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# INVESTIGATIONS OF ANDROGEN-PROSTATIC TISSUE RELATIONSHIPS IN SUPERFUSION EXPERIMENTS USING STEROID CONCENTRATIONS APPROACHING THOSE FOUND *IN VIVO*

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**SUMMARY**

An experimental design, which allows the measurement of parameters of androgen behaviour in prostatic tissue *in vitro*, has been modified to permit the use of concentrations of androgens similar to those found *in vivo*. The fractions of amounts of steroids superfused which enter and are retained in tissues containing few acinar epithelial cells (periurethral tissue) are compared with those in tissues with abundant epithelial cells. Both tissues were derived from human glands showing benign hyperplasia.

In previous publications[1, 2] we have referred to the superiority of superfusion or continuous flow incubations among *in vitro* techniques and to the further advantages of using the experimental design of Gurpide [3, 4] in order to obtain a better measure of the extent to which events actually occur in the cells of tissues during superfusion. We employed these procedures to study various parameters characterizing the *in vitro* behaviour of androgens in prostatic tissue from man and dogs[1, 5]. We observed a relatively poor flexibility of hyperplastic human tissue, as compared with normal, to regulate the retention of androgens. This could be attributed to a deficiency in androgen-metabolizing enzymes or to an excess of non-specific androgen binding sites in the tissue, among other possible causes. Less specialized components of prostatic tissue, such as stroma or muscle, might act as storage sites for androgens. Hyperplasia of such tissues could thus be important in determining the difference observed in the ability of the hyperplastic tissue to regulate its androgen metabolism as compared with normal tissue. In keeping with this suggestion, we observed[2] that periurethral tissue containing only small amounts of acinar epithelium exhibited a relatively very high uptake of androgens as compared with tissue nearer the capsule showing an abundance of epithelial cells.

A disadvantage of our earlier experiments was that steroid concentrations employed were much greater than those found *in vivo*. This was unavoidable using $^{14}$C-labelled steroids of relatively low specific radioactivity. We have now overcome this problem by using tritium labelling at high specific activity for both of the metabolically related steroids used. These steroids are 5α-dihydro[1,2-$^{3}$H]-testosterone and [17α-$^{3}$H]-testosterone. The latter compound may be measured by difference after removing the 17α-$^{3}$H by oxidation at will. The concentrations now employed are shown in Table 1. This new procedure has the added advantage that $^{14}$C-labelled androgens may be used as internal standards to permit determination of procedural losses during the analytical work-up of an experiment. It is no longer necessary to use U.V. or g.l.c. measurements as described in earlier work[2, 5]. It is possible to feed data from counter to computer and thus to obtain parameters of androgen behaviour in the tissue very conveniently.

An outline of the modified method for obtaining concentrations of radioactive steroids in superfusing and superfused medium and in the tissues used is given in Fig. 1.

Table 1. Testosterone (*T*) and 5α-dihydrotestosterone (DHT) concentrations found *in vivo* in plasma and tissue and used *in vivo* in superfusion experiments (ng/ml)

| | Man | | | | Dog | | | |
|---|---|---|---|---|---|---|---|---|
| | Plasma or medium | | Tissue | | Plasma or medium | | Tissue | |
| | *T* | DHT | *T* | DHT | *T* | DHT | *T* | DHT |
| *in vivo* | 5·2 | 0·92 | 0·9 | 6·0 | 0·43 | 0·92 | 0·3 | 3·3 |
| *in vitro* | 1·3 | 0·23 | 0·7 | 5·2 | 0·23 | 0·12 | 1·5 | 3·3 |

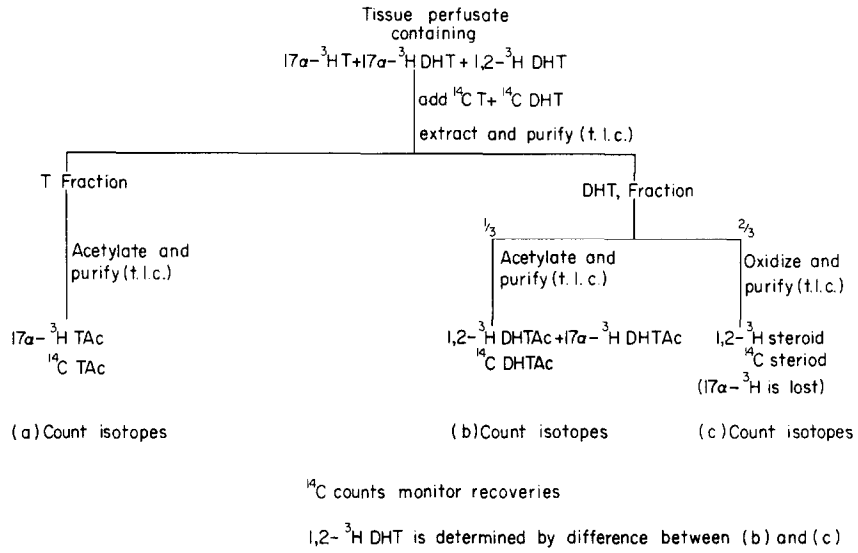


Fig. 1. Outline of method used to determine steroids in superfusion medium and in tissue (T = testosterone, DHT = 5α-dihydrotestosterone).

The main findings in the present series of experiments are as follows:

1. The general pattern of behaviour of androgens studied in canine normal tissue and human hyperplastic tissue *in vitro* is similar to that observed at the higher steroid concentrations used in our earlier experiments.

2. The "uptake" (Ci/Co) of both testosterone and 5α-dihydrotestosterone is in general more marked at the lower than at the higher androgen concentrations (Fig. 2) in keeping with the observations of Wilson and his colleagues[6, 7].

3. The fraction of the amount of testosterone superfused, which enters the tissue ($\alpha_T$), is less than that for 5α-dihydrotestosterone ($\alpha_{DHT}$) in both canine normal prostate and human hyperplastic prostate (Fig. 3).

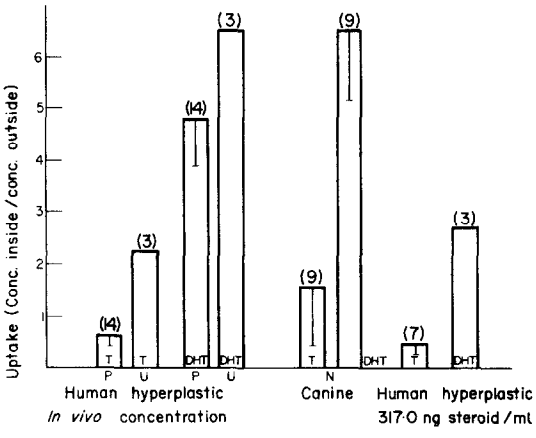


Fig. 2. Uptake or retention of steroids by prostatic tissue, defined as the ratio of the concentration of steroids inside and outside the tissue. DHT = 5α-dihydrotestosterone; T = testosterone; P = prostatic tissue from hyperplastic human glands containing an abundance of acinar epithelial cells; U = prostatic tissue from human glands obtained from regions around the urethra, by transurethral prostatectomy. This contains relatively few epithelial cells. N = normal prostatic tissue obtained from dogs. Histograms on the left show results obtained at *in vivo* concentrations, both superfused steroids being labelled with tritium. Those on the right show results obtained at the higher concentration of 317 ng steroid/ml, one of the steroids being labelled with $^{14}C$ the other with $^{3}H$.

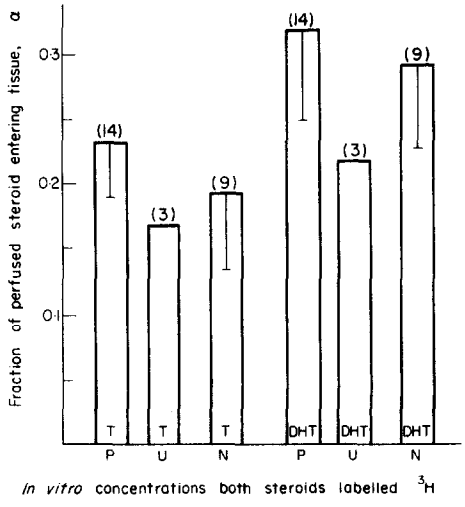


Fig. 3. Fractions of superfused steroids entering the tissue. The histograms on the left show $\alpha_T$, the fraction of superfused testosterone entering; those on the right show $\alpha_{DHT}$, the fraction of 5α-dihydrotestosterone entering. The results shown are for the *in vivo* concentrations now used. These do not differ significantly from the results obtained before at the higher concentration of 317 ng steroid/ml. Numbers in the columns show the numbers of experiments done. The coefficients of variation between the experiments were low and less than 7%. Other abbreviations are given in Fig. 2.

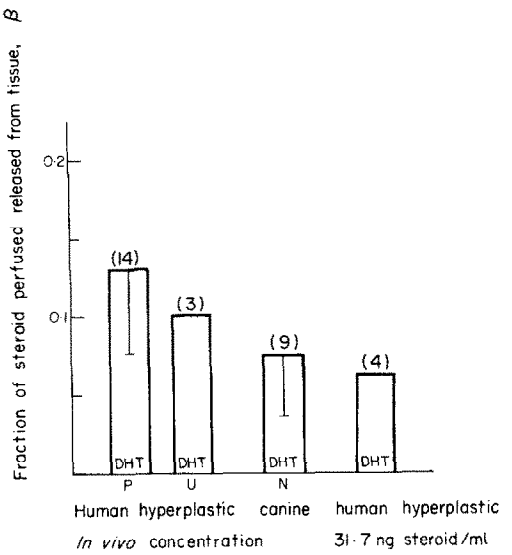


Fig. 4. Fractions of superfused steroids released from the tissue. No significant amounts of testosterone are released ($\beta_T = 0$). Relatively larger amounts of 5α-dihydrotestosterone ($\beta_{DHT}$) are released at the lower (*in vivo* concentrations of steroids used). Abbreviations are given in Fig. 2.

The testosterone entering is almost completely converted to 5α-dihydrotestosterone ($\rho_{TDHT}$ = approximately 1). One might therefore conclude that testosterone is the main source of 5α-dihydrotestosterone which is eventually retained in the tissue. This is in keeping with the view expressed by Mainwaring[8].

4. It is of considerable interest that a significant amount of 5α-dihydrotestosterone ($\beta_{DHT}$) but no significant amount of testosterone ($\beta_T = 0$) is released from the tissue (Fig. 4) showing that, even at the very low concentrations of 5α-dihydrotestosterone used, much is retained in the tissue in an unbound or very readily dissociable form.

5. Also of interest is the observation that tissue from around the urethra, deficient in acinar epithelial cells, shows a consistent higher uptake of testosterone and dihydrotestosterone even at the low concentrations used in the present experiments (Fig. 2).

Obviously, to extend these studies, efforts should be made to separate acinar epithelial cells from stroma and muscle. Franks[9] has described a technique, using a press, for this purpose. Harper[10] has applied a modification of this technique to separate the tissues from the prostate of a patient who received an infusion of [7α-$^3$H]-testosterone before surgery. In this single experiment, 75% of the total radioactivity in the epithelial tissue was associated with 5α-dihydrotestosterone and 36% in the case of the stromal tissue. No radioactivity was associated with testosterone in either tissue. In interpreting these results, consideration must be given to the possibility that the behaviour of stromal tissue in the periurethral part of the gland may differ from that of similar tissue in more peripheral regions.

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## DISCUSSION

*Carstensen*:

Dr. Grant's results, particularly with the dog prostate, are most interesting to us since we found a massive glandular hyperplasia in 3 dogs who were subjected to constant infusion experiments with tritiated testosterone for 8 months. Even after 1–2 months there were microscopic changes in the dog prostates examined, but there was a gross increase in size, 2–3 times the normal size, only in the dogs treated for the longer period (Table 1). One typical example of the gross appearance of the hyperplastic prostate is shown in Fig. 1. The hyperplasia was purely glandular and spread diffusely throughout the gland. No cysts were observed. I believe this differs from the appearance of the human hyperplasia which also affects the stromal tissue. I also believe it differs from the form seen in aged dogs. Since the amount of testosterone administered was so small (Table 1) it seems that the hyperplasia was caused by the intracellular and intranuclear translocation of and irradiation from the tritium.

Table 1. Monthly infusion of [7α$^3$H] -testosterone in male dogs in relation to body weight, age and prostate weight

| | Month | Dog 1 | Dog 2 | Dog 3 | Dog 4 | Dog 5 |
|---|---|---|---|---|---|---|
| μCi | 1 | 56·3 | 30·1 | 69·4 | 61·6 | 55·3 |
| | 2 | | 0 | 0 | 9·6 | 0 |
| | 3 | | | 0 | 0 | 0 |
| | 4 | | | 18·9 | 18·8 | 22·3 |
| | 5 | | | 20·5 | 24·4 | 20·6 |
| | 6 | | | 17·6 | 17·7 | 16·4 |
| | 7 | | | 0 | 0 | 0 |
| | 8 | | | 8·2 | 8·4 | 7·6 |
| total | | 56·3 | 30·1 | 134·6 | 140·4 | 128·9 |
| μg, total | | 0·6 | 0·3 | 1·6 | 1·6 | 1·5 |
| Age, yr | | 4·5 | 1 | 3·5 | 2·0 | 5·0 |
| Body weight, kg | | 25 | 23 | 28 | 25 | 25 |
| Prostate weight, g | | 15·7 | — | 48·3 | 32·3 | 42·5 |

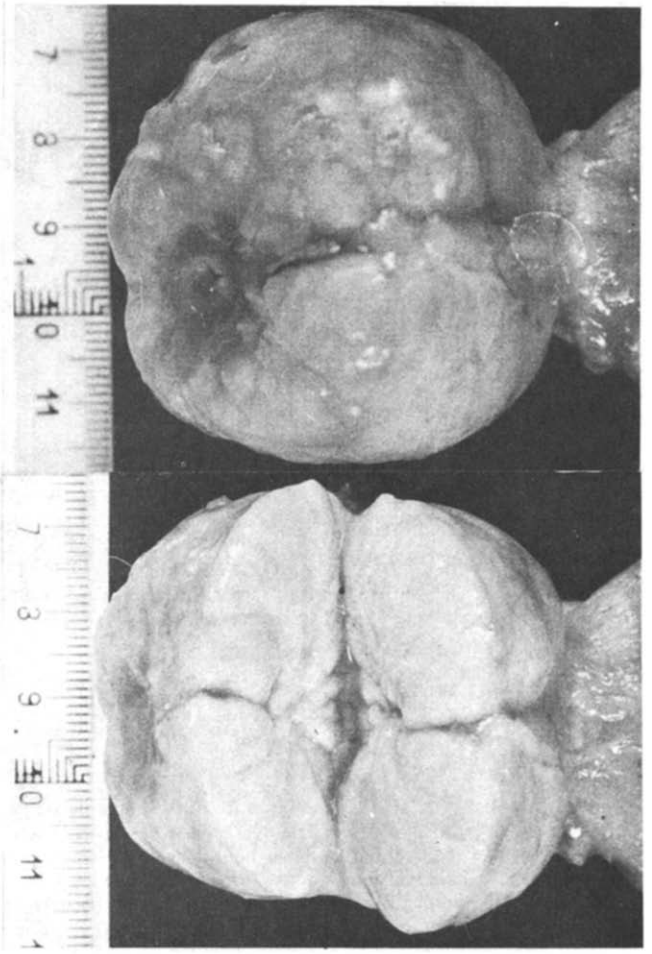

Fig. 1. Hyperplastic dog prostate after [$^3$H]-testosterone treatment.

*Grant*:

We considered the dog prostate as similar to the human prostate both in our earlier and in our present studies. You remarked about the release of androgens from the peripheral tissue. What I was calling the peripheral tissue is the part of the prostate near the capsule. The release from the tissue is so very strikingly different from the outer part of the prostate as it is from the inner part. The point I really wished to make was that the uptake of androgens in a pathological prostate, where hyperplasia is progressing, is greatest round about the urethra and that is the place where hyperplasia starts in man. In the dog as you rightly remarked the pathology is different. In the older dog you get a cystic type of development. The histology looks quite different. The only reason for bringing the dog into our experiments was that the young dog's normal prostate seems to behave very much like the human prostate and thus provides a useful control tissue. I can't really comment on your experiments because when you inject androgen into the dog, undoubtedly you will get a hyperplasia. Wilson and his colleagues have demonstrated this and have shown that the content of dihydrotestosterone in the tissue goes up when they have hyperplasia.

*Carstensen*:

Did your experiments on the dogs indicate that they retained more of the androgen?

*Grant*:

No, I don't think that I would say that dog prostate retains more androgen than the human prostate, but I do say that even in the dog the tissue close to the urethra where there is very little acinar epithelial cells, does retain, if you like take up, more steroid. This uptake becomes progressively greater in man as the gland becomes hyperplastic. Our theory is that it is the increasing uptake of this androgen in this region which is the cause of the trouble.

*Carstensen*:

I would like to comment that the amount of androgen that we gave was so small that the androgen by itself could not have produced any effect on the prostate.

*Grant*:

Yes, your studies are different from ours and I didn't have time to mention very recent work by the Cardiff group at the Tenovus laboratory where Dr. Harper persuaded the urological surgeons to give tritiated testosterone half an hour before the surgeons removed the man's prostate. They found that the radioactivity in the epithelial cells was mainly DHT after an injection of tritiated testosterone. There was more DHT in the epithelial cells than in the stromal cells, but it's a different type of observation from our observation. It's more like your observation and I recommend that you have a look at that last paper of theirs (*J. Endocr.* **60** (1974) 117).

*Rao*:

Dr. Grant, you showed in one of your slides that you could press out the prostate and get a mass of cells. You also said they took up dyes and if you let them stand they might be expected to recover. How would you actually go about getting these cells recovered when you know that they have already been pretty much damaged?

*Grant*:

This is a thing which we have not yet got final information on. The advice we get from the cell biologists is that when you damage these cells by squeezing the whole tissues (and the cell biologists tell us that really the only way to obtain cells of a clearly characterized type is by some mechanical process, by dissection or by squeezing to separate the tissues from the prostate, and they show dye uptake). If you take these cells immediately after you get them, they are supposed to be intact. Under the light microscope they look intact, but if you incubate them with labelled testosterone, you find that you can increase the reduction of testosterone by adding pyridine nucleotides to the incubation mixture. Now you all know that intact, healthy cells do not take up pyridine nucleotides. Preliminary information is that if you keep these cells in organ culture media for 24–48 h, these cells no longer take up dye and they are less influenced by pyridine nucleotide. The difficulty we face is that we cannot use these "rafts or sheets" of cells in superfusion experiments where we're interested in things like the entry and uptake of steroids while their membranes are damaged. Therefore, we must try to allow them to recover. We hope we can do this by keeping them for 24–48 h under suitable sterile conditions.