Testosterone Control of Nucleic Acid Content and Proliferation of Epithelium and Stroma in Rat Seminal Vesicles

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Tissue wet weight, nucleic acid content and epithelial and stromal cell numbers were measured in the seminal vesicles of sexually mature male rats. After castration, tissue weight and RNA decreased rapidly and in parallel to reach, after 14 days, values only 15–20% of those in control (not castrated) animals. During this period, DNA decreased to a much lesser extent (by about 40%), but this change in DNA correlates well with the observed loss of cells from the epithelium. Testosterone *in vivo* promoted an immediate resynthesis of RNA, the value characteristic of control animals being reached within 80 h. Delays occurred in the hormone-induced regain of tissue weight (30 h) and DNA (40 h), each of which preceded proliferation of the epithelium (40–50 h). The cells of the stroma were unaffected by these changes in the androgenic status of the animal. It is suggested that these proliferative changes in the epithelium cannot account for the previously reported induction by testosterone of basic secretory proteins in this tissue.

Protein synthesis in the accessory sexual tissues of the male rodent is dependent on circulating testosterone (for reviews see Price & Williams-Ashman, 1961; King & Mainwaring, 1974). As a model system for androgen action we have been studying the hormone-dependent synthesis of basic proteins secreted by the rat seminal vesicle (Higgins *et al.*, 1976). Castration results in an immediate, pronounced and rapid decline (half-time approx. 2 days) in the rates of synthesis of two of these basic proteins, an effect that is rapidly reversed by testosterone *in vivo*.

It is established that changes in the androgenic status of the animal alter the pattern of cellular proliferation in the accessory tissues (Price & Williams-Ashman, 1961; Kassenaar *et al.*, 1962; Coffey *et al.*, 1968; Ritter, 1969; Kerr & Searle, 1973; Lesser & Bruchovsky, 1973; Morley *et al.*, 1973). This being so, testosterone could control the synthesis of vesicular basic proteins indirectly merely by modulating the number of cells engaged in their production rather than by direct action on the protein-synthetic machinery of individual cells. To distinguish between these possibilities it is essential to determine the effects of hormonal manipulation on cellular proliferation in this tissue during those times when the rates of synthesis of the basic proteins undergo marked change. Thus for testosterone to act indirectly via proliferation, cell numbers would have to decrease by half within 2 days after castration and increase immediately after hormonal treatment of castrated animals.

There have been numerous studies of proliferation in rodent accessory tissues (see above). Unfortunately many have involved tissues other than the seminal vesicle (Kochakian, 1963; Coffey *et al.*, 1968; Kerr & Searle, 1973; Lesser & Bruchovsky, 1973; Isotalo & Santti, 1975) or species other than the rat (Deanesly & Parkes, 1933; Ortiz *et al.*, 1956; Allen, 1958; Kassenaar *et al.*, 1962; Morley *et al.*, 1973; Alison *et al.*, 1974, 1976). Even where the seminal vesicle of the rat has been examined, these studies are not directly applicable to the problem under consideration, because the critical time-periods were not examined in sufficient detail (Moore *et al.*, 1930; Rabinovitch *et al.*, 1951; Porter & Melampy, 1952; Ritter, 1969) or immature animals were used (Overholser & Nelson, 1935; Rudolph & Samuels, 1949; Sheppard *et al.*, 1965). The present study was undertaken in an attempt to define the action of testosterone on cell proliferation in the seminal vesicle of sexually mature rats.

Materials and Methods

Adult male Sprague–Dawley rats (200–300 g) from this Institute were used. Bilateral orchidectomy (scrotal route) under Fluothane anaesthesia was done on the morning of the first day of the experiment. Testosterone propionate (1.25 mg) in 0.25 ml of arachis oil ('Androject'; Intervet Laboratories Ltd., Morden, Surrey, U.K.) was injected subcutaneously in the flank region twice each day (at about 8 a.m. and 5 p.m.). Animals (in pairs in two experiments, in

Fig. 1. Effect of changes in androgen status on seminal-vesicle tissue weight, nucleic acids, cell numbers and basic-protein synthesis

Animals were castrated on day 0 and at the intervals shown groups were killed. Tissue wet weight (a) was measured on a micro-balance and then one seminal-vesicle lobe was assayed for RNA (b) and DNA (d). The other lobe was pinned out to uniform length (3.5 cm) and fixed in Bouin's fluid. Transverse sections ($5 \mu\text{m}$) were cut and cells counted in epithelium (e) and stroma (f). Then 14 days after castration, remaining groups received twice-daily injections of testosterone (1.25 mg). The different symbols refer to three separate experiments. Lines were drawn by eye, except that regression analysis of the data in (e) and (f) was done with an Olivetti P602 micro-computer by treating the periods of hormone withdrawal and stimulation separately. Incorporation *in vitro* of [^{35}S]methionine (c) into seminal-vesicle basic protein-S (■) and seminal-vesicle basic protein-F (□) is taken from Higgins *et al.* (1976).

groups of four in the third) were killed by cervical dislocation, and each lobe of the paired seminal vesicles was clamped off and removed separately after the coagulating gland and connective tissue had been dissected away. One lobe from each animal of the group was weighed on a micro-balance and frozen at -70°C . The other lobe of each animal in the group was pinned out by its extreme ends to a uniform length of 3.5 cm and fixed in Bouin's picro-formol for 24 h. To minimize epithelial damage and compression artifacts, no attempt was made to express secretions. Wax sections ($5\ \mu\text{m}$) were cut transversely about 1.0–1.5 cm from the anterior end and stained with Cole's Haematoxylin and Eosin. By using a microscope fitted with a prism projection head (Gillett and Sibert, London S.W.11, U.K.), the sections were projected (magnification $\times 195$) on to drawing paper and the epithelium was traced out together with the stromal area. From these drawings, a map measurer and a planimeter were used to measure the epithelial length and stromal area respectively. To calculate the number of stromal cells per section (i.e. all non-epithelial cells), an eyepiece graticule and an oil-immersion objective (magnification $\times 940$) were used to count at least 300 nuclei in three or four randomly selected regions of the stroma. A similar number of epithelial nuclei were counted in three or four epithelial lengths marked by recognizable topographical features. By identifying these features on the drawings, the number of epithelial cells per unit length was determined and hence the total number of epithelial cells in each transverse section. In the first of the three separate experiments reported here it was ascertained that identical results were obtained from sections taken from posterior or anterior ends of the lobes.

Nucleic acid determination

Anomalous low readings were observed with the diphenylamine colour reaction for DNA (Burton, 1956) whenever appreciable quantities of the cold- HClO_4 -precipitable fraction of seminal-vesicle secretion were present. Quantitative removal of the secretion was not possible, owing to its tendency to coagulate (Higgins *et al.*, 1976) and the probability that loss of epithelial cells would accompany manual expression of the secretion. Accordingly DNA and RNA were measured by differential extraction. The frozen glands were thawed in 10 vol. of 0.25 M- HClO_4 at 4°C , chopped finely with scissors and disrupted at 4°C with a laboratory blender (Silverson Machines Ltd., Chesham, Bucks., U.K.) by using 1 min at slow speed and 30 s at full speed. Precipitated material was collected (10000 g for 10 min), washed once with cold 0.25 M- HClO_4 (10 vol.) and then resuspended in 10 vol. of 0.3 M- NaOH for 18 h at 37°C . HClO_4 (2.5 M) was added to give a final concentration of 0.25 M and the suspension was cooled

to 0°C . Insoluble material was re-extracted with 0.3 M- NaOH (5 vol., 2 h, 37°C) and was then digested for 20 min at 70°C in 10 vol. of 0.5 M- HClO_4 . The suspension was cooled to 0°C , insoluble material was again removed and re-extracted with hot acid (5 vol.). The E_{260} values of the combined alkali-soluble and the combined hot-acid-soluble fractions were read. RNA and DNA originally present in the tissue were calculated from these values by using calibration curves respectively for purified calf liver RNA [type IV; Sigma (London) Chemical Co., Kingston-on-Thames, Surrey, U.K.] and calf thymus DNA (Sigma; type I). Two extractions with alkali and hot acid served to release at least 95% of the available material.

Results

Atrophy of the seminal vesicles was rapid after bilateral orchidectomy (Fig. 1a). Gland size was decreased by half within 3 days, and after 10–14 days had stabilized at about 20% of the value in intact (not castrated) animals. As expected, testosterone *in vivo* initiated a reversal of this atrophy after a delay of about 30 h (Fig. 1a). Accompanying the decrease in vesicle size was an almost exactly parallel decrease in RNA (Fig. 1b) and protein (results not shown). Net RNA synthesis recommenced almost immediately when testosterone was given to long-term (14-day) castrated animals (Fig. 1b) and preceded gland enlargement. After 80 h the RNA content of the vesicles had reached and then exceeded that of intact controls.

These pronounced changes were not accompanied by such large changes in DNA content. This nucleic acid decreased by 40% 14 days after castration, and a significant increase over this value did not take place until some 40 h of hormone treatment had elapsed (Fig. 1d). The DNA content characteristic of normal glands was regained after 70–80 h.

Assuming that normal tissue is populated by cells containing the diploid amount of DNA, a decrease in DNA presumably indicates net cell death. Hence we can conclude that 14 days after castration about 40% of the cells of the seminal vesicle have died. On the other hand, the testosterone-induced increase in DNA in castrated animals can be less easily interpreted as indicating cell proliferation, since the DNA-replication period of the cell cycle occurs before mitosis, and further there could conceivably be other transient changes in cell ploidy. It is therefore necessary to examine cell numbers directly during periods of hormonal manipulation.

Since it is within the epithelial cells of the vesicles that synthesis of the secretory proteins occurs (Moore *et al.*, 1930; Cavazos & Melampy, 1954; Price & Williams-Ashman, 1961), hormonal effects

on these cells must be distinguished from those on the cells of the stroma.

In the normal rat seminal vesicle, epithelial cells outnumber stromal cells by two to one (Figs. 1e and 1f). After castration, progressive death of epithelial cells (Fig. 1e) took place (60% loss in 14 days), with no significant change in the stroma (Fig. 1f). Testosterone failed to promote a significant increase in epithelial cell numbers until nearly 50h had elapsed (Fig. 1e). The epithelial cell content typical of normal glands was attained by about 90h, although it is possible that epithelial cells were overproduced before this (70–80h). Not surprisingly, the hormone had no effect on the stroma (Fig. 1f).

Discussion

Androgen withdrawal by castration affects only the epithelial cells (but not the stroma) of the rat seminal vesicle, decreasing them by 60% within 14 days. By then they constitute less than half of the total cells, instead of about two-thirds as in the intact animal. Cell death in the epithelium correlates well with the observed decrease in total DNA (40% in 14 days). In the rat (Rabinovitch *et al.*, 1951) and the mouse (Kassenaar *et al.*, 1962) much more modest decreases in seminal-vesicle DNA have been reported, but in the latter species Alison *et al.* (1974, 1976) found this nucleic acid to be decreased by 60–70%. Changes in cell numbers were not described.

The rapid atrophy of the seminal vesicles was clearly much more extensive (80% decrease) than would be expected for the extent of cell death found. Presumably hypertrophy noted by others (Moore *et al.*, 1930; Burkhardt, 1942; Price & Williams-Ashman, 1961) accompanies the proliferative changes. The measurements of RNA show clearly that hormone withdrawal does involve extensive loss of at least this cellular component.

Two basic secretory proteins were studied by Higgins *et al.* (1976). At 10–14 days after castration the synthesis rates were decreased by 75% for one protein and 95% for the other (Fig. 1c), changes that are too great (and also too rapid) to be accounted for by castration-induced epithelial cell death (Fig. 1e). In fact, decrease in basic-protein synthesis correlated well with decreased RNA content (Fig. 1b) and tissue weight (Fig. 1a).

Induction of these basic proteins occurred immediately after castrated animals were treated with testosterone, considerably in advance of significant increases in epithelial cell number (Fig. 1e) or DNA content (Fig. 1d). Thus induction of basic-protein synthesis by testosterone (Higgins *et al.*, 1976) takes place at least initially without need for epithelial-cell proliferation, and this too is much better correlated with the increase in RNA (Fig. 1b). Others have reported rapid androgen-induced synthesis of RNA

in rat seminal vesicle (Wicks & Kenney, 1964; Sheppard *et al.*, 1965; Isotalo & Santti, 1975). Certainly induction of specific proteins by oestrogens in the chick oviduct (Means *et al.*, 1972; Rhoads *et al.*, 1973) and by glucocorticoids in liver and hepatoma cells (Scott *et al.*, 1972; Schutz *et al.*, 1973; Steinberg *et al.*, 1975) appears to involve increases in active messenger ribonucleic acids specific for those proteins without cell proliferation.

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