Sodium, potassium, calcium, magnesium, zinc, citrate and chloride content of human prostatic and seminal fluid

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Summary. The ionic composition of human prostatic fluid varied greatly between individuals, reflecting the secretory activity of the gland and the presence or absence of prostatic inflammatory disease. In normal prostatic fluid the major anion was citrate, while chloride concentrations were lower. Their counterions were mainly sodium and potassium, together with calcium, magnesium and zinc. Prostatic secretions from men with prostatitis comprised mainly sodium and chloride. The electrolytes were closely correlated to each other (except for sodium, which was essentially invariant at about 145 mM). The molar changes per mole of citrate were about 0.52, potassium; −0.53, chloride; 0.17, calcium; 0.14, magnesium; and 0.09, zinc. The pH was also associated with citrate, decreasing from 8.0 to 6.2 as the citrate increased. These various ionic changes can be explained as responses to citrate secretion, without the need to propose specific transport mechanisms for the other ions measured. The marked effect of prostatic inflammation on the composition of prostatic fluid can be seen as being due mainly to decreased secretion rather than active modification.

Introduction

Human seminal plasma contains citrate, zinc, calcium and magnesium, secreted mainly by the prostate gland, in much higher concentrations than in other body fluids. This has prompted a number of studies and the concentrations of these ions in seminal and prostatic fluid are well documented (Mann & Lutwak-Mann, 1981). Less attention has been paid to sodium, potassium and chloride, particularly in prostatic secretion. Sodium (153 mM), potassium (48 mM) and chloride (38 mM) have been reported for prostatic fluid (Huggins, Scott & Heinen, 1942) and these results are generally quoted (e.g. Zaneveld & Tauber, 1981; Isaacs, 1983). Seminal plasma sodium and potassium have commonly been found to be in the range 110–120 mM (Na) and 20–30 mM (K) (Huggins et al., 1942; Skandhan & Mazumdar, 1981). From the published data it is not possible to establish the relative contribution of the prostate to seminal sodium, potassium and chloride content, the variation in their concentrations or their relationship to each other and other ions.

It is possible, however, to conclude that citrate, calcium, magnesium and zinc are positively correlated to each other and to other secretory products such as acid phosphatase, aminopeptidase and ATPase and that their levels show great inter-individual variation, being markedly reduced by the presence of inflammatory prostatic disease (Bostrom & Anderson, 1971; Colleen, Mardh & Schytz, 1975; Fair & Cordonnier, 1978; Hommonai, Matzkin, Fainman, Paz & Kraicer, 1978; Kavanagh & Darby, 1982, 1983; Kavanagh, Darby & Costello, 1982). There are no reports available of the simultaneous study of all these electrolytes in a large series of prostatic and seminal fluid samples. Such a study is essential for the comprehensive assessment of the various ionic inter-relationships. A model for the androgenic control of prostatic citrate secretion at the level of the membrane-bound Na/K ATP-dependent pump has been proposed (Farnsworth, 1982). This incorporates a co-transport mechanism for sodium and citrate, which are claimed to be equally abundant in prostatic fluid (Farnsworth, 1980).
Materials and Methods

Expressed prostatic fluid was obtained by rectal massage from patients suffering from a variety of urological disorders. Such samples are relatively free of contamination by spermatozoa, urine or vesicular fluid (Kavanagh, 1983). The pH of the sample was noted immediately using Camlab Duotest pH papers and any remaining sample not required for bacteriological examination was frozen as soon as possible, normally within 2 h. Before analysis the samples were thawed, and aliquants were placed into separate containers and refrozen. Thus freezing/thawing operations were minimized. Samples were considered to be normal (from patients with healthy prostates) if there were no symptoms or history indicating prostatic disease and urine and prostatic fluid samples contained $< 10^5$ leucocytes/ml or $< 10$ leucocytes per high-power field ($\times 560$), respectively, and no bacteria could be isolated by routine bacteriological investigation.

Semen samples were obtained from men attending a fertility investigation clinic and seminal plasma was prepared by centrifugation (700 g, 20 min). In the absence of a series of samples from men of proven recent fertility, normal semen in this study is defined as having a volume of 2–6 ml, $\geq 20 \times 10^6$ spermatozoa/ml, $\geq 40\%$ motility, $\leq 40\%$ abnormal forms, normal viscosity (assessed subjectively) and a characteristic speed $\geq 30 \mu$m/sec (measured by a laser/Doppler method (Naylor, Martin & Chantler, 1982). Normal semen samples were also required to have normal prostatic and vesicular contributions (zinc, 1:2–3:8 mm; fructose, 6:7–33 mm) (Eliasson, 1982).

Calcium, magnesium and zinc were measured by atomic absorption spectroscopy at sensitivities of 1:0, 0:1 and 0:4 µm respectively. Sodium and potassium were measured by flame emission. All were found at more than 4 times their minimum detection values. Samples were diluted 200 or 1000-fold with 0:1 m-nitric acid for zinc assay. For calcium, magnesium, sodium and potassium they were diluted 1000-fold or greater if required with distilled water. Chloride was measured by specific ion electrode (Eil Electronics) at 25°C in a 20- or 40-fold dilution with 0:05 m-acetic/ammonium acetate buffer. To minimize sample requirements, a sample/AgCl-electrode chamber (volume = 0:4 ml) was connected to the reference electrode by a 0:2% agar/1 m-$(\text{NH}_4)_2\text{SO}_4$ bridge. Care was taken to rinse and dry the sample chamber between readings (which were performed in duplicate). The potential difference between the electrodes was proportional to the logarithm of the chloride concentration between 0:1 and 10 mm. Citrate was measured by a coupled enzyme assay (Kavanagh & Darby, 1982).

Citrate, zinc and pH were measured in 529 prostatic fluid samples. Of these 135 were also analysed for chloride, calcium, magnesium, sodium and potassium, 42 for chloride and 7 for calcium, magnesium, sodium and potassium.

Possible interference by each component in each assay system was examined. Over the relevant concentration ranges the only effect noted was enhanced sodium levels in response to potassium. This effect was so slight that no correction was made (140 µm-sodium was enhanced 4% by 150 µm-potassium).

Recoveries of added standards to prostatic secretion were 109% (sodium), 110% (potassium), 99% (calcium), 97% (magnesium), 108% (zinc), 101% (chloride), 97% (citrate). The % coefficients of intra-assay variation ($n = 10$) were 2:7, 2:9, 7:8, 5:7, 7:8, 4:9 and 4:0, respectively.

Results

Comparison of the measured electrolytes in ‘normal’ prostatic and seminal fluid (Table 1) confirms a largely prostatic origin for citrate, calcium, magnesium and zinc and suggests that the prostate is a major source of seminal potassium. The chloride concentration was almost identical in both groups, while sodium was slightly lower in seminal fluid, indicating similar contributory roles for these ions by the prostate and seminal vesicles. However, the two groups of normal samples are not strictly comparable, so more detailed conclusions cannot be drawn from these results.
Table 1. Summary statistics for ionic concentrations (mM) in normal human prostatic and seminal fluid

<table>
<thead>
<tr>
<th></th>
<th>Prostatic fluid</th>
<th>Seminal fluid</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Median</td>
</tr>
<tr>
<td>Citrate</td>
<td>159</td>
<td>94.2</td>
</tr>
<tr>
<td>Zinc</td>
<td>152</td>
<td>9.0</td>
</tr>
<tr>
<td>Calcium</td>
<td>34</td>
<td>18.5</td>
</tr>
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<td>Magnesium</td>
<td>34</td>
<td>16.3</td>
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<tr>
<td>Potassium</td>
<td>34</td>
<td>63.9</td>
</tr>
<tr>
<td>Sodium</td>
<td>34</td>
<td>149</td>
</tr>
<tr>
<td>Chloride</td>
<td>38</td>
<td>35.7</td>
</tr>
</tbody>
</table>

Text-fig. 1. Cumulative frequencies of sodium and citrate concentrations in prostatic fluid. 
○, sodium (n = 142); ■, citrate, prostatitis patients (n = 87); ▲, citrate, normal samples (n = 162); ●, citrate, all samples (n = 649).

Sodium concentrations in the prostatic fluid samples were approximately normally distributed with a narrow spread, values only rarely falling outside the range 120–170 mM (Text-fig. 1). There was no evidence of any relationship to inflammatory prostatic disease. Citrate concentrations were much more widespread and were closely related to the presence or absence of prostatitis (Text-fig. 1). The same was true of all the other ions studied.

Inspection of scattergrams of prostatic fluid concentration of each ion against each other suggested linear relationships for all except sodium. Correlation analysis confirmed the significance of these observations (Table 2). Increased citrate was accompanied by increased potassium, calcium, magnesium and zinc, and by decreased chloride (Text-fig. 2). The pH of the samples was also strongly correlated to citrate, ranging from about 8.0 at low/zero citrate to pH 6.2 at high citrate concentrations. It has been shown elsewhere that citrate, zinc and pH in prostatic secretion are also strongly correlated to secretory enzyme activities such as acid phosphatase, aminopeptidase and ATPase and all are strongly influenced by the presence or absence of prostatic inflammation (Kavanagh & Darby, 1982, 1983; Kavanagh et al., 1982). Consistent and significant relationships were also found between these enzymes, prostatitis and calcium, magnesium, potassium and chloride (data not shown).
Table 2. Correlation coefficients ($r$) and their significance ($P$) for ionic components of prostatic fluid

<table>
<thead>
<tr>
<th>Component</th>
<th>$r$</th>
<th>$P$</th>
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<tr>
<td>Zinc</td>
<td>0.87</td>
<td>*</td>
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<tr>
<td>Calcium</td>
<td>0.81</td>
<td>0.70</td>
</tr>
<tr>
<td>Magnesium</td>
<td>0.88</td>
<td>0.84</td>
</tr>
<tr>
<td>Potassium</td>
<td>0.78</td>
<td>0.69</td>
</tr>
<tr>
<td>Sodium</td>
<td>0.04</td>
<td>0.08</td>
</tr>
<tr>
<td>Chloride</td>
<td>-0.61</td>
<td>NS</td>
</tr>
<tr>
<td>pH</td>
<td>-0.64</td>
<td>-0.62</td>
</tr>
</tbody>
</table>

Citrate     Zinc     Calcium    Magnesium  Potassium  Sodium  Chloride

Number of samples, $\geq 135$.
NS, not significant ($P > 0.1$); *$P < 0.001$.

Text-fig. 2. Relationships of components of prostatic fluid to citrate (by linear regression, $y$ (mM) = $a$ citrate (mM) + $b$). Coefficients of the equations; $y = Na^+$, $a = 0.03$, $b = 142.3$; $y = K^+$, $a = 0.52$, $b = 14.2$; $y = Ca^{2+}$, $a = 0.17$, $b = 2.7$; $y = Mg^{2+}$, $a = 0.14$, $b = 2.1$; $y = Zn^{2+}$, $a = 0.09$, $b = 0.4$; $y = Cl^-$, $a = -0.53$, $b = 95.1$.

It is clear from Text-figs 1 and 2 that the sodium and citrate concentrations, while sometimes similar, are not generally equal as has been suggested (Farnsworth, 1980, 1982).

Discussion

The regression analysis (Text-fig. 2) enables prediction of approximate prostatic fluid ionic compositions at different citrate concentrations (Text-fig. 3). There is a strong resemblance between blood plasma values and those of prostatic fluid, extrapolated to zero citrate, suggesting that in the absence of active secretion the fluid present in the glandular acini may be quite similar to ultrafiltrated blood plasma. The composition of prostatic fluid might therefore be explained as the result
of modifying secretory activity superimposed on this starting material. The formation of other secretions, e.g. saliva and pancreatic juice, is also believed to be achieved by modification of an initially plasma-like primary fluid (Prince, 1977) and a similar model has been proposed for the formation of canine prostatic fluid (Isaacs, 1983). It is clear from Text-fig. 2 that the dominant ionic process in the formation of human prostatic fluid is citrate secretion. Such an activity cannot be considered in isolation, and some quantitative and qualitative consequences of citrate secretion are now considered.

Citrate has a high affinity for calcium, magnesium and zinc (log $K$ $\geq$ 3.4; $M^{2+} + HL_3^{-} \leftrightarrow MHL^{-}$; 0.1 M ionic strength; 20°C; Sillen & Martel, 1964, 1971). These metals might therefore be co-transported as a citrate complex or a transmembrane citrate gradient could drive their passive or facilitated diffusion. Either of these mechanisms is likely to result in calcium, magnesium and zinc energy-independent secretion, each related linearly to secreted citrate levels. This fits well with the observed results (Text-fig. 2), and there is good evidence for a direct association of prostatic citrate and zinc (Arver, 1982; Kavanagh, 1983). As the citrate concentration is more than double the total divalent metal concentration, not all the citric acid groups will be metal associated. At prostatic fluid pH values of more than 6.2 the non-complexed carboxylic acid groups would be almost fully dissociated (pK1 = 5.6–5.8; 0.1 M ionic strength; 25°C; Sillen & Martel, 1971; Perrin, 1979). Secreted citrate will thus be strongly anionically charged.

These citrate ions will have a low membrane permeability so their secretion will cause a movement of diffusible ions in order to satisfy the new Donnan equilibrium conditions. The tendency will be to exchange extracellular anions (mainly chloride) with intracellular diffusible cations (mainly potassium). Only limited changes in sodium would be expected because of its low intracellular concentration and its lower permeability than potassium. The resulting ion movements would reflect the relative ionic permeabilities and the availability of facilitated diffusion mechanisms, but could be energy independent. Potassium and chloride changes would be expected to be linearly related to the change in citrate and account for the overall non-diffusible charge transferred.

**Text-fig. 3.** Ionic composition of blood plasma (Gamble, 1954) compared to that of prostatic fluid at 0 mM-citrate and 90 mM-citrate. Prostatic fluid concentrations calculated from regression data (Text-fig. 2) and m equiv./l calculated as though all components are completely ionized. Prostatic protein charge was estimated assuming 30 mg protein/ml and pH values of 8.0 and 6.7 at 0 mM- and 90 mM-citrate (Van Slyke et al., 1928). Unidentified species required for balancing are shown by queries.
The observed charge associated with the potassium, chloride and sodium shifts amounts to about 1·1 equiv./mol citrate which, together with the 0·8 equiv./mol citrate accounted for by calcium, magnesium and zinc, leaves a further 1·1 equiv./mol to be explained. This must be made up of undissociated hydrogen and secretion of unidentified non-diffusible cations. On entry into the prostatic acinus the hydrogen would dissociate, much of it being buffered by bicarbonate and protein, causing the pH to fall.

The charge due to prostatic fluid proteins will only be a small proportion of the total anionic charge, even at high pH values. Bicarbonate is likely to be of much greater significance. There are only a few, conflicting, reports on prostatic or seminal fluid bicarbonate (Huggins et al., 1942; Mann & Lutwak-Mann, 1981; Zaneveld & Tauber, 1981), but if it is not a major component at low citrate levels then some other basic component must be present to account for the high pH observed under these conditions. The maximum available buffer base at zero citrate can be calculated from the discrepancy between the measured anions and cations (72 m equiv./l; Text-fig. 3). While this is necessarily only an approximation it allows estimation of the relative composition of the unexplained 1·1 equiv. cations associated with each mole of citrate.

If for instance 50 m equiv. of the anionic charge/l at zero citrate are available for buffering and they are exhausted by about 160 mm-citrate (97% of samples had ≦ 160 mm-citrate (Text-fig. 1)), then this suggests that the citrate is secreted in association with about 0·3 equiv. hydrogen/mol, leaving 0·8 equiv./mol to be supplied by co-transported, non-diffusible cations. As the change in pH and buffer anions would not be linear with citrate this estimate only represents an average over the entire range of citrate values.

The missing cations might be supplied by polyamines, of which human semen contains high concentrations, believed to be secreted by the prostate (Mann & Lutwak-Mann, 1981). Reported values are variable, but generally in the low millimolar range for seminal spermine. The concentration in prostatic secretion would be expected to be about three times that of seminal plasma. An average concentration of 12 mM in normal prostatic fluid samples, which had a mean zinc concentration of 5·4 mM, has been reported (Anderson & Fair, 1976). On the basis of Text-fig. 2 these samples would be expected to have an average citrate concentration in the region of 60 mM. At an estimated cation deficiency of 0·8 equiv./mol citrate this amounts to 48 equiv./l; 12 mM-spermine would contribute 48 m equiv./l.

A model to explain the electrolytic composition of human prostatic fluid therefore emerges. As citrate is secreted into an initially blood plasma-like medium it might take with it, in a complexed form, calcium, magnesium and zinc, and the ionic charge transferred could drive potassium and chloride exchange. Thus the observations expressed in Text-fig. 2 can all be explained. This model is quantitatively complete if some reasonable assumptions on prostatic fluid buffering and polyamine secretion are acceptable.

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References


