Journal of Steroid Biochemistry, 1972, Vol. 3, pp. 427–439. Pergamon Press. Printed in Great Britain

STUDIES ON THE INTERACTION BETWEEN ANDROGEN AND MACROMOLECULES IN MALE ACCESSORY SEX ORGANS OF RAT AND MAN

VIDAR HANSSON, KJELL J. TVETER, OLAV UNHJEM and OLA DJÖSELAND
Institute of Pathological Anatomy, Rikshospitalet, Oslo, Norway

SUMMARY

The androgen-receptor complexes in the ventral prostate cytosol fraction are found in different molecular forms, depending on the experimental conditions. By gel filtration two different complexes are found. One is excluded and the other is retained on a column of Sephadex G-100. By experiments *in vivo* a 6–6·5 S androgen-receptor complex is observed on sucrose gradients. This complex dissociates into a 3·5–4 S complex after dialysis against 0·5 M NaCl (in buffer) for 20 h. The androgen-binding proteins extracted from the nuclear pellet with 0·5 M NaCl move as a sharp peak on sucrose gradients, with a sedimentation constant of 3 S.

The epididymis of adult castrated rats also contains similar proteins, which bind 5α-dihydrotestosterone (5α-DHT) with high affinity and low capacity. The 5α-DHT binding protein in the cytosol fraction of epididymal homogenates is slightly retained on a column of Sephadex G-100, and moves with a sedimentation rate of about 4–4·5 S (mean 4·3 S) on sucrose gradients. Similar results are obtained both at high and low ion strength. The binding of [^{3}H]5α-DHT to this protein is easily depressed by small amounts of non-labelled 5α-DHT. 15 min after the injection of [^{3}H]testosterone *in vivo*, about 90% of the radioactivity bound to proteins moves as 5α-DHT on t.l.c. 1 h after the injection of 60 μCi[^{3}H]5α-DHT *in vivo*, a considerable part of the radioactivity is found in the nuclear fraction of homogenized epididymal tissue.

Human hyperplastic prostatic tissue also contains similar androgen-binding proteins. The binding of androgens to these macromolecules is inhibited by potent anti-androgenic compounds such as SK & F 7690 and cyproterone. Some evidence for direct binding of [^{3}H]cyproterone-acetate in the ventral prostate cytosol fraction is presented.

INTRODUCTION

RECENT investigations indicate that 5α-dihydrotestosterone (5α-DHT) is of primary importance in the mediation of the androgenic message to susceptible cells. Testosterone is rapidly converted to this compound by the accessory sex organs of both rat[1–6] and man[7–9]. One hour after the administration of [^{3}H]testosterone *in vivo*, about 70% of the total radioactivity in the prostate is represented by [^{3}H]5α-DHT[5].

The selective uptake of androgen by the rat ventral prostate is probably due to the interaction of 5α-DHT with specific androgen-binding proteins (receptors). Such binding sites are located both in the prostatic cytosol[10–13] and in the nuclei[2, 11, 14, 15].

In our laboratories we have studied the uptake, metabolic conversion, the cellular and intracellular localization and the binding of androgen in the male accessory sex organs. Recent surveys on these subjects are given, *inter alia*, by Ofner (1968), Liao and Fang (1969), Tveter (1970) and Unhjem (1970) and Williams Ashman and Reddy (1971)[16–20].

The present report describes our latest investigations on the ventral prostate and the epididymis of the rat. Further, we have studied the uptake and binding of androgen by human benign nodular prostatic hyperplasia (BNPH) and the effect of some potent anti-androgens on androgen-receptor interaction.

RAT VENTRAL PROSTATE

The administration of [^{3}H]5α-DHT *in vivo* to castrated male rats, gives essentially the same accumulation of radioactivity in the ventral prostate and epididymis as after the injection of [^{3}H]testosterone[21, 22]. This indicates that 5α-DHT, the predominant intracellular androgenic metabolite, is able to enter the prostatic cells from circulating blood (Fig. 1). These findings are consistent with the well known stimulating effect of 5α-DHT on the prostate after systemic administration.

The "soluble" receptors in the rat ventral prostate cytoplasm may exist in different molecular forms, due either to polymerization or to association with other cellular components. The larger complexes were formed in hypotonic media and had a sedimentation constant of about 8–10 S. These 8–10 S complexes were, however, not constantly found. In some experiments only 6–6·5 S complexes were formed without evidence of any 8–10 S peak (Figs. 2 and 3). When the protein content of the cytosol fractions was low (below 10 mg/ml), we often did not find any distinct 8–10 S peak at all, but a more diffuse labelling in the lower half of the gradient tube. These "larger" complexes were always excluded as a sharp peak from Sephadex G-100 gel beds (Fig. 4a). These 8–10 S complexes have previously been demonstrated[10, 12, 13]. We are, however, not aware of anyone describing 6–6·5 S complexes in the rat ventral prostate cytosol.

The ventral prostate cytosol receptor appeared as one single excluded peak as evidenced by Sephadex G-100 chromatography, when either prostatic homogenates, 600 *g* supernatant fractions (× 10 min) or 1500 *g* supernatant fractions (× 20 min) were incubated with [^{3}H]5α-DHT *in vitro* (Fig. 4a, b, c). All homogenizations were performed in 0·1 M Tris-HCl buffer, and the cytosol fraction prepared by ultracentrifugation for 60 min at 105,000 *g* at 3°C. Likewise, when [^{3}H]5α-DHT or [^{3}H]testosterone were given *in vivo*, and the prostates

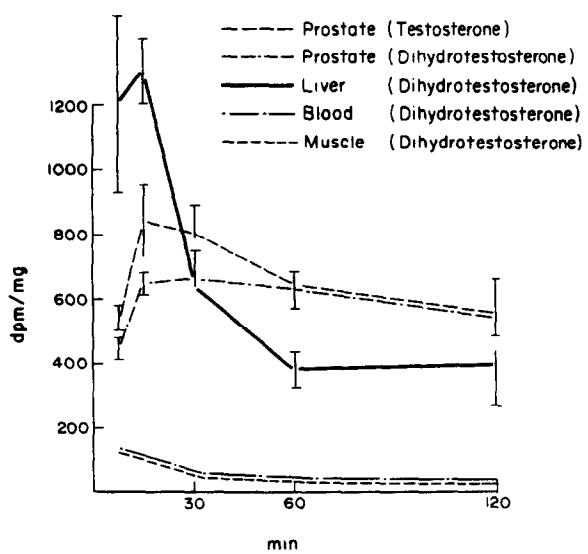

Fig. 1. Uptake of radioactivity by various organs of the rat, following the administration of [^{3}H]testosterone and [^{3}H]5α-DHT *in vivo*. The animals were injected with 15 μCi/100 g body weight, and killed in groups of four at the times indicated. The results are expressed as DPM/mg wet weight ± Standard error of the mean (SEM).

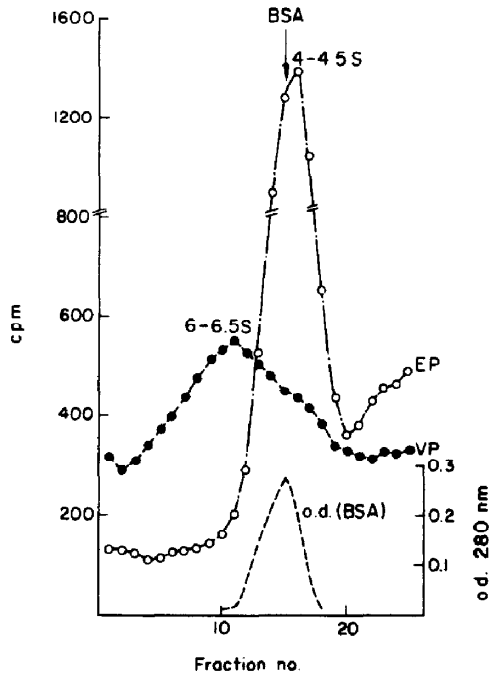

Fig. 2. Sucrose gradient (5–20%, w/v) analysis (38,000 rev./min, 18 h at 2–3°C) of cytosol fractions from the epididymis (EP) and ventral prostate (VP) 15 min after intramuscular injection *in vivo* of 100 μCi [^{3}H]testosterone. BSA = bovine serum albumin.

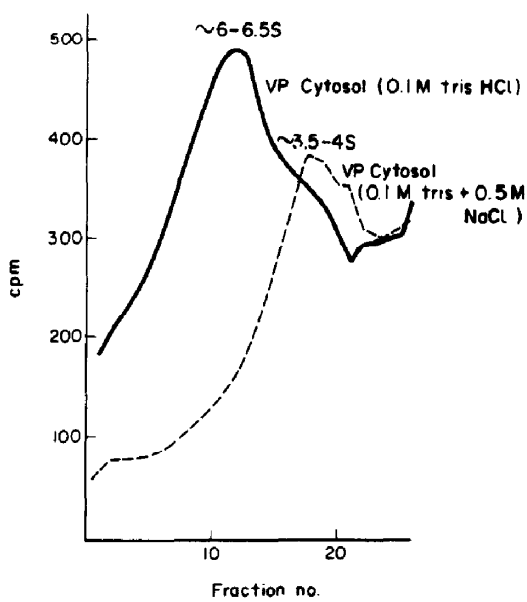

Fig. 3. Sucrose gradient analysis (38,000 rev./min, 18 h at 2–3°C) of ventral prostate cytosol fraction in 0·1 M Tris-HCl buffer or made 0·5 M in NaCL. The animals were injected *in vivo* with 100 μCi [^{3}H]testosterone per animal, and killed 15 min later. By increasing the ion strength to 0·5 M NaCl, the 6–6·5 S peak disappears, and the main part of the protein-bound radioactivity moves at a sedimentation rate of about 3·5–4 S.

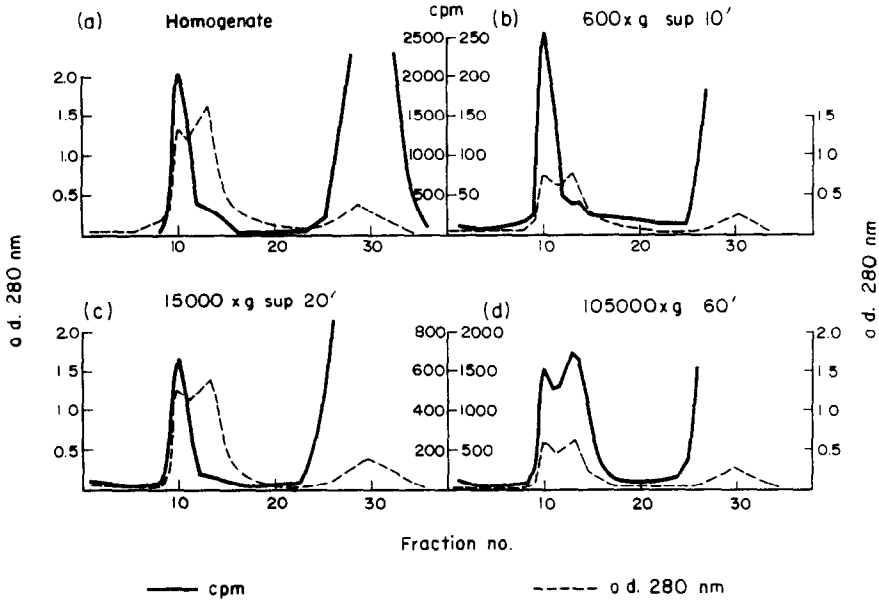

Fig. 4. Sephadex G-100 gel filtration of ventral prostate cytosol fractions after labelling the prostate as homogenates (A), as 600 *g* supernatant fraction (B), as 15,000 *g* supernatant fractions (C) and as 105,000 *g* supernatant fraction (D) with [^{3}H]5α-DHT (30 min at 0°C).

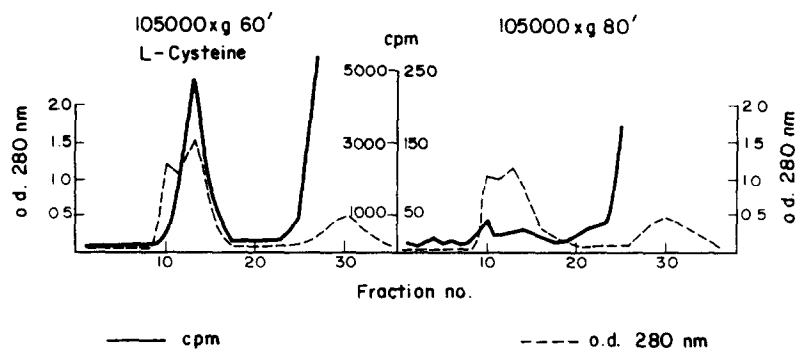

Fig. 5. Sephadex G-100 gel filtration of a ventral prostate cytosol fraction, after incubation 30 min at 0°C with [^{3}H]5α-DHT. The cytosol fractions were made 0·05 M with L-Cysteine (A) or centrifuged 80 min in the ultracentrifuge before incubation with [^{3}H]5α-DHT *in vitro* (B).

homogenized in the same hypotonic Tris-HCl buffer, gel filtration of the cytosol fractions (105,000 *g* for 60 min) gave identical results (Fig. 4). On the other hand, When a 0·1 M Tris-HCl cytosol fraction was incubated directly with [^{3}H]5α-DHT, the androgen-binding proteins appeared as two peaks after gel filtration on Sephadex G-100. One peak was excluded and the other was retained on the column (Fig. 4d). When L-cysteine, used as SH-stabilizing agent, was added to the homogenization medium and the column buffer, no radioactivity was excluded, and all the androgen binding proteins were retained by the gel bed (Fig. 5 left). By increasing the centrifugation time in the ultracentrifuge (at 105,000 *g*)

from 60 to 80 min, only traces of the androgen-binding proteins remained in solution. Most of the receptors were sedimented by this procedure (Fig. 5 right).

When a hypotonic cytosol fraction (0·1 M Tris HCl) from ventral prostate homogenates was centrifuged at 105,000 *g* for 2 h, after labelling of the receptors *in vitro*, the androgen-receptor complexes were sedimented as examined by gel filtration on Sephadex G-25. When the cytosol fractiow was made 0·5 M with NaCl before this centrifugation, the androgen protein complexes remain in solution, and did not sediment. By increasing the ion strength of the cytosol fractions to 0,5 M NaCl, a smaller androgen-protein complex in the cytosol fraction was formed. This androgen-receptor complex sedimented on sucrose gradients between 3·5–4 S (Fig. 3). These findings are consistent with reports of Baulieu *et al.* (1971) and Liao (1971), that aggregation of the prostatic cytosol receptors may be prevented by increasing the ion strength of the homogenization media used [23, 24]. Thus, the formation of the different molecular forms of the "soluble" receptor in the ventral prostate cytosol fraction is influenced as well by the ion strength of the media used, as by the mode of labelling the prostate (*in vivo*, *in vitro*, homogenates, or subcellular fractions) or by chemical compounds that may prevent aggregation (e.g. L-cysteine).

In other experiments, ventral prostate tissue, after labelling *in vivo* with [^{3}H]testosterone, was homogenized in 0·1 M Tris-HCl buffer containing 0·5 *M* NaCl. The tissues were gently homogenized and extracted for 30 min. When a 20,000 *g* supernatant fraction (× 60 min) from this homogenate was subjected to gel filtration, a major part of the radioactivity was slightly retained by a column of Sephadex G-100 (Fig. 6). Sucrose gradient analyses of this supernatant fraction demonstrated a broad peak of radioactivity between 3 and 4 S. When the gradient tube was divided into smaller fractions, two peaks occurred, one at about 3 S and one at 3·6 S (Fig. 7). This combined homogenization-extraction procedure solubilize both the cytoplasmic and nuclear receptors and the results are quite similar to those obtained by Fang *et al.* (1969)[11].

The nuclear receptors of the rat ventral prostate are easily extracted with

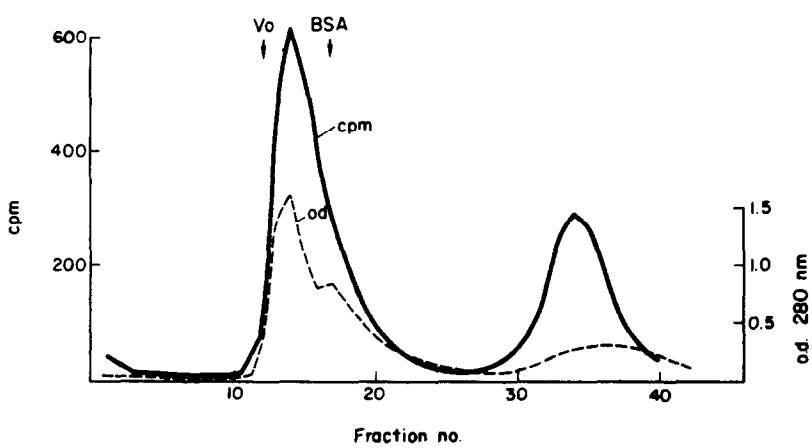

Fig. 6. Gel filtration (Sephadex G-100) of a 20,000 *g* supernatant fraction (× 60 min) from ventral prostate homogenate, after homogenization and extraction directly in 0·5 M Tris-HCl buffer containing 0·5 M NaCl. The tissues were labelled by injecting the animals with 100 μCi [^{3}H]testosterone *in vivo*, followed by decapitation 15 min later.

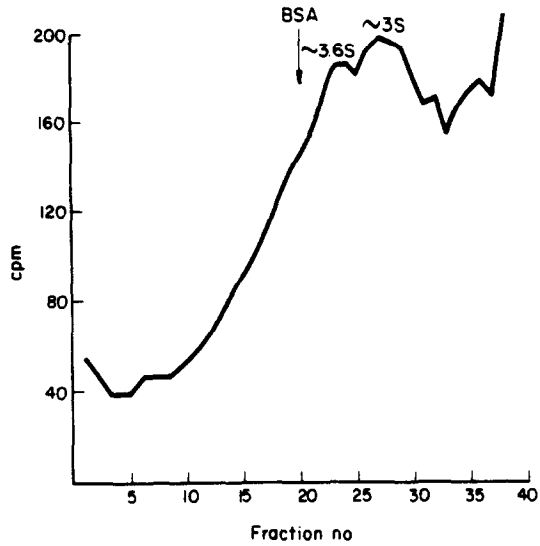

Fig. 7. Sucrose gradient analysis (38,000 rev./min, 18 h at 2–3°C) of a 20,000 *g* supernatant fraction (× 60 min) from ventral prostate homogenate, after homogenization and extraction directly in 0·1 M Tris-HCl buffer containing 0·5 M NaCl. The tissues were labelled by injection of 100 μCi [^{3}H]testosterone *in vivo*, and the animals were killed after 15 min.

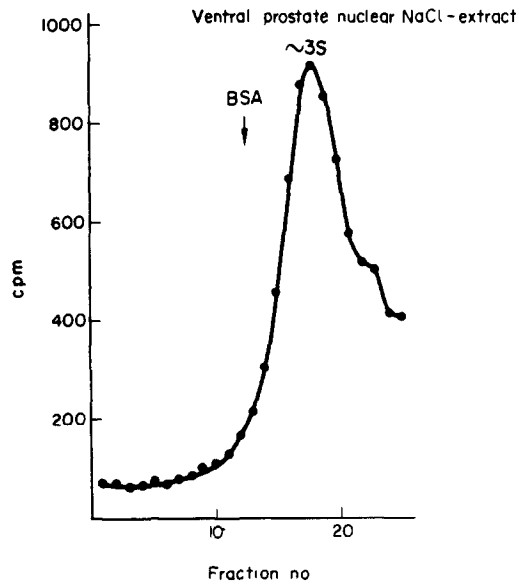

Fig. 8. Sucrose gradient analysis (38,000 rev./min. 18 h at 2–3°C) of a nuclear NaCl extract from ventral prostate cell nuclei. The animals were injected *in vivo* with [^{3}H]testosterone (100 μCi/animal) and killed 1 h later. The androgen-receptor complexes from the ventral prostate cell nuclei are moving as a distinct peak at about 3 S.

0·5–1 M NaCl. These nuclear androgen-binding proteins, which are possibly of cytoplasmic origin[11, 25] are retained on a column of Sephadex G-100[21]. On sucrose gradients these nuclear receptors sedimented as a distinct peak with a sedimentation rate of about 3 S (Fig. 8).

RAT EPIDIDYMIS

The rat epididymis also contains androgen-binding macromolecules both in the cytoplasm and in the nuclei[22]. The cytoplasmic epididymal receptors were slightly retained on a column of Sephadex G-100 (Fig. 9), and moved slightly faster than albumin by gel filtration. On sucrose gradients they moved as a sharp peak with a sedimentation constant of about 4–4·5 S (Fig. 2). 15 min after the injection of [^{3}H]testosterone *in vivo*, more than 90% of the radioactivity bound to these receptors moved as 5α-DHT on thin layer chromatography. The binding of 5α-DHT to these soluble epididymal receptors was depressed by non-labelled 5α-DHT, indicating a "high-affinity low-capacity binding system".

The androgen-binding protein in the epididymal cytosol fraction did not have the same tendency to aggregation as the soluble receptor in the ventral prostate cytoplasm, and moves as a sharp peak between 4–4·5 S both at high and low ion strength.

The binding of 5α-DHT to specific receptors in the epididymis is in agreement with the androgen dependency of this organ. As for the prostate and

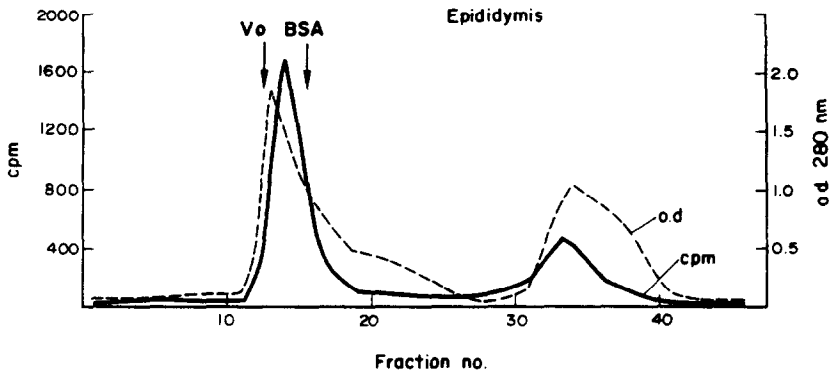

Fig. 9. Sephadex G-100 gel filtration of a cytosol fraction of epididymal homogenates in 0·1 M Tris-HCl buffer containing 0·5 M NaCl. The androgen-protein complexes are slightly retained on a column of Sephadex G-100.

seminal vesicles, the height and the histochemistry of the epithelial cells, as well as the secretory activity are strongly contingent upon the presence of appropriate amounts of circulating androgens. Likewise, withdrawal of androgenic hormones rapidly causes atrophy and regressive changes[26, 27].

Subcellular fractionation studies of epididymal homogenates demonstrate that a significant part of the radioactivity is associated with the nuclear fraction. One h after the injection of [^{3}H]testosterone (Table 1) or [^{3}H]5α-DHT about $\frac{1}{3}$ of the radioactivity was localized in the nuclear fraction. A nuclear accumulation of androgen seems to be characteristic of androgenic target cells, and in the ventral prostate about 55% of the radioactivity is associated with the nuclear fraction [18]. Marker enzyme determinations (glucose-6-phosphatase and acid phosphatase) of our subcellular fractions confirm that our nuclear fractions are relatively clean of cytoplasmic contamination. Due to high amounts of fibrous and muscular tissue in this organ, subcellular fractionation of the epididymis is not so easily done as for the ventral prostate of the rat.

Table 1. Subcellular localization of radioactivity in different organs of the rat, 1 h after intramuscular injection of 100 μCi [^{3}H]testosterone

	c.p.m.	% of total	Recovery
Epididymis			
600 *g* pellet	268804	30	
105000 *g* sediment	82640	9·1	82·7%
105000 *g* supernat.	554055	61	
Seminal vesicles			
600 *g* pellet	90160	60·5	
105000 *g* sediment	9396	6·3	80%
105000 *g* supernat.	49553	33·2	
Kidney			
600 *g* pellet	36576	8·4	
105000 *g* sediment	72263	16·7	83·4%
105000 *g* supernat.	323136	74·9	
Muscle			
600 *g* pellet	1560	6·8	
105000 *g* sediment	954	4·2	98·8%
105000 *g* supernat.	20460	89·0	

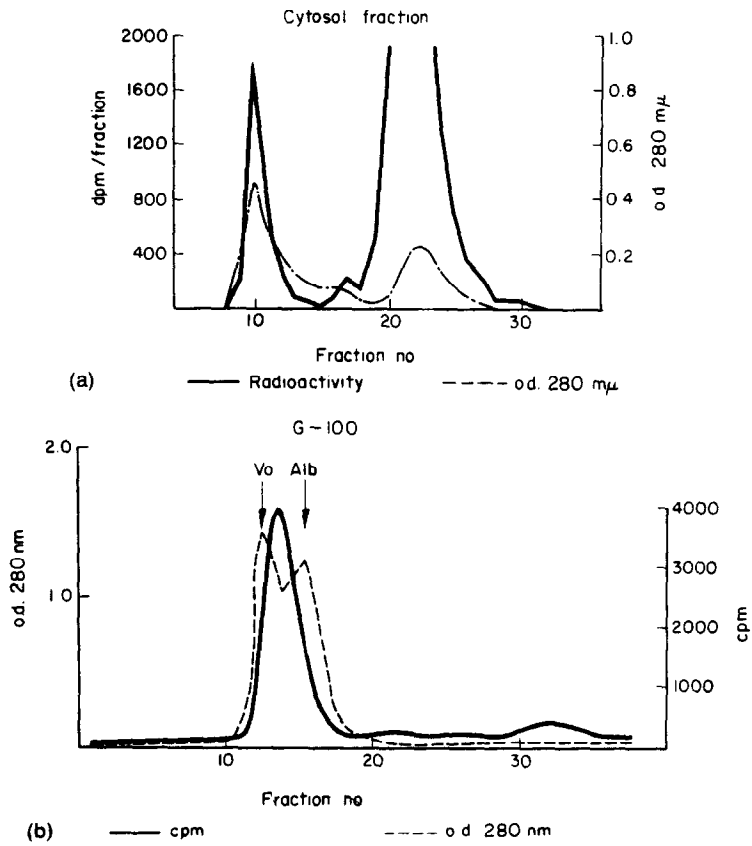

Fig. 10. (A) Sephadex G-100 gel filtration of a cytosol fraction from BNPH, after incubation *in vitro* with $7{\cdot}3 \times 10^{-10}$ M [^{3}H]testosterone for 2 h at 37°C. (B) Sephadex G-100 gel filtration of human male serum (diluted 1/10 ×) incubated 30 min and 30°C with 10^{-9} M [^{3}H]5α-DHT. The radioactivity in the BNPH cytosol fraction is always excluded from the G-100 gel columns, while the TeBG is slightly retained.

Table 2. Uptake and retention of radioactivity in slices from BNPH and the pyramidalis muscle, after incubation for 2 h in Eagle's medium with [^{3}H]testosterone ($7{\cdot}3 \times 10^{-10}$ M), followed by washing in a hormone-free medium for different times at 25°C. The results are given as d.p.m./mg tissue ± S.E.M.

Patient no.	No washing			Washing time in minutes: 30 min			60 min		
	Prostate	Muscle	Ratio	Prostate	Muscle	Ratio	Prostate	Muscle	Ratio
1	3565 ± 260	3855 ± 171	0·92	3090 ± 135	763 ± 56	4·1	2775 ± 123	287 ± 70	9·7
2	3984 ± 323	2995 ± 127	1·33	2008 ± 153	775 ± 20	2·6	2042 ± 128	450 ± 33	4·5
3*	1720 ± 58	1588 ± 65	1·1	1157 ± 94	436 ± 92	2·7	1063 ± 36	316 ± 23	3·3
4*	2238 ± 199	1588 ± 65	1·4	1534 ± 61	436 ± 92	3·52	1282 ± 95	316 ± 23	4·1
Mean ratio	1·2 ± 0·11			3·2 ± 0·25			5·4 ± 1·45		

*Patients 3 and 4 were compared to the same muscle reference.

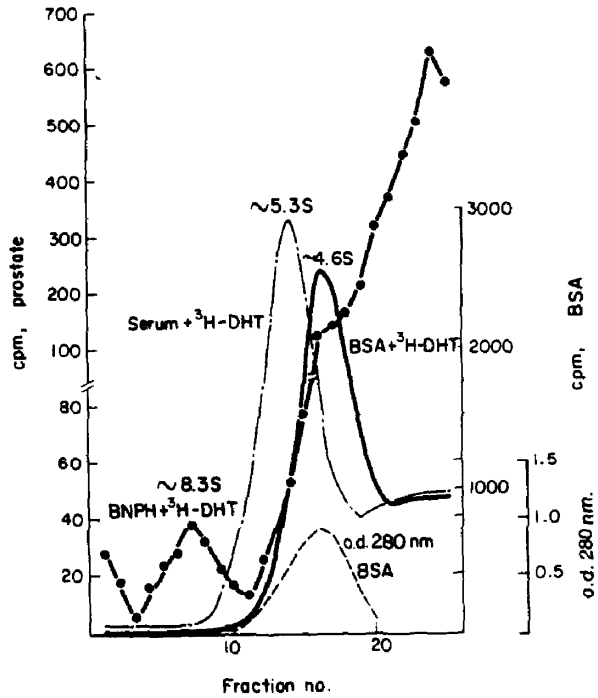

Fig. 11. Sucrose gradient analyses of BNPH cytosol fraction incubated 24 h with [^{3}H] 5α-DHT at 0°C. Human serum incubated with [^{3}H]5α-DHT (5·3 S) and albumin (BSA) (4·6 S) are used as references.

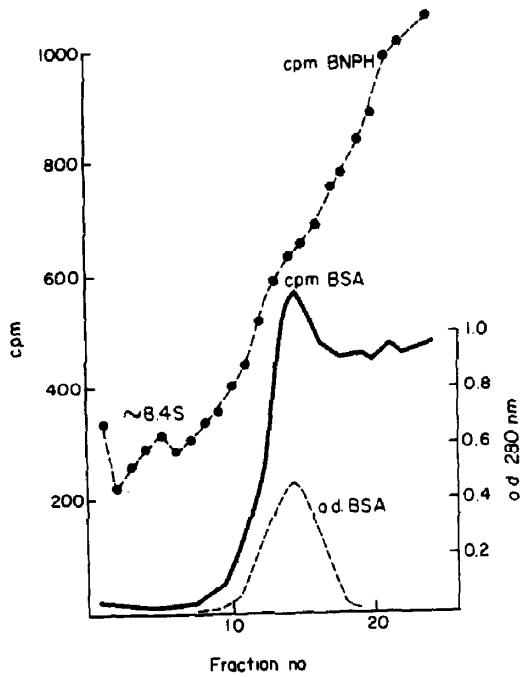

Fig. 12. Sucrose gradient analysis of the same cytosol fraction as in Fig. 11, concentrated six times with pressure dialysis at 0°C for 24 h, against 0·1 M Tris-HCl buffer containing 5×10^{-10} M [^{3}H]5α-DHT.

HUMAN BENIGN NODULAR HYPERPLASIA (BNPH)

We have also investigated the human BNPH[28, 29], and have found by experiments *in vitro* that BNPH is able to accumulate androgen (Table 2). Androgen-binding proteins, different from the androgen-binding proteins in human plasma (testosterone-oestradiol-binding globulin=TeBG and transcortin=CBG), have been demonstrated both in the 105,000 *g* supernatant fraction and in the NaCl extracts of the nuclear fractions of homogenized tissue.

The soluble androgenic receptor in the cytosol fraction was excluded from a Sephadex G-100 gel bed, in contrast to TeBG which was slightly retained on a similar column (Fig. 10a and b). Analyses of such cytosol fractions (0·5 ml) on sucrose gradients give a small but quite reproducible peak between 8 and 8·5 S (Figs. 11 and 12).

The radioactivity solubilized after extraction with 0·5 M NaCl gives a broad peak of radioactivity partly excluded and partly retained by the column of Sephadex G-100[28].

Thus, the results obtained on the human prostate are so far almost identical

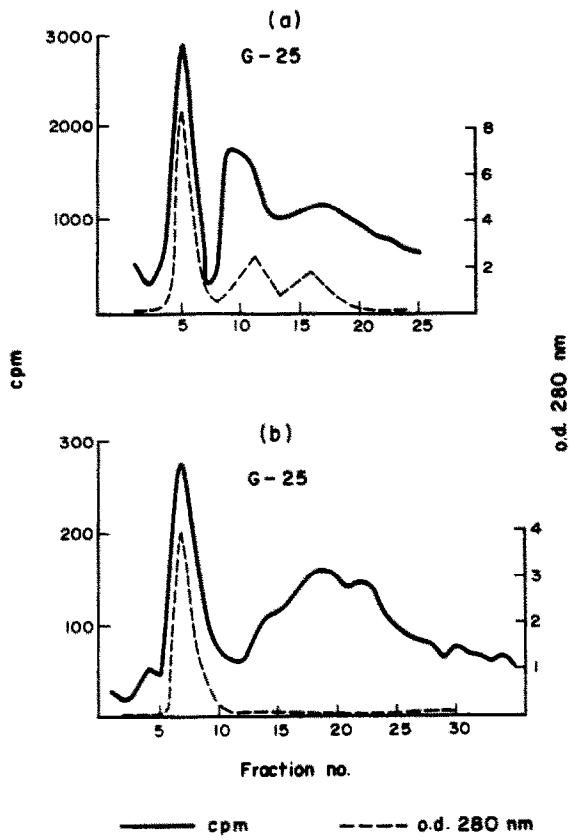

Fig. 13. (A) Sephadex G-25 gel filtration of a ventral prostate cytosol fraction after incubation of slices with [^{3}H]cyproterone acetate (82·5 mCi/mmol) for 24 h at 3°C in Eagle's tissue culture medium. (B) Re-filtration of the macromolecular fraction from the first gel-filtration (Fraction 5, Fig. 13a) after standing 2 h at room temperature. A significant part of the radioactivity is bound to proteins in the ventral prostate cytosol even after the second gel-filtration.

with those of the rat ventral prostate, and might indicate a fundamental similarity with regard to the uptake and retention of androgen by the rat and human prostate. These findings also appear to be in accordance with the idea that androgenic hormones play an important role in the development of prostatic hyperplasia[6, 9].

ANTIANDROGENS

This selective uptake and binding of 5α-DHT in both the rat and human prostate are strongly depressed by some potent anti-androgenic compounds (cyproterone, cyproterone-acetate, and 17α-methyl β-nortestosterone (SK&F 7690)[29–33]. By incubation of rat prostate slices with [^{3}H]cyproterone-acetate for 24 h at 3°C, followed by Sephadex G-25 gel filtration of a cytosol fraction, a considerable part of the radioactivity was bound to proteins excluded from the column (Fig. 13a). A great part of the protein-bound [^{3}H]cyproterone-acetate, resisted even refiltration after standing 2 h at room temperature (Fig. 13b). These findings are in agreement with earlier suggestions that the anti-androgenic activity of these compounds may be due, at least partly, to competition between 5α-DHT and the anti-androgen for the same cellular binding sites[31].

REFERENCES

1. N. Bruchovsky and J. D. Wilson: *J. biol. Chem.* **243** (1968) 2012.
2. N. Bruçhovsky and J. D. Wilson: *J. biol. Chem.* **243** (1968) 5953.
3. K. M. Anderson and S. Liao: *Nature, (London)* **219** (1968) 277.
4. E. E. Baulieu, I. Lasnitski and P. Robel: *Nature, (London)* **219** (1968) 1155.
5. K. J. Tveter and A. Aakvaag: *Endocrinology* **85** (1969) 683.
6. R. E. Gloyna and J. D. Wilson: *J. clin. Endocr.* **29** (1969) 970.
7. W. W. Farnsworth and J. R. Brown: *J. Amer. med. Ass.* **183** (1963) 436.
8. W. W. Farnsworth and J. R. Brown: *Natn. Cancer Inst. Monogr.* No. 12, Bethesda, Maryland (1963) p. 323.
9. P. K. Siiteri and J. D. Wilson: *J. clin. Invest.* **49** (1970) 1737.
10. O. Unhjem, K. J. Tveter and A. Aakvaag: *Acta endocr. (Kbh.)* **62** (1969) 153.
11. S. Fang, K. M. Anderson and S. Liao: *J. biol. Chem.* **244** (1969) 6584.
12. W. I. P. Mainwaring: *J. Endocr.* **45** (1969) 531.
13. E. E. Baulieu and I. Jung: *Biochem. biophys. Res. Commun.* **38** (1970) 599.
14. W. I. P. Mainwaring: *J. Endocr.* **44** (1969) 323.
15. O. Unhjem: *Acta endocr. (Kbh.)* **63** (1970) 69.
16. P. Ofner: *Vitamins and Hormones* **26** (1968) 237.
17. S. Liao and S. Fang: *Vitamins and Hormones* **27** (1969) 17.
18. K. J. Tveter: *Thesis*: Universitetsforlaget, Oslo, 1970.
19. O. Unhjem: *Thesis*: Universitetsforlaget, Oslo, 1970.
20. H. G. Williams-Ashman and A. H. Reddy: *Ann. Rev. Physiol.* **33** (1971) 33.
21. V. Hansson, K. J. Tveter and A. Attramadal: *Acta endocr. (Kbh.)* **67** (1971) 384.
22. V. Hansson and K. J. Tveter: *Acta endocr. (Kbh.)* **66** (1971) 745.
23. S. Liao, J. L. Tymoczko, T. Liang, K. M. Anderson and S. Fang: *Adv. Biosci.* **7** (1971) 155.
24. E. E. Baulieu, I. Jung, J. P. Blondeau and P. Robel: *Adv. Biosci.* **7** (1971) 179.
25. A. W. Steggles, T. C. Spelsberg, S. R. Glasser and B. W. O'Malley: *Proc. natn. Acad. Sci. (U.S.A.)* **68** (1971) 1479.
26. L. F. Cavazos: *Anat. Rec.* **132** (1958) 209.
27. T. Mann: *Biochemistry of the Semen and the Male Reproductive Tract.* Merheuen, London (1964) p. 53.
28. V. Hansson, K. J. Tveter, A. Attramadal and O. Torgersen: *Acta endocr. (Kbh.)* **68** (1971) 79.
29. V. Hansson and K. J. Tveter: *Acta endocr. (Kbh.)* **68** (1971) 69.
30. S. Fang and S. Liao: *Molec. Pharmacol.* **5** (1969) 428.
31. J. M. Stern and A. J. Eisenfeld: *Science* **166** (1969) 233.
32. K. J. Tveter: *Acta endocr. (Kbh.)* **66** (1971) 352.
33. K. J. Tveter, O. Unhjem, A. Attramadal, A. Aakvaag and V. Hansson: *Adv. Biosci.* **7** (1971) 193.

DISCUSSION

Liao: In the last slide you showed, was it radioactive cyproterone?

Hansson: Yes, tritiated cyproterone acetate with a S.A. of 82·5 mCi/mmol.

Liao: Have you tried to calculate the number of cyproterone molecule bound per amount of protein to see whether it is in the same order of magnitude as in the case of 5α-dihydrotestosterone.

Hansson: We have not calculated this, but the radioactive cyproterone-acetate is about 500 times less labelled than the ordinary high labelled dihydrotestosterone. However, in these experiments we therefore increased the amount of protein (receptors) put onto the Sephadex columns by about 20 times, compared to similar experiments with highly labelled dihydrotestosterone. The radioactive peak excluded from the Sephadex columns under these circumstances should therefore be roughly about 25 times less than normal, if the dissociation rate during the gel filtration is the same. I think that non-specific binding to albumin and other proteins will dissociate by repeated gel-filtration as in this experiment, but of course I can't claim that all the radioactivity bound to proteins after the second gel filtration is only due to the receptor proteins. However, competition experiments with different non-labelled steroids would tell us more about the specificity of the binding.