferens and ventral prostate of adult rats after castration (means \pm S.E.M., $n = 3$)

Organ	Treatment	Weight (mg)	DNA (μ g/organ)	RNA (μ g/organ)	Protein (μ g/organ)	Incorporation of [³ H]phenylalanine (c.p.m./mg protein)
Caput epididymidis	Intact control	549.6 \pm 28.4	1458.1 \pm 153.8	50.33 \pm 5.1	9258.1 \pm 118.0	2216 \pm 126
	Castrated	94.8 \pm 7.2****	63.1 \pm 41.2****	11.1 \pm 0.6***	676.7 \pm 85.5****	447 \pm 163****
Cauda epididymidis	Intact control	574.3 \pm 32.5	1938.5 \pm 307.0	50.3 \pm 5.1	1248.0 \pm 78.0	1123 \pm 171
	Castrated	95.6 \pm 9.7****	41.7 \pm 1.0***	9.5 \pm 3.2****	283.7 \pm 73.8****	312 \pm 149*
Vas deferens	Intact control	195.0 \pm 12.3	598.3 \pm 171.3	39.2 \pm 2.7	7080.2 \pm 90.0	1763 \pm 349
	Castrated	65.3 \pm 3.2****	63.3 \pm 4.1**	16.1 \pm 3.0****	583.3 \pm 40.9****	280 \pm 114**
Ventral prostate	Intact control	444.0 \pm 7.5	1280.0 \pm 21.0	208.3 \pm 14.8	7890.3 \pm 79.1	7607 \pm 716
	Castrated	37.3 \pm 4.2****	37.7 \pm 4.3****	7.2 \pm 1.3****	325.3 \pm 39.8****	509 \pm 193****

* $P < 0.05$; ** $P < 0.02$; *** $P < 0.01$; **** $P < 0.001$; compared with intact control.

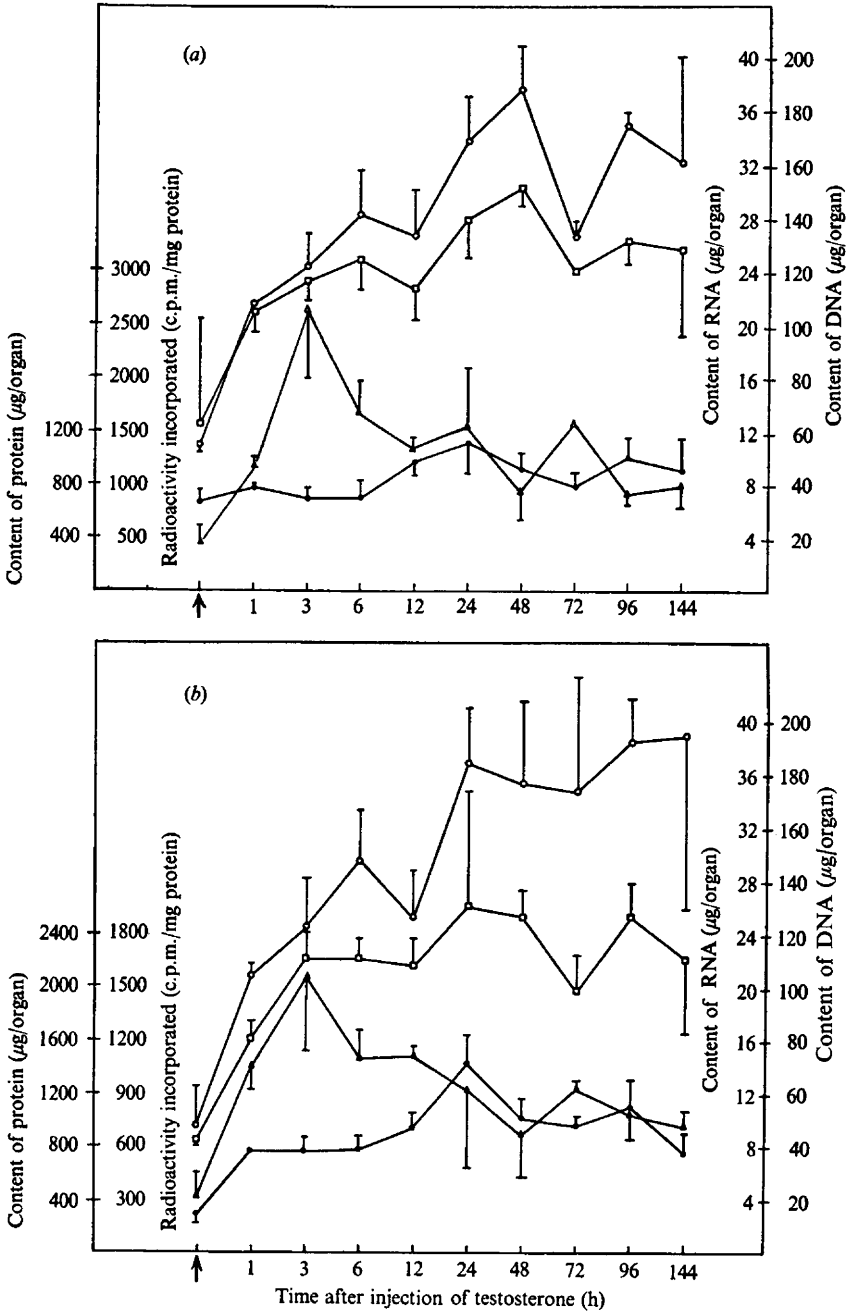


Fig. 1. Changes in the content of DNA (□), RNA (○) and protein (●), and the incorporation of [³H]phenylalanine into proteins (▲) in (a) caput epididymidis and (b) cauda epididymidis of adult castrated rats. ↑ Rats castrated for 30 days. Testosterone (400 μg) was administered subcutaneously at various intervals before autopsy as shown in figure. [³H]Phenylalanine (50 μCi) was administered intravenously 3 h before autopsy. Values are means ± s.e.m., n = 3 separate determinations.

and remained at this level till 144 h with minor fluctuations which were not significant ($P > 0.05$; Fig. 1*a*).

Corpus epididymides

The content of DNA increased significantly to reach a peak at 24 h ($P < 0.01$) and remained at the same level until 72 h; it decreased thereafter to castrated control levels by 96 h ($P > 0.05$). The content of RNA, however, showed a significant increase only at 48 h ($P < 0.05$); at other time intervals, the values were not significantly different. The content of protein, on the contrary, was unaffected by the single dose of testosterone at any of the time intervals studied (Table 2).

Cauda epididymides

The content of DNA, RNA and protein increased significantly within 1 h after administration of testosterone ($P < 0.05$) and reached peak levels by 24 h (Fig. 1*b*). The contents of DNA and RNA remained significantly above the castrated control values until the termination of the experiment: however, the content of RNA at 144 h was not significantly different from the castrated control values ($P > 0.05$). The content of protein decreased progressively from the peak value to reach, by 144 h, levels which were still significantly above those of the castrated controls ($P < 0.05$).

Vas deferens

The dose of testosterone administered did not cause any increase in the content of RNA and DNA in the vas deferens while an increase in the content of protein was noticed at 48, 96 and 144 h ($P < 0.05$; Fig. 2).

Ventral prostate

The content of DNA, RNA and protein increased significantly within 1 h after hormone injection ($P < 0.02$; Fig. 3). The levels of RNA and DNA reached a maximum at 12 and 96 h respectively and were maintained until the termination of the experiment. However, the content of protein reached a peak at 48 h and decreased gradually to castrated levels by 72 h and remained at the same level with wide numerical variations up to the end of the experiment (144 h) ($P > 0.05$).

The dose of testosterone administered did not increase the content of DNA, RNA and protein in any of the organs to intact control levels.

Incorporation of [³H]phenylalanine

Incorporation of [³H]phenylalanine into protein in the epididymis, vas deferens and ventral prostate increased significantly within 1 h after hormone administration (Figs 1, 2 and 3). The caput and cauda epididymides, vas deferens and ventral prostate showed a similar pattern of change since maximum incorporation occurred at 3 h; this was followed by a gradual decrease up to 96 h and was maintained at the same level up to 144 h. However, the incorporation of [³H]phenylalanine into proteins of the corpus epididymidis was maximal at 48 h and decreased sharply thereafter to reach the lowest levels by 144 h (Table 2). In the different segments of the epididymis and in the vas deferens, the precursor incorporated (c.p.m./mg protein) at peak time intervals was numerically higher than that in the intact controls; however, in the ventral prostate maximal incorporation was significantly below that of intact controls ($P < 0.01$). The uptake of radioactivity in the total acid-soluble fractions from the caput, corpus and cauda epididymides, vas deferens and ventral prostate did not show any significant change in the different groups. The precursor pool was therefore not subjected to further analysis.

Table 2. Changes in the weight, contents of DNA, RNA and protein, and the incorporation of [³H]phenylalanine into the corpus epididymidis of adult castrated rats at various times after a single s.c. administration of 400 µg testosterone (means ± S.E.M., n = 3)

Groups	Weight (mg)	DNA (µg/organ)	RNA (µg/organ)	Protein (µg/organ)	Radioactivity incorporated (c.p.m./mg protein)
Intact control	85.6 ± 6.4	282.7 ± 62.7	50.3 ± 5.1	2530.0 ± 80.0	2882 ± 657
Castrated control	21.0 ± 2.6	19.3 ± 3.6	3.9 ± 1.7	122.6 ± 26.8	279 ± 87
Castrated + testosterone (1 h)	23.3 ± 1.6	27.5 ± 6.1	5.5 ± 0.8	188.0 ± 4.9	1490 ± 179
Castrated + testosterone (3 h)	13.3 ± 4.9	22.5 ± 3.5	6.1 ± 2.4	127.0 ± 21.9	2127 ± 313
Castrated + testosterone (6 h)	19.3 ± 1.5	27.1 ± 5.8	8.9 ± 1.3	109.3 ± 9.9	2624 ± 75
Castrated + testosterone (12 h)	16.3 ± 3.4	19.2 ± 4.1	9.5 ± 3.4	132.0 ± 24.2	2965 ± 487
Castrated + testosterone (24 h)	26.7 ± 1.6	45.0 ± 1.8	9.4 ± 1.8	229.3 ± 91.7	2596 ± 830
Castrated + testosterone (48 h)	24.7 ± 2.2	39.2 ± 5.4	10.0 ± 0.0	192.0 ± 42.8	3373 ± 576
Castrated + testosterone (72 h)	19.3 ± 2.9	35.8 ± 2.0	8.4 ± 2.0	122.7 ± 25.7	2549 ± 776
Castrated + testosterone (96 h)	26.0 ± 6.2	30.8 ± 7.4	10.3 ± 2.7	205.3 ± 50.8	1193 ± 211
Castrated + testosterone (144 h)	25.0 ± 4.7	35.8 ± 7.4	8.5 ± 0.9	220.6 ± 59.5	992 ± 74

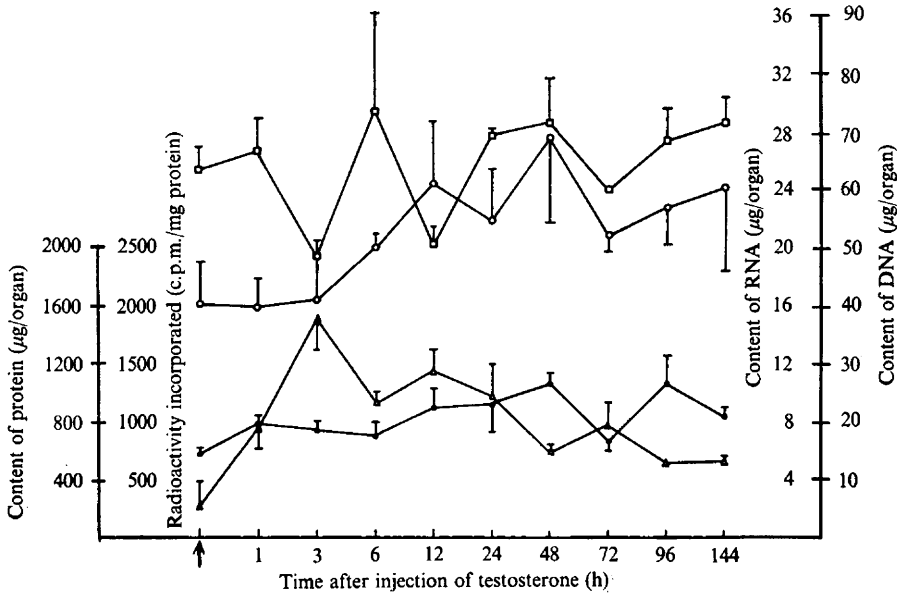


Fig. 2. Changes in the content of DNA (□), RNA (○) and protein (●), and the incorporation of [³H]phenylalanine into proteins (Δ) in the vas deferens of adult castrated rats. ↑, Rats castrated for 30 days. Testosterone (400 µg) was administered subcutaneously at various intervals before autopsy. [³H]Phenylalanine (50 µCi) was administered intravenously 3 h before autopsy. Values are means ± S.E.M., *n* = 3 separate determinations.

DISCUSSION

Regression of the accessory organs in rats after castration is maximal by 30 days and no further involution occurs thereafter (Gupta, 1974). The present studies showed the presence of basal levels of DNA, RNA and protein and minimal incorporation of [³H]phenylalanine into proteins of rats castrated for 30 days. These results indicate the existence in castrated rats of a residual population of cells which are perhaps resistant to androgen deprivation. Lesser & Bruchovsky (1973) observed that in rat prostate, only 15% of the cells representing a relatively constant population remain from 6 to 14 days after castration.

A differential androgen threshold hypothesis has been postulated by Prasad *et al.* (1973) indicating that the epididymis has a higher threshold requirement of androgens for the maintenance of its function than the accessory glands. The temporal sequence of metabolic changes induced by a single administration of testosterone highlights further the existence of a differential response among the accessory sex organs to modulation of their secretory function by androgens. Significant increases in the content of DNA, RNA and protein occur in the cauda epididymidis and ventral prostate within 1 h of hormone administration; however, the caput epididymidis appears to be less sensitive to the injected androgen since the content of protein and DNA increased only at 24 h. This may be due to the differences in the factors that regulate their functions; the functional integrity of the caput epididymidis is regulated largely by the presence of testicular fluid and spermatozoa while that of the cauda epididymidis and ventral prostate is governed by the levels of androgens in the circulation (Prasad *et al.* 1973). The absence of testicular fluid/spermatozoa in the caput epididymidis of the castrated rat used in the present study explains the decreased responsiveness of this organ to testosterone administration. Among the three segments of the epididymis, the corpus epididymidis appeared least sensitive to stimulation by androgens since it

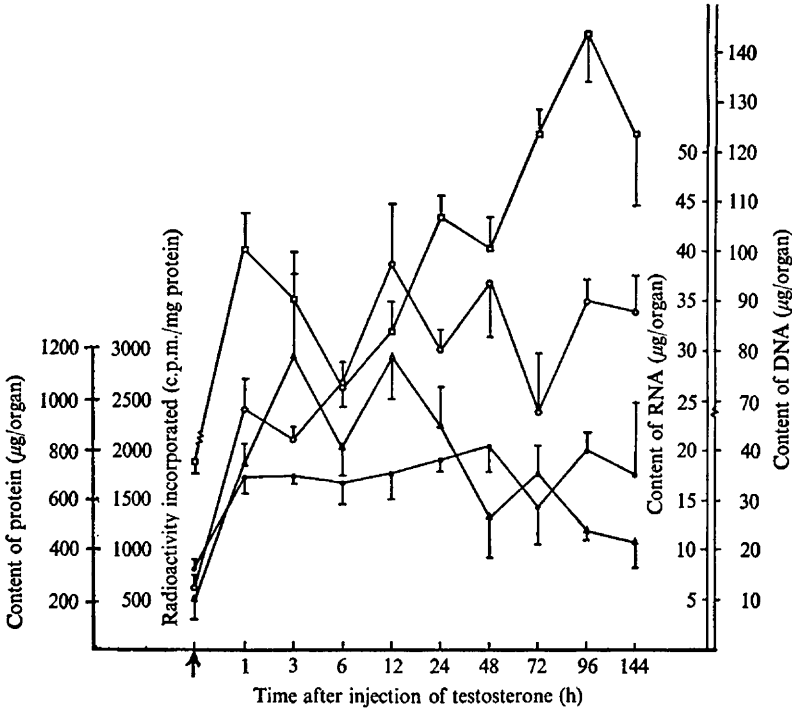


Fig. 3. Changes in the content of DNA (□), RNA (○) and protein (●), and the incorporation of [³H]phenylalanine into proteins (△) in the ventral prostate of adult castrated rats. †, Rats castrated for 30 days. Testosterone (400 µg) was administered subcutaneously at various intervals before autopsy. [³H]Phenylalanine (50 µCi) was administered intravenously 3 h before autopsy. Values are means ± S.E.M., n=3 separate determinations.

showed no increase in the content of protein; the increase in the content of RNA occurred only at 48 h.

Among the sex accessories, the vas deferens was least responsive to testosterone. Recent evidence on the ability of the vas deferens to incorporate large amounts of [³H]testosterone (Appelgren, 1969; Hansson & Tveter, 1971; Thampan, Dinakar, Arora, Prasad & Duraiswami, 1974), the ability of spermatozoa to survive for long periods in vasectomized men (Roy & Taneja, 1974) and the restoration of fertility in men following epididymo-vasostomy (Bedford, Calvin & Cooper, 1973), highlight the dynamic nature of this organ. The present study indicates that the vas deferens requires much higher amounts of androgens than the epididymis for the reinitiation of its regressed metabolic machinery. Whether the stimulation of the vas deferens is, in any way, regulated by the presence of spermatozoa needs further investigation.

A single dose of testosterone did not increase the weights of any of the organs studied. This may be due to the relative insensitivity of this parameter and/or due to the nature of the androgen administered. Dihydrotestosterone appears to be more potent than testosterone in increasing the weights of accessory organs (Schmidt, Noack & Voigt, 1972; Lesser & Bruchovsky, 1973; Tuohimaa, Oksanen & Niemi, 1973; Gupta *et al.* 1974). It is also likely that increase in growth of tissues may occur at a much later time sequence in the action of androgens than changes in biochemical parameters. Lesser & Bruchovsky (1973) observed rapid growth of regressed rat prostate after a 7-day lag period whereas increase in DNA

synthesis occurred much earlier, namely within 3 days after androgen administration. Fujii (1971) observed an increase in incorporation of ^3H -labelled amino acids into proteins of the seminal vesicles of castrated, immature rats 14 h after testosterone administration while no increase in the weight of the organ was apparent.

An acute dose of testosterone, while sufficient to restore to normal the incorporation of [^3H]phenylalanine into proteins of all the organs, did not increase to control levels the net amounts of DNA, RNA or protein. This emphasizes the need for prudent selection of suitable parameters while studying androgen action and the potency of different androgenic compounds. While protein synthesis may be rapidly stimulated by the single dose of testosterone to levels comparable to those in control animals, the effect elicited was not sufficient to cause an increase in new RNA and protein to levels comparable to those of intact rats.

The maximal incorporation of [^3H]phenylalanine into protein after testosterone administration occurred within 3 h in the caput and cauda epididymides, vas deferens and ventral prostate while the biochemically estimated peak levels of RNA and protein occurred much later. Frieden & Ku (1971) showed that stimulation of [^{14}C]orotic acid incorporation occurred in mouse kidney within 6 h of a single dose of testosterone propionate followed by an increase in the incorporation of labelled amino acids into proteins *in vitro* (1–5 days); increase in total DNA, RNA and protein content was apparent only after 5–10 days. Chung & Coffey (1971) reported peak prostatic DNA synthesis 3 days after testosterone propionate administration whereas the incorporation of [^3H]leucine into the isolated protein fraction was 24–48 h before maximal DNA synthesis.

The absence of any significant change in the uptake of precursor into the acid-soluble fraction of the epididymis, vas deferens and ventral prostate during the experimental period indicates that the increased incorporation of [^3H]phenylalanine into protein of these tissues is not due to an enhanced rate of entry of the labelled precursor and consequent changes in pool size.

No mitotic figures were present in the epididymis and ventral prostate of castrated, hormone-treated rats in spite of an increase of DNA content. Cavazos & Melampy (1954) and Chung & Coffey (1971) showed that the nuclear volume of the cells of the prostate decreased after castration and was restored by androgen replacement. DNA synthesis accompanied by an increase in nuclear volume has been reported by Chung & Coffey (1971). It is likely that the dose of testosterone administered in the present study increased the content of DNA but did not stimulate cell proliferation; furthermore, it is possible that testosterone may not be an effective hormone to induce mitosis (Baulieu, Lasnitzki & Robel, 1968; Lesser & Bruchovsky, 1973).

The testosterone administered in the present study did not restore the content of DNA, RNA and protein to intact control levels. Lesser & Bruchovsky (1973) comparing the effects induced by a single dose of dihydrotestosterone with multiple doses, observed that the magnitude of response was greater with multiple injections. Blaquier (1973) observed a better stimulation of the incorporation of amino acids into acid-insoluble protein when the epididymal tubules were cultured in a medium containing androgens for 7 days than in those tubules cultured for 3 days. Since the objective of the present study was to compare the metabolic effects resulting from a single administration of testosterone in different organs, the possible additive effects of multiple administration were not investigated.

The present study envisages the synthesis of new proteins by the accessory sex organs of castrated rats after stimulation with testosterone. Rapid synthesis of new protein has been recorded after treatment of adult rats with the anti-androgen cyproterone acetate (Rajalakshmi & Prasad, 1975) or during inhibition of androgen production as a result of experimentally induced cryptorchidism (Rajalakshmi & Prasad, 1974). The new proteins synthe-

sized in response to stimulation by androgens may be associated with the restoration of the structural integrity/secretory activity of the epididymal cells and must be differentiated from the proteins synthesized after hormone withdrawal or inhibition of hormone action which may be involved in autolytic processes (Rajalakshmi & Prasad, 1974, 1975). Differences in the synthesis of different classes of RNA in the ventral prostate of rats have been observed after administration of oestrogens or androgens (Bashirelahi, Chader & Villee, 1969). Further investigations are in progress to determine the nature of the classes of RNA and protein synthesized in the epididymis after androgen stimulation/withdrawal.

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