136 (2659)

The solubility product of tertiary calcium phosphate and its importance in biological systems.

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Calcium metabolism in general is unquestionably connected with the solubility of $Ca_3(PO_4)_2$ and this in turn with the ions of H_3PO_4 and Ca^{++} through the solubility product principle. This is particularly true for the deposition and absorption of bone as well as for the many related pathological conditions. Inasmuch as $Ca_3(PO_4)_2$ cannot precipitate unless the ion product $[Ca^{++}]^3 \times [PO_4^{---}]^2$ exceeds the equilibrium value of this product (K_{sp}), it is necessary to evaluate K_{sp} under various conditions in order to determine the degree of saturation or undersaturation before proceeding to an investigation of the other factors involved in calcium metabolism. It appears from our studies that the K_{sp} of the tertiary phosphate of calcium is of more biological importance than the secondary phosphate $CaHPO_4$. Data have been obtained on the latter and will be communicated later.

In a system containing orthophosphates, a knowledge of the hydrogen ion concentration and of the total phosphorus content makes it possible to calculate the concentration of ($PO_4^{\equiv}$) ion with reasonable accuracy, by using the formula:

$$[PO_4^{\equiv}] = \frac{[P]K_1K_2K_3}{[H^+]^3 + [H^+]^2K_1 + [H^+]K_1K_2 + K_1K_2K_3}$$

where [P] represents the molar concentration of phosphorus as phosphate, $[H^+]$ that of hydrogen ion, and K_1, K_2, and K_3 the 1st, 2nd, and 3rd ionization constants of phosphoric acid, respectively. This formula is derived from the equations defining these three ionization constants:

$$(1) \quad \frac{[H^+] \times [H_2PO_4^-]}{[H_3PO_4]} = K_1$$

$$(2) \quad \frac{[H^+] \times [HPO_4^{=}]}{[H_2PO_4^-]} = K_2$$

$$(3)\quad \frac{[H^{+}] \times [PO_4^{\equiv}]}{[HPO_4^{=}]} = K_3$$

and the equation:

$$(4)\quad [P] = [H_3PO_4] + [H_2PO_4^{-}] + [HPO_4^{=}] + [PO_4^{\equiv}]$$

which expresses the fact that the molar concentration of total phosphorus [P] is equal to the sum of the molar concentrations of the unionized and ionized forms of phosphoric acid.

A knowledge of the $PO_4^{\equiv}$ ion concentration and the Ca^{++} ion concentration enables one to calculate the value of the solubility product constant for tertiary calcium phosphate.

$$[Ca^{++}]^3 \times [PO_4^{\equiv}]^2 = K_{sp}$$

in systems which are in equilibrium with solid $Ca_3(PO_4)_2$. The system studied was prepared by titrating orthophosphoric acid with lime water, and determining the pH electrometrically after periods—extending in some cases to 8 months—had elapsed to insure equilibrium. Ca and P were determined for the solution; the composition of the solid phase was repeatedly checked by chemical analyses of the precipitates in equilibrium with the solution.

The true solubility or thermodynamic product for $Ca_3(PO_4)_2$ which is a constant under all conditions is the product of the activities or active masses of the Ca^{++} and $PO_4^{\equiv}$ ions which may be written:

$$[{}^{a}Ca^{++}]^3 \times [{}^{a}PO_4^{\equiv}]^2 = {}^{a}K_{sp}$$

At the present time, however, methods for measuring these individual ion activities with any degree of accuracy are not available; we have therefore confined ourselves to a study of the variation of the *stoichiometric* solubility product constant of the salt $Ca_3(PO_4)_2$:

$$[Ca^{++}]^3 \times [PO_4^{\equiv}] = K_{sp}$$

from which one can evaluate the mean activities of the ions of the salt.

It was found that the stoichiometric constant is greatly affected by the salt content of the solution. Thus, in a system containing only calcium salts and sodium orthophosphates at high dilution, variations from 3×10^{-30} ($pK_{sp} = 29.5$) to 3×10^{-32} ($pK_{sp} = 31.5$) are obtained at 38° C. The chief variable responsible for these marked changes is apparently the concentration of primary

phosphate in the solution. The effect of foreign salts of different valence types on the solubility product constant is shown by the following table:

	K_{sp} at 38° C.	pK_{sp}	ΔpK_{sp}	f $Ca_3(PO_4)_2$
No foreign salt. Extrapolated to infinite dilution.	$1. \times 10^{-32}$ (about)	32.+	—	1.00
M NaCl 8	2.44×10^{-30}	29.61	2.4	.33
M Na_2SO_4 8	4.73×10^{-29}	28.33	3.7	.18
M $MgSO_4$ 10	5.97×10^{-26}	25.22	6.8	.04

The symbol pK_{sp} refers to the negative log of the stoichiometric K_{sp} of tertiary calcium phosphate as defined above, analogous to pH^+. Larger values of pK_{sp} indicate that $Ca_3(PO_4)_2$ is more soluble. ΔpK^+_{sp} is the difference between the true value; *i. e.*, the value of K_{sp} at infinite dilution where the gas laws hold rigidly, and the stoichiometric value at a given salt concentration. ΔpK_{sp} therefore is proportional to the activity of coefficient (f) of the salt by the general relation.

$$\Delta pK_{sp} = -\nu \log f \text{ (salt} = -5 \log f \text{ } (Ca_3(PO_4)_2)$$

ν is the number of ions composing the salt.

Although the solubility product for $Ca_3(PO_4)_2$ is greatly affected by the addition of salts and proteins, it remains remarkably constant when a medium of constant salt composition is maintained. Thus in a solution containing inorganic salts in approximately the concentration found in blood serum, the solubility product constant at 38° C. was found to be very close to 6×10^{-28} ($pK_{sp} = 27.2$), and in blood serum itself a product of about 1×10^{-26} ($pK_{sp} = 26.0$) was regularly found at this temperature.

These marked salt effects are not surprising in the light of the studies of Bronsted and LaMer.[1] From a consideration of the high valences of the ions of the saturating salt, the magnitude of the effects may be predicted from the equation which they derived and tested. In the present case their equation 34 can be reduced to the simple form

[1] Bronsted and LaMer, *J. Am. Chem. Soc.*, 1924, xlvi, 555.

$$\Delta pK_{sp} = 3\sqrt{\mu} + \beta\mu$$

where μ is the ionic strength of the solution as defined by G. N. Lewis[2] and β is an empirical constant depending primarily upon the size of the ions involved. The data show that the magnitude of the salt effect is in as good agreement as could be expected in view of the difficulties involved in studying the system and the unsymmetric nature of the valence types of the salts used. A critical investigation of the theory of Debye and Hückel upon which the equation is founded shows that a definite theoretical reason exists for the extension of the theory in this respect. Accurate data on appropriate salts are now being obtained for a test of a more general equation resulting from a consideration of the effects of unsymmetric valence types.

Since the stoichiometric solubility product remains constant in a medium like blood serum in which relatively small changes in the salt concentration (ionic strength) occur, it is obvious that this constant can be used to determine whether blood serum is supersaturated or undersaturated with $Ca_3(PO_4)_2$. Calculations which we have made of the ion product:

$$(Ca^{++})^3 \times (PO_4\equiv)^2$$

indicate that human serum is normally very much supersaturated with $Ca_3(PO_4)_2$. The ion products in normal serum and in cases of active and healing rickets are shown in the following table:

	$(Ca^{++})^3 \times PO_4\equiv)^2$	P (ion product)
Active Rickets	3.5×10^{-26} to 8.4×10^{-25}	25.45 to 24.08
Normal (adults)	6.2×10^{-25} to $1. \times 10^{-24}$	24.20 to 24.00
Healing Rickets	6.5×10^{-25} to 4.8×10^{-24}	24.18 to 23.32

Even in active rickets the serum would seem to be considerably supersaturated, although not to the extent of normal serum.

When normal serum is shaken continuously at 38° C., no reduction in the ion product $[Ca^{++}]^3 \times [PO_4\equiv]^2$ occurs, even after 2 weeks. When, however, this shaking is carried out in the presence of solid $Ca_3(PO_4)_2$ a gradual deposition of this salt

[2] Lewis and Randall: "Thermodynamics and Free Energy of Chemical Substances," New York, 1923. Page 364.

occurs, and in about a week values close to that of the solubility product constant are obtained. This slow precipitation has been observed also in inorganic solutions. The rate of precipitation apparently depends upon how much the value of the solubility product is exceeded; *i. e.*, the rate of reaction is proportional to the free energy change (—ΔF) or driving force of the process.

Thus one might expect to find that in active rickets there exists not complete arrest of calcification, but rather a marked diminution in the rate of calcification, resulting from the diminution of the ion product in blood serum. Some observations recently made on rats by Dr. Shipley[2] indicate that this is the case. If rats are kept a sufficiently long time upon a rickets-producing diet, a fine deposition of calcium in the metaphysis is often found. Moreover in human rickets a complete arrest of calcification is seldom if ever found.

The tendency of tertiary calcium phosphate to remain supersaturated in solutions for long periods of time would seem to be of considerable biological importance. It is by this mechanism that the blood is able to hold quantities of calcium sufficient to prevent tetany.

A more detailed report of these experiments will shortly be published.

137 (2660)

A new color test for differentiating neoarsphenamine from sulfarsphenamine.

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Although sulfarsphenamine, which was first introduced clinically in France as Sulfarsenol, is closely related to neoarsphenamine, a distinct chemical difference exists between the two drugs, the former being a derivative of sulfurous acid while the latter is a derivative of the hypothetical sulfoxylic acid.

Publications of Macallum,[1] deMyttenaere[2] and others indicate

[2] P. G. Shipley, personal communication.