THE UPTAKE OF TESTOSTERONE AND ZINC IN VITRO BY THE HUMAN BENIGN HYPERTROPHIC PROSTATE

M. JOAN REED* AND S. R. STITCH

Division of Steroid Endocrinology,
Department of Chemical Pathology, School of Medicine,
Leeds, LS2 9NL

(Received 19 October 1972)

SUMMARY

The uptake of $^{65}$Zn and [1,2-$^3$H]testosterone by minced tissue of human benign hypertrophic prostates and the subcellular distribution of radioactivity were examined. The nature of steroid and $^{65}$Zn binding by the cytosol (105000 g supernatant) fraction was investigated by gel filtration, ion-exchange chromatography and electrophoresis.

It was found that steroid binding after incubation at 4 °C was specific. One or two regions of steroid binding were observed after gel filtration of the cytosol using Sephadex G-200, depending upon incubation conditions. Binding of $^{65}$Zn was found in the low molecular weight peak after G-200 gel filtration. Equimolar CdCl$_2$ and $^{65}$ZnCl$_2$ were incubated with [1,2-$^3$H]-testosterone and minced tissue and the cytosol was subjected to gel filtration. Compared with control values, without CdCl$_2$, reduction of $^{65}$Zn binding by about 50% occurred, while binding of $^3$H-labelled steroid was unaffected. Electrophoresis and ion-exchange chromatography showed that $^{65}$Zn and $^3$H-labelled steroid were bound to different proteins. A sample of the zinc-binding protein was prepared by ion-exchange chromatography and the homogeneity was checked by electrophoresis.

INTRODUCTION

Recently, the mode of action of testosterone has been widely studied, especially in the rat ventral prostate. The presence of androgen receptors in the cytosol and nuclei of this gland (Mainwaring, 1969; Unjhem, Tveter & Aakvaag, 1969), and the existence of the ‘active androgen’ dihydrotestosterone (17β-hydroxy-5α-androstan-3-one) (Anderson & Liao, 1968; Bruchovsky & Wilson, 1968; Mainwaring, 1969; Unjhem et al. 1969), have been demonstrated. Mainwaring (1970) has separated the 5α-reductase and androgen receptor components in the nucleus and cytosol. The presence of androgen receptors has also been demonstrated in the human prostate (Hansson, Tveter, Attramadal & Torgersen, 1971). Siiteri & Wilson (1970) observed

* Present address: Cookridge Radiation Research Centre, Cookridge Hospital, Leeds 16.
a fivefold increase in the dihydrotestosterone content of hypertrophic tissue compared with normal tissue, and suggest that it may be a causal agent in this condition.

The high zinc content of the rat dorsolateral prostate has long been recognized (Mawson & Fischer, 1952); known metalloenzymes account for only a fraction of this zinc (Vallee, 1959). Since the division of the human prostate into lobes is still controversial (Price, 1963; McNeal, 1970), the glandular distribution of zinc has been investigated (Kerr, Keresteci & Mayoh, 1960), as has the zinc content in various disease states (Mawson & Fischer, 1952; Whitmore, 1963). Uptake of $^{65}$Zn \textit{in vivo} is high (Prout, Sierp & Whitmore, 1959) and is androgen-dependent.

The prostatic secretion, the production of which is androgen-dependent (Huggins, 1946), is an important part of the seminal plasma and has a high zinc content. Zinc is necessary for sexual development (Sandstead, Prasad, Schultet, Farid, Miale, Bassilly & Darby, 1967; Caggiano, Schnitzler, Strauss, Baker, Carter, Josephson & Wallach, 1969) and fertility (Birnbaum, Hall & Lee, 1961), but the metabolic or structural role of zinc in this system is unknown.

Much of the seminal zinc is protein-bound and the secretory process can be accompanied by cell rupture (Mann, 1964), suggesting that this zinc comes from the prostate gland. Using histochemical methods, secretory granules containing zinc have been observed in the secretory epithelium of the human prostate (Maquinay, Timmermans & Gerebtzoff, 1963).

The relationship between zinc and testosterone has not been investigated at the cellular level. This paper describes investigations of the nature of uptake of testosterone and zinc by the human prostate and the intracellular fate of these compounds.

**MATERIALS AND METHODS**

**Radiochemicals and steroids**

$^{65}$ZnCl$_2$ in 0.1 M-HCl (sp. act. 20000 mCi/mg) and [1,2-$^3$H]testosterone (sp. act. 44.1 Ci/mM) were both obtained from the Radiochemical Centre, Amersham. [1,2-$^3$H]Dihydrotestosterone (sp. act. 44.0 Ci/mM) was manufactured by New England Nuclear Corporation and obtained from Micro-Bio Laboratories Ltd, 46 Pembroke Road, London, W. 11.

Non-radioactive steroids were kindly donated by Organon Laboratories Ltd.

**Other reagents**

Bovine serum albumin was obtained from Armour Pharmaceutical Co. Ltd, Eastbourne, and Aquacide I from Calbiochem Ltd, Wyndham Place, London. All reagents were of A.R. grade except barbitone (diethylbarbituric acid) and sodium barbitone (laboratory reagent grade, BDH), toluene (for scintillator II, laboratory reagent grade, Griffin & George Ltd) and PPO and dimethyl POPOP (scintillation grade, Nuclear Enterprises, Sighthill, Edinburgh). Visking dialysis tubing was purchased from the Scientific Instrument Centre Ltd, London.

**Tissue**

Benign hypertrophic human prostatic tissue, removed by retropubic prostatectomy, was obtained from St James’s Hospital, Leeds, and was kept on ice. Benign tissue was used since (a) it is readily available, and (b) its content and utilization of zinc do
Testosterone and zinc in human prostate

not differ significantly from normal prostatic tissue (Mawson & Fischer, 1952; Maquinay et al. 1963).

Paper chromatography

Homogeneity of radioactive steroids was checked regularly by chromatography on a Bush A system for 9 h, followed by scanning on a Nuclear Chicago Actigraph II radiochromatogram scanner.

Counting of radioactivity

Aqueous samples (0·5 ml) were mixed with 10 ml scintillator I (Gray & Shaw, 1965). Organic samples were evaporated to dryness and dissolved in 5 ml scintillator II (4 g butyl PBD/litre toluene). The samples were counted in a Packard Tricarb scintillation spectrometer (model 4322) at an efficiency of 21% (scintillator II) and 12% (scintillator I) for tritium, and 5% (scintillator I) for $^{65}$Zn.

We suggest that the $\gamma$ emission of $^{65}$Zn is measured in this system, via the Compton effect.

Quenching was determined by the internal standards method and was found to have no significant effect in these experiments.

Incubation of whole tissue

The tissue was washed with buffer, weighed and minced with scissors. It was incubated in Tris-HCl buffer (50 mmol/litre, pH 7·4) incorporating $^{65}$ZnCl$_2$ with ZnCl$_2$ carrier ($2 \mu$Ci/10 ml at a sp. act. of 1·6 $\mu$Ci/µg) and [1,2-3H]testosterone which had been evaporated to dryness under nitrogen and redissolved in two drops of redistilled propylene glycol. The amounts of tissue, [1,2-3H]testosterone and $^{65}$Zn normally used for cytosol studies were 10 g tissue, $2 \mu$Ci [1,2-3H]testosterone and $2 \mu$Ci $^{65}$Zn in 10 ml buffer. For differential centrifugation, the amounts used were 2·5 g tissue, $5 \mu$Ci [1,2-3H]-testosterone and $2 \mu$Ci $^{65}$Zn.

Incubations were carried out in a shaking incubator at 37°C for 1½ h. (From previous experiments, this time had been found to be optimal.) The buffer was removed by a pipette at the end of the incubation, and the tissue was washed with three 10 ml volumes of ice-cold buffer (non-labelled). It was homogenized in ice in a Waring blender by $5 \times 10$ s ‘bursts’ in buffer (0·5 ml/g tissue) and then centrifuged in an MSE Superspeed 40 at 105000 $g$ for 60 min at 4°C.

Cell fractionation

The homogenate, prepared as above but incorporating 0·25 m-sucrose, was filtered through coarse nylon gauze. Crude nuclear, mitochondrial, microsomal and cytosol fractions were prepared according to the procedure of Kowarski, Shalf & Migeon (1969). Each fraction was resuspended in buffer and recentrifuged. These fractions were extracted with two 3-ml volumes of diethyl ether and the extract was evaporated to dryness and counted in scintillator II. (This extraction removed 98% of $^3$H-labelled steroid.) After extraction, the particulate fractions were transferred in toto to 10 ml scintillator I and counted for $^{65}$Zn. A sample (0·5 ml) of the extracted 105000 $g$ supernatant (cytosol) fraction was counted for $^{65}$Zn.
Preparation and storage of cytosol

The tissue was treated in one of two ways: it was either (a) incubated, homogenized and centrifuged as described above, or (b) homogenized without prior incubation, then centrifuged at 105000 g as described above.

The cytosol, if not used immediately for incubations, was either stored at −15 °C (viable for up to 4 weeks) or, in case (b), lyophilized (viable for at least 18 months in a desiccator at 4 °C).

Labelling of cytosol prepared by method (b)

Samples (1 or 2 ml) of the cytosol preparation were incubated with 65ZnCl₂ in 50 ml Tris–HCl buffer (pH 7·4) and/or [l,2-3H]testosterone, evaporated under nitrogen and redissolved in one drop of propylene glycol. This was incubated for either (a) 45 min at 25 °C, or (b) 18 h at 4 °C, and chromatographed on Sephadex columns.

Gel filtration

Sephadex G-25

The gel was equilibrated in excess Tris–HCl buffer (50 ml, pH 7·4) at 4 °C for at least 4 h before being packed into 1 × 20 cm glass columns on a glass wool support. Fractions (1 ml) were collected manually, diluted to 3 ml, and radioactivity and optical density at 280 nm were measured.

Binding of 65Zn and [l,2-3H]testosterone was observed by incubating 0·05 µCi 65ZnCl₂ and 0·125 µCi [1,2-3H]testosterone with 1 ml cytosol as described above (at 37, 25 or 4 °C) and subjected to Sephadex G-25 chromatography as described above.

Further investigation was carried out by incubating duplicates of 1, 2, 3, 4 and 5 ng non-radioactive testosterone each with 1 ml cytosol and 0·125 µCi [1,2-3H]testosterone and a control, containing no non-radioactive testosterone, at 4 °C for 18 h. Each sample was then subjected to Sephadex G-25 chromatography.

Specificity of binding by the cytosol was investigated by incubating in duplicate 2 ng each of progesterone, dihydrotestosterone and testosterone at 4 °C for 18 h, employing the principle of competitive protein binding. Each was subjected to G-25 gel filtration as described and the total radioactivity coinciding with each peak absorbing at 280 nm was calculated.

Treatment with a proteolytic enzyme was carried out at 4 °C for 18 h. Cytosol (1 ml) was incubated with 0·125 µCi [1,2-3H]testosterone, 0·05 µCi 65ZnCl₂ and 10 mg pepsin at pH 6. Controls, containing no enzyme, were also incubated.

Sephadex G-200

The gel was equilibrated at 4 °C in excess buffer for at least 3 days. A Pharmacia column (gel size 2·5 × 38 cm), eluted at constant pressure, was used. Cytosol was chromatographed after preparation by the methods described above. Unlabelled cytosol (2 ml) was incubated with 0·1 µCi 65ZnCl₂ and 0·25 µCi [1,2-3H]testosterone at 25 °C or 4 °C as described previously.

Fractions of 3 ml were collected using an Ultrapac drop counter. The optical density at 280 nm was measured, the 3H-labelled steroid present was extracted with 2 × 3 ml ether and was counted in scintillator II. Samples (0·5 ml) of each fraction were then counted in scintillator I for 65Zn.
Electrophoresis of cytosol which had been incubated with $^{65}$ZnCl$_2$ and [1,2-3H]-testosterone at 4°C was carried out on Whatman no. 1 paper (cut into strips measuring $4 \times 27$ cm) in barbitone buffer at 350 V for 5 h. Samples of normal male serum were run at the same time for comparison. Barbitone buffer (pH 8-6, 60 mmol/l) was made up as an aqueous solution containing 7·36 g barbitone and 41·2 g sodium barbitone in 4 litres, preserved with 20 ml 5% thymol in ethanol.

The strips were stained by either (a) soaking in Lissamine Green (0-2% in 3% acetic acid) and destaining in 3% acetic acid, or (b) spraying with ninhydrin [1% (w/v) in acetone].

Unstained strips were cut into 1-cm sections and counted in aqueous scintillator for tritium and $^{65}$Zn.

**Ion-exchange chromatography**

DEAE-cellulose-Sephadex A-50 was equilibrated with Tris-HCl buffer (25 mmol/l, pH 7-4) at 4°C with repeated buffer changes until constant pH was attained. The slurry was poured on to a glass wool support in a column giving gel dimensions of $1 \times 28$ cm. A 1-cm layer of Sephadex G-25 was then added to prevent disturbance of the surface of the ion-exchange gel during the experiment. Gradient elution was carried out under constant pressure using 25 mM-Tris-HCl buffer. From fraction 10 to fraction 40, an ionic gradient from zero to 0·3 m-NaCl was employed at pH 7-4. Fractions (3 ml) were collected.

The fractions incorporating the final (lowest molecular weight) protein peak obtained after Sephadex G-200 chromatography were combined and dialysed for 18 h against Aquacie I to reduce the volume. The resulting preparation (2–3 ml) was then incubated at 4°C for 18 h with 0·25 μCi [1,2-3H]testosterone and 0·1 μCi $^{65}$ZnCl$_2$. Unbound steroid and $^{65}$Zn were removed by passing the incubated preparation through a Sephadex G-25 column (as described previously), allowing the eluate to drop directly on to the ion-exchange column.

The ionic strength of the final buffer was insufficiently high to elute serum albumin from the gel.

This method was used to prepare the zinc-binding protein analysed by Heathcote & Washington (1973). The fractions incorporating the protein peak were combined, reduced in volume by dialysis against Aquacie I, then dialysed against distilled water for 24 h. The solution was lyophilized before analysis.

**Polyacrylamide gel electrophoresis**

Volumes of 0·2 ml of the protein solution were subjected to electrophoresis before lyophilization.

Electrophoresis was carried out in glass tubes (internal diameter $5 \times 85$ mm) which contained a large-pore spacer gel (2·5% acrylamide, length 7 mm) above a small-pore running gel (7% acrylamide, length 70 mm). The protein sample was applied in 40% sucrose solution and the run carried out in Tris-glycine buffer (6·0 g Tris and 28·8 g glycine/l, pH 8·3) diluted ten times immediately before use. Bromophenol blue (0·001%) was incorporated in the sample as a tracking dye. The run, at
4 mA/gel, was continued until the tracking band was within 5–10 mm of the lower end of the gel.

Dialysis

On dialysing for 24 h a 2 ml sample of the prostatic cytosol labelled with $^{65}$Zn against Tris-HCl buffer, radioactivity due to $^{65}$Zn was reduced to background level in the cytosol.

RESULTS

Cell fractionation

The results of the cell fractionation are shown in Fig. 1. Losses in radioactivity arose from (a) activity which remained in the incubation buffer and (b) activity which was taken up by cells which were not broken by homogenization and therefore discarded.

The uptake of $^{65}$Zn by the supernatant fraction was lower than that by the crude nuclear fraction. This corresponds with the reported distribution of endogenous zinc levels (Mawson & Fischer, 1952; Kar & Chowdhury, 1966). The uptake of $^3$H-labelled steroid followed a different pattern; the nuclear uptake was exceeded by the supernatant uptake. There appear to be no reports in the literature of subcellular distributions of tritium in the human prostate after uptake of [$^3$H]testosterone, but work on the rat ventral prostate shows considerable variations, depending upon experimental procedure (Mangan, Neal & Williams, 1967; Tveter, 1969). In view of the differences in structure and function between the rat ventral prostate and the human
benign hypertrophic prostate, it would be unwise to make close comparisons until the androgen-binding system of the human prostate has been clarified further. Moreover, the absence of added cofactor in this experiment would limit the reduction of

\[ ^{3} \text{H}\text{testosterone} \rightarrow ^{3} \text{H}\text{dihydrotestosterone} \]

according to the availability of endogenous reduced NADP and, hence, nuclear labelling.

The cytosol was selected for further study as this would include the secretory granules, presumed to contain the zinc of the prostatic secretion.
The functions of zinc in the prostatic cell are likely to be more diverse than those of testosterone: therefore, the distribution of $^{65}$Zn need not necessarily reflect involvement in the secretory function in which we are interested.

_Gel filtration on Sephadex G-25_

After gel filtration on Sephadex G-25 of cytosol labelled at any of the three temperatures with either $^{65}$ZnCl$_2$ or [3H]testosterone, two radioactive peaks were observed. The radioactivity which was eluted with the protein peak was assumed to represent binding, and that in the second peak to represent unbound ligand. Typical elution profiles after incubation at 4°C are shown in Figs 2(a) and 2(b).

![Graph](https://via.placeholder.com/150)

Fig. 3. Competition of [3H]testosterone with unlabelled testosterone for protein binding sites in prostatic cytosol. The reduction in bound d.p.m. observed at each point represents the degree of competition.

Figure 3 shows the curve obtained after plotting decrease in bound radioactivity (d.p.m.) against incorporated non-radioactive testosterone. When radioactive steroid is diluted by non-radioactive steroid, the specific activity is effectively reduced and proportionately fewer radioactive molecules are bound if the protein is specific and of limited capacity. ‘Non-specific receptors’ tend to be of high capacity: therefore, the principles of competitive protein binding do not apply.

Figure 4 shows the displacement of bound $^3$H-labelled steroid by competing non-radioactive steroids. Progesterone does not compete with testosterone for binding sites but dihydrotestosterone does – a response which is also compatible with androgen specificity of the binding protein at 4°C.

Treatment of the cytosol with pepsin followed by chromatography on Sephadex G-25 caused breakdown of the peaks absorbing at 280 nm compared with those of the controls. Bound radioactivity in this region was also considerably reduced.

_Gel filtration on Sephadex G-200_

The elution profile resulting from chromatography of the prostatic cytosol after incubation of whole tissue is shown in Fig. 5. Two peaks of tritium corresponding
with protein peaks were obtained, and $^{65}\text{Zn}$ was found almost entirely in the final (low molecular weight) peak, although there was sometimes some labelling of the central peak. The elution volume of the latter corresponded with that of serum albumin, so it is suggested that this may be due to the binding of $^{65}\text{Zn}$ by serum albumin in the preparation.

Fig. 4. Study of specificity of steroid binding by human prostatic cytosol proteins. The histograms represent the d.p.m. of $^3\text{H}$-labelled steroid bound after incubation of $[^3\text{H}]$testosterone with cytosol and each of the steroids testosterone (T), dihydrotestosterone (DHT), and progesterone (P). The vertical lines indicate the range of the duplicates.

Incorporation of equimolar concentrations of cadmium (as CdCl$_2$) with $^{65}\text{ZnCl}_2$ in the incubation medium resulted in reduced zinc binding by the cytosol as shown in Fig. 6. Steroid binding was not affected.

The profile obtained after incubation of cytosol with $[^3\text{H}]$testosterone and $^{65}\text{ZnCl}_2$ at 4 and 25 °C is shown in Fig. 7. Only the low molecular weight region was labelled with $^3\text{H}$ after incubation at 4 °C, but there was some labelling in the higher molecular weight region at 25 °C. Labelling with $^{65}\text{Zn}$ did not alter with the method of incubation.

Incubation of 17 000 d.p.m. [1,2-$^3\text{H}$]dihydrotestosterone with 2 ml prostatic cytosol at 4 °C resulted in labelling in the same two regions that were labelled after incubation of whole tissue with $[^3\text{H}]$testosterone. This suggests that metabolism of testosterone to dihydrotestosterone may occur under certain conditions in vitro, probably being dependent upon temperature and/or the presence in the system of cell organelles, particularly nuclei. Temperature sensitivity of rat ventral prostate receptors has been reported by Mainwaring (1969).

The possibility was considered that tritium labelling in the intermediate peak was due not to steroid binding by a specific prostatic protein but to $[^3\text{H}]$testosterone binding by serum albumin as a contaminant, similar to the $^{65}\text{Zn}$ binding by serum albumin.
albumin suggested earlier in this section. However, after incubation of \(^{3}H\)testosterone with serum albumin under similar conditions, followed by chromatography on Sephadex G-200, no labelling of the serum albumin was observed.

Elution of unbound \(^{3}H\)testosterone and \(^{65}Zn\)Cl\(_2\) in a separate experiment showed that both were eluted with the same elution volume as the low molecular weight protein peak.

![Graph](image)

**Fig. 5.** Profile of human prostatic cytosol proteins eluted from a Sephadex G-200 column after incubation of 10 g prostatic tissue with 2 \(\mu\)Ci each of \(^{3}H\)testosterone and \(^{65}Zn\)Cl\(_2\) in Tris-HCl buffer at 37 \(^\circ\)C. Absorbance (E) at 280 nm (○); radioactivity due to \(^{65}Zn\) (●); radioactivity due to \(^{3}H\) (●). The arrow indicates the position of elution of unbound \(^{65}Zn\) and \(^{3}H\)testosterone.

**Paper electrophoresis**

The pattern observed after staining electrophoresis strips with Lissamine Green was essentially similar to the pattern of normal male serum, but more bands were apparent from the prostate cytosol after staining with ninhydrin. This is probably a result of the method of staining; soaking in an aqueous solution of Lissamine Green elutes low molecular weight proteins, while the heating and spraying treatment for ninhydrin staining would tend to ‘fix’ all molecules on to the paper, so that all were stained.

Separation of \(^{65}Zn\)- and \(^{3}H\)-labelling, corresponding with stained protein bands, was observed, showing that the zinc- and steroid-binding proteins in the final G-200 peak are separable by difference in charge.

Electrophoresis of \(^{65}Zn\)-labelled human serum showed \(^{65}Zn\) binding in the serum albumin region.

Results obtained using different times for electrophoresis indicated that the peak of radioactivity was always associated with the same two bands, which could always be distinguished by the different colours formed with ninhydrin.
**Testosterone and zinc in human prostate**

**Ion-exchange chromatography**

The elution profile after ion-exchange chromatography of the low molecular weight peak from Sephadex G-200 gel filtration is shown in Fig. 8. As observed after paper electrophoresis, the zinc- and steroid-binding proteins were separated by difference in charge.

Only one protein band, stained with Amido Schwarz (naphthol blue-black, 1 g/100 ml 7% acetic acid), was obtained on polyacrylamide gel electrophoresis. This suggests that the protein is probably homogeneous.

![Graph](image)

**Fig. 6.** Profile of human prostatic cytosol proteins eluted from a Sephadex G-200 column after incubation of 10 g prostatic tissue with 2 µCi each of \(^{3}H\)testosterone and \(^{65}Zn\) and equimolar Cd as CdCl\(_2\) in Tris-HCl buffer at 37 °C. Absorbance \((E)\) at 280 nm (○); radioactivity due to \(^{65}Zn\) (○); radioactivity due to \(^{3}H\) (○). The arrow indicates the position of elution of unbound \(^{65}Zn\) and \(^{3}H\)testosterone.

Elution of unbound \(^{3}H\)testosterone and \(^{65}Zn\)Cl\(_2\) in a separate experiment, under the same conditions, showed that \(^{65}Zn\) was eluted five fractions earlier than bound \(^{65}Zn\); \(^{3}H\)testosterone was eluted shortly after unbound \(^{65}Zn\) and 12 fractions earlier than bound steroid.

The use of electrophoresis and ion-exchange chromatography showed that both \(^{65}Zn\)- and \(^{3}H\)-labelled steroid were bound after incubation at 4 °C.

**DISCUSSION**

It was concluded from these experiments that there are probably at least two steroid-binding proteins and one zinc-binding protein in the cytosol of the human prostate. The zinc-binding protein can be separated from the steroid-binding protein in the low molecular weight region on the basis of molecular charge.
Mainwaring (1969) refers to 'specific' and 'non-specific' binding of administered testosterone, the radioactivity in the low molecular weight region being considered to be 'non-specific'. Unjhem & Tveten (1969), however, also observing uptake by

the rat ventral prostate, state that, after incubation of cytosol with \(^{3}H\)testosterone at 0°C, the radioactivity is eluted with 'low molecular weight' substances, which may indicate that they consider the testosterone to be unbound. However, the
experiments reported here show that (a) although the elution volumes of $^{65}$Zn and tritium on G-200 gel filtration after incubation at 4 °C are similar to the elution volumes of $^{65}$Zn and [3H]testosterone in the absence of cytosol, electrophoresis and ion-exchange chromatography prove that there is binding in this region, and (b) tests of specificity of testosterone binding at 4 °C indicate that it is specific under these conditions.

At 4 °C, labelling is found only in the lowest molecular weight peak after Sephadex G-200 gel filtration, whereas labelling at 25 and 37 °C occurred also in the intermediate peak. After incubation with [3H]dihydrotestosterone, labelling occurred in both peaks. It is, therefore, suggested that the labelling in the intermediate peak represents that observed by Hansson et al. (1971), and the labelling in the lowest molecular weight peak consists of both bound and unbound steroid, the binding here being specific, as observed in experiments with Sephadex G-25 filtration. Binding in the intermediate peak will occur after incubation with dihydrotestosterone itself or after reduction of testosterone to dihydrotestosterone, the incubation temperature, presence of cell organelles and probably the supply of reduced cofactors being important.

The vital role of zinc in sexual development and fertility, especially in man (Birnbaum et al. 1961; Sandstead et al. 1967; Caggiano et al. 1969), suggests that zinc is concerned with maintenance of the sex organs – a theory supported also by the destructive effects of cadmium (Pařížek, 1957) – and possibly spermatogenesis and sperm metabolism. Somers & Underwood (1969) noted defective protein and nucleic
acid metabolism in the zinc-deficient rat testis, and, based on experiments on the sexual organs of the pig (Prasad, Oberleas, Miller & Luecke, 1971), it has been suggested that zinc plays a role in the structural integrity of RNA and DNA.

The nature of zinc binding by the protein, while being clarified further by the work of Heathcote & Washington (1973), seems likely to be of only moderate firmness; binding occurs under mild conditions in vitro and $^{65}$Zn is removable by dialysis, but the binding is strong enough to withstand ion-exchange chromatography and electrophoresis. Cadmium is in the same group (II B) as zinc and it reduces zinc uptake by whole tissue, indicating some specificity of the system. If the function of the protein-bound zinc is to provide zinc for the metabolic requirements of the spermatozoa, then a readily dissociable, rather than a firmly bound, form of zinc would be appropriate. This would be a 'metal–protein complex' rather than the more firm 'metalloprotein' (Vallee & Wacker, 1970).

The observation that cadmium reduced $^{65}$Zn binding by the cytosol is interesting with regard to the damaging effects of cadmium on the reproductive tract of many animals (Pańzek, 1957; Aughey, 1970), the high incidence of prostatic carcinoma in chemical workers who are in contact with cadmium (Scott, 1970), and the ability of cadmium to inhibit enzymes by replacement of zinc in the molecule (Vallee, Riordan & Coleman, 1963).

As shown here, testosterone and zinc do not bind to the same protein, but more precise conclusions about the mode of action of testosterone on zinc uptake cannot be drawn from these experiments. It is suggested that experiments in vitro upon discrete cell types of the human prostate would be of value.

We thank the Medical Research Council who provided financial support for this work. The authors are grateful to Mr Smith, Urological Surgeon, of St James's Hospital, Leeds, for kindly providing tissue removed at operation, and to Professor P. N. Campbell of the Department of Biochemistry, University of Leeds, for advice. Dr J. G. Heathcote and Dr R. J. Washington of the University of Salford carried out an amino acid analysis of the zinc-binding protein; their results are published in the subsequent paper. Non-radioactive steroids were kindly donated by Organon Laboratories Ltd.

REFERENCES


Testosterone and zinc in human prostate


