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ORIGINAL PAPER

Simplified estimates of ion-activity products of calcium oxalate and calcium phosphate in mouse urine

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Abstract This study aimed at formulating simplified estimates of ion-activity products of calcium oxalate (AP_{CaOx}) and calcium phosphate (AP_{CaP}) in mouse urineto find the most important determinants in order to limit the analytical work-up. Literature data on mouse urine composition was used to determine the relative effect of each urine variable on the two ion-activity products. AP_{CaOx} and AP_{CaP} were calculated by iterative approximation with the EQUIL2 computerized program. The most important determinants for AP_{CaOx} were calcium, oxalate and citrate and for AP_{CaP} calcium, phosphate, citrate, magnesium and pH. Urine concentrations of the variables were used. A simplified estimate of AP_{CaOx} (AP(CaOx)-index$_{MOUSE}$) that numerically approximately corresponded to $10^8 \times AP_{CaOx}$ was given the following expression:

$$\text{AP(CaOx)-index}_{\text{MOUSE}} = 0.70 \times \text{Calcium}^{1.05} \times \text{Oxalate}^{0.95}(0.90 - 0.0225 \times \text{Citrate}) + (6.6 \times 10^{-8} \times \text{Citrate}^{3.98})$$

For a series of urine samples with various composition the coefficient of correlation between AP(CaOx)-index$_{MOUSE}$ and $10^8 \times AP_{CaOx}$ was 0.99 ($p = 0.00000$). A similar estimate of AP_{CaP} (AP(CaP)-index$_{MOUSE}$) was formulated so that it approximately would correspond numerically to $10^{14} \times AP_{CaP}$ taking the following form:

$$\text{AP(CaP)-index}_{\text{MOUSE}} = \frac{0.05 \times \text{Calcium}^{1.17} \times \text{Phosphate}^{0.85} \times \text{Magnesium}^{0.18} \times (\text{pH} - 4.5)^{6.8}}{\text{Citrate}^{0.76}}$$

For a series of variations in urine composition the coefficient of correlation was 0.95 ($p = 0.00000$). The two approximate estimates shown in this article are simplified expressions of AP_{CaOx} and AP_{CaP}. The intention of these theoretical calculations was not to get methods for accurate information on the saturation levels in urine, but to have mathematical tools useful for rough conclusions on the outcome of different experimental situations in mice. It needs to be emphasized that the accuracy will be negatively influenced if urine variables not included in the formulas differ very much from basic concentrations.

Keywords Mouse · Urine concentrations · Calcium oxalate · Calcium phosphate · Ion-activity products · EQUIL2 · Theoretical calculations · Citrate · Phosphate · pH

Introduction

Animal experiments with the aim of studying the risk of in vivo crystallization of calcium oxalate (CaOx) and calcium phosphate (CaP) in urine require information on the ion-activity products. Although CaP in this regard does not represent a specific calcium phosphate crystal phase, it can be used to reflect the ion-activity products of various such salts [1]. Reasonably accurate calculations of ion-concentrations and ion-activities can be made with computerized iterative approximation according to Robertson [2], or with

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programs such as EQUIL2 [3], Sequil [4] and JESS [5]. It is thus necessary to feed into these programs a large number of analytical data. Such an extensive analysis of urine composition is not always possible or practically achievable, particularly in small laboratory animals.

Simplified estimates therefore previously were derived for the ion-activity products of CaOx [6–8] and CaP in human urine [1] as well as for these ion-activity products in rat urine [9]. In this article we report the corresponding calculations for mouse urine and present approximate estimates of these ion-activity products.

Methods

Based on data found in the literature [10–13] and from analyses carried out in our own animals we used a urine composition as shown in Table 1 as a basis for the further calculations. The urine variables were all expressed in mmol/L and that was also the units that we decided to apply to the final simplified expressions of the ion-activity products.

The relative effect on the ion-activity products of the concentration of each urine variable was studied by varying one variable over a wide range while keeping the others constant according to the baseline concentrations. The ion-activity products were calculated by means of ion-activities derived from runs in the EQUIL2 program [3] as follows:

$$\mathrm{AP}_{\mathrm{CaOx}} = a_{\mathrm{Ca}^{2+}} \times f_2 \times a_{\mathrm{Ox}^{2-}} \times f_2$$
$$\mathrm{AP}_{\mathrm{CaP}} = a_{\mathrm{Ca}^{2+}} \times f_2 \times a_{\mathrm{PO}_4^{3-}} \times f_3$$

The expression a in these formulas is used for the ion activities and f_2 and f_3 are the activity coefficients for ions with valences 2 and 3, respectively. $\mathrm{AP}_{\mathrm{CaP}}$ is used as a general expression of various calcium phosphate crystal phases encountered in urine.

Table 1 Basic concentrations of important variables in mouse urine

Urine variable	mmol/L (except for pH and volume)
Calcium	3.3
Oxalate	0.35
Citrate	18
Magnesium	15
Phosphate	36
pH	6.0
Sodium	142
Potassium	225
Sulphate	44
Ammonium	8
Chloride	113
Pyrophosphate	–
Carbon dioxide	–
Urate	0.5
Creatinine	2.5
Volume (mL/24 h)	2.3

Results

Calcium oxalate

For the different variables (VAR) a relationship with the ion-activity product was derived according to the general formula:

$$\mathrm{AP}_{\mathrm{CaOx}} = \mathrm{A} \times \mathrm{VAR}^{x}$$

This type of expression was possible to apply for all variables except for citrate which due to the wide concentration range had a more complex relationship. Exponent x and factor A for the different urine variables are summarized in Table 2.

Table 3 shows how a tenfold increased concentration of each urine variable affects $\mathrm{AP}_{\mathrm{CaOx}}$. This table also gives information on the range for which this effect was calculated.

For calcium oxalate, the most important urine variables were *calcium*, *oxalate*, *citrate* and—if concentration values are not used—the urine *volume*. The relationship between these variables and $\mathrm{AP}_{\mathrm{CaOx}}$ is shown in Fig. 1a–c. It is evident that for calcium there is almost a linear relationship between the concentration and $10^8 \times \mathrm{AP}_{\mathrm{CaOx}}$. For oxalate a similar linearity is recorded for concentrations up to 5 mmol/L, but at higher oxalate concentrations there is slight deviation. The average exponent for oxalate concentrations as high as 10 mmol/L (concentrations which might be very unusual) is 0.95. A linear relationship was also recorded for citrate at least as long as the citrate concentration does not exceed 20–25 mmol/L. With that

Table 2 Factor A and exponent x obtained from calculation of $\mathrm{AP}_{\mathrm{CaOx}}$ as a result of variation of the individual variable (VAR)

Urine variable	Factor A	Exponent x
Calcium	0.143	1.05
Oxalate	1.43	0.95
Citrate	5.83	−0.98*
Magnesium	6.33	0.18
Phosphate	0.679	−0.09
Sodium	0.67	−0.06
Potassium	1.06	−0.14
Sulphate	0.635	−0.07
Ammonium	0.51	−0.01

$\mathrm{AP}_{\mathrm{CaOx}} = A \times \mathrm{VAR}^{x}$

* Valid only for citrate values up to 25 mmol/L

Table 3 Increased (+) or decreased (−) AP_{CaOx} caused by a tenfold increased VAR-concentration

Urine variable	Effect of a tenfold increased variable concentration, expressed as times change of AP_{CaOx}
Calcium (1–10 mmol/L)	11.0 (+)
Oxalate (0.2–2.0 mmol/L)	9.6 (+)
Citrate (5–50 mmol/L)	5.1 (−)
Magnesium (4–40 mmol/L)	1.2 (+)
Phosphate (5–50 mmol/L)	1.2 (+)
pH (5–6) (expressed as hydrogen ion concentration)	1.3 (−)
Sodium (40–400 mmol/L)	1.3 (−)
Potassium (50–500 mmol/L)	1.4 (−)
Sulphate (10–100 mmol/L)	1.2 (−)
Ammonium (4–40 mmol/L)	1.0 (−)
Volume (0.2–2.0 mL)	7.7 (−)

The range in which this calculation was made is indicated

restriction a formula for AP(CaOx)-index$_{\text{MOUSE}}$ would take the following form:

$$\text{AP(CaOx)-index}_{\text{MOUSE}} = \frac{\text{A} \times \text{Calcium}^{1.05} \times \text{Oxalate}^{0.95}}{\text{Citrate}^{0.98}}$$

But a more general expression needs to account also for higher citrate concentrations and this is the reason for a more complex expression:

$$\begin{aligned}\text{AP(CaOx)-index}_{\text{MOUSE}} &= 0.70 \times \text{Calcium}^{1.05} \\ &\quad \times \text{Oxalate}^{0.95}(0.90 - 0.0225 \times \text{Citrate}) \\ &\quad + \left(6.6 \times 10^{-8} \times \text{Citrate}^{3.98}\right)\end{aligned}$$

When a series of 63 different combinations of urine variables was used to derive both AP(CaOx)-index$_{\text{MOUSE}}$ estimates with the formula shown above and AP_{CaOx} from EQUIL2 calculations, it was evident from Fig. 2 that there was a statistically strong correlation between the two parameters ($r = 0.99$; $p = 0.00000$). The factors and exponents in the AP(CaOx)-index$_{\text{MOUSE}}$ were chosen so

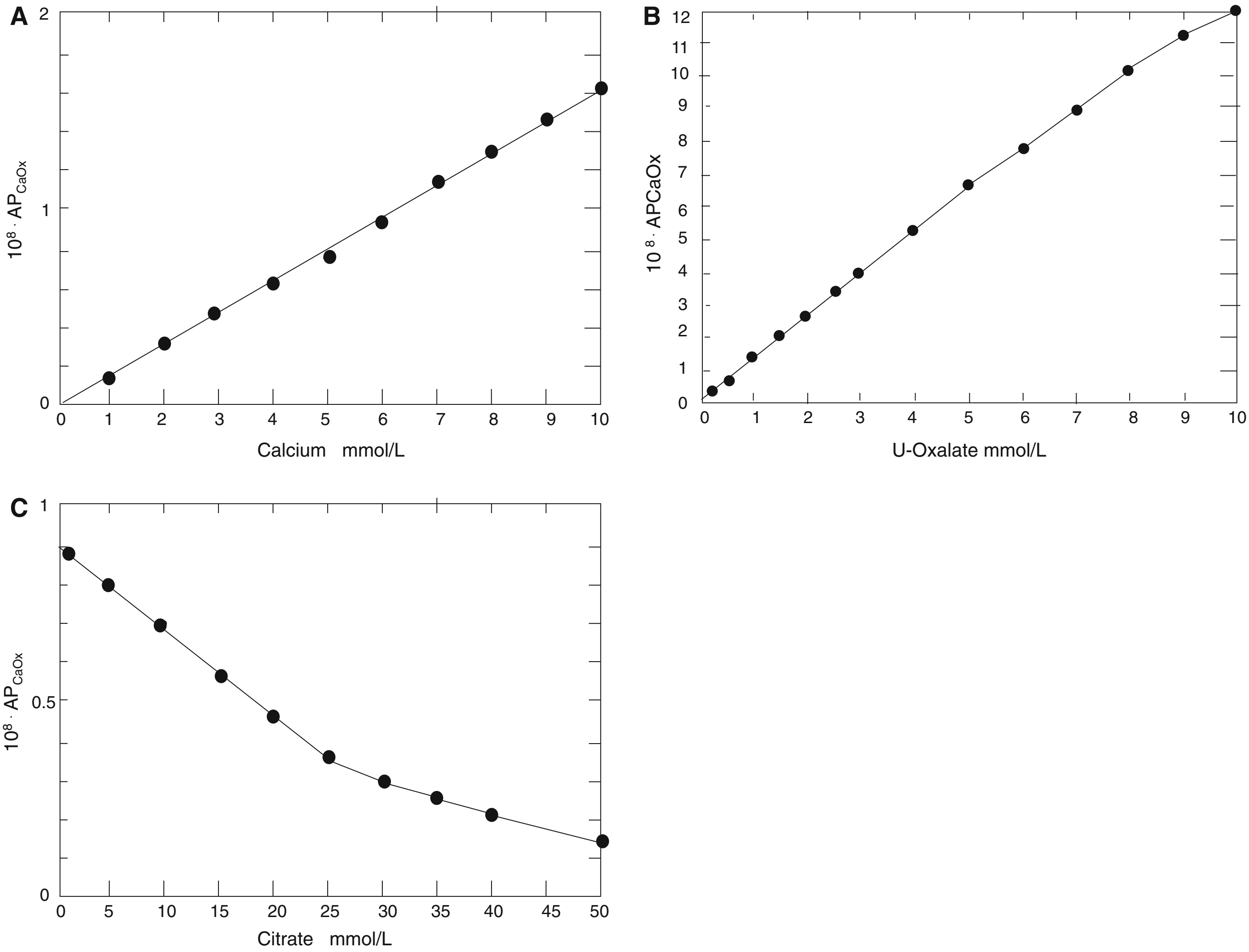

Fig. 1 Relationship between $10^8 \times AP_{CaOx}$ and urine concentrations of **a** calcium, **b** oxalate and **c** citrate

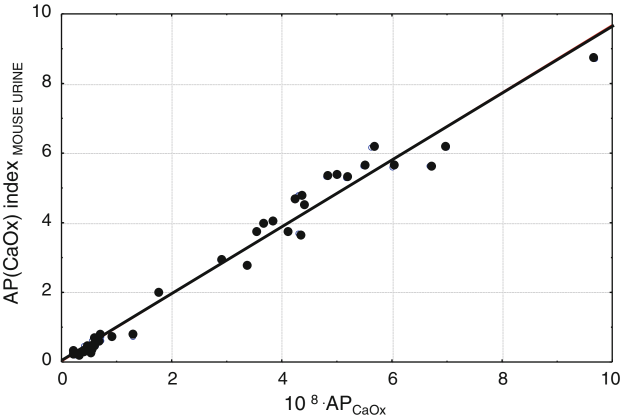

Fig. 2 Relationship between $10^8 \times AP_{CaOx}$ and AP(CaOx)-index$_{MOUSE}$

that the index numerically would correspond approximately to $10^8 \times AP_{CaOx}$. The equation of the relationship shown in Fig. 2 is AP(CaOx) index = $(0.97 \times 10^8 \times AP_{CaOx}) + 0.009$

To elucidate whether combined increments in calcium and urine pH had an important influence on AP_{CaOx}, calculations also were made for calcium concentrations in the range of 1–10 mmol/L at pH 6.5, 7.0, 7.5 and 8. As shown in Fig. 3 the AP_{CaOx} values were very similar and only at the highest calcium concentrations were small differences noticed.

Calcium phosphate

As for calcium oxalate the general formula applied for expressing the relationship between urine variables and AP_{CaP} was as follows:

$$AP_{CaP} = B \times VAR^{y}$$

Values of exponent *y* and factor *B* for each urine variable are summarized in Table 4.

Table 5 shows the influence on AP_{CaP} by tenfold increased concentrations of each urine variable.

The most important determinants of AP_{CaP} were *calcium*, *citrate*, *magnesium*, *phosphate*, *pH* and—if concentration variables are not used—the *urine volume*. The relationship between these individual variables and AP_{CaP} is shown in Fig. 4a–e.

A definite formula for AP(CaP)-index$_{MOUSE}$ took the following form:

$$\text{AP(CaP)-index}_{MOUSE} = \frac{0.05 \times \text{Calcium}^{1.17} \times \text{Phosphate}^{0.85} \times \text{Magnesium}^{0.18} \times (\text{pH} - 4.5)^{6.8}}{\text{Citrate}^{0.76}}$$

Sixty-three different combinations of urine variables were used to compare AP(CaP)-index$_{MOUSE}$ and AP_{CaP} values and it is evident from Fig. 5 that there was a

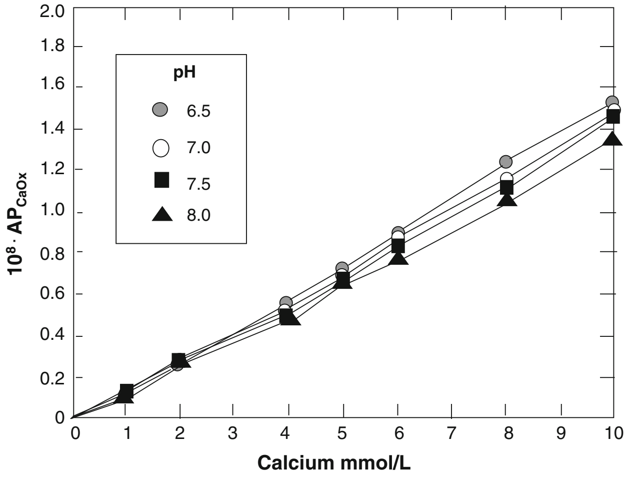

Fig. 3 Relationship between different concentrations of calcium and AP_{CaOx} at pH 6.5, 7.0, 7.5 and 8.0

Table 4 Factor *B* and exponent *y* obtained from calculation of AP_{CaP} as a result of variation of the individual variable (VAR)

Urine variable	Factor *B*	Exponent *y*
Calcium	3.04	1.165
Oxalate	11.67	−0.01
Citrate	4.0	−0.76
pH	pH − 4.5*	6.8
Magnesium	6.33	0.18
Phosphate	0.52	0.85
Sodium	12.5	−0.01
Potassium	9.87	0.036
Sulphate	16.8	−0.09
Ammonium	11.7	−0.001

$AP_{CaP} = B \times VAR^{y}$

* In accordance with previous simulations [1]

Table 5 Increased (+) or decreased (−) AP_{CaP} caused by a tenfold increased VAR-concentration

Urine variable	Effect of a tenfold increased variable concentration, expressed as times change of AP_{CaP}
Calcium (1–10 mmol/L)	13.6 (+)
Oxalate (0.2–2.0 mmol/L)	0.96 (−)
Citrate (5–50 mmol/L)	8.24 (−)
Magnesium (4–40 mmol/L)	2.68 (+)
Phosphate (5–50 mmol/L)	4.2 (+)
pH (expressed as hydrogen ion concentration)	23.5 (pH 6–7) (+) 49.4 (pH 5–6) (+)
Sodium (40–400 mmol/L)	1.0 (+)
Potassium (50–500 mmol/L)	1.1 (+)
Sulphate (10–100 mmol/L)	1.3 (−)
Ammonium (4–40 mmol/L)	1.0 (+)
Volume (0.2–2.0 mL)	17.3 (−)

The range in which this calculation was made is indicated

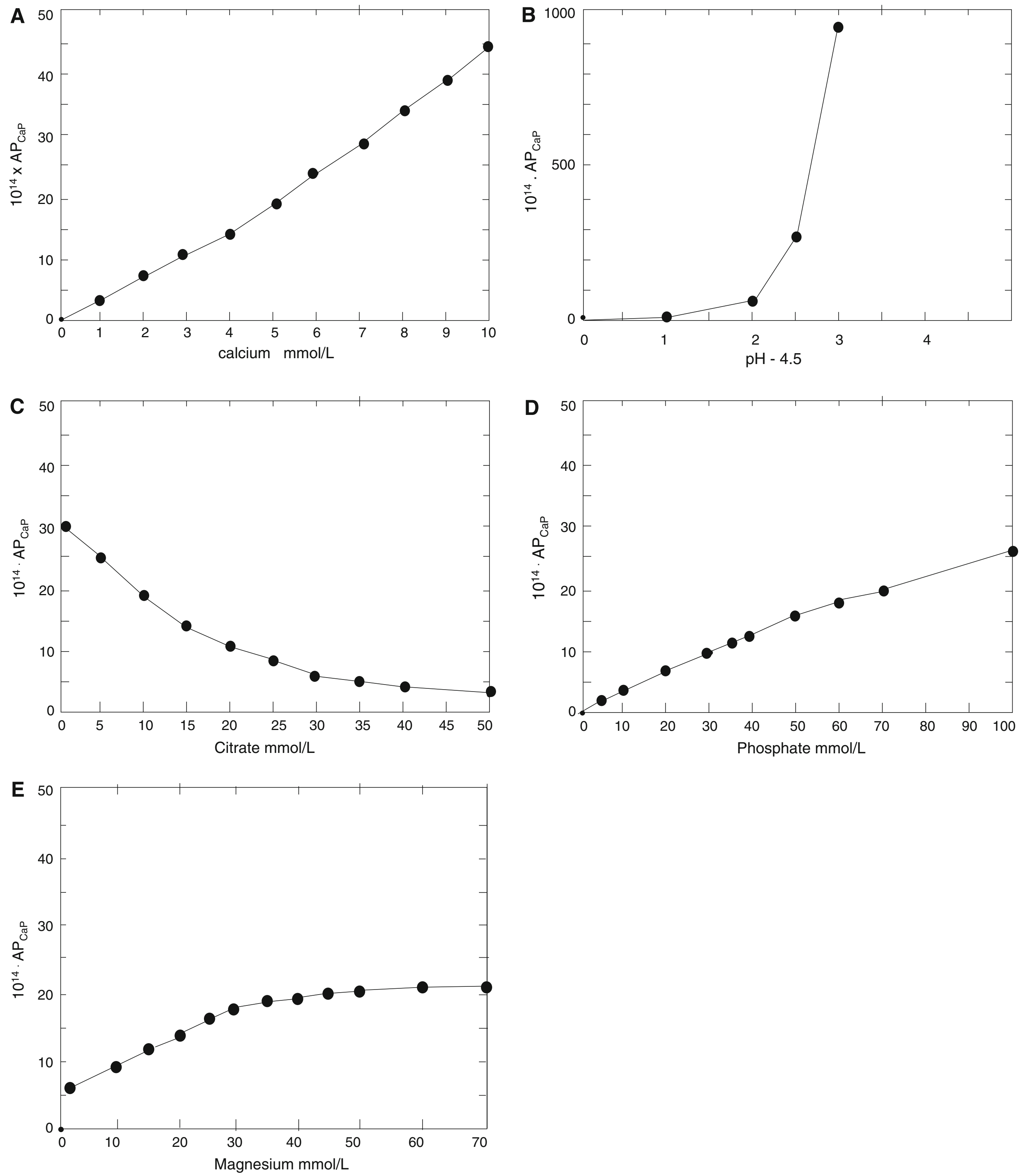

Fig. 4 Relationship between $10^{14} \times AP_{CaP}$ and urine concentrations of **a** calcium, **b** pH 4.5, **c** citrate, **d** phosphate and **e** magnesium

reasonably good correlation between the two parameters ($r = 0.95$; $p = 0.00000$). The correspondence between the two parameters was, however, not as good as that seen for calcium oxalate (Fig. 2). Inasmuch as the AP_{CaP} is very sensitive to variations in pH it is usually not that important to get an exact estimate of the level of AP_{CaP}. The factors and exponents in the AP(CaP)-index$_{MOUSE}$ were chosen so that the index numerically should correspond approximately

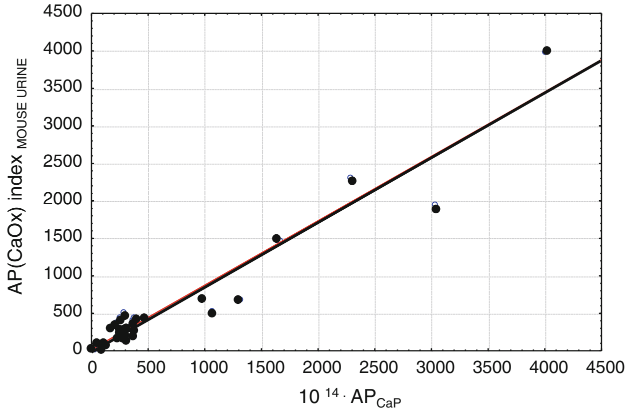

Fig. 5 Relationship between $10^{14} \times AP_{CaP}$ and AP(CaP)-index$_{MOUSE}$

to $10^{14} \times AP_{CaP}$. The equation of the relationship shown in Fig. 5 is AP(CaP)-index = $(0.73 \times 10^{14} \times AP_{CaP}) + 46.9$.

Discussion

It is important to note that the simplified expressions presented above do not exactly correspond to the more accurately calculated ion-activity products of CaOx and CaP. One factor that might lead to less accurate estimates of the ion-activity products is if the urine variables, not included in the formula, deviate very much from the basic values in Table 1. Magnesium might have some influence on AP_{CaOx}, but it was excluded from the simplified formulas in order to get expressions with as few variables as possible because the effect was considered relatively small. If, however, the magnesium level varies over a wide range it is important to keep this simplification in mind. On the other hand, the effect on AP_{CaP} was more pronounced and therefore we included magnesium in AP(CaP)-index$_{MOUSE}$.

In previously formulated indices [1, 6–9], the total excretion of urine variables during defined periods of time was used together with measurements of the urine volume during the same period. In the indices given in this article we used the concentration of each variable and information on the duration of the collection period is thus not necessary, neither is any correction factor for that. The main reason for this strategy was that in mouse urine it might be very difficult to accurately assess the excretion of individual variables during a defined period of time. Even small errors in the recovery of urine will have a great impact on the subsequent calculations if total excretion values are used.

The reason for the complicated equation necessary for AP(CaOx)-index$_{MOUSE}$ was the wide range of citrate concentrations that might be encountered in mouse urine. The relationship between the citrate concentration and AP_{CaOx} decreased linearly up to a citrate concentration of 25 mmol/L. This linearity is, however, lost for higher citrate concentrations and in order to avoid two separate formulas for low and high citrate concentrations the presented mathematical solution was chosen.

In the process of index formulation and in order to get a relative accuracy at high levels of ion-activity products, the indices necessarily were made slightly less sensitive to small variations of variable concentrations at low ion-activity products. From an experimental point of view it is, however, probably most important with formulas that take into account situations that are encountered at high levels of supersaturation with CaOx and CaP. We accordingly confirmed that AP(CaOx)-index$_{MOUSE}$ was valid for oxalate concentrations as high as 10 mmol/L, although concentrations at that high level are unlikely encountered even under extreme experimental conditions.

Urine pH has a pronounced influence on AP_{CaP}, but its influence on AP_{CaOx} is very small also when the calcium concentration is varied at pH-levels up to 8.0 (Fig. 3). The slight differences at high calcium concentrations and high pH are not of the magnitude that they justify a pH variable in AP(CaOx)-index$_{MOUSE}$. There might of course be other reasons for measuring pH in urine of stone-forming animals, but as a factor of importance for estimating the supersaturation with CaOx it is not.

There is, undoubtedly, a difference between anions and cations when the variables in Table 1 are considered and a pH-dependent ion-gap might exist. Variations in the concentration of chloride have not significantly affected the calculations. Macromolecular organic acids and anionic compounds contribute to maintain electro-neutrality, but these ionic compounds are not accounted for in the EQUIL2 program. It is important to be aware of shortcomings in this regard when the estimates described in this article are used for conclusions on physico-chemical properties in urine.

The presented indices seem to be sufficiently accurate for rough and approximate conclusions on how variations in urine composition affect the ion-activity products. If, however, more exact information is required it is still necessary to apply the more laborious techniques available [2–5].

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