Journal of Steroid Biochemistry, 1972, Vol. 3, pp. 375–384. Pergamon Press. Printed in Great Britain

RECEPTORS AND BINDING OF ANDROGENS IN THE PROSTATE

A. AAKVAAG, K. J. TVETER, O. UNHJEM and A. ATTRAMADAL
The Hormone and Isotope Laboratory, Aker sykehus, The Institute of Pathological Anatomy and The Institute of Surgical Research, Rikshospitalet, Oslo, Norway

SUMMARY

The interaction between androgens and prostatic and other androgen-dependent tissues has been investigated in castrated male rats *in vivo* following administration of [1,2-^{3}H] testosterone of high specific activity. Radioactive androgens were concentrated and retained in such tissues for a number of hours. The receptors in the ventral prostate appeared to have a limited capacity, as administration of 20 μg non-radioactive testosterone simultaneously with the tritiated testosterone significantly reduced the uptake of radioactivity, and 500 μg completely abolished it. Synthetic androgens, antiandrogens, progesterone and corticosterone also reduced the uptake.

Autoradiography of the prostatic tissue revealed a selective labelling of the glandular epithelium, with most of the radioactivity associated with the nuclei. This conclusion was supported by subcellular fractionation of homogenized prostatic tissue.

Isolation and identification of the radioactive steroids confirmed previous observations that 5α-dihydrotestosterone was the quantitatively most important radioactive compound in the various accessory sex organs. 5α-dihydrotestosterone accounted for 50–72 per cent of the radioactivity, whereas 3–17 per cent was recovered as unchanged testosterone. Following the administration of [1,2-^{3}H]-androstenedione, accumulation of radioactivity was also observed in androgen-dependent tissues, and about 50 per cent of the radioactivity was identified as 5α-dihydrotestosterone.

By Sephadex® G-100 gel filtration of a 105·000 g supernatant of a homogenate of the ventral prostate obtained one or two hours after the administration of [1,2-^{3}H]-testosterone, the major fraction of the radioactivity was associated with macromolecules excluded from the gel. The radioactivity bound to the macromolecules was extractable with ether, suggesting a non-covalent binding, and 5α-dihydrotestosterone accounted for more than 90 per cent of the radioactivity. This cytosol androgen-macromolecule complex was destroyed by proteolytic enzymes and SH-reagents, but was unaffected by RNase and DNase.

Incubations with [1,2-^{3}H]-5α-dihydrotestosterone and a 105·000 g supernatant of the ventral prostate suggested the existence of two different species of cytosol macromolecules with binding affinity for 5α-dihydrotestosterone, one of which was excluded from the gel during Sephadex® G-100 gel chromatography, whereas the other was slightly retained. In the experiments with [1,2-^{3}H]-testosterone administration *in vivo* the radioactive 5α-dihydrotestosterone was mostly bound to the former.

Attempts to isolate [^{3}H]-5α-dihydrotestosterone in muscle tissue following [1,2-^{3}H]-testosterone administration were unsuccessful, suggesting that 5α-dihydrotestosterone may not be involved in the action of androgens on such tissue.

INTRODUCTION

THE RESPONSE to testosterone in so-called 'target tissue' is a rapid effect: prostatic RNA polymerase activity is significantly increased as early as 1 hr after testosterone administration to castrated male rats[1]. Since it may be assumed that this effect results from some contact between the hormone and the prostate, it is obviously of central importance to know the early fate of testosterone when the steroid reaches the prostate and the other androgen dependent tissues. The increased protein synthesis resulting from the androgen influence suggests a stimulation of the function of the cell nuclei. There must therefore be some type of a

hormonal signal between the testosterone that reaches the prostatic cell via the blood, and the cell nucleus.

The pioneer work by Jensen and his colleagues in Chicago on the selective uptake and binding of oestradiol in the uterus opened up a new field of research, and suggested that a similar type of interaction between the androgens and the androgen-dependent tissues might take place [2]. Somewhat in disagreement with this concept was the inability to demonstrate any accumulation or retention of testosterone in the prostate, and it was therefore suggested that the metabolic effect of the androgen on the prostate might not be dependent upon the same type of uptake as observed for oestradiol in the uterus [3]. We felt that the lack of androgen accumulation was a rather important point that ought to be reinvestigated. Regardless of the results of that investigation, it seemed important also to study the early fate of testosterone in the prostate.

The present paper represents a short review of some of the work we have been doing over the last few years along these lines in the rat.

MATERIALS AND METHODS

All the experiments *in vivo* have been carried out on male rats weighing about 250–300 g castrated 24 h before the experiment, except when otherwise stated. *In vitro* experiments were performed with various types of preparations of prostatic tissue obtained from the same type of animals.

The methods used have all been described in detail previously, and the reader is referred to these publications [4–11].

RESULTS AND DISCUSSION

Uptake of testosterone by the prostate in vivo

[1,2-^{3}H]testosterone of high specific activity (46·5 Ci/mmol) was given to rats 3 days after castration, and the amount of radioactivity in the various tissues during the first 16 h following the injection can be seen in Fig. 1. The uptake curve for the ventral prostate was strikingly different from those for blood or muscle, but much lower than those for liver. Similar curves were observed for the other prostatic lobes and accessory sex organs (Fig. 2). Whereas the peak concentration in blood and muscle was observed as early as 7½ min after injection, the uptake in the accessory sex organs kept on increasing to reach a maximum 1–2 h after the injection, and the activity was retained for a considerable period of time.

In intact animals the uptake of radioactivity was about 1/4 of that in 1-day castrated animals, and administration of 20–500 μg of non-radioactive testosterone simultaneously with the tritiated testosterone significantly reduced or completely abolished the uptake in the prostate. This implies that the prostate has a limited capacity for taking up testosterone, and explains why previous workers [12–14], who did not have testosterone of sufficiently high specific activity, have failed to demonstrate clearly a selective uptake.

Once the selective uptake of testosterone in the accessory sex organs was established, it was of importance to know where in the organ the activity was located, and in what chemical form. At the time these studies were initiated, the information available from the literature was inconclusive. By analogy with the studies on the uterus, one might assume that the activity was located primarily in the nucleus, in an unmetabolized form, as testosterone. Previous

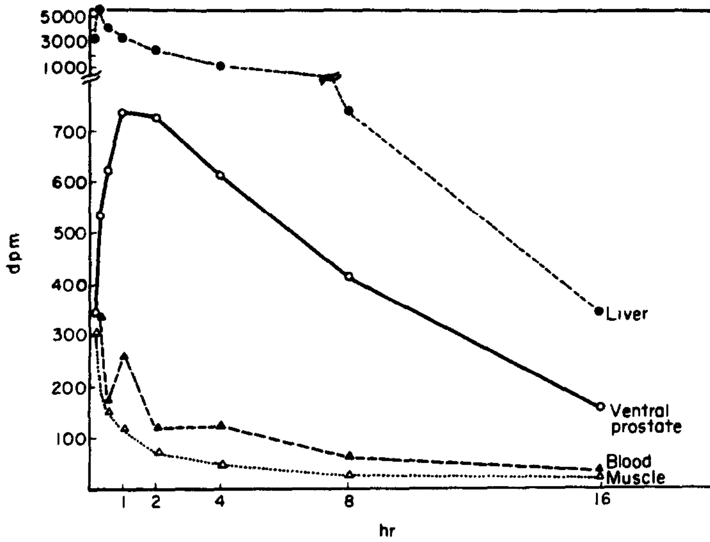

Fig. 1. Distribution of radioactivity in various tissues of male rats castrated 3 days prior to intramuscular injection of 60 μCi of [1,2-^{3}H]-testosterone per 100 g body weight. Radioactivity is expressed as d.p.m./mg wet weight, and as d.p.m./μl for blood. Each point represents the mean of 5 animals. From [7].

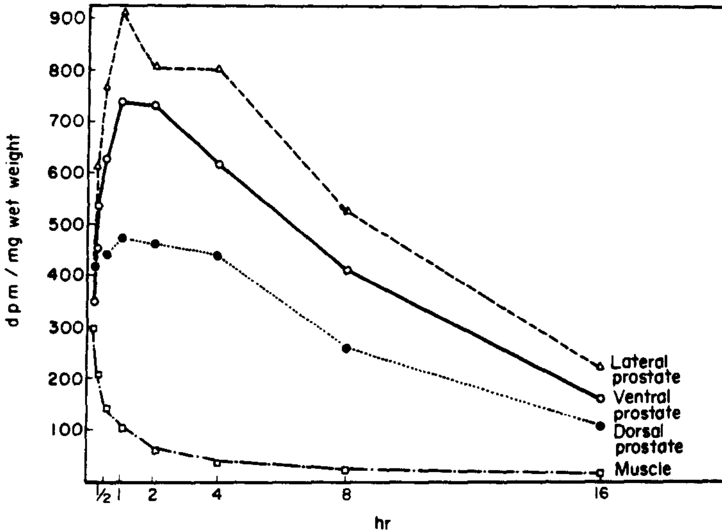

Fig. 2. Distribution of radioactivity in prostatic lobes and muscle tissue of rats castrated 3 days prior to intramuscular injection of 60 μCi of [1,2-^{3}H]-testosterone per 100 g body weight. The radioactivity is expressed as d.p.m./mg wet weight. Each point represents the mean of 5 animals. From [4].

attempts to identify radioactive metabolites in the rat prostate following administration of radioactive androgens had, however, given conflicting results [13, 14]. Androstenedione and etiocholanolone had been claimed to be the principal components.

Autoradiography of the prostate taken out 1 hr after the administration of 250

μCi of [1,2-^{3}H]-testosterone, using the technique developed by Attramadal[15], clearly demonstrated that the radioactivity was primarily located in the nuclei of the glandular epithelium[8]. The nuclear uptake of testosterone was confirmed by subcellular fractionation of the prostate following [1,2-^{3}H]-testosterone administration[5]. More than 50 per cent of the radioactivity was associated with the prostatic nuclear fraction, and about 30 per cent with the final supernatant fraction. During the progress of these investigations the publication by Bruchovsky and Wilson[16] appeared, wherein it was shown that the larger part of the radioactivity in the prostate in similar experiments was made up of 5α-dihydrotestosterone, and we had obtained data in support of these findings[6]. By crystallization to constant specific activity between 49 and 72 per cent of the radioactivity in the various accessory sex organs 1 h after the administration of [^{3}H]-testosterone was identified as [^{3}H]-5α-dihydrotestosterone[6]. Between 6·4 and 23 per cent of the radioactivity was found to be unchanged testosterone[6].

It is reasonable to assume that the nuclear uptake of 5α-dihydrotestosterone following testosterone administration is a central process in the action of testosterone. It would therefore be of considerable interest to know the effect of various types of antiandrogens and androgens on this uptake, and thereby get some insight into the mechanism of action of such compounds. We have studied this by giving 1-day castrated rats one of the following compounds by intraperitoneal injection 10 min prior to the administration of [1,2-^{3}H]-testosterone: androstenedione, 17α-methyltestosterone, 17α-methyl-β-nortestosterone (SK&F 7690), progesterone, corticosterone, oestradiol, and diethylstilboestrol[6]. The oestrogens did not affect the uptake of radioactivity in the ventral prostate, whereas the two androgens reduced the uptake to about 1/3, and the other compounds, progesterone, corticosterone and 17α-methyl-β-nortestosterone, to about 2/3 of the control values. None of the substances had any effect on the uptake in muscle tissue[6].

The blocking effect of androstenedione is probably related to its properties as a weak androgen. The androgen receptors of the prostate have low capacity, and a significant conversion of androstenedione to dihydrotestosterone, probably required for the androgenic action, will block the receptors for [^{3}H]-dihydrotestosterone.

Recent studies have supported this suggestion; administration of [1,2-^{3}H]-androstenedione to 1 day castrated rats leads to prostatic uptake of [^{3}H]-5α-dihydrotestosterone[17]. It may thus be assumed that 17α-methyl-testosterone in a similar way is being converted to 17α-methyl-5α-dihydrotestosterone that will occupy the receptors for [^{3}H]-5α-dihydrotestosterone.

Oestrogens and the synthetic antiandrogen 17α-methyl-β-nortestosterone appear to display their antiandrogenic effects in different ways, since the oestrogens did not have any effect on the uptake of radioactivity, whereas the antiandrogen induced a reduction to about 2/3. This animal preparation may therefore be of significant value in testing out the properties of various types of antiandrogens.

The inhibitory effect of progesterone on the uptake is interesting in light of the beneficial effect of this steroid on benign prostatic hyperplasia. Progesterone is a weak androgen[18] and at the same time an antiandrogen[19], which may be in accordance with these findings. The effect of progesterone has recently been confirmed and extended by Stern and Eisenfeld[20], who suggested that the reduced uptake might in part be attributed to an inhibition of the enzymatic con-

version of testosterone to 5α-dihydrotestosterone. They based their suggestion upon the observation that the ratio testosterone/dihydrotestosterone in the prostate was unaffected by testosterone or cyproterone administration, but increased significantly following progesterone.

The etiology of the benign prostatic hyperplasia is unknown, and the beneficial effect of progesterone may not necessarily be related to the inhibitory effect on the testosterone uptake. It is, however, noteworthy that human hypertrophic tissue contains about 5 times more dihydrotestosterone than does normal tissue [21], and dihydrotestosterone administration to dogs leads to accelerated growth of the prostate [22]. It is therefore not unconceivable that the therapeutic effect of large doses of progesterone to such patients is due to an inhibition of nuclear uptake of dihydrotestosterone.

In the literature there are conflicting opinions about the effect of corticosterone on the accessory sex organs [23, 24]. There is, however, rather conclusive evidence that cortisol is an antiandrogen in the rat [24]. Our observations on the effect of corticosterone on the uptake of androgen in the prostate are in agreement with this concept.

Binding of 5α-dihydrotestosterone to fractions of prostatic tissue

In order for the androgen to arrive at the cell nucleus testosterone has to leave the blood, enter the cytoplasm of the cell against a concentration gradient and move into the nucleus against another concentration gradient. Somewhere along that line testosterone is being converted to 5α-dihydrotestosterone. Since the metabolite is present in so much higher concentration than testosterone, in the cytoplasm as well as in the nucleus, it is tempting to assume that the conversion takes place at a very early stage.

Cytoplasmatic binding substances. Prostatic tissue taken out 1 h after the administration of [1,2-^{3}H]-testosterone to castrated rats has been investigated [10]. Following homogenization in Tris-HCl buffer and centrifugation at 105·000 *g* for 1 h the supernatant was chromatographed on Sephadex® G-100 at room temperature. A representative chromatogram is seen in Fig. 3. The radioactivity was partly associated with macromolecules excluded from the gel,

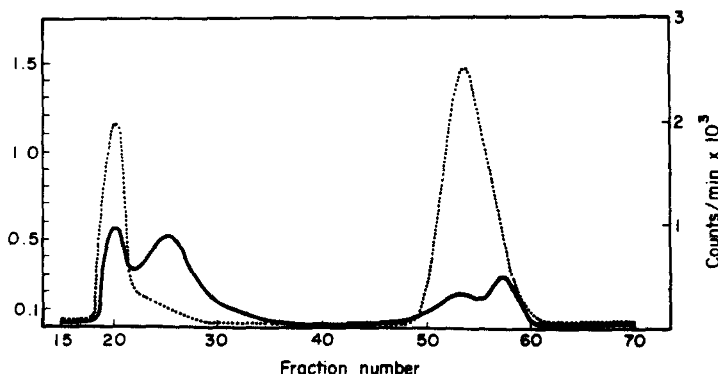

Fig. 3. Representative elution profile obtained by passage of a 105,000 *g* supernatant from rat ventral prostate labelled *in vivo* with [1,2-^{3}H]testosterone through a column of Sephadex G-100 gel. Continuous line represents optical density at 280 nm, dotted line c.p.m. per 5 ml fraction. From [10].

whereas most of the radioactivity was retained and eluted corresponding to material of small molecular weight. It should also be noted that corresponding to a protein slightly retained by the gel there was a 'shoulder' of radioactivity. Gel filtration of a similar supernatant obtained from muscle, liver or kidney did not reveal any binding of radioactivity to macromolecules.

Slices of the ventral prostate have been incubated *in vitro* with [1,2-^{3}H]-testosterone at 37°C in Eagle's tissue culture medium. Following incubation the tissue was homogenized and a 105·000 *g* supernatant was prepared, which was subsequently chromatographed on Sephadex G-100. The elution pattern was about identical to that obtained from the tissue *in vivo*[11]. When the incubation was carried out at 0°C, no binding to macromolecules was observed, indicating that some metabolic transformation of the testosterone was required for the complex formation, and it was subsequently shown that the substance bound to the macromolecule was 5α-dihydrotestosterone[11].

When a 105·000 $g \times 60$ min supernatant of the homogenized ventral prostate was incubated for 30 min at 0° or 37°C with [1,2-^{3}H]-5α-dihydrotestosterone, binding to two different macromolecules was observed[25]. By Sephadex G-100 gel filtration of the incubation mixture one macromolecule-androgen complex was excluded from the gel, corresponding to the elution pattern observed *in vivo*, and one complex was slightly retained (Fig. 4).

These two complexes correspond to those described by Fang and Liao[26]. They observed two proteins in the cytosol that would bind dihydrotestosterone, and designated the two α- and β-proteins. They differed in various ways, in binding characteristics, specificity, heat stability, solubility in ammonium sulphate solution, and most important, in their ability to promote nuclear uptake of dihydrotestosterone. Fang and Liao[26] found that their β-protein formed the so-called complex II with dihydrotestosterone and stimulated the nuclear uptake of the androgen by isolated prostatic nuclei. From gel filtration studies this β-protein appears to be the same protein we found associated with dihydrotestosterone in the supernatant following [1,2-^{3}H]-testosterone *in vivo*[10].

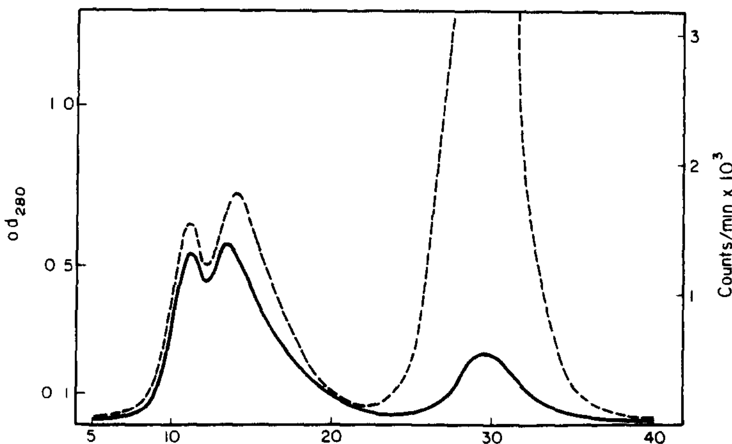

Fig. 4. Representative elution profile obtained by gel filtration on Sephadex G-100 of a 105,000 *g* supernatant prepared from rat ventral prostate homogenates. The supernatant had been incubated with [1,2-^{3}H]-5α-dihydrotestosterone. Continuous line represents optical density at 280 nm, dotted line c.p.m. per 3 ml fraction. From [25].

This protein therefore seems to be the intracellular transport protein for dihydrotestosterone. The other protein, the α-protein, will inhibit nuclear uptake of dihydrotestosterone[26], and the significance of this protein remains unclear. It is probably not a plasma protein, since rats do not seem to have a protein in the plasma which binds to testosterone and dihydrotestosterone[27].

There has been some confusion with regard to sedimentation constants during ultracentrifugation in a sucrose gradient of the specific dihydrotestosterone-binding protein in the cytosol. Values around 3–5 S and 8–10 S have been reported by various groups of investigators[11, 28, 29]. More recent information seems to indicate that the solution to the problem is polymerization of the substance, since the 8–10 S can be converted to 3–5 S by treatment with 0·5 M KCl[30].

The binding activity of the macromolecule was destroyed by treatment with proteolytic enzymes and different SH-reagents. Treatment with DNase or RNase did not, however, affect the binding properties of the macromolecule[11].

Nuclear binding of dihydrotestosterone. Several lines of evidence show that the androgen is taken up by the nucleus of the prostate, and the important questions in this connection are: what is the nature of the androgen, how does it get into the nucleus, and what is the receptor, if any, in the nucleus? Today we know most of the answers, we think. Anderson and Liao[31] demonstrated in 1968 that following incubation of minced prostatic tissue with [7α-^{3}H]-testosterone 70–80 per cent of the radioactivity in the nucleus could be identified as dihydrotestosterone. Dihydrotestosterone was also isolated in the nucleus following administration of tritiated testosterone *in vivo*[31]. These results have later on been confirmed by others[9].

Incubation *in vitro* of slices of the ventral prostate with [1,2-^{3}H]-testosterone gave labelling of nuclei with about 78 per cent of the activity being dihydrotestosterone and the rest testosterone[25, 32]. Treatment of a suspension of nuclei prepared from such incubations with Tris-HCl buffer removed 24 per cent of the radioactivity, 28 per cent of the protein and only traces of the DNA from the nuclei. Extraction with Tris-HCl buffer with 1M NaCl released another 60 per cent of the radioactivity, 38 per cent of the protein and 82 per cent of the DNA. Gel filtration on Sephadex G-100 of the first Tris-HCl extract demonstrated that all the radioactivity was eluted in the small molecular region, whereas the radioactivity in the Tris-NaCl extract was partly eluted in the macromolecular fraction, and partly with the small molecular components (Fig. 5). About 90 per cent of the radioactivity eluted in this macromolecular fraction was identified as dihydrotestosterone by thin layer and gas–liquid chromatography. These findings are all in agreement with the conclusion of Anderson and Liao[31] that most of the radioactivity in the nucleus is dihydrotestosterone, which is tightly bound to the nuclear chromatin.

For this binding to the chromatin Steggles *et al.*[33] have recently demonstrated that the androgen has to be associated with a specific cytosol receptor, and only chromatin from a specific target tissue will bind the androgen-receptor complex.

With regard to the mechanism of the nuclear uptake, I expect Dr. Liao to cover this aspect of androgen binding in the prostate in detail.

Effect of antiandrogens. Theoretically an antiandrogen might interfere with any of the steps between the cellular uptake of testosterone and the chromatin binding of dihydrotestosterone, and it appears that different types of antiandro-

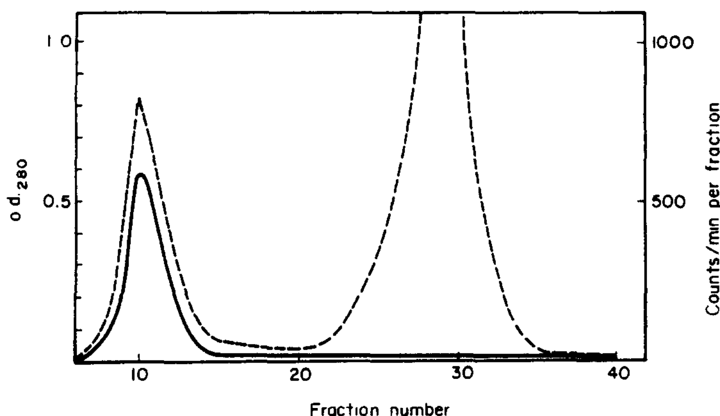

Fig. 5. Sephadex G-100 gel filtration of a NaCl extract of rat ventral prostate cell nuclei. The nuclei were isolated following incubation of a homogenate with [1,2-^{3}H]-dihydrotestosterone. Continuous line represents optical density at 280 nm, dotted line c.p.m. per 3 ml fraction. From [32].

gens may have different modes of action. Progesterone seems to inhibit the reduction of testosterone to dihydrotestosterone[20], whereas 17α-methyl-β-nortestosterone inhibited the complex formation between [^{3}H]-dihydrotestosterone and the cytosol and thus the nuclear uptake[34]. Cyproterone seems to have a similar effect[35], whereas the antiandrogenic effect of oestrogens may have a different mode of action[6]. This system may thus be well suited for testing out antiandrogens and their mode of action.

The effect of androgens in other tissues. We did not succeed in isolating dihydrotestosterone in muscle tissue, in spite of rather large amounts of tissue[17]. It should be noted that 37 per cent of the radioactivity in the muscle following [1,2-^{3}H]-testosterone administration was identified as testosterone, a higher percentage than observed for any other tissue examined[17]. The stimulatory effect of androgens on muscle tissue may therefore be mediated via some other mechanism than the one suggested for the prostate. If this suggestion is correct, it may be hoped that a more significant dissociation of the androgenic effect from the anabolic effect of steroids than hitherto achieved can be obtained for use in clinical medicine.

REFERENCES

1. S. Liao, K. R. Leininger, D. Sagher and R. W. Barton: *Endocrinology* **77** (1965) 763.
2. E. V. Jensen, T. Suzuki, T. Kawashima, W. E. Stumpf, P. W. Jungblut and E. R. DeSombre: *Proc. Natn. Acad. Sci. (U.S.A.)* **59** (1968) 632.
3. E. V. Jensen: *Excerpta med. (Amst.) Int. Congr. Ser.* **83** (1965) 420.
4. K. J. Tveter: *Acta endocr. (Kbh.)* **60** (1969) 60.
5. K. J. Tveter: *Endocrinology* **85** (1969) 597.
6. K. J. Tveter and A. Aakvaag: *Endocrinology* **85** (1969) 683.
7. K. J. Tveter and A. Attramadal: *Acta endocr. (Kbh.)* **59** (1968) 218.
8. K. J. Tveter and A. Attramadal: *Endocrinology* **85** (1969) 350.
9. O. Unhjem: *Acta endocr. (Kbh.)* **63** (1970) 69.
10. O. Unhjem and K. J. Tveter: *Acta endocr. (Kbh.)* **60** (1969) 571.
11. O. Unhjem, K. J. Tveter and A. Aakvaag: *Acta endocr. (Kbh.)* **62** (1969) 153.
12. W. H. Pearlman and M. R. J. Pearlman: *J. biol. Chem.* **236** (1961) 1321.
13. B. W. Harding and L. T. Samuels: *Endocrinology* **70** (1962) 109.
14. K.-O. Mosebach, H. Jühe and W. Dirscherl: *Acta endocr. (Kbh.)* **54** (1967) 557.
15. A. Attramadal: *Histochemie* **19** (1969) 110.
16. N. Bruchovsky and J. D. Wilson: *J. biol. Chem.* **243** (1968) 2012.

17. K. J. Tveter and A. Aakvaag: *Acta endocr. (Kbh.)* **65** (1970) 723.
18. R. I. Dorfman: *Nat. Cancer Inst. Monograph* **12** (1963) 161.
19. Q. Jehan, V. P. Kamboj and A. B. Kar: *Ind. J. exp. Biol.* **8** (1970) 68.
20. J. M. Stern and A. J. Eisenfeld: *Endocrinology* **88** (1971) 1117.
21. P. K. Siiteri and J. D. Wilson: *J. clin. Invest.* **49** (1970) 1737.
22. R. E. Gloyna, P. K. Siiteri and J. D. Wilson: *J. clin. Invest.* **49** (1970) 1746.
23. E. L. Klaiber, C. Lloyd, A. Solomon and D. M. Broverman: *Endocrinology* **83** (1968) 387.
24. L.-E. Tisell: *Acta endocr. (Kbh.)* **69** (1972) 359.
25. O. Unhjem: *Acta. endocr. (Kbh.)* **65** (1970) 517.
26. S. Fang and S. Liao: *J. biol. Chem.* **246** (1971) 16.
27. Raynard and Mercier-Brodard, quoted by E. E. Baulieu, I. Jung, J. P. Blondeau and P. Robel: *Adv. Biosci.* **7** (1971) 179.
28. S. Fang, K. M. Anderson and S. Liao: *J. biol. Chem.* **244** (1969) 6584.
29. W. I. P. Mainwaring: *J. Endocr.* **45** (1969) 531.
30. E. E. Baulieu, I. Jung, J. P. Blondeau and P. Robel: *Adv. Biosci.* **7** (1971) 179.
31. K. M. Anderson and S. Liao: *Nature (London)* **219** (1968) 277.
32. O. Unhjem: *Acta endocr. (Kbh.)* **65** (1970) 533.
33. A. W. Steggles, T. C. Spelsberg, S. R. Glasser and B. W. O'Malley: *Proc. Nat. Acad. Sci. (U.S.A.)* **68** (1971) 1479.
34. K. J. Tveter: *Acta endocr. (Kbh.)* **66** (1971) 352.
35. S. Fang and S. Liao: *Mol. Pharmacol.* **5** (1969) 428.

DISCUSSION

Tuohimaa: In your last two slides, the radioactivity is expressed per protein fraction. If you express the radioactivity per mg of protein, what is the pattern then? Is there some specific fraction which contains higher specific radioactivity?

Aakvaag: I haven't done this but it seems that the amount of radioactivity per mg of protein, one may call it specific activity is constant throughout the peak.

Kellie: I understand you to say that you subjected the cytosol fraction to Sephadex gel filtration and that you did this at room temperature. If in fact you were successful, would you not separate small molecules from large molecules and would not the complex be expected to dissociate under these conditions?

Aakvaag: If the binding affinity is low, dissociation of the complex will take place; for instance, if albumin-cortisol complex is chromatographed on Sephadex, dissociation will take place. In this case, however, re-chromatography of the steroid-macromolecule complex did not reveal significant dissociation.

Pasqualini: Regarding your very important conversion of testosterone to 5α-dihydrotestosterone, especially in the nucleus, do you have an idea if this takes place before the formation of the complex, or can the testosterone complex be reduced directly to the 5α-dihydrotestosterone complex?

Aakvaag: It appears that the complex is being formed after the conversion to dihydrotestosterone, since what we consider as the protein that brings the steroid into the nucleus doesn't bind testosterone anywhere as it does dihydrotestosterone.

Siiteri: You mentioned that you get some 50% of androstenedione appearing in prostate as dihydrotestosterone but did not present data concerning this point, and I was interested to hear a little more about this. Dr. Wilson and I have tried to demonstrate the conversion of androstenedione to dihydrotestosterone by a variety of means and have shown only a very minor conversion. In our studies which you alluded to, in which we found very much higher concentrations of dihydrotestosterone in hypertrophic prostatic tissue, we certainly considered androstenedione as a logical precursor, but this has not turned out to be the case in our hands.

Aakvaag: We gave androstenedione of the same high specific activity (roughly

50 Ci/mmol) *in vivo* to 1-day castrated rats (exactly the same animal preparation as we used for the testosterone studies) and took out the organs 1 h after the administration of androstenedione, and as I said, we were able to isolate about 30% of the radioactivity as dihydrotestosterone by means of paper chromatography and crystallization to constant specific activity. The figures were definitely lower than observed with testosterone, in which case 50–70% of the radioactivity was 5α-dihydrotestosterone. Because of this considerable difference, we wanted to find out what other radioactive steroids were present in the tissue. We were, however, unable to identify these steroids. All the radioactivity was extractable with ether, suggesting that the steroids were unconjugated.

Siiteri: I hasten to point out that there may well be species differences here. When we measured the endogenous level of androstenedione itself, in the prostate of both the dog and human, it was the same as it was in muscle or other control tissues, so that at least in these species, we feel that androstenedione does not contribute significantly to the tissue level of dihydrotestosterone. This of course may be different in the rat.

Vermeulen: May I ask you what was the percentage of radioactivity you recovered in the prostate?

Aakvaag: This has not been calculated, but the percentage is low.

Morfin: I had the same question as Dr. Siiteri: I would just add that one would be surprised for androstenedione to generate 5α-dihydrotestosterone, since androstenedione has always been considered to be a weak androgen. We have shown in dog, *in vivo*, that androstenedione was not characteristic as an androgen for target tissues like the prostate, but that it was for non-target tissues like the urinary bladder.

Liao: The percentage of a particular radioactive metabolite in a tissue might be very much dependent on the length of time elapsed after the radioactive steroid was injected into the experimental animal. If only the radioactive 5α-dihydrotestosterone can be retained by the nucleus for a prolonged length of time and one measures at a long time (1 h) after the injection, then one probably will find 5α-dihydrotestosterone as the major radioactive metabolite in the whole tissue. If one checks at a short time (10 min) the picture may be completely different. Have you studied this?

Akvaag: We isolated 5α-dihydrotestosterone only 1 h after administration, we didn't do any isolation work on other time periods, because we wanted to have as much radioactivity as we could in the tissue. With regard to the uptake of androstenedione, the inhibition of testosterone uptake observed when we gave non-radioactive androstenedione is, of course, in agreement with the concept that androstenedione is really taken up in the prostate and being converted. Also in the experiment where we observed the inhibition of uptake in the prostate, no effect on the uptake in other tissues like muscle and liver was observed.

Grant: I have a comment on the problem raised by Dr. Siiteri: I think the answer you get depends on the conditions you have. With dog and with human hyperplastic prostate, in superfusion experiments at the steady state, we found an equilibrium between androstenedione and testosterone, and we find that androstenedione is in fact converted to dihydrotestosterone.

Exley: Could I ask you if you've used the propionate or any derivative ester of testosterone and what the uptake of that would be? Have you used the propionate?

Aakvaag: No.