Subcellular distribution of zinc in the benign and malignant human prostate: evidence for a direct zinc androgen interaction

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Abstract. The subcellular distribution of zinc and its interaction with androgens has been examined in the benign and malignant human prostate. Endogenously, most of the zinc was associated with the nuclear fraction but significant concentrations were also found in the cytosol. Furthermore, the epithelium contained more zinc than that found in either the stroma or the intact gland. Zinc concentrations were lower in the subcellular fractions of the cancerous tissue when compared to hyperplastic specimens.

In vitro uptake of zinc into prostatic homogenates was rapid and at equilibrium the binding was stable for both the 4°C and the 37°C incubations. At low zinc concentrations (≤ 5 nm) the uptake was higher in the nucleus, whereas at higher concentrations, the cancerous tissue exhibited a greater capacity for the metal which was predominantly retained by the cytosol. Our data suggest the presence of a saturable zinc retention mechanism in the nucleus. The zinc uptake was found to be independent of any added androgen. In contrast, the total androgen uptake by the prostate was significantly enhanced by the addition of zinc. This effect was not due to increases in the nuclear and cytosolic receptor binding since zinc inhibited the binding of the androgen to these receptors.

It has long been recognized that the human prostate gland contains a high concentration of zinc (Habib 1978). These concentrations are significantly lower in cancerous tissues (CaP) when compared to either the normal or hyperplastic (BPH) gland (Gyorkey et al. 1967; Habib 1978, 1980). Another factor which may affect the levels of the metal in the prostate is the stromal/epithelial composition of the tissue which not only varies from one gland to another but also between segments obtained from the same patient. Some early autoradiographic studies have suggested that high levels of zinc would be found in the cytoplasm of the epithelial cells (Siegel et al. 1961) but this was not supported by more recent experiments indicating that the zinc was accumulating in the nucleus (Dhar et al. 1973).

The in vivo uptake of zinc by the prostate in both human and experimental animals is increased by androgens and decreased by oestrogens (Millar et al. 1957; Schoonees et al. 1970). It is however, not possible at this stage to ascertain whether steroids were having a direct influence on the zinc uptake, or were inducing their effect by means of an indirect mechanism.

Much of the evidence on the role and distribution of zinc in the prostate gland has been gained from rat tissue but the gross differences in the histology and pathology between human and rat prostate gland make comparisons between the two species tenuous. The present investigations were therefore undertaken in order to establish a comprehensive study of the distribution of zinc in the human prostate gland and to elucidate the nature of the metallo/hormonal relationship of the prostate. These experiments were not only confined to the intact gland but were also pursued on the stromal/epithelial components of the benign and malignant tissue.

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Materials and Methods

Radioisotopes and other chemicals

Carrier-free $^{65}$Zn (0.5 Ci/mg), 5α-dihydro [1,2,4,5,6,7-3H]testosterone (94 Ci/mmol) were all obtained from the Radiochemical Centre, Amersham, UK [17α-methyl-3H]-methyltrienolone (87 Ci/mmol) from New England Nuclear Ltd., Dreieich FRG. Non-radioactive steroids were obtained from Sigma, Poole, Dorset UK and all other chemicals were of an analytical reagent grade except for the zinc chloride (Specpure grade, Fisons Ltd., Leics. UK) and HCl (Aristar grade, BDH Chemical Ltd. Poole, Dorset, UK). The buffer systems used in the present study were as follows: Buffer 1 contained 10 mM tris and 1 g/l methylcellulose. Buffer 2 contained 10 mM tris and 1.5 mM EDTA. Buffer 3 contained 10 mM tris, 10 mM Na MO$_2$ and 10% (v/v glycerol). All the buffers were adjusted to a pH 7.4 with 0.1 N HCl.

Tissue preparation and incubations

Prostatic tissue was obtained by transurethral resection (TUR) and the specimens were transported to the laboratory in ice-cold saline solution and used immediately. Fragments from each specimen were examined histologically to determine the pathological state.

The epithelial and stromal fractions were prepared by the Cowan et al. (1976) method with a few modifications as detailed in our previous paper (Habib et al. 1981). The purity of the stroma and epithelium was assessed by light microscopy and the results were confirmed by biochemical assays using acid phosphatase (Mahan & Doctor 1979) and hydroxyproline (Videman et al. 1981) as markers for establishing cell purity.

Subcellular fractionation was achieved by centrifuging the tissue at 400 × g and the resultant pellet was resuspended in 5 volumes of Buffer 1 and rehomogenised. The homogenates were subsequently centrifuged at 800 × g to obtain the crude nuclear pellet and the supernatant fraction was spun again at 15 000 × g to produce the mitochondrial pellet. Subsequent centrifugation of the resultant supernatant at 105 000 × g provided the microsomal fraction and the cytosol. The purity of the subcellular fractions was checked by using marker enzymes: glucose 6-phosphatase for the microsomes (De Duve et al. 1955), malate dehydrogenase for the mitochondria (Beattie et al. 1963) and lactate dehydrogenase for the cytosol (Beaufay et al. 1959).

The measurement of endogenous zinc followed the procedure outlined by Habib et al. (1976).

The in vitro uptake of zinc was assessed by homogenising the tissue in Buffer 1 and incubating the resultant pellet with increasing amounts of zinc (30 000 CPM $^{65}$Zn + 50 µM to 500 µM cold zinc) in triplicate at 4°C and 37°C for various times. The incubations were terminated by centrifuging the incubation mixtures at 800 × g and washing the resultant pellets three times in Buffer 2 containing 10 mM EDTA to remove residual and non-specifically bound zinc (Giles & Cousins 1982). The levels of $^{65}$Zn specifically bound to the tissue were assessed by means of a gamma scintillation counter. To study the effects of androgens on the uptake of $^{65}$Zn, 10 µl ethanolic solutions containing either testosterone or DHT (50 µM, 500 µM and 5 mM) were added to a 100 µl Buffer 1 solution containing increasing amounts of zinc (50 000 CPM $^{65}$Zn + 50 µM to 500 µM cold zinc) and these were, in turn, mixed with freshly dispersed BPH and CaP tissues and incubations were allowed to proceed under the conditions outlined in the results section.

![Fig. 1.](https://www.bioscientifica.com)
Fig. 2.
The effect of time on the uptake of zinc by 8 benign prostatic tissues following incubation at 4°C (○—○) and 37°C (●—●). The concentration of zinc used was 500 µM and each incubation was performed in triplicate. Values represent mean ± SEM.

Fig. 3.
The effect of zinc concentration on the uptake of zinc into 8 BPH (●—●) and 6 CaP (○—○) following 1 h incubation at 37°C. Each incubation was performed in triplicate and values represent mean ± SEM.
In order to estimate the effect of zinc on the total androgen uptake, aliquots of the dispersed tissue were incubated with either [3H]testosterone or [3H]dihydrotestosterone (20,000 CPM + 5–500 μM of cold androgen) in the presence of increasing concentrations of zinc (0–50 mM) for 2 h at 37°C. At the end of the incubation the steroids were extracted and counted. We also investigated the effects of zinc on androgen receptor binding. Receptors were estimated by the method of Smith et al. (1983) with the omission of EDTA and DTT from the incubation mixtures (Buffer 3).

Results

Subcellular distribution of endogenous zinc

The results in Fig. 1 indicate that the bulk of the zinc was associated with the nuclear fraction of all tissue components. Nonetheless, significant amounts were also associated with the cytosol. The levels of zinc in the mitochondria and microsome were minimal. Subcellular fractions prepared from epithelial cells contained more zinc than those prepared from either the intact gland or the stroma.

Zinc concentrations in the nucleus and cytoplasm of the hyperplastic tissue were significantly greater than those measured in the corresponding CaP fraction (P < 0.05). No significant differences were detected between mitochondrial and microsomal fractions of the two tissue types (P > 0.05).

Uptake of exogenous zinc — time and temperature studies

The results in Fig. 2 demonstrate that the uptake of exogenous zinc by the prostate tissue is rapid at both 4°C and 37°C. The maximal zinc uptake was achieved after 30 and 45 min for the 37°C and 4°C incubations respectively. Once equilibrium was achieved the binding of the zinc remained stable throughout the duration of the experiment (20 h).

Zinc concentration studies

Fig. 3 suggests that when zinc is added at low concentrations (5 mM or less) the uptake of metal into BPH is 58% greater than that taken up by the cancerous specimens. However, at higher doses the amount of zinc associated with cancerous tissue exceeds that which was measured in the hyperplenced specimens by an average of 43%. Fig. 4 illustrates the subcellular distribution of zinc taken up by the tissue following 1 h incubation at 37°C.

Incubation with 5 mM zinc produces a subcellular distribution pattern similar to that observed in the endogenous system, with the bulk of the zinc associated with the nuclear fraction, but also, significant amounts were measured in the cytosol. However, when the tissues were incubated with high concentrations of zinc, the nuclear levels were elevated only marginally, whilst the bulk of the metal was now associated with the cytoplasm.

The effect of androgens on the uptake of zinc

Our data suggest that testosterone and dihydrotestosterone fail to influence the uptake of zinc into
either BPH or the cancerous tissues under any of the conditions employed in the present study (data not presented).

The effect of zinc on the uptake of androgens
Fig. 5 demonstrates that the uptake of androgens by the tissue was significantly increased by concentrations of zinc in excess of 10 mM in a dose-related manner ($P < 0.05$). This effect was independent of the pathological state of the gland.

The effect of zinc on the specific binding of androgens to the cytosol and nuclear fractions
The results outlined in Fig. 6 demonstrate that zinc inhibits the specific binding of [3H]R1881 and [3H]DHT to both the cytosol and nuclear androgen receptors prepared from either the BPH or the CaP specimens; the inhibition was manifested at concentrations of zinc of 5 mM or greater ($P < 0.05$).

Discussion
The present study highlights once again the fundamental distribution that must be made between endogenous measurements and in vitro uptake studies. The data outlined in this paper clearly demonstrates that the subcellular distribution of
endogenous and exogenous zinc do not always follow the same pattern. The distribution of zinc was found to be similar only when the in vitro incubations were carried out in the presence of low zinc concentrations (<5 mM). Under these conditions the amount of zinc associated with nuclear fractions exceeds that measured in the cytosol from both BPH and CaP specimens.

Furthermore, the zinc content of the fractions prepared from BPH tissues were always greater than those measured in the corresponding CaP specimens; this was previously reported (Dhar et al. 1973; Gyorkey et al. 1967; Habib 1978; Habib et al. 1979). If however, the uptake studies were performed in the presence of a higher cation concentration (>5 mM) the capacity of the cancerous tissue to take up the zinc far exceeds that the BPH, thus confirming an earlier observation made by Gyorkey & Sato (1968). It would therefore appear that the increase in zinc uptake manifested by the cancerous tissue at high cation concentrations was related to a change in the subcellular distribution of the zinc. Firstly we have the initial uptake of zinc, mostly confined to the nucleus and

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**Fig. 6.**
The effect of zinc on the specific binding of radiolabelled R1881 and radiolabelled DHT to the cytosolic and nuclear receptor. o—o BPH, —— CaP. Values represent mean ± SEM.
on reaching an average concentration of 5 nmol zinc/g tissue, nuclear saturation is achieved and the bulk of the exogenous zinc is directed towards the cytoplasm (Fig. 4). Although the initial zinc uptake by the nucleus of the CaP specimens is significantly lower than that exhibited by the benign tissue (Fig. 3) the final nuclear zinc concentrations for the two tissue types was similar (Fig. 4). Since nuclear saturation precedes any major zinc uptake by the cytoplasm, our measurements indicate that the localisation of zinc in the nucleus is of a higher affinity but lower capacity. The mechanism responsible for the preferential zinc uptake is not known, but specific binding molecules may be involved (Reed & Stitch 1973).

Another interesting feature of the present report regards the failure of androgens to induce increases in the total zinc uptake: this is in conflict with earlier trends (Millar et al. 1957; Schooness et al. 1970) which may have been instigated by an indirect mechanism. We also note that the zinc uptake studies were not affected by the supplementation of the incubation medium with the testosterone or dihydrotestosterone, whereas androgen uptake was significantly increased in the presence of zinc; this effect was not mediated by the androgen receptors in the tissue since this binding was inhibited by the cation. Although similar patterns were observed in other animals, the effect is species dependent (Dube & Tremblay 1974; Liao et al. 1975) and underlines some of the problems one encounters when comparing animal studies and experiments on human tissue.

Clearly, further studies are required to elucidate the mechanism responsible for these conflicting patterns, nonetheless, the present study suggests that zinc plays a major role in maintaining the function and well-being of the human prostate.

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References


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