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# The Determination of Ultrafiltrable Calcium and Magnesium in Serum

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## ABSTRACT

Ultrafiltrate of human serum was investigated in order to evaluate the serum content of calcium and magnesium. The acid and base concentrations and pH of the serum was altered through titration with HCl- or NaOH-solutions. The $Pco_2$ was varied in the titrated serum using different carbon dioxide tensions. This was performed when serum was filtered in a recycling system. It is shown that the analysis of calcium and magnesium have to be done under anaerobical conditions or at standardized pH and $Pco_2$ situations, as the concentrations vary with both pH and $Pco_2$. The concentration ratio between ultrafiltrate and serum for calcium and magnesium was found to be 0.56 and 0.74 respectively at pH=7.41 and $Pco_2$=40 mmHg.

## INTRODUCTION

It is now generally assumed that the ionized rather than the total calcium in serum is the fraction responsible for many of the physiological effects of calcium. It is also the general opinion that the ionized calcium is the fraction under hormonal control.

Calcium in serum can be separated into three fractions, the protein-bound, the fraction which exists in complexes like citrate, carbonate, lactate and phosphate and the ionized cacium fraction. The two latter fractions form an ultrafiltrable part, which is about 55% of the total serum calcium. The ionized calcium fraction is of particular interest since this part has a main function in all calcium metabolism and since this fraction is supposed to be physiological active. In normal conditions the ratio between ionized calcium and total serum calcium probably is rather constant. In pahtological conditions the ratio is supposed to vary, why the total serum calcium will be a bad indicator of the ionized calcium concentration.

The protein-bound serum magnesium fraction contributes to about 30% of the total serum magnesium concentration. The ultrafiltrable magnesium fraction consists mainly of ionized magnesium which is assumed to be biologically active. A small magnesium fraction is bound to phosphate, citrate and may also form other complexes (12).

From the above it is of interest to find a method suitable for clinical practice for the assay of concentrations of ionized calcium and magnesium in serum. Few reports have been published on this matter. In the literature the papers so far mainly have been focused on the use of a calcium electrode. In the reports published, two ways have been used, either direct measurement of the calcium ion activity by means of a calcium electrode (4) or by analysis of an ultrafiltrate produced either under pressure (9, 10) or by ultracentrifugation (2, 8). The above-mentioned methods are time consuming. It has also been difficult to maintain constant original patient condition such as temperature, pH, carbon dioxide tension.

The aim of the present investigation was to study the effect of pH and $P_{CO_2}$ on the ultrafiltrable calcium and magnesium ion concentratios of serum. Serum was filtered under pressure using different $CO_2$ tensions as well as different pH values achieved by adding acid or base. The ultrafiltrate was analysed for calcium and magnesium.

## METHODS

### Preparation of the samples

Pooled serum samples from several normal blood donors were used throughout the whole investigation. To 150 ml serum volumes 1.05M hydrochloric acid or 0.97M sodium hydroxide solutions were added according to Table I in order to reach different levels of base excess (11).

TABLE I

| BE (mM) | -15.75 | -10.50 | -5.25 | 0 | +4.85 | +9.70 | +14.55 |
|---|---|---|---|---|---|---|---|
| 1.05M HCl | 2.250 ml | 1.500 ml | 0.750 ml | | | | |
| 0.97M NaOH | | | | | 0.750 ml | 1.500 ml | 2.250 ml |

Table 1. Addition of acid or base to a 150 ml volume of serum

The serum samples were then divided into 10 ml portions, which were stored in glass vials with screw caps, at -18°C. All serum samples were frozen only once. Pilot experiments showed that repeated freezing of the samples changes both the pH and the buffer capacity. Freezing only once on the other hand, did not change the results.

Equilibration of the samples

Serum samples to which acid or base had been added in order to obtain different base excess levels were equilibrated for five minutes with gas mixtures containing 1.5, 3, 5 or 12% $CO_2$ followed by pH measurements. The pH values and the corresponding carbon dioxide tensions were then plotted in an Astrup diagram (1). The rest of the gas mixtures consisted of oxygen (except for the gas mixture containing 5% $CO_2$ where nitrogen was used instead of oxygen). The equilibrations were performed at room temperature and 0.5 ml samples were used each time.

In order to standardize the pH measurements the readings of the pH-meter were done after 15 sec. The main reason for this was that the microglass electrode had a certain memory probably due to the bicarbonate content of the samples (3). Since each individual equilibration was performed with two different gas mixtures in two equilibration chamber simultaneously (Fig. 1), the first two measured pH values from the first "legs" ($C_1$ in Fig. 1) and the first pH value from the second "legs" ($C_2$ in Fig. 1) have been excluding in the calculations of the results, in order to avid the memory effect of the microglass electrode.

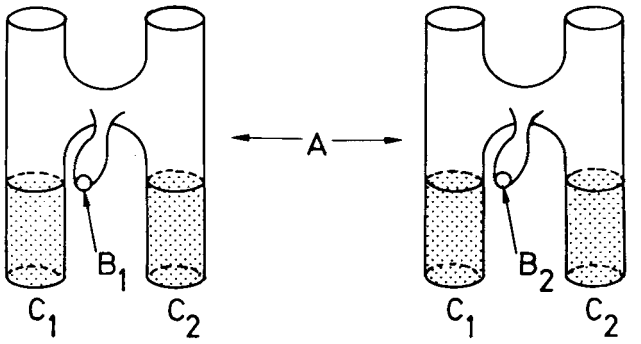


Figure 1. The equilibration equipment (A). B represents the gas inlet and C the equilibration "legs". The sample is shown as a shadowed area.

The temperature during the equilibrations varied between 20 and 29°C. All pH values have been corrected to 25°C ( pH = 0.003 x (25 - t°C) (5).

Ultrafiltration

For the ultrafiltration a Diaflo membrane (Type PM10, Amicon Corp. Lexington Mass. USA) was used. The serum samples passed at room temperature by means of a pump across the membrane and by means of an over-pressure a filtrate was obtained (see Fig. 2). The

filter equipment was connected to a gas tank by which the desired over-pressure was obtained and also connected to a mercury manometer by means of which the pressure could be adjusted.

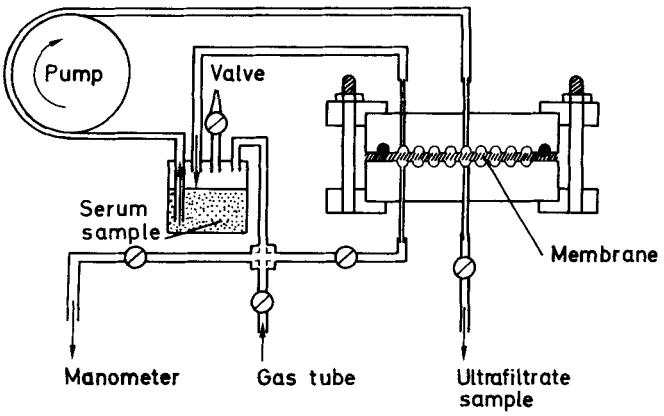


Figure 2. The principle of filtration equipment.

Over-pressure corresponding to three different carbon dioxide tensions namely, 80, 40, and 20 mmHg were used These over-pressures, which varied between 711 to 805 mmHg was calculated according to the formula below

$$P_{CO_2} = (P_{ov} + P_{bar} - P_{H_2O}) \cdot CO_2$$

Where $P_{ov}$ = over pressure
$P_{bar}$ = barometric pressure
$P_{H_2O}$ = aqueous vapour pressure
$CO_2$ = carbon dioxide concentration in per cent

A series with two ultrafiltrations for each serum with different base excess and different carbon dioxide tensions was performed, by which two 2 x 750 µl filtrate from each sample of 10 ml of serum were collected during two 40 min periods. The ultrafiltrates were analyzed for calcium and magnesium with atomic absorption spectrophotometry (Perkin Elmer Model 303) for calcium at a wave length of 422.7 nm and magnesium at 285.2 nm. Standard solutions and the preparation of samples were prepared according to Perkin Elmer (7), where ultrafiltrate of 250 µl was used.

## RESULTS

### pH-measurements

The pH values corrected to 25°C were used for the calculation of the equation of the

TABLE II

| BE (mM) | -15.75 | -10.50 | -5.25 | 0 | +4.85 | +9.70 | +14.55 |
|---|---|---|---|---|---|---|---|
| k | -1.275 | -1.250 | -1.173 | -1.157 | -1.149 | -1.110 | -1.138 |
| l | 10.673 | 10.644 | 10.180 | 10.180 | 10.173 | 9.928 | 10.209 |
| n | 112 | 91 | 97 | 96 | 98 | 107 | 118 |
| r | 0.998 | 0.999 | 0.999 | 0.999 | 0.999 | 0.982 | 0.999 |

Table 2. Calculated values for the equations of the straight buffer lines, where log $P_{CO_2}$ = K• pH + l, r = correlation coefficient and n = number of pH measurements.

straight buffer lines (see Table 2). Figure 3 shows the results from the equilibrations with different gas mixtures of the different serum samples with different base excess levels. The buffer lines obtained from the figure were used for the estimation of the pH values which the samples obtained during the filtrations.

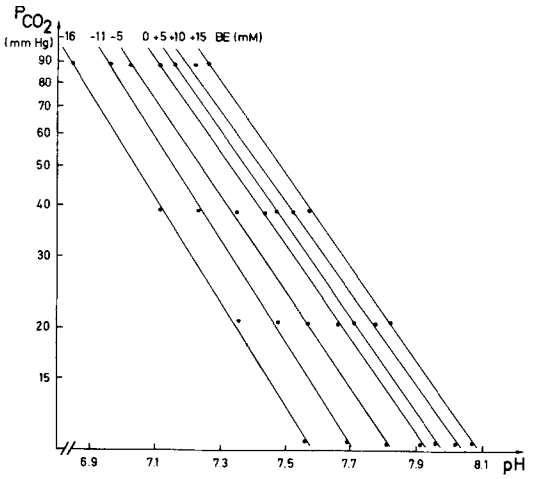


Figure 3. Serum buffer lines at different base excess values (BE), where the $P_{CO_2}$ is plotted as a function of the pH.

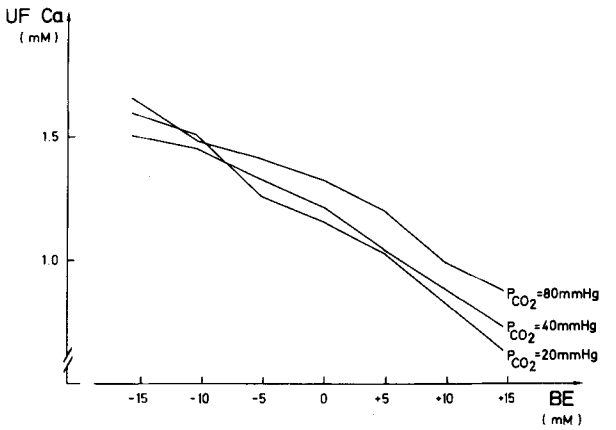


Figure 4. The calcium concentration of the ultrafiltrates in relation to the base excess at various $P_{CO_2}$.

Calcium concentrations in the ultrafiltrates.

Figure 4 illustrates the average calcium concentrations collected from all measurements of the calcium concentrations in the ultrafiltrate plotted against base excess. The values obtained from equal carbon dioxide tensions have been connected. From the figure it is obvious that the calcium concentration of the ultrafiltrates decreases when the base excess increases. This is true for all carbon dioxide tensions. The relation between the ultrafiltrable calcium concentration and the pH and $P_{CO_2}$ is shown in the Figures 5 and 6. Figure 5 illustrates the calcium concentration in the ultrafiltrate in relation to the pH during the filtration using different carbon dioxide tensions. The mean values of the ultrafiltrate collected during the first and second collecting periods are shown respectively. It is quite obvious that the calcium concentrations are lower during the first collecting periods. For all the used carbon dioxide tensions an increase of the pH of about 0.45 units corresponds to a reduction of the calcium concentration in the ultrafiltrates of about 0.7 mM.

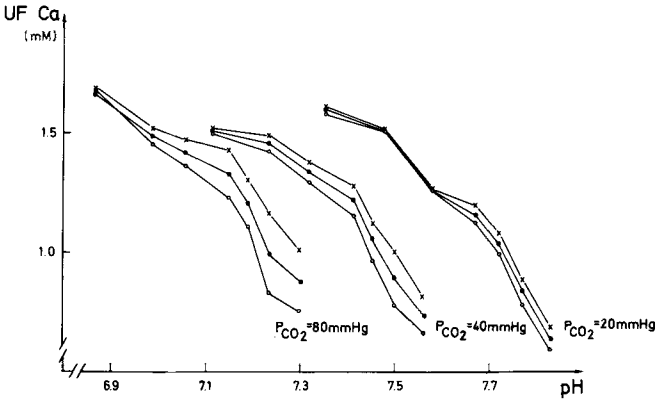


Figure 5. The calcium concentration of the ultrafiltrates in relation to the pH values of the serum samples during the filtration using different $P_{CO_2}$. Mean values from the first (x), second (o) and whole ( • ) collecting period.

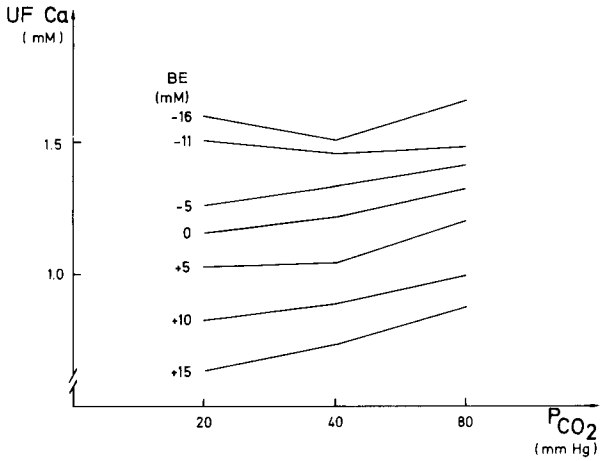


Figure 6. The calcium concentration of the ultrafiltrates related to the corresponding $P_{CO_2}$ during ultrafiltration at the various base excess levels.

Figure 6 illustrates the calcium concentration in relation to the carbon dioxide tension in the ultrafiltrate using sera with different base excess. An increase of $P_{CO_2}$ from 20 to 80 mmHg corresponds to an increase of the calcium concentration of the ultrafiltrate of maximum 0.2 mM.

The analysis of the total serum calcium concentration showed an average concentration of 2.15 mM. From Figure 5 the concentration of the ultrafiltrable calcium concentration is found to be 1.21 mM at $P_{CO_2}$= 40 mmHg and pH 7.41, which means that the ultrafiltrable fraction of calcium in this case corresponds to 56% of the total calcium concentration in serum.

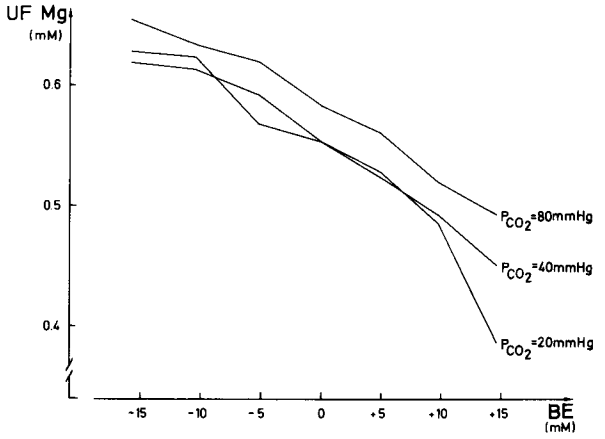


Figure 7. The magnesium concentration of the ultrafiltrates in relation to the base excess at various $P_{CO_2}$.

Magnesium analysis

The results of the magnesium analysis are analogous with those from calcium and only the absolute values are different from the calcium ones.

Figure 7 shows the average values from all the analyses of the ultrafiltrates related to the base excess levels. From the figure it is obvious that the magnesium concentration in the ultrafiltrates decreases when the base excess level increases at a constant carbon dioxide tension. This is true for all the investigated carbon dioxide tensions.

Figure 8 shows the magnesium concentration of the ultrafiltrates in relation to pH during the filtrations at different carbon dioxide tensions. From the curves it is clear that the first initial collected filtrates always had a lower magnesium concentration. For all carbon dioxide tensions used, a pH increase of about 0.45 units corresponds to a decrease of the magnesium concentration of the ultrafiltrate of about 0.2 mM.

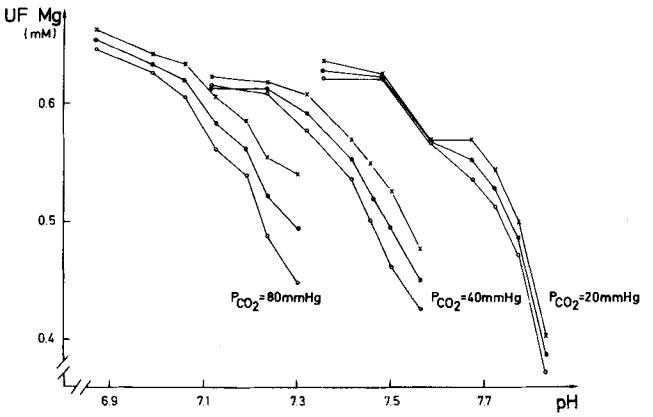


Figure 8. The magnesium concentration of the ultrafiltrates in relation to the pH values of the serum samples during the filtration using different $P_{CO_2}$. Mean values from the first (x), second (o) and whole (•) collecting period.

Figure 9 shows the magnesium concentration of the ultrafiltrates in relation to the $P_{CO_2}$ of the ultrafiltrates from each base excess level. An increase of the $P_{CO_2}$ from 20 to 80 mmHg corresponds to an increase in the magnesium concentration of the ultrafiltrate of maximum 0.1 mM.

Analysis of the serum gave a total magnesium mean concentration of 0.742 mM. From Figure 8 the magnesium concentration of the ultrafiltrate at $P_{CO_2}$=40 mmHg and pH 7.41 was found to be 0.552 mM. The ultrafiltrable fraction of serum magnesium was in this case found to be 74% of the total magnesium concentration.

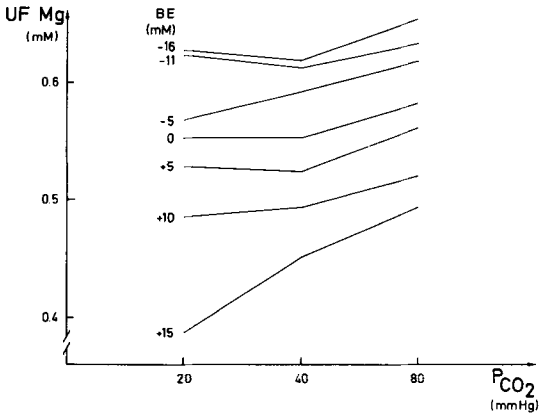


Figure 9. The magnesium concentration of the ultrafiltrates related to the corresponding $P_{CO_2}$ during ultrafiltration at the various base excess levels.

## DISCUSSION

In this invetigation it is shown that the ultrafiltrable fraction of serum calcium and serum magnesium is both dependent on the pH and the $P_{CO_2}$. The size of this dependence has also been able to determine. In the literature only a pH dependence has previously been described (for instance 2, 6). A hypothetical $P_{CO_2}$ dependence has only been commented but as far as we have found never been investigated before (6, 13). Similar studies where the calcium and magnesium concentrations of ultrafiltrates of serum have been measured are in good accordance with the results described above (12).

From several practical viewpoints it is important to know that the concentration of ultrafiltrable calcium and magnesium is dependent on both pH and $P_{CO_2}$. From analytical viewpoint it is important to ultrafiltrate serum at a standardized pH and $P_{CO_2}$ in order to obtain relevant and comparable results. Is ultrafiltration performed at a standardized pH and $P_{CO_2}$ the venous sampling anaerobicly seems not to be necessary. From clinical viewpoint the results have to be judged in connection with assay of the acid base status of the patient. In most cases it seems to be adequate to estimate the effect of the pH and $P_{CO_2}$ from the relations in the figures shown above.

Sources of error

Pedersen (6) has studied how the handling and the storing of the samples may affect the calcium binding and magnesium binding capacity of the serum proteins as well as of the other not filtrable serum components. The freezing and the thawing of the serum samples did not affect either ultrafiltrable or ionized calcium concentration. A change of temperature from 37°C to room temperature due to the fact that the experiemnts were performed at room temperature may be a source of error if the conclusions should be

transferred to in vivo conditions. Studies using an electrode published by Ladenson (4) showed for instance that the values were two to three per cent lower using a temperature of 37°C compared to the values at 25°C.

Since the serum volume during the filtrations has been fixed and limited a total concentration increase of the serum samples of about 15% has been obtained. This increase may therefore be the reason why the results between the two sampling periods were higher in the finally collectd fraction compared to the first collected one as concerns both ultrafiltrable calcium as ultrafiltrable magnesium. A possible Donnan effect across the membrane may result in changed permeability during the filtration and this could also be a possible factor which give rise to the concentration differences between the two filtration fractions.

The concentrations of the ionized form of serum calcium and serum magnesium are the most interesting from biological point of view. The ultrafiltration technique described here will not give a measure of the ionized fraction but an ultrafiltrable one which contains both the ionized and complex bound calcium and magnesium. During normal conditions the concentration of free ions is the main part of the ultrafiltrable fraction and the complex bound fraction represents only 5 to 15 per cent (12). During pathological conditions the complex bound fractions may probably vary much more. Therefore it is necessary to combine the ultrafiltration technique with the determination of the complex bound fraction in order to obtain the concentrations of ionized calcium and magnesium. The described technique is in principle suitable for clinical praxis but a final analysis of the ultrafiltrate is necessary to obtain the concentration of free ions, for instance a spectrophotometric method which only is sensitive for free ions.

In order to obtain ultrafiltrate enough for duplicate analysis a serum volume of 5 ml and a filtration time of about 15 min is necessary. Several samples may be ultrafiltered at the same time if more than one filtration chamber is available. The time consumption is about the same as that which is necessary for corresponding analysis by the use of electrodes but so far this has not been quite reliable. As concerns magnesium such electrodes for the estimation of free magnesium ions are not available.

ACKNOWLEDGEMENT

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