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DIFFERENTIAL EFFECTS OF pH, pCO_2, AND PROTEIN CONCENTRATION ON PLASMA CALCIUM ION LEVEL AND ALGORITHM FOR CALCULATING ITS STANDARDIZED VALUE

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Determination of the plasma Ca^{++} concentration, an important parameter of calcium homeostasis in the body, has become possible only recently as a result of the introduction of ion-selective electrodes [2, 7, 11]. To interpret the measured values of the ionized calcium concentration (Ca_i^{++}) they are reduced to pH 7.4 by means of equations obtained by approximating experimental relationships $Ca^{++} = f(pH)$. The equation for pH 7.4 is the one most widely used [12]:

$$\lg \left(Ca^{2+}\right)^{st}_{pH\,7.4} = \lg Ca_i^{2+} - 0.24\left(7.4 - pH_i\right), \quad (1)$$

but it has a number of limitations: It was obtained for average values of concentrations of albumin A = 0.656 mM and total protein P = 37 g/liter [11], and is valid for cases when variation of pH and, correspondingly, of Ca^{++} is due entirely to equilibration of the sample with gas mixtures with different pCO_2 values. Equation (1) is not intended to describe real situations.

This paper describes an attempt to determine the precise algorithm for calculating the standardized calcium $(Ca^{++})^{st}$ by analyzing differential effects of pH, pCO_2, and A(P) of the result of determination.

EXPERIMENTAL METHOD

Heparinized grouped normal plasma (heparin added in a dose of 2 U/ml) was equilibrated in a tonometer (IL-237, USA) with carbogen containing 1.5, 3.9, 5.6, and 7.8% CO_2. The Ca^{++}

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*Deceased.

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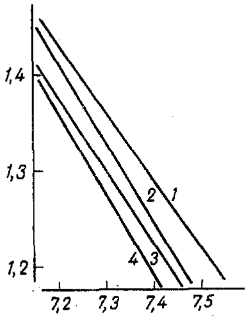

Fig. 1

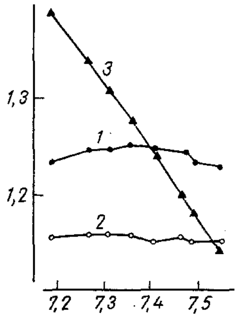

Fig. 2

Fig. 1. Dependence of Ca^{++} concentration on pH at different values of pCO_2. Abscissa, values of pH; ordinate, Ca^{++} concentration (in mM). Values of pCO_2: 1) 14.89, 2) 27.82, 3) 42.64, 4) 54.86 mm Hg.

Fig. 2. Comparative evaluation of algorithms for calculating Ca^{++}. 1) Calculation by Eq. (1); 2) by equation (2b); 3) experimental titration curve for pCO_2 = 27.85 mm Hg, A = 0.41 mM, P = 53.9 g/liter. Remainder of legend as to Fig. 1.

concentration and pH were determined simultaneously on an ICA-I analyzer (Radiometer, Denmark). The plasma samples were transferred from the tonometer to the analyzer under anaerobic conditions. The CO_2 saturation of the samples was monitored by measuring pH and pCO_2 on an ABL-2 analyzer (Radiometer). Values of pH between 7.0 and 7.8 (when pCO_2 = const) were obtained by adding different quantities of 1.5 M $NaHCO_3$ to plasma, previously diluted with ultrafiltrate, obtained from the original plasma on CF-50A filters (Amicon, USA), and acidified to pH 5.9-6.1. The pH of the acidified plasma was between 6.5 and 6.9. The ratio of the volume of added $NaHCO_3$ and sample did not exceed 1:100. The number of samples with different pH values for each value of pCO_2 was 12. Samples with different A(P) were obtained by diluting the original plasma with its ultrafiltrate in ratios giving fluctuations in P up to 50 g/liter; P was determined by the biuret method and A by the reaction with bromcresol green. The total calcium concentration was determined by atom-absorption spectrophotometry (IL-351, USA). In all samples it was 3.0 mmole/liter.

EXPERIMENTAL RESULTS

The functions $Ca^{++} = f(pH)$ were approximated by regression lines. In all cases high coefficients of correlation (r) were observed, namely from 0.980 to 0.999. Typical relationships obtained at different pCO_2 levels for plasma with A = 0.51 mmole/liter, P = 63.0 g/liter are illustrated in Fig. 1. The remaining results are given in Table 1. In it values of $(Ca^{++})^{st}_{pH\,7.40}$, calculated from the corresponding regression lines, are shown. The results are evidence of a significant differential contribution of pCO_2 to $(Ca^{++})^{st}_{pH\,7.40}$, which confirms the need for allowing for it in the algorithms of calculation. The same conclusion can also be drawn as regards allowing for effects of A(P). These effects can be divided into two components: One determines dependence of the coefficients of the regression lines $Ca^{++} = f(pH)$, the other, dependence of $(Ca^{++})^{st}_{pH\,7.40}$ on pCO_2 and A(P).

It can be concluded from analysis of the results that standard conditions must also include the requirements pCO_2 = 40 mm Hg and A = 0.6 mM (P = 70 g/liter). It is stated in the literature that pCO_2 [4, 6, 8, 9] and also A(P) [3, 5, 9] have differential effects on the characteristics of the function $Ca^{++} = f(pH)$. However, the quantitative aspect of the problem has not been analyzed in detail. This has led to a certain modification of the method of determination of $(Ca^{++})^{st}_{pH\,7.40}$, according to which before measurements the plasma must be equilibrated with a gas mixture with pCO_2 = 40 mm Hg [9, 11]. This procedure increases the accuracy of Eq. (1) and rules out the effect of pCO_2 of the original sample on the result of determination of $(Ca^{++})^{st}_{pH\,7.40}$. It also rules out the possibility of determining values of Ca^{++}_i, which have diagnostic importance of their own. With the results given in Table 1 it is possible to obtain an equation for reducing Ca^{++}_i to standard conditions (pH 7.40; pCO_2 = 40 mm Hg, P = 70 g/liter, A = 0.6 mM). For this purpose straight regression lines $(Ca^{++})^{st}_{pH\,7.40} = f(pCO_2)$ were plotted for different values of A(P). Analysis showed that the effect of protein on the

TABLE 1. Parameters of Regression Lines for Ca^{++} = f(pH) at Different pCO_2 Levels

pCO_2, mm Hg	Regression equation	r	P, g/liter	A, mM	$(Ca^{++})^{st}$, mmoles/liter (pH 7.40)
16,1	$Ca^{2+} = 6,9 - 0,758$ pH	0,992	71,5	0,61	1,29
40,75	$Ca^{2+} = 7,62 - 0,865$ pH	0,999			1,21
53,6	$Ca^{2+} = 7,84 - 0,9$ pH	0,998			1,189
14,9	$Ca^{2+} = 6,7 - 0,73$ pH	0,998			1,29
27,85	$Ca^{2+} = 7,15 - 0,8$ pH	0,999	63,0	0,51	1,249
42,65	$Ca^{2+} = 7,56 - 0,854$ pH	0,997			1,237
54,86	$Ca^{2+} = 7,67 - 0,876$ pH	0,999			1,185
15,55	$Ca^{2+} = 5,6 - 0,585$ pH	0,98			1,279
27,85	$Ca^{2+} = 6,4 - 0,69$ pH	0,999	53,9	0,41	1,249
41,75	$Ca^{2+} = 6,49 - 0,72$ pH	0,98			1,17
54,37	$Ca^{2+} = 6,57 - 0,738$ pH	0,99			1,108

TABLE 2. Parameters of Regression Lines for K = $f(pCO_2)$ at Different A(P) Values

Equation	r	P, g/liter	A, mM/	K $pCO_2 = 40$
$K = -0,7 - 0,0038\ pCO_2$	0,994	71,5	0,61	−0,852
$K = -0,685 - 0,0037\ pCO_2$	0,982	63,0	0,51	−0,833
$K = -0,553 - 0,0037\ pCO_2$	0,918	53,9	0,41	−0,698

regression coefficients within the range studied is not statistically significant ($P > 0.05$). The result is described by the equation $(Ca^{++})^{st}_{pH\,7.40} = 1.3375 - 0.0032\ pCO_2$ ($r = 0.89$), on the basis of which the contribution of pCO_2 to $(Ca^{++})^{st}_{pH\,7.40}$ can be found and it becomes possible to calculate it at pCO_2 = 40 mm Hg. The last values were used to estimate the effect of A(P) on $(Ca^{++})^{st}$. Dependence on A and P is described by the corresponding regression lines: $(Ca^{++})^{st} = 1.37 - 0.0307\ A$ ($r = 0.84$); $(Ca^{++})^{st} = 1.44 - 0.0035\ P$ ($r = 0.8$). The coefficient of the regression line for case P (−0.0035) virtually coincides with the value (−0.00357) obtained in [9] at pCO_2 = 40 mm Hg.

Estimation of the contributions of pCO_2 and A(P) to coefficients of the regression lines for Ca^{++} = f(pH) was done on the basis of analysis of the dependence of these coefficients on the above values. The results for different levels of A(P) are given in Table 2.

Here also are given values of K at pCO_2 = 40 mm Hg, by which the contribution of proteins to the coefficients of the regression lines for Ca^{++} = f(pH) were determined. These contributions, for A and P, are described by the equations: $KA = -0.391 - 0.788\,A$ ($r = 0.92$); $KP = -0.24 - 0.0088\,P$ ($r = 0.93$).

The choice of A = 0.6 mM as standard conditions was determined so as to ensure that condition KA = KP was satisfied at P = 70 g/liter.

The resultant calculated equations have the form:

$$\left(Ca^{2+}\right)^{st}_{P} = Ca^{2+}_{i} - [0.86 - 0.0088\,(70 - P) - 0.0037\,(40 - pCO_2)]\cdot(7.40 - pH) - 0.0032\,(40 - pCO_2) - 0.0035\,(70 - P)\ldots \quad (2^{a})$$

$$\left(Ca^{2+}\right)^{st}_{A} = Ca^{2+}_{i} - [0.86 - 0.79\,(0.6 - A) - 0.0037\,(40 - pCO_2)]\cdot(7.4 - pH) - 0.0032\,(40 - pCO_2) - 0.307\,(0.6 - A)\ldots \quad (2^{b})$$

An example of the calculation of Ca^{++}_{i} by Eqs. (1) (curve 1) and (2^{b}) (curve 2) for the titration curve (curve 3), obtained by adding different quantities of 1.5 M $NaHCO_3$ to the acidified plasma, is shown in Fig. 2. The results are evidence of more exact correction of Ca^{++}_{i} in accordance with the suggested algorithm (Eq. 2). Allowing for the effect of A (instead of P) usually gives better results for the whole range of pH and pCO_2, for calcium in the plasma is mainly (up to 80%) bound with albumin [1]. Differences in the values of $(Ca^{++})^{st}$

calculated by Eqs. (1) and (2^b) are due mainly to the contributions of the components of dependence of $(Ca^{++})^{st}_{pH7.40}$ on pCO_2 and A(P), corrections for which are not provided in Eq. (1). Under conditions close to standard, values of Ca^{++} calculated by Eqs. (1) and (2^b) virtually coincide. Improvement of correction by Eq. (2), especially at pH < 7.20, can be explained by allowing for effects of pCO_2 and A(P) on coefficients of the regression lines for $Ca^{++} = f(pH)$. Unsatisfactory correction according to Eq. (1) in this pH region also was observed in an experimental study [10].

It was not one of the aims of the investigation to study the mechanisms of the effect of pCO_2 on the Ca^{++} level, pH = const. The possible causes of this effect are: 1) formation of protein complexes with CO_2, accompanied by conformational changes with a corresponding increase in the affinity of Ca^{++} for protein (an increase in the binding constant and in the number of binding sites); 2) a change in the HCO_3^- concentration and, correspondingly, in the concentration of $CaHCO_3^+$ complex. The effect of a change in Ca^{++} at pH = const will be more marked if it is assumed that calcium binds with protein in the form $CaHCO_3^+$. Experimental confirmation of the differential effects of pH, pCO_2, and A(P) on Ca^{++} provides a new outlook on the problem of its normal values. Calculation by Eq. (2) leads to corrections of ±0.3 mmole/liter to Ca_i^{++} during changes in pH, pCO_2, and A(P) within physiological limits, evidence of their significant contribution to the range of normal values of Ca_i^{++}. By allowing for natural fluctuations of pH, pCO_2, and A(P) in plasma obtained from healthy human blood, their effect on the $(Ca^{++})^{st}$ level can be ruled out, the precise limits of the normal value of $(Ca^{++})^{st}$ can be determined, and the diagnostic value of this parameter can be enhanced.

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