Journal of Steroid Biochemistry, Vol. 11, pp. 625 to 630
Pergamon Press Ltd 1979. Printed in Great Britain

METABOLISM AND ACTION OF STEROID HORMONES ON HUMAN BENIGN PROSTATIC HYPERPLASIA AND PROSTATIC CARCINOMA GROWN IN ORGAN CULTURE

ILSE LASNITZKI
Strangeways Research Laboratory, Worts' Causeway, Cambridge CB1 4RN, England

SUMMARY

The metabolism of testosterone was examined in normal human prostate, benign prostatic hyperplasia (BPH) and prostatic carcinoma in organ culture. Dihydrotestosterone was the principal metabolite in the normal and hyperplastic prostate and in most of the carcinomas, although over half of the carcinomas produced less DHT than the benign tissue. There was no correlation between their degree of differentiation and their metabolic pattern but carcinomas from the oldest patients formed significantly greater amounts of 17-keto-metabolites. The effect of testosterone, its active metabolites and oestradiol-17β on the growth and differentiation of BPH and prostatic carcinoma in organ culture were correlated with changes in DNA and RNA synthesis, studied by autoradiographic methods. In the absence of hormones, explants of BPH grew well but showed signs of squamous transformation. Treatment with androgens prevented the metaplasia and preserved the secretory activity of the epithelium, while oestradiol caused cellular necrosis. In the BPH, DNA synthesis was depressed by oestradiol and unaffected by the androgens, except for 3α-androstanediol which increased it. RNA synthesis was increased by the androgens and reduced by oestradiol. In the carcinoma, the androgens as well as oestradiol depressed DNA synthesis while RNA synthesis was increased by the androgens but not affected by oestradiol. In both BPH and carcinoma, the stromal cells and the epithelium responded similarly to hormonal treatment.

Investigations into the effects of steroid hormones on benign prostatic hyperplasia (BPH) and carcinomas aim to explore the role of the hormones in the genesis of the tumours and also to evaluate the hormonal response of existing tumours as an aid in their clinical management.

In animal experiments the nutritional, immunological and hormonal status of the host will alter the basic effects of hormones and complicate the interpretation of results. In an *in vitro* system these factors are eliminated and the direct action of hormones can be studied at the cellular level under well controlled experimental conditions.

Hormone concentration and duration of exposure can be easily manipulated and an easy replication of experiments makes it possible to obtain both qualitative and quantitative data.

In the work described here organ culture has been used as a method of choice. In this system, the two main tissue components, epithelium and stroma and their anatomical relationship are, under suitable conditions, preserved *in vitro*. In human BPH and prostatic carcinoma the stroma often forms a substantial part of the tissue and the action of hormones on the epithelium may depend on an intact epithelial–stromal relationship. Moreover, recent evidence suggests that the stroma is metabolically active [1] and that it may mediate the effect of androgens on the epithelium [2].

In addition, the system avoids the disadvantage of time lag and cell selection inherent in the use of cell cultures and fragments of BPH or carcinomas can be explanted shortly after removal from the patient.

TESTOSTERONE METABOLISM

The hormonal response of the two tissues may be influenced by differences in their testosterone metabolism and the formation of metabolites was, therefore, measured in organ cultures of normal human prostate [3], in BPH and in different types of prostatic carcinomas [4]. The carcinomas were classified as moderately differentiated, if the epithelium was arranged in acinar structures. Tumours which consisted of strands or clusters of cells without organisation were classified as poorly differentiated.

Figure 1 shows the formation of testosterone metabolites in a normal prostate gland from a 19-year-old man. The metabolism is preponderantly reductive: large amounts of dihydrotestosterone (DHT) are formed while those of androstanediol, androstanedione and androsterone are very low.

A comparison of testosterone metabolism in BPH and in moderately and poorly differentiated carcinomas shows that it is reductive in both types of tissue (Fig. 2). The principal metabolite is DHT in all the benign specimens and carcinomas, but the proportion of DHT formed in seven of the carcinomas is lower than that in the benign tissues. The differences in DHT formation appear unrelated to the stage of differentiation of the carcinomas. This also holds true for the formation of 17-keto metabolites (Fig. 3). Four of the carcinomas form significantly higher amounts of androstanedione than BPH irrespective of their stage of differentiation. However, if the differences are examined according to the age of

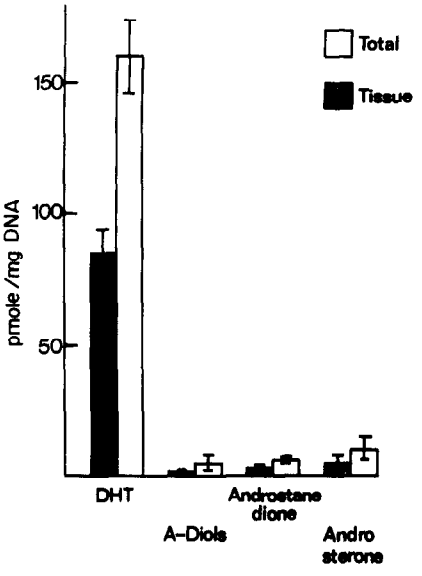

Fig. 1. Metabolism of testosterone in prostate gland from a 19-year-old man. The amounts of metabolites and their standard deviations are expressed as pmol/mg DNA. (DHT: 5α-dihydrotestosterone, A-diols: 3α- and 3β-androstanediols, total: tissue and medium).

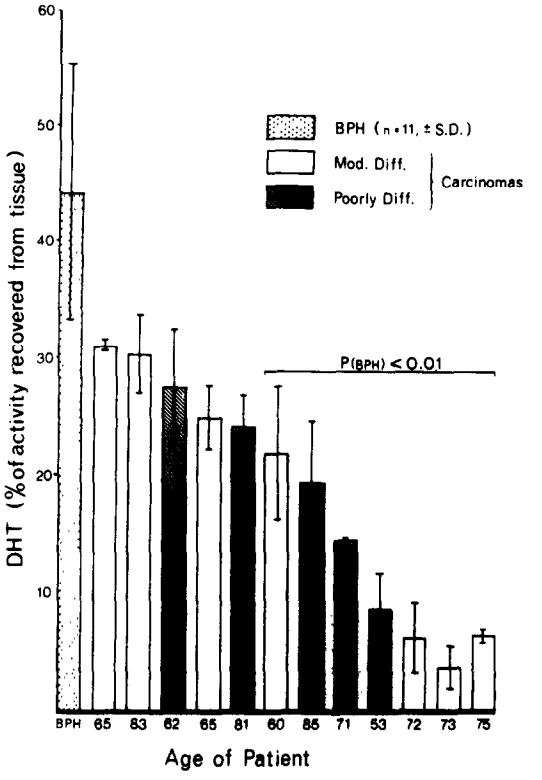

Fig. 2. Formation of 5α-DHT in BPH and in moderately and poorly differentiated carcinomas. Results are expressed as percent activity ± standard devation.

the patients, there seems to be a positive correlation between low DHT values with high androstanedione production in tumours obtained from the 70–80 year age group (Fig. 4). In contrast, BPH does not show any variation with age.

The mechanism involved in this shift towards a more oxidative pathway with age is uncertain. Nozu and Tamaoki[5] have reported that in ventral rat prostate the content of 5α-reductase declines with age and it is possible that in human carcinoma, though not in benign hyperplastic tissue, the synthesis of the enzyme is similarly diminished.

ACTION OF HORMONES

Hormonal studies of human BPH and carcinoma in organ culture are of a relatively recent date (Table 1) and suggest that the growth of BPH is no longer hormone dependent [6–10]. The tissue retained its normal architecture and growth pattern in the absence of testosterone, while addition of androgens or anti-androgens did not alter its morphology or enzyme activity.

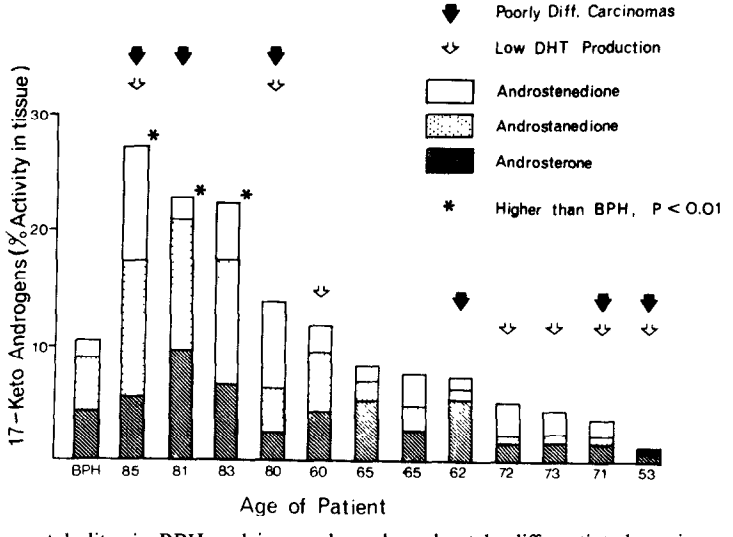

Fig. 3. 17-Keto metabolites in BPH and in poorly and moderately differentiated carcinomas. Results are expressed as percent activity ± standard deviation.

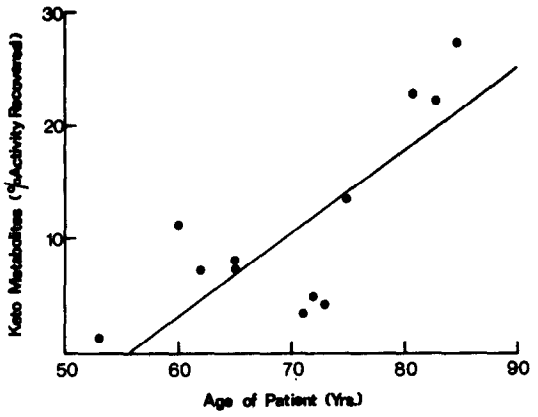

Fig. 4. Relationship between proportion of testosterone metabolized to 17-oxo-derivatives in carcinomas and the age of the patient.

In contrast, testosterone was seen to promote differentiation in prostatic carcinoma. MacMahon *et al.*[10] reported that the hormone increased the secretory apparatus of the epithelium in a differentiated carcinoma and induced acinar formation in an anaplastic tumour.

In androgen deprived target tissues the first consequence of testosterone treatment is an increase in RNA synthesis [11]. RNA synthesis seems, therefore, a more sensitive criterion of androgen action than alteration or maintenance of structure, and the effect of steroids on RNA synthesis determined in organ cultures of BPH [12] has been compared with that measured in carcinoma and correlated with changes in DNA synthesis and alteration of morphology.

Both BPH and carcinoma were obtained by transurethral resection (TUR) and were explanted within 2–3 h after removal from the patient. They were grown for 4–6 days by a modified Trowell technique [13] in medium 199 [14] supplemented with 10% calf serum. Testosterone, DHT, 3α-androstanediol, 3β-androstanediol, androstendione and oestradiol-17β were added at a concentration of 3.0 μg/ml medium.

DNA and RNA synthesis were studied by autoradiography in explants labelled with ^{3}H-thymidine or ^{3}H-uridine and expressed as the percentage of labelled cells or the average number of grains per cell. The incorporation of ^{3}H-uridine into RNA was assessed separately in epithelium and smooth muscle cells.

BENIGN PROSTATIC HYPERPLASIA

Histological observations

Before explantation the benign prostatic tissue showed glandular and stromal elements in varying proportions. The epithelium was arranged in aleveoli lined, usually, with one row of columnar or cuboidal cells. In some areas, the alveolar lumen was dilated and the cells flattened, in others the epithelium had multiplied and become stratified. The stroma consisted of collagenous and smooth muscle fibres.

After 6 days growth in non-supplemented medium the epithelium of many alveoli proliferated and formed several rows. The hyperplastic epithelium became stratified and formed tonofibrils bridging the intercellular gaps.

Testosterone or DHT did not entirely suppress this epithelial hyperplasia although in many areas the normal architecture of the epithelium was maintained. In others, alveoli lined with several rows of cells were present. However the hyperplastic epithelium retained its secretory character, exuded much secretory matter into the lumen and showed no evidence of squamous transformation, such as tonofibrils.

Oestradiol did not reduce epithelial cell proliferation but the superficial secretory cells were shed into the lumen and not replaced. In addition, foci of

Table 1. Organ culture of BPH and prostatic carcinoma

Authors		Source	Method	Results
Schrodt & Foreman (1971)	BPH	Retropubic resection	Modified Trowell technique	Structural maintenance without androgens, necrosis after addition of testosterone propionate.
McRae *et al.* (1972)	BPH	Open prostatectomy	Leighton tubes	Testosterone raises DNA synthesis slightly, does not affect glucose utilisation.
Harbitz, Falkanger & Sander (1974)	BPH	Open prostatectomy	Trowell technique	Maintenance of structure and normal enzyme pattern without androgens. Testosterone, dihydrotestosterone, oestradiol, progesterone and cyproterone do not alter morphology, enzyme pattern or DNA synthesis.
MacMahon & Thomas (1973)	BPH	T.U.R.	Modified Trowell technique	Structural maintenance without androgens. Testosterone or stilboestrol do not alter morphology.
MacMahon *et al.* (1972)	Ca	T.U.R.	Modified Trowell technique	Structural maintenance without androgen. Testosterone increases structural and cytological differentiation. Stilboestrol does not alter morphology.

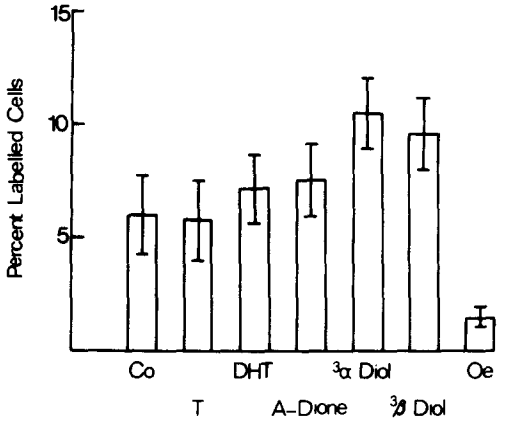

Fig. 5. The effect of steroid hormones on incorporation of [^{3}H]-thymidine in epithelium of human BPH expressed as percentage of labelled cells ± standard deviation. (Co: control, T: testosterone, DHT: dihydrotestosterone, A-dione: androstenedione, 3α- and 3β-diols: 3α- and 3β-androstanediols, Oe: oestradiol).

degenerate cells with vacuolated cytoplasm had appeared within the hyperplastic epithelium.

DNA synthesis

With the exception of 3α-androstanediol the androgens did not raise the incorporation of [^{3}H]-thymidine into DNA over that seen in the controls while oestradiol diminished it (Fig. 5). The increase induced by 3α-androstanediol, though slight, is just significant.

RNA synthesis

In treated and untreated explants the epithelium incorporated the tracer substantially (Fig. 6). The percentage of labelled cells was comparatively high in the controls being 74%; testosterone and DHT increased it to 88% and 92% respectively, while oestradiol had no affect. These differences were more pronounced if the grain counts were considered. Testosterone and DHT raised them by approximately 50% over those in the controls, whereas oestradiol reduced them by 35%.

It seems likely that the increase of RNA synthesis by testosterone and DHT is directly linked with the maintenance of secretory activity by these two androgens.

The stroma was morphologically similar in controls and hormone treated explants but the cells of the smooth muscle appeared to be more heavily labelled in androgen treated tissue. This observation was supported by the counts. Although the number of labelled cells was similar in controls and androgen treated tissue, the average grain counts per cell were raised by testosterone and dihydrotestosterone (Fig. 7). In contrast, oestradiol diminished both the number of labelled cells as well as the grain counts per cell.

PROSTATIC CARCINOMA

The carcinoma examined was an untreated adenocarcinoma. The epithelial components formed a substantial part of the tumour and were arranged in alveoli lined with cuboidal or columnar secretory cells forming papillary projections into the lumen.

After explantation in organ culture the growth pattern became somewhat modified. While in some parts the original architecture of the epithelium was maintained, in others it formed several solid cell clusters.

DNA synthesis

Figure 8 summarises the effect of various androgens and oestradiol on the incorporation of [^{3}H]-thymidine. Unexpectedly, all the androgens depress the uptake of the tracer. Oestradiol depresses it as well but to a smaller degree.

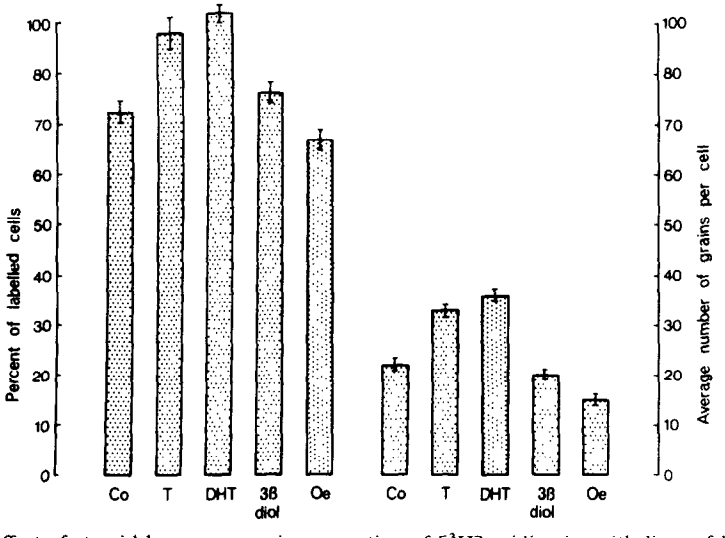

Fig. 6. The effect of steroid hormones on incorporation of [^{3}H]-uridine in epithelium of human BPH expressed as percentage of labelled cells or average number of grains per cell. Standard deviations are indicated. Abbreviations as in Fig. 5.

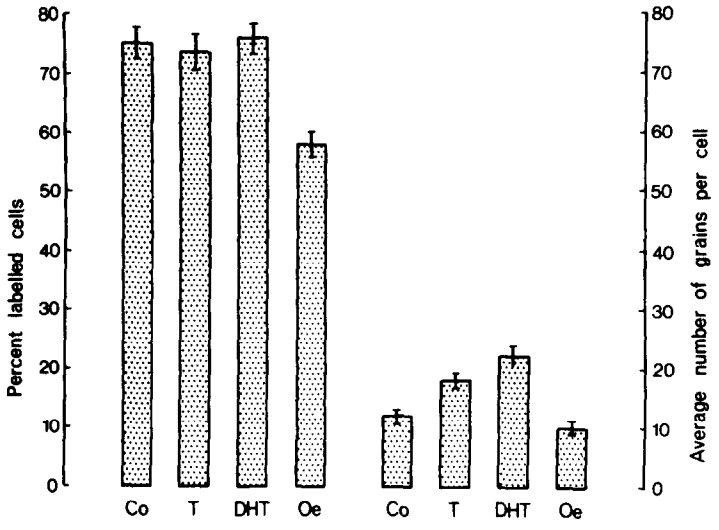

Fig. 7. The effect of steroid hormones on incorporation of [^{3}H]-uridine in smooth muscle cells of human BPH expressed as percentage of labelled cells and average number of grains per cell. Standard deviations are indicated. Abbreviations as in Fig. 5.

RNA synthesis

In contrast, uridine incorporation is, as in the benign tissue, increased by the androgens while oestradiol does not affect it. Epithelium and stroma respond in a similar way.

Figure 9 shows the incorporation of [^{3}H]-uridine into RNA in epithelium and smooth muscle cells expressed as the percentage of labelled cells. In the absence of hormones the uptake is substantial in both components but testosterone, DHT and androstenedione raise it further. The incorporation per cell is equally stimulated by the three androgens (Fig. 10). In contrast, explants exposed to oestradiol show similar values to the controls, both as regards number of labelled cells and grain counts.

To compare the hormonal response of benign prostatic hyperplasia and prostatic carcinoma with that of the normal rat prostate which remains androgen dependent and responsive *in vitro* [15], the effect of testosterone, DHT, 3α-androstanediol and oestradiol on DNA and RNA synthesis was determined (Fig. 11). Of the three androgens, only DHT increased the incorporation of [^{3}H]-thymidine while the other two androgens did not change it. The uptake of [^{3}H]-uridine was considerably lower in rat prostates grown in control medium than in the human tissues kept under identical conditions. The stimulation of RNA synthesis by the androgens was correspondingly higher and they increased the uptake two fold and more. Treatment with oestradiol affected neither the incorporation of [^{3}H]-thymidine nor that of [^{3}H]-uridine.

To conclude: although BPH and carcinoma can be maintained in the absence of hormones they still remain hormone-responsive. In both tissues, the androgens promote RNA synthesis while oestradiol reduces DNA synthesis.

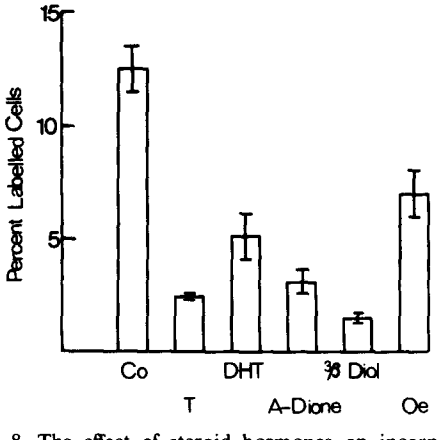

Fig. 8. The effect of steroid hormones on incorporation of [^{3}H]-thymidine in human prostatic carcinoma expressed as percentage of labelled cells ± standard deviation. Abbreviations as in Fig. 5.

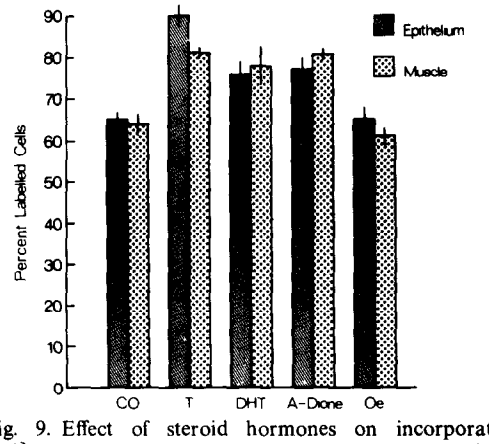

Fig. 9. Effect of steroid hormones on incorporation of [^{3}H]-uridine in epithelium and smooth muscle cells of human prostatic carcinoma, expressed as percentage of labelled cells ± standard deviation. Abbreviations as in Fig. 5.

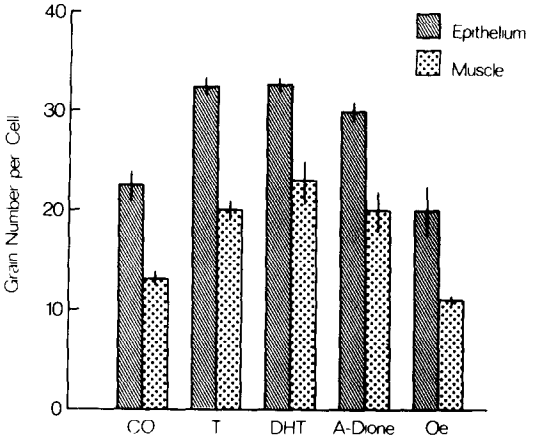

Fig. 10. Effect of steroid hormones on incorporation of [^{3}H]-uridine in epithelium and smooth muscle cells of human prostatic carcinoma expressed as average grain number per cell ± standard deviation. Abbreviations as in Fig. 5.

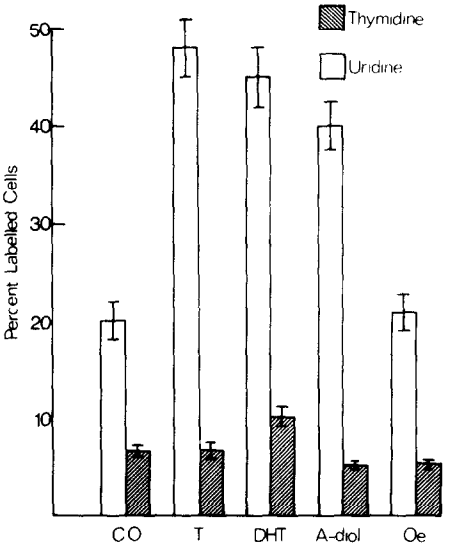

Fig. 11. Effect of steroid hormones on incorporation of [^{3}H]-thymidine and [^{3}H]-uridine in rat prostatic epithelium expressed as a percentage of labelled cells and its standard deviation. Abbreviations as in Fig. 5.

The effects of the androgens on DNA synthesis are less clear cut. With the exception of 3α-androstanediol they do not affect DNA synthesis in the BPH and all androgens depress it in the adenocarcinoma. This latter result is unexpected and contrary to established concepts. More work is needed to confirm it and to elucidate further the mode of action of androgens on DNA synthesis and cell proliferation in both BPH and prostatic carcinoma.

Acknowledgement—This work has been supported by a Leverhulme Emeritus Fellowship.

REFERENCES

1. Cowan R. A., Cowan S. K., Grant J. K. and Elder H. Y.: Biochemical investigations of separated epithelium and stroma from benign hyperplastic prostatic tissue. *J. Endocr.* **74** (1977) 111–120.
2. Cunha G. R.: Epithelial-stromal interaction in development of the urogenital tract. *Intern. Review of Cytology* **47** (1977) 137–144.
3. Lasnitzki I.: Unpublished results.
4. Bard D. R. and Lasnitzki I.: The influence of oestradiol on the metabolism of androgens by human prostatic tissue. *J. Endocr.* **74** (1977) 1–9.
5. Nozu K. and Tamaoki B. I.: Intranuclear and intermicrosomal distributions of 3-oxo-5α-steroid: $NADP^+$ Δ^4-oxidoreductase in rat ventral prostate. *Biochem. biophys. Acta* **348** (1974) 321–333.
6. Schrodt G. R. and Foreman C. D.: *In vitro* maintenance of human hyperplastic prostate tissue. *Invest. Urol.* **9** (1971) 85–94.
7. McRae C. U., Ghanadian K., Fotherby K. and Chisholm G. D.: The effect of testosterone on the human prostate in organ culture. *Br. J. Urol.* **45** (1973) 156–162.
8. Harbitz T. B., Falkanger B. and Sander S.: Benign prostatic hyperplasia of the human prostate exposed to steroid hormones in organ culture. *Acta Path. Microbiol. Scand. Sect. A Suppl.* **248** (1974) 89–93.
9. MacMahon M. J. and Thomas G. H.: Morphological changes of benign prostatic hyperplasia in culture. *Br. J. Cancer* **27** (1973) 323–335.
10. MacMahon M. J., Butler A. J. V. and Thomas G. H.: Morphological responses of prostatic carcinoma to testosterone in culture. *Br. J. Cancer* **26** (1972) 388–400.
11. Liao S. and Fang S.: Receptor proteins and the mode of action of androgens on gene transcription in the ventral prostate: *Vitams Horm.* **27** (1969) 17–90.
12. Lasnitzki I., Whitaker R. H., Withycombe J. F. R.: The effect of steroid hormones on the growth pattern and RNA synthesis in human benign prostatic hyperplasia in organ culture. *Br. J. Cancer* **32** (1975) 168–178.
13. Lasnitzki I.: Action and interaction of hormones and 3-methylcholanthrene on the ventral prostate gland of the rat *in vitro*. *J. natl. Cancer Inst.* **35** (1965) 339–348.
14. Morgan J. F., Morton H. J. and Parker R. C.: Nutrition of animal cells in tissue culture. *Proc. Soc. exp. Biol. Med.* **73** (1950) 1–8.
15. Lasnitzki I.: The action of androgens on rat prostate glands in organ culture. In *Organ Culture for Biomedical Research.* (Edited by M. Balls and M. A. Monnickendam). Cambridge University Press. Cambridge (1976) pp. 241–256.