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The Ultrastructure of the Accessory Sex Organs of the Male Rat. 7. The Effect of an Anti-androgenic Compound, SK and F 22340

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Summary. The influence of a potent anti-androgenic compound, SK and F 22340, on the ultrastructure of the lateral and ventral prostate and the seminal vesicles of the rat has been studied. After treatment for 18 days there was macroscopic atrophy of the organs with decreased amount of secretory material. There was reduction of the height and width of the epithelial cells and loss of cytoplasm. The most distinct changes were found in the cytoplasm and comprised a quantitative reduction of the rough endoplasmic reticulum (RER) and of free ribosomes; the Golgi apparatus was reduced and the number of secretion granules were diminished. All the alterations observed are apparently similar to the changes caused by bilateral orchidectomy.

Key words: Prostate-rat, anti-androgens, electron microscopy.

Introduction

Treatment of prostatic cancer with oestrogens was introduced in clinical medicine after the pioneer work of Charles Huggins and co-workers (18, 16). Serious complications, such as cardiovascular disorders and feminization have, however, to some extent restricted the usefulness of this treatment. The development of specific anti-androgenic compounds with a reaction mechanism different from that of oestrogen therefore seems to represent an important improvement in the effort to treat prostatic diseases. Whereas oestrogen primarily acts via the pituitary gland by diminishing the formation of gonadotrophic hormones, with subsequently reduced synthesis of testicular androgens, the anti-androgenic compounds seem to have a more specific effect on the prostate and the seminal vesicles (25, 9, 27, 28, 29). While there are numerous reports dealing with bioassay studies of anti-androgens (21), the influence of these compounds on the fine structure of the prostate and the seminal vesicles does not seem to have been studied in detail.

The present paper, which continues previous reports on the ultrastructure of the prostatic complex (7) describes the effect of a potent anti-androgenic compound, SK & F 22340, on the ultrastructure of the ventral and lateral prostate and the seminal vesicles of the rat.

Materials and Methods

Seven adult albino rats (Charles River CDF strain) 4-6 months old were used.

Four rats received 15 mg of the anti-androgenic compound SK & F 22340 (Smith, Kline and French Overseas co., Philadelphia, PA.) daily as subcutaneous injections for 18 days. The remaining three animals served as controls and received the same amount of the solvent vehicle daily for the same period.

All the animals were sacrificed two days after the last injection. Under nembutal and ether anaesthesia the iliac arteries on both sides were ligated to block the blood flow to the lower extremities. The aorta was cannulated with a plastic

catheter of calibre no. 4 proximal to the renal arteries, and the tip of the catheter was then placed just above the aortic bifurcation. Rinsing and fixation of the pelvic organs were achieved by brief perfusion through the catheter with dextran followed by 1.7% glutaraldehyde in 0.1 M phosphate buffer, pH 7.3, for 20 min, using a perfusion pressure of about 150 mm Hg. Great care was taken to avoid manipulation of the prostate and the seminal vesicles during perfusion. The pelvic organs were then excised en bloc and fixed for an additional period of 2 hrs by immersion in the perfusion fluid.

After fixation the different prostatic lobes were gently dissected and cut into small cubes under the stereo microscope. Only the tip of the lateral prostate was used, since the intermediate region of the dorsolateral prostate contains a mixture of dorsal and lateral tissue (14). For the same reason,

Fig. 1

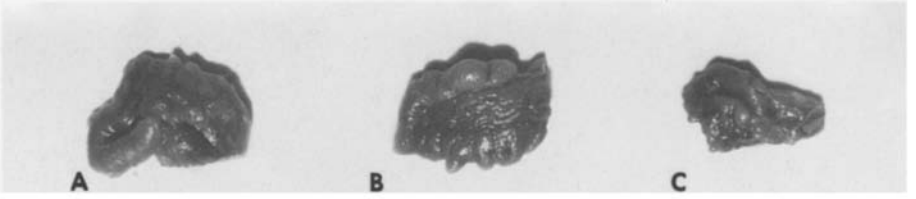

Fig. 2

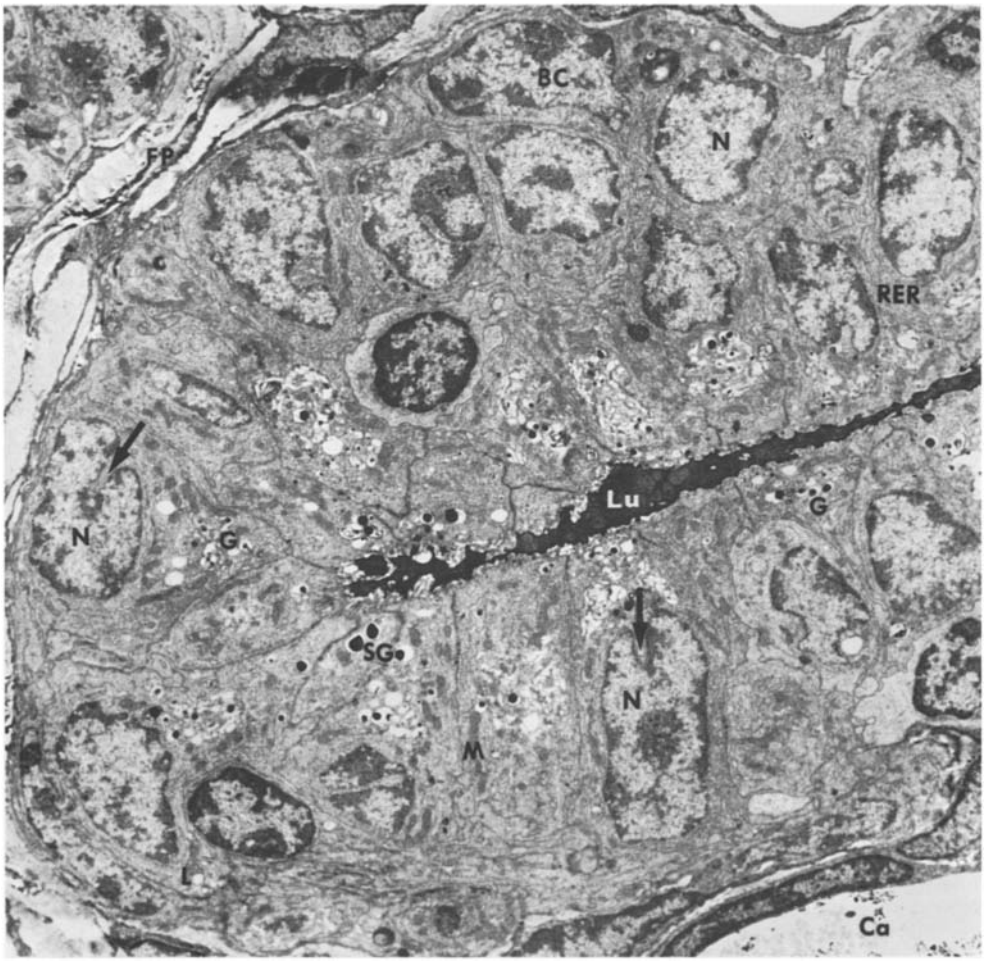

Fig. 1. Photograph of the free dissected seminal vesicles and part of the coagulating gland. A) Solution treated, B) Normal untreated, C) SK and F 22340 treated for 18 days. Note the reduction of the size of the organ in the SK and F 22340 treated animal (C). x 1.62

Fig. 2. Survey electron micrograph of an acinus of the seminal vesicles. There is a marked reduction of the lumen (Lu) with only a small amount of secretory material (Black). There is a marked reduction of the cell height and width, general loss of organelles and secretory granules (SG). There is reduced amount of mitochondria (M), rough endoplasmic reticulum (RER) and diminished Golgi area (G). Note the indentations of the nuclei (arrow) N = nucleus, Ca = capillary, Fp = fibrocyte prosesses surrounding the acinus. BC = basal cell. L = lipid droplet. x 4370

tissue from the dorsal lobe was taken from an area as close to the midline as possible. Six blocks were processed from each lobe of the various animals, and all the specimens were rinsed for 10 min in 0.15 M phosphate buffer (pH 7.3) and post-fixed in OsO_4. The blocks were rapidly dehydrated in graded series of acetone and embedded in Vestopal W (22).

Ultrathin sections were cut on an LKB Ultratome III. The sections were examined in a Siemens Elmiskop Ia electron microscope. From the same plastic blocks sections were prepared for light microscopy. The sections were stained on a heating stage with an aqueous solution of 0.1% Toluidine Blue adjusted to pH 8.9 with 0.067 M Na_2HPO_4.

Results

All the observations in the present investigation were compared with the results obtained in the study of the normal, (Dahl, Kjaerheim & Tveter,

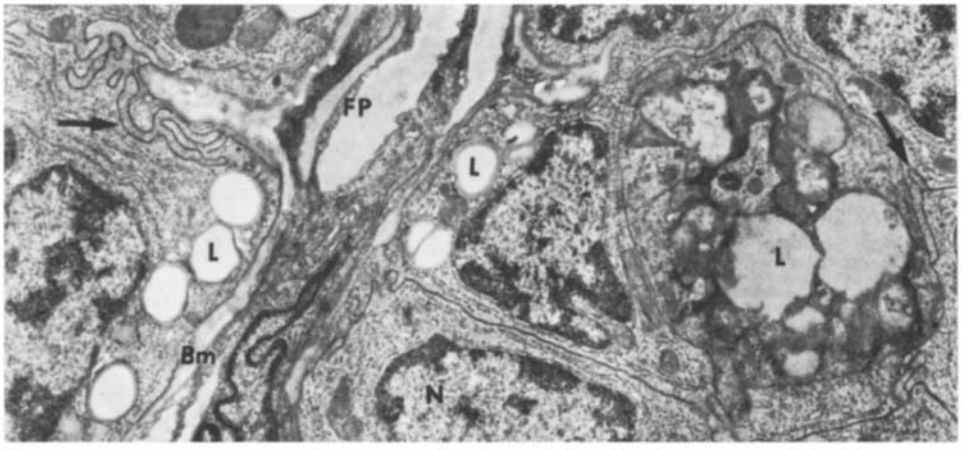

Fig. 3

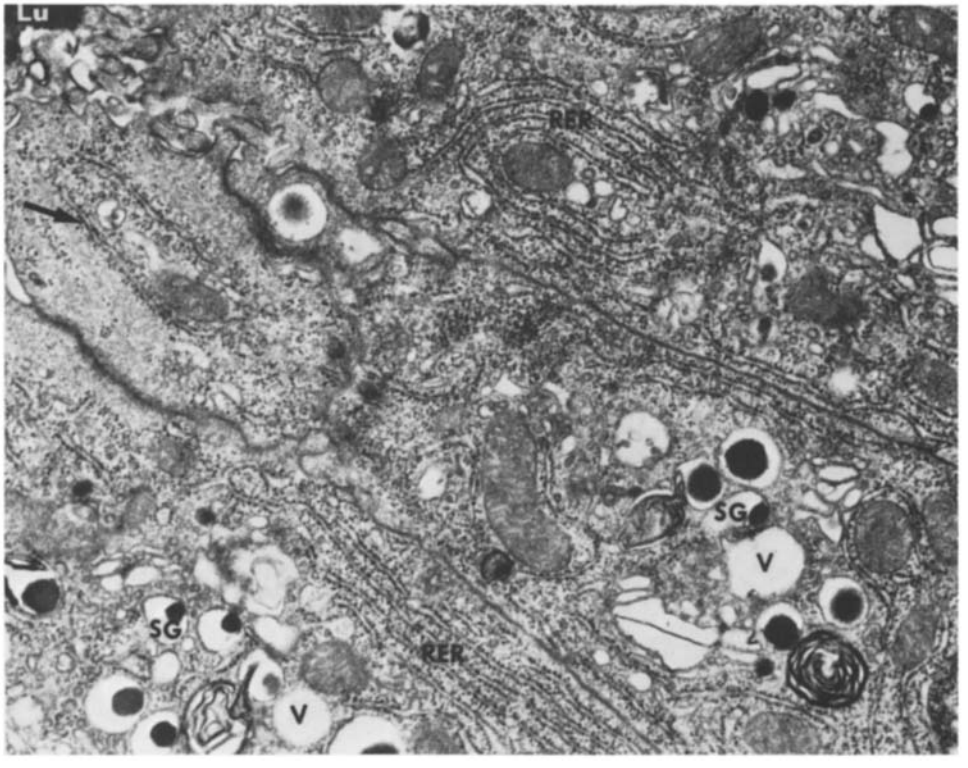

Fig. 4

Fig. 3. Cells from the basal part of two neighbouring acini of the seminal vesicle. In addition to reduction of the cytoplasm and organelles, there is an accumulation of lipid droplets (L) in the epithelial cells. N = nucleus, Bm = basement membrane, Fp = fibrocyte process. Interdigitations of two neighbouring cells is arrowed. x 11.250

Fig. 4. An oblique section of the apical part of epithelial cells of the seminal vesicles. In some areas (the right half of the picture) there is still some rather well developed rough endoplasmic reticulum (RER), while other cells which border the lumen (Lu) left upper corner are almost deprived of this organelle (arrow). Note the empty secretory vacuoles (V). Secretory granules (SG). x 21.600

1973) castrated (4, 5) and testosterone treated rats (7). From these previous observations, it was obvious that injections of SK & F 22340 subcutaneously for 18 days did not influence the general condition of the animals. They were all in good condition at the time of sacrifice. There was a marked involution of the accessory genital organs with macroscopic atrophy (Fig. 1) and reduced amount of secretory material.

Although all the prostatic lobes showed alterations of the fine structure after administration of the anti-androgenic compound, there were quanti-

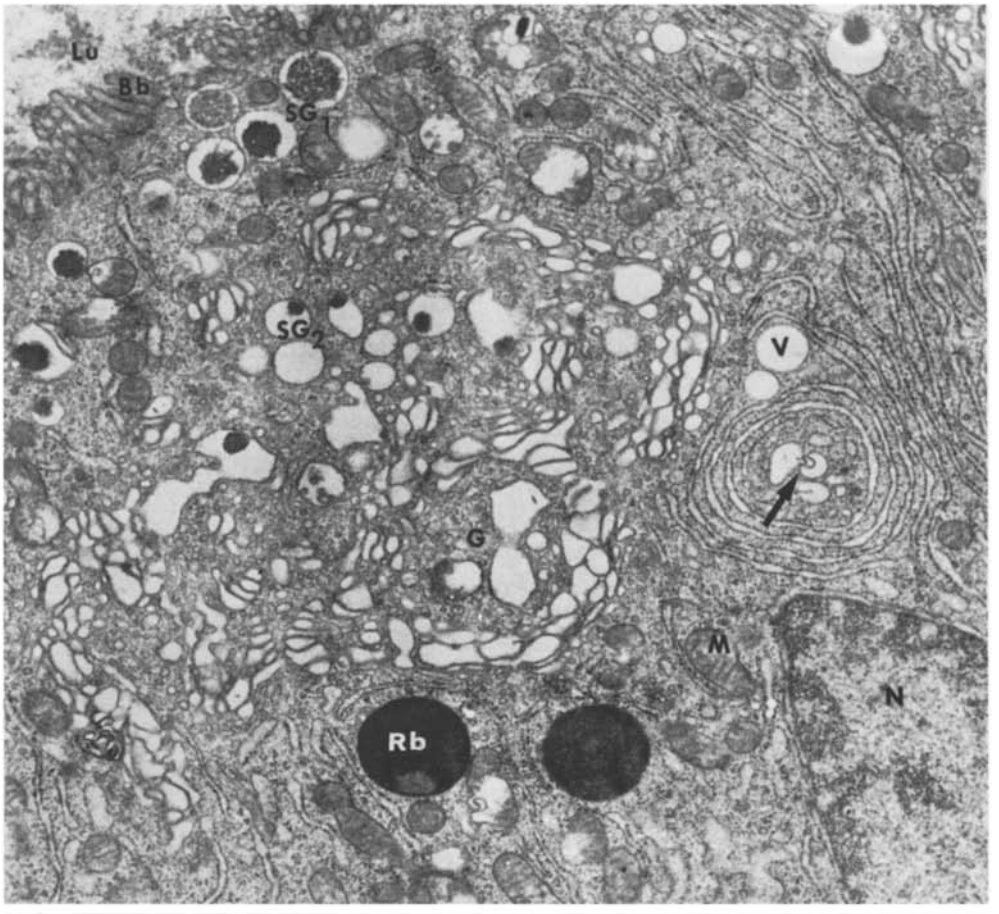

Fig. 5

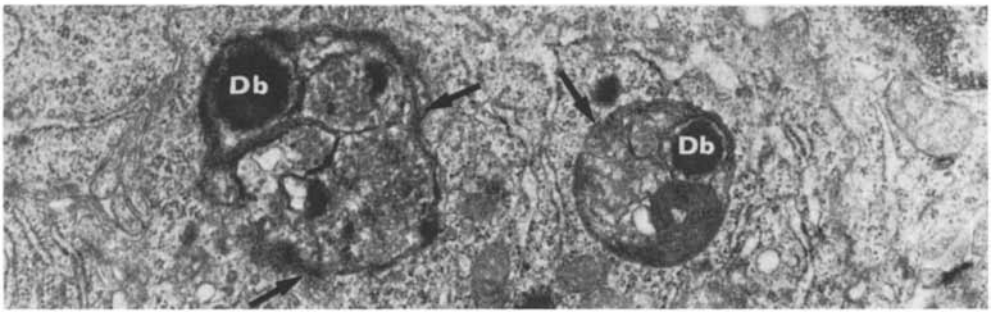

Fig. 6

Fig. 5. In this micrograph from the apical part of epithelial cells of the lateral lobe, the Golgi area (G) is still rather well developed, but almost totally without any secretory material. Towards the acinar lumen (Lu) different types of secretion granules (SG_1, SG_2) are seen. The brush border (Bb) is disrupted and with smaller and more slender microvillis than normally found. A whorl formation with centrally located vacuoles (arrow) is seen to the right. Note the large residual bodies (Rb). N = nucleus, V = empty vacuoles in the Golgi apparatus, M = mitochondrion. x 13.500

Fig. 6. Detail micrograph from the lateral lobe demonstrating the initial stage of an autophagic vacuole with segregation of a portion of the cytoplasm (arrows) containing dense bodies (Db) of the lysosome-series and cytoplasmic debris. x 21.600

tative differences both from cell to cell within the single acinus (Figs. 2, 4) and also within the different organs. This was reflected by some differences in the size and the number of organelles of the epithelial cells, obliquity of the sections taken in consideration (Figs. 2, 4). Basically, however, the administration of SK & F 22340 caused similar changes in the three different organs. To avoid a repetitive detailed description of the cellular structures as they appeared in each lobe, only the most general, salient features will be described in common in all the three organs.

There was a general reduction of both the cell height and width of the epithelial cells (Fig. 2),

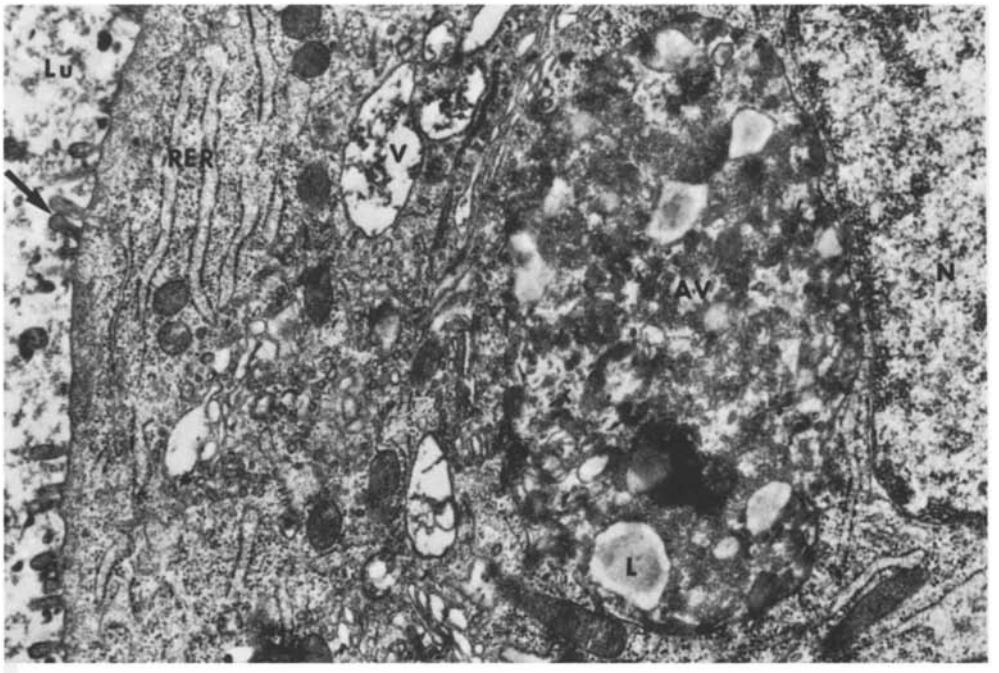

Fig. 7

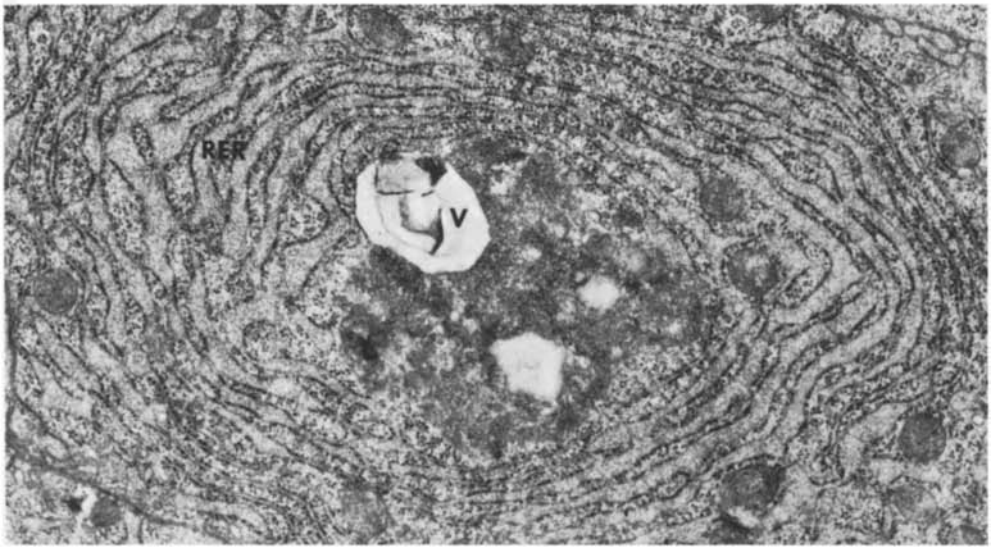

Fig. 8

Fig. 7. This micrograph demonstrates a portion of an epithelial cell from the ventral lobe. There is a marked reduction of the rough endoplasmic reticulum (RER): Vacuoles (V) with flocculent material and a large autophagic vacuole (AV) in the Golgi area containing lipid droplets (L) and cell debris. N = nucleus (cfr. Fig. 6), Lu = acinar lumen. Note the sparcety of microvilli (arrow). x 22.500

Fig. 8. Detail from an epithelial cell of the ventral prostate. The cisternae of the rough endoplasmic reticulum (RER) still have an almost normal appearance, but are arranged in whorls, and in the centre rather large vacuoles (V) and amorphous material indicating cellular debris are seen (cfr. Fig. 6). x 21.600

with loss of cytoplasm, indentations of the nuclei (Fig. 2), diminished Golgi area with empty vacuoles (Figs. 2, 5) indicating reduced activity. Generally there was a marked reduction of the rough endoplasmic reticulum (RER) (Figs. 2, 4) and reduced amount of secretion granules (Figs. 2, 4, 6). Furthermore, the secretion granules also tended to be smaller than normal.

More specifically, the RER formed whorl formations (Figs. 5, 7), with centrally located vacuoles (Figs. 5, 7) or dense bodies of the lysosomal series. Residual bodies (Fig. 5) were found in an increased amount. A rather conspicious finding was the presence of various types of autophagic vacuoles (Figs. 5, 6) containing vesicles, membranes, dense bodies and cell debris (Fig. 6).

In addition to these general atrophic changes, specific alterations characteristic of the various lobes were also observed. The well developed brush border of the lateral prostate which in the normal rat is a characteristic feature for this lobe, was reduced (Fig. 5). The epithelial cells of the seminal vesicles regularly contained accumulation of lipid droplets (Figs. 2, 3). In the ventral lobe, which in the normal animal is recognized by its large stacks of parallel cisternae of RER, there was a marked reduction of this organelle (Fig. 6).

Discussion

Electron microscopic examination revealed that the accessory reproductive organs contain epithelial cells which have all the characteristic features of protein synthesizing cells (1, 3, 4, 5, 12).

The function of these cells is dependent upon an appropriate amount of circulating androgen (4).

Biochemical data point to the nucleus of the epithelial cells as the primary site of action of the androgenic hormones.

Androgen seems to act directly on the DNA template, and stimulates the formation of different forms of RNA, which, after entering the cytoplasm, regulate the formation of structural and secretory-proteins as well as enzymes (33). The proteins are synthesized by the RNA-containing ribosomes. The ribosomal RNAs will give rise to formation of specific proteins according to their genetic information (11).

The prostate and the seminal vesicles possess the capacity to concentrate and to retain androgen, in contrast to less responsive tissues such as skeletal muscle (27, 28, 29). This high and prolonged uptake of androgen is due to the presence of specific androgen binding substances, or androgen receptors, in the target tissues. Such androgen receptors are located both in the cytoplasm and in the nucelei of the epithelial cells and have been demonstrated in the rat as well as in the human prostate (2, 31, 30, 9, 20). The physiological significance of these receptors is not clear, but all evidence seems to indicate that the interaction of androgen with androgen receptors is intimately related to the biological effects of androgenic hormones (10). The synthetic anti-androgenic compounds are able to depress the uptake of androgen by the prostate and the seminal vesicles and to inhibit the association of androgen with cytoplasmic as well as with the nuclear receptors (30). The anti-androgenic properties of these drugs therefore seem to be related to a direct peripheral effect on the binding sites for androgen in the cytoplasm and nuclei.

In the present study, the effect of the anti-androgenic compound SK & F 22340 on the rat prostate is reflected by macroscopic involution of all the accessory sex organs. Ultrastructurally there are alterations in the fine structure as compared with the control animals, with reduction both in the cell height and width, as well as quantitative and qualitative changes, indicating a decreased function of the organelles of the cells, recognized by a marked reduction of secretory material. In addition to these general changes, more specific alterations of the various lobes were also observed. When the present observations are compared with previous investigations (12, 3, 1, 15) it is evident that the systematic administration of the compound SK & F 22340 to intact non-castrated rats causes changes in the gross anatomy and the fine structure of the whole prostatic complex similar to the alterations seen after orchiectomy (3, 5).

It should be added that in a previous investigation (6) the effects of another potent anti-androgen, viz. Cyproterone acetate, have been discussed. When the micrographs from these two different investigations are compared, it is of interest to note that cyproterone acetate and SK & F 22340 appear to have similar effects on the ultrastructure of the epithelial cells of the accessory reproductive organs of male animals, indicating that both compounds exert their effects through similar mechanisms. There do not appear to be any differences, neither quantitative nor qualitative, between these two compounds as far as their effects on the fine structure of the rat prostate is concerned.

Both carcinoma of the prostate and benign prostatic hyperplasia seem to be causally related to the presence of androgens. It therefore seems worthy to note that both neoplastic and hyperplastic tissues contain cytoplasmic as well as nuclear androgen receptors (30). Anti-androgenic compounds have already proven to be useful drugs in the treatment of both benign and malignant prostatic diseases, (13, 24) although with some side effects (32). Future research will undoubtedly develop new compounds, probably with an even more specific effect on the prostate. In the complete evaluation of such drugs, electron microscopic investigations seem to be a rather valuable method.

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