SOME THEORETICAL CONSIDERATIONS CONCERNING CRYSTALS WITH RELEVANCE TO THE PHYSICAL PROPERTIES OF BONE

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(Received June 12th, 1958)

SUMMARY

Some of the apparently anomalous properties of bone crystals can be understood by relatively simple thermodynamic consideration of the characteristics of a crystal bed in contact with an aqueous solution of its ionic constituents. The small size of bone crystals means that an appreciable proportion of the mineral constituents of bone are in the surface or interfacial phase. This means that the (Gibbs) free energy for the transition of ionic constituents from solution into the crystal bed (or vice versa) depends upon not only the pressure, temperature and mole numbers but also upon the geometrical parameter defining the surface to volume ratio. Under such circumstances the solubility product will not be defined, a conclusion in agreement with the discrepant results of the various attempts to measure such a quantity experimentally. It is also demonstrated that the thermodynamic potential will favor internal rearrangements of the crystal bed such that the surface to volume ratios of the crystals in the bed are decreased giving rise to various effects associated with the maturation of bone. This also provides an interpretation of the discrepancy between the isotopically observed rates of exchange between the supernatant solution and crystal bed and the time required for the crystal bed to come to solubility equilibrium with its supernatant solution. Some thermodynamic distinctions between precipitation and crystal growth are discussed and suggestions presented concerning the kinetics of formation of calcium phosphate microaggregates in the absence of seeding sites.

INTRODUCTION

Since bone is comprised of small mineral crystals deposited in an organic matrix, many of its most important properties will depend upon the intrinsic properties of these crystals. These mineral crystals are known to be made up primarily of calcium and phosphate ions with varying amounts of carbonate, hydroxyl ions, citrate and water. Lesser and variable amounts of Na^+, K^+, Cl^-, F^- and Mg^{++} are also found in the bone salt. The basic structure of the crystal lattice is believed to be that of hydroxyapatite (McLean and Urist[7]).

Although many of the properties of bone crystals depend specifically upon their

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particular chemical constituents, some of their important properties are common to ionic crystals in general. Since there is a considerable amount of variability in the composition of the bone salt (Neuman and Neuman[9]) it can be anticipated that kinetic considerations formulated in terms of a particular lattice arrangement and ionic composition may not be sufficiently general to be of much value at the present time. This paper will be concerned primarily with certain general properties, for the most part obtainable from simple thermodynamic considerations, possessed by populations of crystals containing essentially the same ionic constituents and in contact with a solution of those ions.

CONSIDERATIONS

A population of crystals containing essentially the same ionic constituents and in contact with a solution of those ions will be referred to as a "crystal bed" hereafter. Although the crystals of a crystal bed may be all comprised of the same ionic constituents, with the same elementary ionic lattice units, they will not usually be of uniform size and will exhibit a certain amount of irregularity in shape.

The irregularities in shape may arise from pits, projections, ridges and dislocations that give rise to unpaired electrostatic bonds in the crystal surface and play an important role in the growth of the crystal. In general the ions in the surface layer of a crystal are in a different state from those in the interior so that the surface must be regarded as a separate phase, related to but distinct from the interior phase.

One may regard an elementary crystalline lattice unit as a sort of molecule-like aggregate of ions and speak of a mole of such lattice units. Let μ_s be the chemical potential (Zemansky[10]) of the crystalline constituents in the surface phase and μ_i their chemical potential in the interior phase. These chemical potentials are the partial molar free energies of the lattice units in the surface and interior phases respectively. Since an ion in the surface will not be able to bond electrostatically with as many ions of opposite sign as an ion in the interior, μ_s will usually be appreciably greater than μ_i.

Suppose the material of which the crystal is comprised to be calcium phosphate. Let us say that there are ν_c calcium and ν_p phosphate ions contained in an elementary ionic lattice unit of the crystal, without commitment as to the specific values of ν_c and ν_p except to assert that they are positive integers. Imagine some process of crystallization whereby ν_c moles of calcium ions and ν_p moles of phosphate ions are transferred from a supernatant solution into lattice units of a crystal. Because of the shape requirements of a crystal, a certain number of surface phase lattice units will, of necessity, be formed whenever an interior phase lattice unit is formed. Thus, for each mole of crystal lattice units formed, a certain proportion of the units must be in the surface phase and a certain proportion in the interior phase. The relative amounts will depend upon the surface to volume ratio of the crystal. One can, therefore, write the following "crystallization reaction" equation

$$\underset{\text{solution}}{\nu_c\,\mathrm{Ca}^{++} + \nu_p\,\mathrm{PO}_4^{\equiv}} \rightleftarrows \underset{\text{crystal surface}}{\frac{n_s}{n_x}\,\mathrm{Ca}_{\nu_c}\,(\mathrm{PO}_4)_{\nu_p}} + \underset{\text{crystal interior}}{\frac{n_i}{n_x}\,\mathrm{Ca}_{\nu_c}\,(\mathrm{PO}_4)_{\nu_p}} \tag{1}$$

where n_x, n_i and n_s are the number of moles of lattice units in the crystal, in its interior phase and in its surface phase respectively. The free energy change, ΔF, of the "reaction" will be

$$\Delta F = -\nu_c \mu_c - \nu_p \mu_p + \frac{n_s}{n_x} \mu_s + \frac{n_i}{n_x} \mu_i \tag{2}$$

where μ_c and μ_p are, respectively, the chemical potentials of the calcium and phosphate ions in solution.

If δ is the mean lattice distance between adjacent ions of opposite charge, it is probably adequate, for purposes of the present discussion, to consider the surface phase to have a thickness of the order of magnitude of δ. The surface to volume ratio of a crystal shall be denoted as ξ. Since ξ depends upon both the size and shape of a crystal and these properties are not absolutely uniform among the crystals of the bed, one will generally find that ξ has some probability distribution among the various crystals. Define $\bar{v}$ as the partial molar volume of the lattice units in the crystal. Although $\bar{v}$ will not, rigorously speaking, be the same in the surface and internal phase, it will be satisfactory for the present discussion to consider it so.

Consider a crystal of volume v. There will be $(1 - \xi\delta)\, v/\bar{v}$ moles of lattice units in its interior phase and $\xi\delta\, v/\bar{v}$ moles in its surface phase. The total number of moles of lattice units in the crystal will be $v/\bar{v}$. One can therefore write that

$$\frac{n_s}{n_x} = \xi\delta$$

and

$$\frac{n_i}{n_x} = (1 - \xi\delta) \tag{3}$$

It will be convenient to define

$$\mu = \nu_c \mu_c + \nu_p \mu_p, \tag{4}$$

γ_c and γ_p as the activity coefficients of the calcium and phosphate ions in solution, and μ_o as the standard free energy of these ions in solution.

In dilute solutions γ_c and γ_p can be considered to be given adequately by the DEBYE-HÜCKEL theory (GLASSTONE[4]) and will hence depend only upon the total ionic strength and the temperature of the solution. If (Ca) and (PO_4) are, respectively, the concentrations of calcium and phosphate ions in the supernatant solution, R is the gas constant and T the absolute temperature, one can write μ in the form

$$\mu = \mu_o + RT \ln \gamma_c^{\nu_c} \gamma_p^{\nu_p} + RT \ln (\mathrm{Ca})^{\nu_c} (\mathrm{PO_4})^{\nu_p} \tag{5}$$

Define

$$\mu_o' = \mu_o + RT \ln \gamma_c^{\nu_c} \gamma_p^{\nu_p}. \tag{6}$$

Since μ_o depends only upon the temperature and pressure, one can say that μ_o' will depend only upon the temperature, pressure and total ionic strength of the supernatant solution.

Substituting expressions (3), (4), (5) and (6) into equation (2) one obtains that

$$\Delta F = -\mu_o' - RT \ln (\mathrm{Ca})^{\nu_c} (\mathrm{PO_4})^{\nu_p} + (1 - \xi\delta)\, \mu_i + \xi\delta\, \mu_s \tag{7}$$

For equilibrium between the supernatant solution and the crystal to occur the free energy of the transition must be zero. At equilibrium, therefore,

$$\Delta F = 0 \tag{8}$$

Imposing this condition in equation (7) one finds that

$$(\mathrm{Ca})_e^{\nu_c}\,(\mathrm{PO}_4)_e^{\nu_p} = \mathrm{e}^{\frac{\mu_i - \mu_o'}{RT}}\;\mathrm{e}^{\frac{(\mu_s - \mu_i)}{RT}\,\xi\delta} \qquad (9)$$

where $(\mathrm{Ca})_e$ and $(\mathrm{PO}_4)_e$ refer to the equilibrium concentrations of these ions. One can identify

$$K = \mathrm{e}^{\frac{\mu_i - \mu_o'}{RT}}\;\mathrm{e}^{\frac{(\mu_s - \mu_i)}{RT}\,\xi\delta} \qquad (10)$$

as the "solubility product" for the equilibrium between the crystal and the supernatant solution. Expressions (9) and (10) lead to certain interesting results.

Since the greatest reservoir of calcium and phosphate in the body is in the bone salts and these present an extensive surface of contact to the body fluids, the solubility of calcium phosphate has long been a matter of interest to those concerned with homeostatic regulation of the concentrations of these ions in the tissue fluids. For the foregoing reason, and because of the theoretical importance for a classical view of osteogenesis (ROBISON[9]), quite a few attempts have been made to determine the solubility product, *in vitro*, between bone crystals and calcium and phosphate ions in solution. A number of different values and forms have been given as the appropriate ones, leading NEUMAN AND NEUMAN[8] to agree with HODGES[5] that perhaps no such solubility product exists. So far as one can see, these authors base their conclusion fundamentally upon the discrepant results reported by the various investigators who have attempted such a measurement.

Consider a bed of crystals sufficiently small and/or sufficiently irregular that, for an appreciable proportion of them, ξ is large enough so $(\mu_s - \mu_i)\,\xi\delta/RT$ cannot be neglected. Since the equilibrium value of the product $(\mathrm{Ca})^{\nu_c}\,(\mathrm{PO}_4{}^{\nu_p}$ will depend upon a quantity ξ, it is clear that crystals having different values of ξ cannot be in equilibrium with the same solution simultaneously. If the crystals of the bed are not uniform with respect to ξ, it is not then possible to define a solubility product such that all the crystals of the bed are in equilibrium with the solution. The crystals of bone are very small being only of the order of magnitude of 350 Å long 300 Å wide and 30 Å thick (NEUMAN AND NEUMAN[8]).

With such small sizes the surface to volume ratios ξ will be relatively large and $(\mu_s - \mu_i)\,\xi\delta/RT$ probably appreciable. The value of ξ is almost certainly not uniform among the crystals. One is led to conclude on logical grounds, therefore, that a solubility product does not exist for a bed of bone crystals. As the average crystal size of a bed becomes larger and the crystals more regular, the mean value of ξ will become zero for practical purposes so $(\mu_s - \mu_i)\,\xi\delta/RT$ becomes effectively zero. When only a negligible proportion of the material in the crystal bed resides in crystals for which ξ is appreciably greater than zero, the value of the solubility product becomes

$$K_o = \mathrm{e}^{\frac{\mu_i - \mu_o'}{RT}} = \lim_{\xi \to 0} K \qquad (11)$$

and, since it is independent of ξ, has the same value for all the crystals in the bed. Thus, when the crystals in question are sufficiently large, the solubility product is defined and a unique value can be experimentally measured for it at a given temperature, pressure and total ionic strength.

These results are in accord with a general cautionary note made earlier by FERMI[2] who, in a discussion of thermodynamic systems, states "The geometry of our system is obviously characterized not only by its volume, but also by its shape. However, most thermodynamical properties are largely independent of the shape, and, therefore, the volume is the only geometrical datum that is ordinarily given. It is only in the cases for which the ratio of surface to volume is very large (for example, a finely grained substance) that the surface must also be considered". It would appear that the mineral component of bone is such a system.

One might argue that, although the individual crystals are going into solution or growing, one could pick a solubility product such that the forward and reverse rates were equal and that such a value would, for all practical purposes, fulfil the requirements of the classical solubility product.

Let us choose some value K^* for the solubility product, K, and suppose that $\xi^* > 0$ is the surface to volume ratio of those crystals in true equilibrium with the supernatant solution in which $(\mathrm{Ca})^{\nu_c} (\mathrm{PO}_4)^{\nu_p} = K^*$. Then

$$K^* = K_0\, \mathrm{e}^{\frac{(\mu_s - \mu_i)}{RT} \xi^* \delta} \tag{12}$$

In such a supernatant solution all those crystals with $\xi < \xi^*$ will now start growing and those with $\xi > \xi^*$ will begin to dissolve. The effect of growth of a crystal, without change in shape, will be to make the surface to volume ratio ξ smaller, while the effect of dissolution will be to make it larger. As a result of these processes the frequency distribution of crystals in the bed, having various values of ξ, will be altered with time so that, after a sufficiently long period, there will be no crystals with $\xi > \xi^*$. Thus if ξ^* is such that the rates of growth and dissolution are equal initially then, after a sufficiently long period of time in contact with the supernatant solution, the rate of growth must of necessity become larger than the rate of dissolution. The value of K originally giving a zero net rate of growth for the bed as a whole will now not give a zero rate but a positive one instead. Clearly this is not what one has in mind when one thinks of a solubility product. It is apparent from such considerations that, if an appreciable portion of the crystal bed is comprised of crystals sufficiently small, or irregular, that their values of $(\mu_s - \mu_i)\, \xi\delta/RT$ are not negligible, it is not even possible to choose a practical value of K for the bed in the sense that the rates of dissolution and crystallization are equal and remain so.

Maturation of the crystal bed

It is fairly well known that newly formed crystals, and particularly those precipitated rapidly, tend to be small and somewhat irregular. A bed of such crystals, left in contact with the supernatant solution of the ions of which the crystals are composed, tends to undergo certain modifications with time. This process is referred to as maturation. It is found that old bone has somewhat different properties from bone that is newly mineralized or in which mineralization is still going on. Certain of these differences can perhaps be attributed to the physical process of maturation of the crystal bed of the bone. With this in mind it will be worthwhile to examine certain features of crystal maturation.

The free energy, μ_x, per mole of lattice units in a crystal with surface to volume ratio, ξ, will be

$$\mu_x = \mu_i + (\mu_s - \mu_i)\, \xi\delta \tag{13}$$

Since $\mu_s > \mu_i$ and ξ and δ are always positive, it is clear that μ_x will be a minimum when ξ is a minimum. Consider a crystal of a given volume, *i.e.* containing some given number of lattice units. The lattice units can be "stacked together" in a large number of different ways without deformation of the shape of the elementary lattice unit just as bricks of a given shape can be placed congruent to one another to make houses of various shapes. The arrangement, however, that, for a given number of lattice units in a crystal, minimizes ξ will, for many relatively simple lattice configurations, be a macroscopic replica of the fundamental lattice unit. It is easy to convince oneself of this in the case of a simple cubical lattice such as that of sodium chloride. The validity in the case of more complex lattice units will be merely asserted without proof. If there were no constraints on the relative positions of the ions in the lattice then the situation would be similar to that of a perfect fluid in which the shape giving the minimum value of is a sphere.

So long as the shape is such that ξ has not attained its minimum value then μ_x will not have attained its minimum so that there will exist a thermodynamic potential difference favoring rearrangements in the crystal toward a smaller value of ξ. This process of resculpturing will not only tend to perfect the shape of the crystal but will reduce the number of unsaturated surface binding sites.

It is usually observed experimentally that newly mineralized bone, or those regions of bone undergoing mineralization, is much more prone to adsorb foreign elements, such as radium or strontium, than old bone. The process of maturation of a crystal bed discussed above gives a possible avenue of interpretation for these effects. A given volume of bone crystals, in contact with a liquid phase, will, with time, tend to redistribute its ionic constituents in such a manner that the amount of crystal surface, and consequently the number of surface binding sites, is reduced. With such a reduction in the number of binding sites, the amount of the foreign element bound will consequently be reduced.

It is experimentally observed, by those who are attempting solubility product measurements on bone crystals, that a long time is required for equilibrium to be attained (*e.g.* HOLT *et al.*[6]; CAMPBELL AND GREENBERG[1]).

A possible interpretation of this observation might be as follows. When a bed of bone crystals, of sufficiently small size that the surface to volume ratio is large and not the same for the various crystals of the bed, is initially placed in contact with a solution of calcium and phosphate ions, no true equilibrium is possible. It is not until maturation of the crystal bed has occurred to such an extent that $(\mu_s - \mu_i) \cdot \xi\delta/RT$ can be neglected for all but an insignificant portion of the crystal bed that equilibrium can be attained. Maturation of the bed, however, is a process that requires much longer periods of time than attainment of equilibrium with a crystal bed in which the crystals are homogeneous with respect to size and shape. Thus, one would find time of an entirely different order of magnitude required for such phenomena as isotopic exchange with the surface phase than for the bed to come to equilibrium with respect to net transfer.

Some thermodynamic distinctions between precipitation and crystal growth

Consider the situation when there are *no* crystals initially present in a solution containing calcium and phosphate ions. If the product $(\mathrm{Ca})^{\nu_c} (\mathrm{PO}_4)^{\nu_p}$ is increased there will be some point at which a precipitate will form. In order for a solid aggregate of calcium and phosphate ions to form, in the absence of crystals or seeding loci on which these ions can deposit, it is first necessary for them to form a microaggregate. Such a microaggregate will be able to form electrostatic bonds with even fewer ions of the opposite sign that those ions present in the surface phase of a crystal. One may, therefore, anticipate that the chemical potential of the ions in such a microaggregate will be as large or larger than those in the surface phase of a crystal. Thus, if μ_m is the chemical potential in the microaggregate, one can fairly safely assert that

$$\mu_m > \mu_s \tag{14}$$

For a net positive transfer of ions from solution into a solid phase, in the absence of a crystal bed upon which growth can occur, it must therefore be the case that

$$\mu \geq \mu_m. \tag{15}$$

In order for a crystal with surface to volume ratio ξ to grow, when in contact with the solution, it is only necessary that $\mu > \mu_x$. Since $\mu_m > \mu_s > \mu_x$, it is clear that it is possible to choose a solution with concentrations of calcium and phosphate such that

$$\mu_m > \mu > \mu_x = \mu_i + \xi\delta\,(\mu_s - \mu_i) \tag{16}$$

In the case of calcium phosphate solutions fulfilling the condition of inequality (16), crystals of surface to volume ratio less than ξ will grow but no precipitate will form. One cannot, however, quite agree with the conclusion of NEUMAN AND NEUMAN[8] that "almost any solution containing finite concentrations of calcium and phosphate ions can be considered to be metastable". No solution of calcium and phosphate ions for which $\mu \leq \mu_i$ would be metastable. It is true that, from a practical point of view, the concentrations must be fairly small for this condition to be satisfied, however, from a logical standpoint it is important to note that it can be.

The biological significance of these results is fairly straightforward but crucial. A spread between the values of μ_m and μ_x permits the organism to deposit calcium and phosphate into bone without precipitation of calcium phosphate in the blood vessels or soft tissues. It seems likely that in the normal homeostatic balance the free calcium and phosphate levels in the blood are such that $\mu_m > \mu \geq \mu_x$.

Some suggestions regarding the kinetics of formation of calcium phosphate microaggregates in the absence of seeding sites

In order for a precipitate of calcium phosphate to form, in the absence of crystals or seeding loci at the outset, it is necessary that ion-ion reactions occur to form microaggregates of calcium and phosphate ions. As will be seen in the course of the discussion, the reaction system by which this can happen would appear to be rather complex. A complete solution of the process will therefore not be attempted but some suggestions will be made in the hope that they may be useful to the person inspired to carry the problem through to completion.

One can feel fairly safe in asserting several points at the outset.

(1) The probability of three or more individual molecules (or ions) undergoing simultaneous collision can be neglected. Therefore, an aggregate comprised of a number of calcium and phosphate ions must be built up by a process involving only two body collision processes.

(2) The probability of a step involving the union of two ions of like charge can be neglected.

(3) The probability of a step involving the union of two ions of unlike charge will be relatively large.

In an aqueous solution containing calcium and phosphate ions there will of course be present also hydronium and hydroxyl ions. One can therefore write the following ion ion reactions as processes which may occur

$$\begin{array}{lll}
H^+ & + PO_4^{\equiv} & \rightleftarrows HPO_4^{=} \\
H^+ & + HPO_4^{=} & \rightleftarrows H_2PO_4^- \\
Ca^{++} & + PO_4^{\equiv} & \rightleftarrows CaPO_4^- \\
Ca^{++} & + HPO_4^{=} & \rightleftarrows CaHPO_4 \\
Ca^{++} & + H_2PO_4^- & \rightleftarrows CaH_2PO_4^+ \\
CaPO_4^- & + H^+ & \rightleftarrows CaHPO_4 \\
CaPO_4^- & + Ca^{++} & \rightleftarrows Ca_2PO_4^+ \\
CaPO_4^- & + Ca_2PO_4^+ & \rightleftarrows Ca_3(PO_4)_2
\end{array}$$

etc. etc.

In general one can consider a reactant ion of the composition

$$Ca_r\, H_s\, (PO_4)_t$$

having a net charge

$$Q = (2r + s - 3t)\ \varepsilon$$

where ε is the charge of an electron. Denote the net rate of combination between the *ith* ionic species and *jth* ionic species as v_{ij}. Denote the concentration of the *ith* species as C_i, of the *jth* species as C_j and of the species resulting from this union as C_{ij}. One can then write that

$$v_{ij} = k_{ij}\, C_i\, C_j - \underline{k}_{ij}\, C_{ij}$$

By viewing these ion-ion reactions in terms of absolute reaction rate theory (GLASSTONE, LAIDLER AND EYRING[4]) one can write

$$k_{ij} = e^{-\Delta F^{\ddagger}_{ij}/kT}\ \frac{kT}{\hbar}$$

$$\underline{k}_{ij} = e^{-\Delta \underline{F}^{\ddagger}_{ij}/kT}\ \frac{kT}{\hbar}$$

where k is the Boltzman constant, $\hbar$ is Planck's constant, $\Delta F^{\ddagger}_{ij}$ is the free energy of formation of the activated complex from the reactants, $\Delta \underline{F}^{\ddagger}_{ij}$ the free energy of formation of the activated complex from the product. Denote r_a as the mean distance of separation of the charges on the two ionic reactants when the activated complex is formed, r_o as their mean distance of separation in the product of the reaction and D as the effective microscopic dielectric constant of water. Let us assume that the variation in $\Delta F^{\ddagger}_{ij}$ and $\Delta \underline{F}^{\ddagger}_{ij}$ between the various types of ion species in question can, for the most part, be accounted for by variations in the coulombic interactions between the ions. Taking this view one can write that

$$\Delta F^{\ddagger}_{ij} = \Delta F^{\ddagger}_{1} + \frac{Q_i Q_j}{r_a\, D}$$

$$\Delta \underline{F}^{\ddagger}_{ij} = \Delta \underline{F}^{\ddagger}_{1} + \frac{Q_i Q_j}{D}\left(\frac{1}{r_a} - \frac{1}{r_o}\right)$$

where $\Delta F_1^{\ddagger}$ and $\Delta \underline{F}_1^{\ddagger}$ are the same for all the ion-ion reactions in question. One can therefore write the rate constants in the form

$$k_{ij} = k_1\, e^{-QiQj/r_a DkT}$$

$$\underline{k}_{ij} = \underline{k}_1\, e^{-Q_iQ_j(1/r_a - 1/r_o)/DkT},$$

where

$$k_1 = \left(\frac{kT}{\hbar}\right) e^{-\Delta F_1^{\ddagger}/kT} \text{ and } \underline{k}_1 = \left(\frac{kT}{\hbar}\right) e^{-\Delta \underline{F}_1^{\ddagger}/kT},$$

the rate of reaction between the *ith* and *jth* species would then be

$$v_{ij} = k_1 e^{-Q_iQ_j/r_a DkT}\, C_iC_j - \underline{k}_1\, e^{-Q_iQ_j\,(1/r_a - 1/r_o)/DkT}\, C_{ij}$$

Since estimates for D, k, r_o and r_a are available from other sources this leaves only k_1 and $\underline{k}_1$ as unknown parameters.

If the parameters k_1 and $\underline{k}_1$ were known one could then say what the rate of reaction between any two of the possible ionic constituents would be in terms of their concentrations. Hence, if one knew the initial concentrations and had the additional information that the system was closed, one should, in principle, be able to give the time course of the concentration of any of the ionic aggregate species. Unfortunately as the maximum aggregate sizes increase to the point where they contain more than a very few calcium, hydrogen and phosphate ions, the number of different possible ion-ion reactions becomes very large and the logical analysis formidable. Because of these mathematical difficulties and the doubtful role of such precipitation phenomena in bone mineralization (NEUMAN AND NEUMAN[8]), no attempt will be made to extend the treatment of this reaction system in the present discussion.

ACKNOWLEDGEMENT

The author wishes to thank Dr. WILLIAM NORRIS of the Biological section of the Argonne National Laboratory for discussions that served to stimulate the ideas set forth in this paper.

REFERENCES

[1] W. W. CAMPBELL AND D. M. GREENBERG, *Proc. Natl. Acad. Sci. U.S.*, 26 (1940) 176.
[2] E. FERMI, *Thermodynamics*, Dover Publications, Inc., New York, 1937, p. 2.
[3] S. GLASSTONE, *Textbook of Physical Chemistry*, 2nd ed., D. van Nostrand Co., Inc., New York, 1946, p. 956.
[4] S. GLASSTONE, K. LAIDLER AND H. J. EYRING, *The Theory of Rate Processes*, McGraw-Hill Book Co., Inc., New York and London, 1941.
[5] H. C. HODGES, *Conference on Metabolic Interrelations, Trans. of the Third Conference*, 1951, p. 194.
[6] L. HOLT JR., V. K. LA MER AND H. B. CHOWN, *J. Biol. Chem.*, 64 (1925) 509.
[7] F. C. MCLEAN AND M. R. URIST, *Bone: An Introduction to the Physiology of Skeletal Tissue*, The Univ. of Chicago Press, Chicago, 1955.
[8] W. F. NEUMAN AND M. W. NEUMAN, *Chem. Rev.*, 53 (1953) 1.
[9] R. ROBISON, *Biochem. J.*, 17 (1923) 286.
[10] M. ZEMANSKY, *Heat and Thermodynamics*, 2nd ed., McGraw-Hill Book Co., Inc., New York and London, 1943, p. 322.