Zinc binding properties of human prostatic tissue, prostatic secretion and seminal fluid

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Summary. When studied by gel filtration, zinc in prostatic cytosol (10^5 g, 1 h) was associated with high ($>80\,000$), medium (3000 – $80\,000$) and low molecular weight (<3000) molecules in approximately equal proportions. The molecules of high and medium M_r were not secreted into the prostatic fluid where all the zinc was associated with molecules of low M_r (probably citric acid). After ejaculation much of the zinc is redistributed and becomes bound to molecules of high and medium M_r of vesicular origin.

Introduction

Zinc concentrations in normal human seminal plasma are high in comparison to those in other body fluids and tissues. This zinc is secreted predominantly by the prostate gland (Mann & Lutwak-Mann, 1981). While much attention has been paid to the levels of seminal or prostatic zinc associated with different fertility states or different prostate pathologies, few investigators have considered the zinc-binding properties of seminal fluid. Whether the zinc is free or protein bound could markedly affect its biological activity. Arver (1980) investigated the zinc-binding molecules in seminal plasma by gel filtration and reported that high, intermediate and low molecular weight molecules were present. A surprising observation was a correlation between zinc bound to high molecular weight proteins and fructose, suggesting a vesicular origin for this component. Experiments performed with split ejaculates have tended to confirm this conclusion and support the concept of a post-ejaculatory redistribution of zinc from molecules of low molecular weight to those of high and intermediate M_r values (Arver, 1982a; Arver & Eliasson, 1982).

In the present study the zinc-binding properties of 'pure' prostatic secretion, prostatic cytosol and seminal plasma were compared.

Materials and Methods

Expressed prostatic secretion was obtained by digital rectal massage from men with apparently normal prostates or benign prostatic hyperplasia (Kavanagh, Darby & Costello, 1982). Semen samples were obtained from donors providing specimens for artificial insemination. Seminal plasma was prepared by low speed centrifugation. Prostatic cytosol was prepared from tissue from prostates showing benign hyperplasia obtained by resection or open surgery. This tissue was finely chopped, frozen in liquid N_2 and disrupted in a Braun Melsungen Mikro-Dismembrator (15 mm deflection for 15 sec). The powder was suspended in 10–20 volumes of 0.05 M-Tris-HCl pH 7.5 and

centrifuged at 100 000 *g* at 4°C for 1 h. The supernatant was freeze-dried and re-suspended in 0.5–1 ml buffer.

Zinc concentrations were determined by atomic absorption spectroscopy (Kavanagh & Darby, 1982). Fructose was determined by a resorcinol method (Polakoski & Zaneveld, 1977).

For column chromatography, up to 100 µl of sample containing 10–80 µg zinc were applied to a G-75 Sephadex column (9 × 150 mm). Constant volume fractions of about 0.3 ml (checked gravimetrically) were collected. The fractions were diluted to 3 ml with 0.1 M-nitric acid before zinc determination. Protein elution was monitored by the absorbance at 280 nm.

Results

Purity of prostatic fluid. In a previous study (Kavanagh & Darby, 1982) it was noted that the urea concentration of 69 samples of prostatic fluid was very similar to that of serum. This has now been confirmed in a series extended to 107 samples, of which only 15 would be classified as having above normal serum urea levels. Urinary contamination of expressed prostatic secretion is not therefore considered a significant problem. The fructose concentrations of a series of 57 prostatic fluid samples had a mean of 1.17 mM and a median of 0.40 mM. Taking 30 mM as an upper limit for normal seminal fructose (Mann & Lutwak-Mann, 1981) then prostatic fluid samples having fructose concentrations higher than 1.5 mM (>5% of maximum seminal level) might be considered as contaminated with seminal vesicular secretion. On this basis 8 of the 57 samples tested were contaminated. Of the 5 prostatic fluid samples used in the gel filtration studies 2 formed part of this series of 57. Their fructose concentrations were 0.7 and 1.2 mM.

Gel filtration. Five samples of prostatic cytosol, expressed prostatic fluid and seminal plasma were chromatographed through G-57 Sephadex. A representative zinc elution profile for each sample type is shown in Text-fig. 1, in which the volumes I, II and III correspond to the high,

Text-fig. 1. Representative zinc elution from G-75 Sephadex for prostatic fluid (—), seminal fluid (---) and prostatic cytosol (....).

intermediate and low molecular weight regions used by Arver (1980) (<80 000, 3000–80 000 and <3000 respectively). The zinc in prostatic cytosol was distributed fairly evenly between the 3 types of molecules while that from expressed prostatic fluid entirely associated with the low M_r components. In seminal plasma much of the zinc was bound to molecules of high and intermediate molecular weight (Table 1).

Table 1. Proportion of zinc (mean and range) associated with binding molecules of high, intermediate and low molecular weight (regions I, II and III, respectively, of Text-fig. 1)

	% of total zinc		
	Region I	Region II	Region III
Prostatic cytosol	39.6 (29.0–50.1)	24.4 (13.3–28.8)	35.0 (24.2–57.5)
Prostatic fluid	2.5 (1.1–3.6)	1.4 (0.8–3.1)	96.1 (93.3–98.0)
Seminal fluid	22.5 (17.4–26.4)	24.9 (10.0–34.6)	52.6 (46.0–72.6)

Comparison of the seminal plasma and prostatic fluid results suggests a post-ejaculatory redistribution of zinc from low to higher molecular weight molecules, the extent of which appears to be related to the amount of vesicular fluid present in semen (estimated by fructose concentration) (Text-fig. 2).

Text-fig. 2. Relationship between zinc from different molecular weight regions and fructose concentration from 2 prostatic and 5 seminal fluid samples (●, region I; ○, region II; ▲, region III).

Discussion

It is clear from these results that prostatic cytosol contains molecules of high and intermediate molecular weight which are not secreted into the prostatic acini. These are likely to be mainly epithelial in origin, where they must function intracellularly and may be concerned with accumulation of zinc or intracellular zinc transport. It has been observed that $^{65}\text{ZnCl}_2$, incubated with prostatic cytosol, eluted from G-200 Sephadex in the low molecular weight region (Reed & Stitch, 1973). Further purification led to the isolation of a histidine-rich zinc-binding protein, whose molecular weight was estimated at 32 000 (Heathcote & Washington, 1973). Cells from a human prostatic adenocarcinoma cell line accumulate $^{65}\text{Zn}^{2+}$ which is associated with high and intermediate molecular weight molecules. The molecule of intermediate M_r may be metallothionein (Giles & Cousins, 1982). It has been suggested that the accumulation of prostatic zinc, via specific proteins (whose synthesis would be under androgenic control), could exert a feedback control on zinc uptake by inhibition of prostatic testosterone metabolism (Wallace & Grant, 1975).

The almost complete absence of zinc-binding molecules in prostatic fluid is surprising in view of reports of seminal zinc-binding glycoproteins which may originate from the prostate (Herrmann, 1975; Clavert, Montagnon & Brun, 1980). However both of these were electrophoretic studies and no attempt was made to quantify the proportion of free and bound prostatic fluid zinc. A low molecular weight zinc-containing antibacterial factor has been isolated from prostatic fluid and identified as mainly ZnCl_2 (Fair, Couch & Wehner, 1973). This preparation involved ion exchange chromatography which was likely to separate Zn^{2+} from any natural binding molecules. Arver (1982b) has presented convincing evidence that the zinc-binding molecule of low M_r in seminal fluid is citrate. This is present in prostatic fluid in a 10-fold molar excess over zinc (Kavanagh & Darby, 1982) and both co-eluted from a G-15 Sephadex column. The major zinc-binding molecule of low M_r in human milk is also thought to be citrate (Lonnerdal, Stanislawski & Hurley, 1980; Martin, Licklider, Bushmiller & Jacobs, 1981; Arver, 1982b).

Evidence presented here (Text-fig. 2) and elsewhere (Arver, 1980; Arver & Eliasson, 1982) suggests that the zinc-binding molecules of high and intermediate molecular weight in seminal plasma are of vesicular origin. The intermediate M_r components may be breakdown products of the high M_r molecules which are glycoproteins (Arver & Eliasson, 1982).

The significance of the post-ejaculatory redistribution of zinc is unclear but is suggestive of a process which requires activation or inhibition, the initiation of which needs to be synchronized to ejaculation. The obvious process is coagulation/liquefaction, and it has been suggested that the human coagulum is a glycoprotein matrix stabilized by divalent metal ions (Daunter, Hill, Hennessey & Mackay, 1981). Other post-ejaculatory changes occur in seminal plasma. Well known are the hydrolysis of phosphorocholine by prostatic acid phosphatase and extensive proteolysis (Mann & Lutwak-Mann, 1981). There is also a decrease in ionized calcium associated with an increasing pH and, although calcium is mainly excreted by the prostate, there is more ionized calcium in vesicular fractions than in prostatic fractions of split ejaculate semen (Arver & Sjöberg, 1982).

These post-ejaculatory changes indicate that semen is a dynamic system that should not be considered simply as the sum of individual accessory fluids and spermatozoa.

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