# STEROID RECEPTORS, METABOLISM AND PROSTATIC CANCER

Workshop of the
Society of Urologic Oncology
and Endocrinology
Amsterdam,
27th and 28th April, 1979

Editors:
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# Welcome Address

H. J. de Voogt
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Ladies and Gentlemen,

First of all I would like to extend a cordial welcome to all of you who have accepted our invitation and taken the trouble to travel, some over long distances, to be here today to present what I look forward to as some very interesting papers, or to listen to these and take an active part in the discussions.

Amongst you there is a small group of dedicated biochemists and urologists from different parts of Europe, which has met in the past on several occasions in Leiden and Hamburg. These were rather informal meetings where, in a friendly atmosphere, we could exchange our ideas and experiences on the matter of steroid receptors in prostatic tissue.

From the programme it might be concluded that a European Prostate Cancer Research Group actually exists, but this is not so. However, we have been thinking about bringing such a group into being – if not today then in the near future.

It has always been a pleasure for me to organize these meetings and I got the impression from many of you that you wanted to continue them. However, on the one hand due to the burden of my daily work I did not see an opportunity to continue on my own, while on the other I thought that it might be much more informative to invite other research workers whom we knew of through their publications, personal acquaintance or meetings. I felt we could learn much more in this way and probably even set out lines or paths along which our future research efforts might be directed. I must say that we were aware of the fact that we may have overlooked quite a few people of whom we did not know.

At one point I had the opportunity to discuss this with Schering and indirectly with Prof. Neumann, and I was very glad when they gave me their support in organizing this meeting, which is what I had in mind. Through their generosity we were able to invite people from more remote places in Europe as well as from the U.S. and Canada. Once again, I am very glad that you all reacted favourably to the proposals that I was able to work out together with Prof. Schröder and Dr Teulings, with whom a scientific committee was formed.

From the beginning we have said that, though it seems to be a much

more formal meeting, with official presentations, it should still remain and have the nature of a workshop, which means that there must be ample time for discussion, as well as informal get-togethers during breaks for coffee and meals.

You will therefore find that after each presentation there is a short time for questions which should be more technical or simply clarify points. After each session there is a longer period for more general discussions and I have asked the chairmen of the sessions to look after this very carefully.

Much to my regret I have to tell you that Prof. Mainwaring unfortunately had to cancel his attendance at the last moment. For that reason we had to change the programme: in his place Prof. Neumann will speak. This will give more time for discussion in Saturday morning programme.

With this I officially declare this workshop open and wish you all a good meeting.

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# I

# RECENT ADVANCES ON THE METABOLISM AND MECHANISM OF ACTION OF STEROIDS IN THE PROSTATE

# Combined effects of testosterone, estradiol and hydrocortisone on rat ventral prostate in organ culture

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## Introduction

It is widely accepted that the development of benign prostatic hypertrophy (BPH) requires the presence of testes. BPH has rarely if ever been reported in men castrated in youth or as young adults. However, once BPH has developed to a degree where urinary obstruction is present, the testes seem less important. If elderly men with advanced BPH are castrated, relief of bladder neck obstruction is variable. Some regression of epithelial elements occurs, but the stromal elements of BPH nodules are not affected. The point to be emphasized is that each cell type in the prostate does not necessarily respond in equivalent fashion to sex steroid hormones [1].

The ageing process is, indeed, accompanied by changes in circulating sex hormones. The concentration of circulating testosterone (both total and unbound) is decreased in older men. The concentration of circulating estradiol is almost unchanged. Hence the ratio of unbound estradiol/unbound testosterone appears to be significantly increased in such men [2].

Prostatic hypertrophy resembling the human disease never occurs spontaneously in the rat ventral prostate, nor can it be induced in organ culture by physiological concentrations of testosterone. Indeed when culture takes place in Medium 199 without supplementation, and in the absence of hormone, epithelial cells regress but stromal components are not affected and even tend to proliferate. Testosterone maintains the epithelial cells and prevents the increase of perialveolar and interstitial stroma [3]. We show in this paper that estradiol in conjunction with androgen induces simultaneous hypertrophy of epithelial and stromal elements [4, 5].

## Materials and methods

### *Chemicals*

Estradiol, testosterone and hydrocortisone were obtained from Roussel-Uclaf, France. Medium 199 was obtained from Flow Laboratories Ltd.,

Scotland. Methyl-$^3$H thymidine was from the Radiochemical Centre, Amersham, England.

*Animals*

Male Wistar rats were obtained from Iffa Credo. St Germain sur l'Arbresle, France, and sacrificed at 7 weeks of age.

*Organ culture*

Explants of ventral prostate were cultured in serum-free medium as previously described [3]. After a 24-hour preincubation in hormone-free medium to eliminate any interference by endogenous hormones the explants were transferred to medium containing estradiol, testosterone or hydrocortisone either alone or in combination. Hormones were added to the medium in propylene glycol. Control cultures in hormone-free medium received an equal volume of solvent. After 72 hours all explants were transferred into similar fresh media for a further 72 hours. For each treatment examined a minimum of 10 explants from different animals were cultured. Methyl-$^3$H thymidine (0.2 Ci/mmol, 4 $\mu$Ci per ml) was added to some cultures 22 hours after the transfer to fresh medium.

*Light microscopy and autoradiography*

Explants were fixed in Bouin's aqueous fixative and cut into 5-$\mu$m sections. The preparations were covered with Ilford L4 nuclear emulsion. After a two week exposure at 4°C the emulsions were developed in D 19 B (Kodak) and fixed. Sections were stained with hematoxylin eosin. Other sections were stained with periodic acid-Schiff to reveal the mucopolysaccharides. For evaluation of alkaline phosphatase according to Gomori [6] explants were fixed in 80% ethanol at 0°C.

## Results

Several concentrations of testosterone (1–100 nM), estradiol (1–1,000 nM), and hydrocortisone (10–1,000 nM) were used alone or in combination. Representative experiments semi-quantitatively evaluating hormonal effects on ventral prostate epithelial and stromal elements are summarized in figure 1.

At all concentrations of testosterone $\geqslant$ 1 nM the prostatic explants were maintained in a state comparable to that observed before culture, confirming preceding observations [3] (Figure 2). The glandular epithelium mainly consisted of tall columnar cells, showing well developed rough endoplasmic reticulum, supranuclear Golgi area, secretory granules and microvilli under electron microscopic examination (data not shown). The perialveolar sheath was composed of 1 or 2 layers of smooth muscular cells and of fibroblasts [7].

The interalveolar stroma was reduced to a few dispersed fibroblasts and macrophages. Periodic acid-Schiff and alkaline-phosphatase staining predominated in the epithelial cells [4], whereas thymidine labelling was almost exclusively restricted to perialveolar and interstitial fibroblasts (Figure 3).

In the absence of testosterone alveolar diameter was reduced, the epithe-

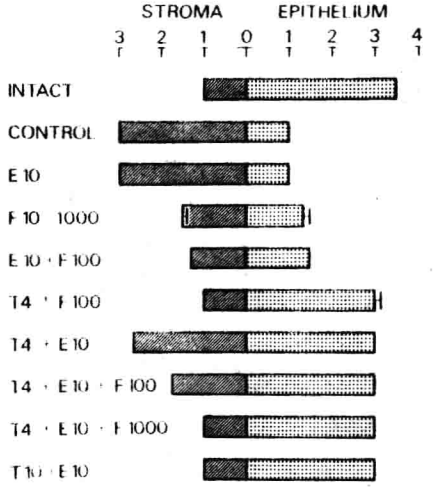


*Fig. 1. Effects of testosterone (T), estradiol (E), and hydrocortisone (F) on rat ventral prostate epithelium and stroma. The numbers following T, E or F are the concentrations of hormones used and are expressed in nM. The arbitrary scales apply to light microscopic, periodic acid-Schiff and alkaline phosphatase staining data, as follows: Epithelium: 4 = fully maintained, 3 = maintained (predominant columnar cells), 2 = partly maintained (predominant cuboidal cells), 1 = not maintained (predominant flat cells). Stroma: 1 = completely regressed, 2 = partly regressed, 3 = not regressed.*

lium was atrophic and its cells flattened, with disappearance of secretory granules and microvilli and, in addition, exhibition of dense bodies. In contrast the perialveolar sheath was thickened, retracted muscular cells showed 'holly-leaf' spines and signs of micropinocytosis. Moreover, the stroma was well developed, with many fibroblasts and active macrophages readily visible together with a dense network of collagen fibers. Periodic acid-Schiff and alkaline phosphatase stains were predominant in the stromal cells, mainly fibroblasts. Thymidine labelling of the nuclei of the same cells also tended to increase.

Regardless of the concentration used, estradiol alone did not alter the morphologic, staining and labelling characteristics observed without hormone.

When estradiol (> 0.1 nM) was added to physiological concentrations of testosterone (1 or 4 nM), the epithelium was well developed and more stimulated than with testosterone alone. In particular, thymidine labelling of nuclei was markedly increased.

However, interstitial tissue was also developed almost as much as in the absence of hormones or in the presence of estradiol alone. All features observed separately in androgen-stimulated epithelial cells and in no-hormone or estradiol-exposed stromal cells were observed when testosterone (1 to 4 nM) and estradiol (1 to 1,000 nM) were present together.

This was no longer the case when estradiol (1 nM to 1,000 nM) was added to supraphysiological concentrations of testosterone (10 nM and more). The latter again provoked a typical androgenic stimulation of epithelial cells, but the stroma was unresponsive to estradiol. Biochemical parameters were

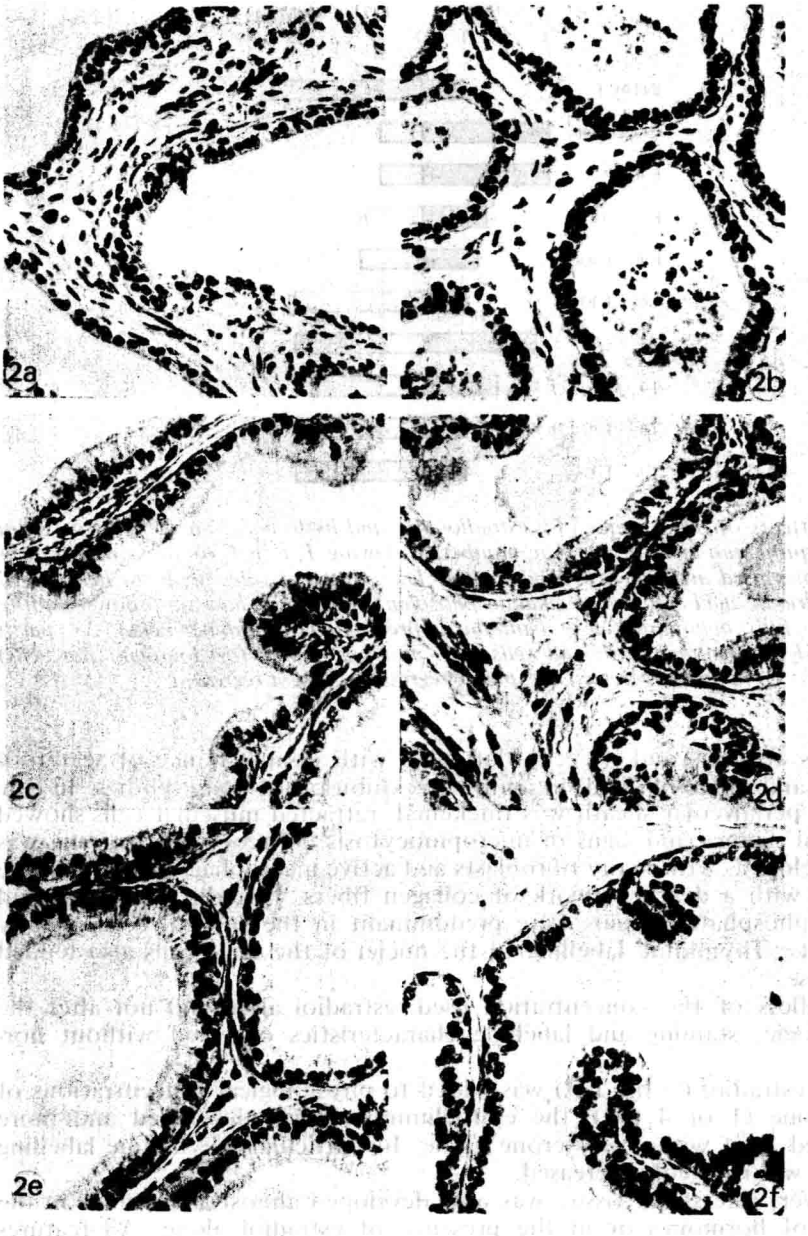


*Fig. 2. Light microscopy of 7-day cultures (T. Feyel-Cabanes). a, without hormone; b, 10 nM estradiol; c, 4 nM testosterone; d, 4 nM testosterone with 10 nM estradiol; e, 100 nM testosterone; f, 100 nM testosterone with 100 nM estradiol. × 300. (from [5]).*

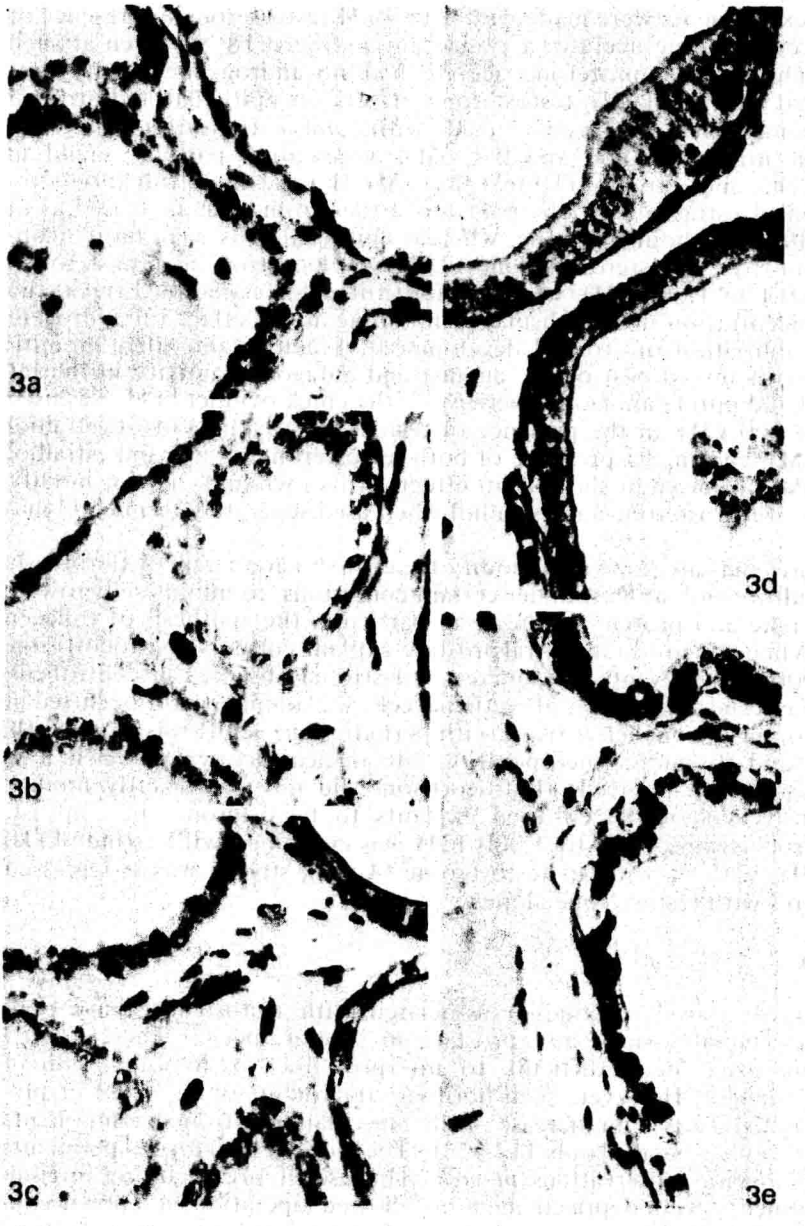


*Fig. 3.* $^{3}H$ *thymidine-autoradiography of 7-day cultures (S. Weiller). a, without hormone; b, 10 nM estradiol; c, 100 nM hydrocortisone; d, 4 nM testosterone; e, 4 nM testosterone with 10 nM estradiol.* × *500.*

also similar to those observed with testosterone alone.

Several experiments were made with 1 to 4 nM testosterone in presence of 1,000 nM cyproterone acetate, a classic antiandrogen [8, 9]. Even at such large concentrations cyproterone acetate had no androgenic activity, but counteracted very markedly testosterone effects on epithelial and stromal cells. Preliminary observations were made with combinations of lower testosterone concentrations (0.01 and 0.1 nM), which only partially maintain epithelial cells, and estradiol (10 to 1,000 nM). Here again no full antiandrogenic action of estradiol was observed, since the stroma was increased as in control cultures without hormone, whereas epithelial cells were more maintained than by testosterone alone. Diethylstilboestrol, a non-steroidal oestrogen (0.1 to 1,000 nM), was also substituted for estradiol. Even at the highest concentration used it displayed no antagonistic effect on androgen-dependent inhibition of stromal development. Finally, tamoxifen, an anti-oestrogen with mixed oestrogenic agonist and antagonist activity in the rat uterus [10] and purely antagonist activity in the chick oviduct [11], was used alone (1 to 100 nM), in the presence of testosterone (1 nM) or of estradiol (1 to 10 nM), and in the presence of both testosterone (4 nM) and estradiol (1 to 10 nM). Tamoxifen showed no effect on its own and did not modify the effects of testosterone and estradiol when used separately or in combination.

Glucocorticoids are known to modify the growth parameters of fibroblasts in tissue culture and, at least under certain conditions, to inhibit cell growth, glucose uptake and protein synthesis, in particular the synthesis of collagen [12, 13]. When added to rat ventral prostate explant cultures, hydrocortisone (10 to 1,000 nM) prevented the increase of stroma observed in control cultures, whereas the stimulation of epithelial cells was minimal, if it occurred at all. The staining of connective tissue with periodic acid Schiff was also greatly decreased, and thymidine incorporation into nuclear deoxyribonucleic acid (DNA) was greatly reduced. Hydrocortisone did not significantly modify the responsiveness of the cultured explants to testosterone. In contrast, when hydrocortisone (100 to 1,000 nM) was combined with estradiol (10 to 100 nM), with or without testosterone (4 nM), stroma was as regressed as in cultures with testosterone alone.

## Discussion

The effects of estradiol alone or associated with testosterone have been studied in seminal vesicles and prostate in several species. These *in vivo* observations have been difficult to interpret due to complex indirect hormonal changes. However, such hormone treatments of castrated or prepubertal animals caused an increase of the muscular and stromal components of the accessory sexual glands [14–23]. The present *in vitro* experiments essentially confirm observations *in vivo*. The use of organ culture in completely defined, serum-deprived medium allowed operation at known concentrations of both hormones and the exclusion of extra-prostatic factors.

The effects of testosterone on ventral prostate can be reproduced partly in an *in vitro* culture system [3]. In the absence of hormone, the regression observed is similar to that after castration. The mechanism for the striking increase in smooth muscle cells, fibroblasts and other stromal cells in culture without testosterone, whether estradiol is present or not, is unknown. Testosterone can apparently repress this increase when present at physiological

concentrations but cannot do so when estradiol is present at the same time. This antitestosterone effect of estradiol is observed only on stroma and not on epithelial cells, which remain fully stimulated. However, at supraphysiological concentrations testosterone overrides the antagonistic effect of any concentration of estradiol and the stroma remains repressed by the androgen. These results suggest a greater sensivity of stromal than epithelial cells to the antiandrogenic effects of estradiol.

Several authors have suggested the presence of oestrogen receptor in rat ventral prostate [24, 25]. Oestrogen receptor has recently been unequivocally identified (M. Ginsburg and I. Jung-Testas, personal communication) although in small amounts (less than 10% of the concentration of androgen receptor). However the oestrogenic effects on rat ventral prostate observed in organ culture do not show the hormone specificity of oestrogen receptors. Diethylstilboestrol, which displays oestrogenic activity and high binding affinity for oestrogen receptors (Table) [26], could not replace estradiol, at least in the range of concentrations tested and tamoxifen, an antioestrogen acting at the oestrogen receptor level in several systems [10, 11, 27], did not antagonize estradiol action nor did it display any estradiol-like activity. We suggest it is possible that estradiol acts via the androgen receptor because it binds to this receptor with high affinity [28] while both diethylstilboestrol and tamoxifen show only negligible affinity (Table). This could explain the relatively large concentration of estradiol needed. If the estradiol effect takes place at the androgen receptor level, it still has to be found out whether androgen receptor in epithelial or stromal cells or both is concerned. Autoradiographic experiments have shown that androgen receptors are located mainly if not exclusively in epithelial cells of rat ventral prostate [28, 29, 30]. Therefore, it is not impossible that the inhibitory effect of testosterone on interstitial stroma and, consequently, estradiol activity is indirect. It must also be kept in mind that a full antiandrogenic response is difficult to obtain with estradiol, in contrast to cyproterone acetate. Estradiol in fact enhances testosterone action on epithelial cells, an effect never observed with pure antiandrogens.

Estradiol inhibits testosterone 5α-reductase. Selective inhibition by natural oestrogens such as estradiol as opposed to the synthetic oestrogen diethylstilboestrol could explain the difference observed between the 2 oestrogens. However, both compounds have been shown to give similar inhibition of prostatic 5α-reductase [3]. Moreover, testosterone is more effective in repressing stromal growth than its metabolite androstanolone [3].

*Table. Affinity of natural and synthetic hormones for receptors*

| | Affinity for | |
|---|---|---|
| | Oestrogen receptor | Androgen receptor |
| Estradiol | ++++ | ++ |
| Diethylstilboestrol | ++++ | ± |
| Tamoxifen | ++ | ± |
| Androstanolone or testosterone | ± | ++++ |

Composite table summarizing the affinity of different compounds for known androgen and oestrogen receptors [26, 28]. ++++: Dissociation constant ($K_D$) ∿ 0.1 nM; ++: $K_D \simeq 10$ nM; ±: $K_D \geqslant 1$ μM.