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The Surface Chemistry of Bone. VIII. On the Mechanism of Ionic Exchange[1]

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The ionic exchange properties of bone mineral, as represented by a sample of hydroxy apatite, have been studied under a variety of conditions using radiophosphate and radiocalcium as tracers. The time course of the over-all exchange process appears to occur in a series of steps corresponding to the physical location of the exchangeable phosphate groups in the crystals themselves, *i.e.*, the hydration shell, the lattice surface and interior lattice positions.

Many of the chemical and physiological properties of bone have been attributed recently to the surface chemistry of the mineral crystals of bone.[2] Thus far, the principal contributions in the development of this field have been derived from the study of the ionic exchange of the crystal surfaces of bone preparations with the aid of isotopes.[3-6] Unfortunately, the methods used[3a,b] have been tedious and lacked the precision required to elucidate the actual mechanisms involved.

An apparatus recently described by Schweitzer and Nehls[7] has been applied to a comprehensive study of the exchange process with hydroxy apatite—the prototype mineral of bone.[2] The results permit a kinetic analysis which confirms and extends earlier studies of Kolthoff and collaborators[8] of the surface chemistry of a number of inorganic precipitates.

Materials.—While the bone mineral shows considerable variation in composition, it always exhibits the X-ray diffraction pattern of hydroxy apatite.[9] To eliminate possible variations, a single, well-characterized[10] preparation of commercial hydroxy apatite was utilized for these studies. In some instances, as designated, another apatite was also investigated for comparison. Both apatite preparations have been characterized by X-ray diffraction and have been carefully analyzed.[10]

The apparatus employed in these studies was like that of Schweitzer and Nehls[7] except for a special sampling device.[11] This sampling device which is operated by a pressure reduction afforded by either a mercury leveling bulb or a 50 ml. syringe deserves detailed attention. It has recently been shown that basic calcium phosphates and, thus, presumably, bone crystals show a marked tendency to form colloidal suspensions.[12] Some fine, sintered-glass filters gave a good separation of the solid from the solution; however, other filters of the same type proved unsatisfactory. It is necessary to determine the proper filter by comparing the filtrate with supernatant solutions of an equivalent sample centrifuged at 80,000 × gravity, a separating force which insures the removal of all suspended particles.[12] A fine filter of nominal maximum pore size of 5 μ has been found quite satisfactory, while a filter having pores of 14 μ gave inconsistent results.

Experimental

Procedure.—To 700 ml. of a test solution (for most purposes a potassium phosphate–calcium chloride solution containing 5 μg. of P and 10 μg. of Ca per ml. which has been adjusted to *p*H 7.2 by the addition of NH_4OH has been found to be optimal) is added one gram of the apatite or bone preparation to be studied. The crystal suspension is stirred for at least 24 hours to attain solubility equilibrium. Then, 1 ml. of carrier-free radio phosphate (P^{32}) solution is added. In some instances high specific activity Ca^{45} was used instead. The level of radioactivity is previously adjusted in this aliquot to give on final dilution approximately 1000 counts per min. per ml. in the suspension liquid. Because the exchange reaction is very rapid, it is necessary to determine the initial level of radioactivity by a separate dilution of an identical aliquot of P^{32} to 700 ml. with the buffer solution containing no crystals. In these experiments, the radioactive P^{32} was assayed using 1-ml. aliquots and a thin mica-window G–M counting tube equipped with a commercial scaler. Ca^{45} was determined similarly by drying 0.1-ml. aliquots on aluminum foils to increase counting efficiency.

The course of the exchange is followed by periodic removals of one-ml. samples of filtrate through the sampling tube. Each sample is then assayed for radioactivity. A final large portion of filtrate is removed to provide for the accurate analysis of total concentrations of calcium and phosphate at the solubility equilibrium.[12] These aliquots were analyzed for phosphate by the method of Fiske and SubbaRow[13] and for calcium by a titration with disodium ethylenediamine tetraacetate.[14]

The Kinetics of the Exchange Process

The Surface Hypothesis.—It is helpful to consider other known facts concerning the hydroxy apatite solution system in relation to the data to be obtained by this approach. In an aqueous medium, the crystals possess a hydration layer containing exchangeable ions[15] and the crystals themselves possess a net positive charge.[9,15] While many details are lacking, the system may be represented schematically as in Fig. 1 where it is seen that an exchangeable ion may occupy a number of positions: (1) in the bulk solution; (2) in the

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(1) This paper is based on work performed under contract with the United States Atomic Energy Commission at the University of Rochester Atomic Energy Project, Rochester, New York.

(2) W. F. Neuman, "Metabolic Interrelations," 2nd Josiah Macy Conf. 1950, p. 32.

(3) (a) M. Falkenheim, W. F. Neuman and H. C. Hodge, *J. Biol. Chem.*, **169**, 713 (1947); (b) W. F. Neuman, M. W. Neuman, E. R. Main and B. J. Mulryan, *ibid.*, **179**, 335 (1949).

(4) M. Falkenheim, E. Underwood and H. C. Hodge, *ibid.*, **188**, 805 (1951).

(5) W. F. Neuman and B. J. Mulryan, *ibid.*, **185**, 705 (1950).

(6) G. Hevesy, "Radioactive Indicators," Interscience Publishers, Inc., New York, N.Y., 1948.

(7) G. K. Schweitzer and J. W. Nehls, *J. Phys. Colloid Chem.*, **55**, 215 (1950).

(8) I. M. Kolthoff and A. S. O'Brien, THIS JOURNAL, **61**, 340 (1939).

(9) W. F. Neuman and M. W. Neuman, *Chem. Revs.*, **53**, 1 (1953).

(10) W. F. Neuman, Atomic Energy Report, UR-238 (1953).

(11) J. H. Weikel, Jr., and W. F. Neuman, Atomic Energy Report, UR-228 (1952).

(12) G. J. Levinskas, Thesis, University of Rochester, 1953.

(13) C. H. Fiske and Y. SubbaRow, *J. Biol. Chem.*, **66**, 375 (1925).

(14) A. E. Sobel and A. Hanok, *Proc. Soc. Exptl. Biol. Med.*, **77**, 737 (1951).

(15) W. F. Neuman, T. Y. Toribara and B. J. Mulryan, THIS JOURNAL, **75**, 4239 (1953).

hydration layer; (3) in the crystal surface; (4), (5), (6)—and a number of positions in the lattice interior.

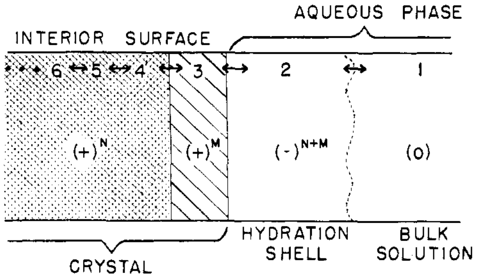

Fig. 1.—A schematic representation of a cross-section of the crystal:solution interface.

With no foreknowledge of the subject, one might reasonably expect that an exchange of ions between (1) and (2) would be a fast diffusion, between (2) and (3) might be a slower process and interchanges *within* the crystals definitely would occur even more slowly. It seemed possible, therefore, that the rates of exchange between these "compartments" would be sufficiently different to permit separate kinetic analyses, even though all steps of the exchange are interrelated.

As expected, the disappearance of radiophosphate and radiocalcium from solution appears to have taken place in a series of separate and distinct steps. This was not readily evident from an examination of a plot of solution activity against time. When, however, the log of the solution activity (counts/min./ml.) was plotted *vs.* time, there could be seen a series of lines of progressively decreasing slope (Fig. 2). Due to the speed of the early portions of this exchange reaction, the first portion of the curve can only be approximated. This can readily be understood when it is noted that, in many cases, the extent of the exchange at two minutes is nearly one-half that at four hours.

In the course of these studies the steps of the reaction have been numbered consecutively with that portion of the exchange of phosphorus, and the corresponding step in the calcium exchange, which occurs between roughly one and six hours, being step II. All that precedes this is noted as step I. Steps II and III which have rates applicable to a kinetic study are the portions of the exchange reaction to be considered in these investigations.

In deriving a rate expression to describe this process it is necessary to remember that an exchange reaction is reversible. It has been pointed out that isotopic exchanges follow first-order kinetics regardless of the mechanism or of the order of the individual reactions with respect to the actual chemical constituents.[16] Thus the net rate equation is $dx/dt = R[(ay - bx)/ab]$ in which a is the number of exchangeable phosphate ions in the solid, b is the number of exchangeable phosphate ions in the solution, x is the number of labeled phosphate ions in the solid, y is the number of labeled phosphate ions in the solution, and R is the gross rate exchange.[16] But since

$$y - y_e = x_e - x$$

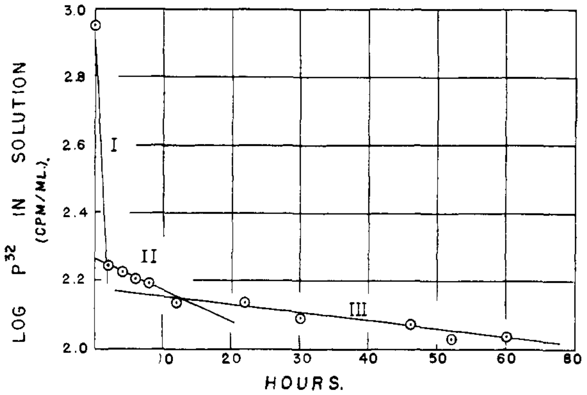

Fig. 2.—Data illustrating that the over-all exchange process occurs as a series of reactions exhibiting first order kinetics.

and

$$x_e/y_e = a/b$$

in which the subscript e represents equilibrium values

$$dx/dt = (R/ab)[(a + b)(x_e - x)]$$

This integrates into

$$\ln[x_e/(x_e - x)] = (R/ab)(a + b)t$$

or

$$\log_{10}(y - y_e) = -Kt + \ln x_e$$

where K is a constant equal to $[R(a + b)/2.3\ ab]$.

This rate equation applies only to a single process, whereas in the present instance, there are at least three separate reactions occurring simultaneously. If it is assumed that the reactions exhibit widely different rates of reaction and therefore, for practical purposes, the specific activity of the solution, SA_{solution}, = $SA_{\text{hydration shell}}$ in the analysis of step II and $SA_{\text{solution}} = SA_{\text{hydration shell}} = SA_{\text{surface}}$ in the analysis of step III, it is permissible to apply the equation to the separate portions of the over-all curve.

Thus, a plot of the log of the solution activity of isotope in solution (proportional to y) *vs.* time, as in Fig. 2, would give a curve comprised of at least three approximately linear portions *provided* y_e *is negligible with respect to* y in each case. Actually, y_e was not negligible (20–30% of y in step II and approximately 10% of y in step III) but the curvature to be expected was within experimental error. Because of the uncertainties in this kinetic analysis, no quantitative inferences are drawn from the data; only qualitative interpretations could be made.

In the subsequent discussion, the negative of the slope, taken from a semi-logarithmic plot of the course of the reaction, is referred to as the "K value."

Effect of Ionic Strength.—In Fig. 3 are plotted the variations in K values for steps II and III when ionic strength was varied by additions of KCl to the buffer solution. The data from both steps II and III are consistent with the Brønsted equation

$$\log k = B + Z_a Z_b \sqrt{\mu}$$

where k is the specific rate constant, B is a complex constant, μ is the ionic strength and $Z_a Z_b$ is the

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[16] A. A. Frost and R. G. Pearson, "Kinetics and Mechanism" John Wiley and Sons, Inc., New York, N. Y., 1953, p. 179.

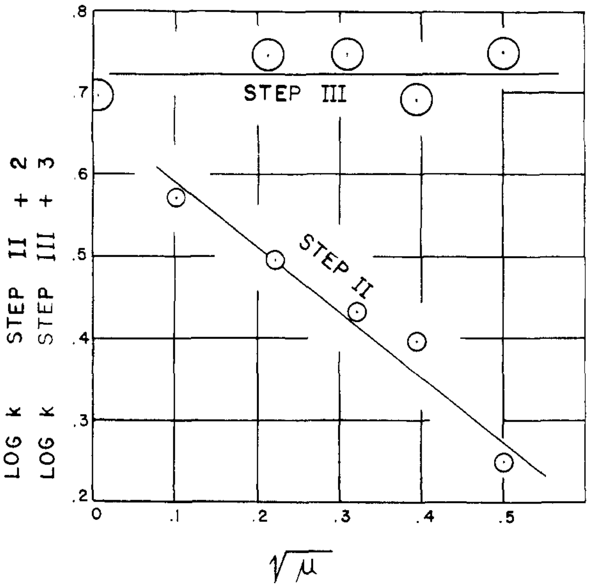

Fig. 3.—The effect of ionic strength on the K values of steps II and III.

product of the ionic charge of the reactants. Step II is markedly affected by ionic strength. Step III is independent of ionic strength, a fact from which it may be concluded that the reactants are out of solution and *within* the crystal surface since the product, Z_aZ_b, is not likely to be zero in this case. This conclusion is valid regardless of the complexities involved in the evaluation of the K value.

In these terms, the most reasonable interpretation of the data given above and subsequently is that

Step I represents the reaction (1) ⟷ (2);
Step II represents the reaction (2) ⟷ (3);
Step III, *et seq.*, represent the reactions (3) ⟷ (4) *et seq.*

as depicted in Fig. 1.

Effect of Temperature.—As shown later, there is reason to believe that the rate limiting step in step II of the exchange reaction is the "diffusion" or "escape" of the phosphate ion from the crystal surface to the hydration shell against a potential gradient imposed by the positive charge on the crystal. The K values for both steps II and III were determined at various temperatures; the resulting data were plotted in Fig. 4 in terms of the Arrhenius equation.[17] The curve for step II down to 12° was approximately linear. The sharp drop between 12 and 4° suggests a change in mechanism in this temperature region.

The K values for step III were too variable to establish a relationship.

Effect of Phosphate Concentration.—Within the limits of experimental error, the K value for step II was independent of both the concentration of non-isotopic phosphate (from 3 to 50 μg.P/ml.) and the concentration of radiophosphate (from 600 to 1200 c.p.m./ml.) in the bulk solution. This is consistent with the view that step II represents an exchange between ions in the crystal surface and the hydration shell and is therefore independent of the concentration of phosphate in the bulk solution.

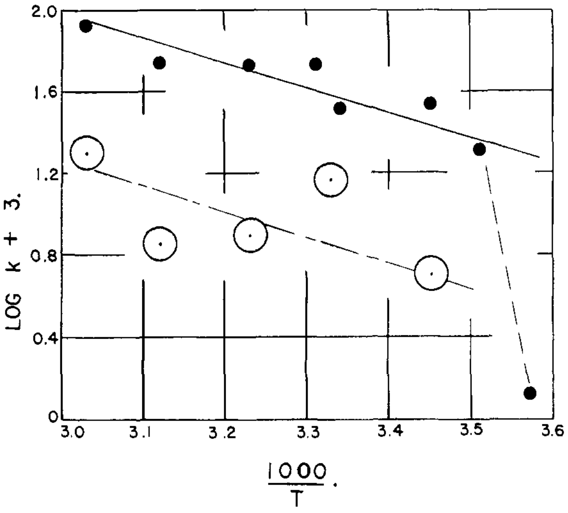

Fig. 4.—The effect of temperature on K values for steps II and III plotted according to the Arrhenius equation: ●, step II; ○, step III.

The Extent of the Exchange Process

Though total isotopic equilibration between liquid and solid phases is not reached in the course of these experiments, the log of the solution activity plotted against time elapsed gives a series of approximately linear curves, Fig. 2, indicating a series of reactions, well separated in time. Extrapolation of the line, step II, to zero time gives the value for the activity in the solution at the completion of all previous steps of the over-all reaction, step I. In the subsequent discussion the percentage of phosphate exchanged, % P_{ex}, has been evaluated from the extrapolation of step II by means of the equations

$$P_{ex} = \frac{(R_i - R_e) \times P_s}{R_e} \qquad (5)$$

$$\% \ P_{ex} = \frac{P_{ex}}{P_t} \times 100\% \qquad (6)$$

where R_i is the initial level of radioactivity in solution; R_e is the extrapolated value; P_s is the total quantity (mg.) of phosphate in the solution, P_{ex} is the quantity (mg.) of phosphate in the solid phase that has exchanged; and P_t is the total phosphate (mg.) present in the solid phase. These equations are similar to derivations previously employed.[1,8,18] All results in subsequent studies have been expressed as % P_{ex} and, in terms of the hypothetical model given in Fig. 2, this term is a measure of the phosphate residing in the hydration shell only.

Pre-equilibration Period.—Regardless of the conditions chosen for the exchange, some time is required for the solid phase to reach a solubility equilibrium. If radioactive phosphate is added to the system during the period in which the solid is dissolving, the uptake of radioactivity will follow an irregular course with time.[11] Any period of stirring over 18 hours was found to give solubility

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(17) W. J. Moore, "Physical Chemistry," Prentice-Hall, Inc., New York, N. Y., 1950, p. 528.

(18) W. F. Neuman and B. J. Mulryan, *J. Biol. Chem.*, **193**, 237 (1951).

equilibria. The uptake curve with time is then smooth and regular and the results are reproducible as in Table I.

TABLE I

A COMPARISON OF SURFACE AND EXCHANGE MEASUREMENTS ON TWO APATITE PREPARATIONS

Apatite (Ca/P)	Surface area	Stirring rate, r.p.m.	Step I K values, hr.$^{-1}$ $\times 10^2$	P_{ex}, %	Mean ∓ av. dev., %
		200	1.26	0.92	
		200	1.54	.88	
Kahlbaum	16.8	300	1.57	.94	0.89
(1.53)		300	1.66	.81	∓4.8
		200	4.40	3.37	
		200	4.65	3.49	3.45
L Apatite	67.8	1600	4.22	3.20	∓3.4
(1.66)		1600	4.65	3.73	

These data cannot be generalized, however; it seems possible that, with other preparations, longer periods of pre-equilibration may be required.

Stirring Rate.—Though conditions will vary with the size and pitch of the stirring blade as well as with the design, any rate sufficient to keep the solid in a uniform suspension proved adequate. At very high speeds, technical difficulties are encountered, particularly with the ground glass seal which shows a tendency to overheat and occasionally to bind. Under the conditions employed in these experiments both the amount of exchange and the rate of the exchange were found to be independent of the rate of stirring, as illustrated in Table I.

Solid to Solution Ratio.—Recent studies on the solubility of basic calcium phosphate have shown a marked effect of the solid to liquid ratio in the relative amounts of calcium and phosphate dissolved.[3,19,20] With this sample of hydroxy apatite it has been shown that as the solid to solution ratio is increased, greater amounts of calcium are dissolved with little change in the amount of phosphate dissolved.[12] As seen in Fig. 5, the composition of the surface does change. The % P_{ex} rises to a peak with increasing solid and then declines. This means that for accurate comparisons the solid to solution ratio must be kept constant in any given series of experiments.

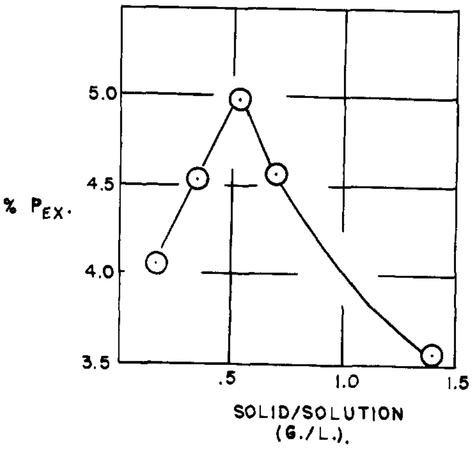

Fig. 5.—Variation in % P_{ex} with varying solid to solution ratio.

The Displacement of Calcium by Sodium.—It has been noted that bone salt can undergo heteroionic as well as isoionic exchange.[9] Recent evidence indicates a mole for mole exchange of sodium for calcium.[9,21] If this does occur, a displacement of the divalent calcium ion by the univalent sodium ion will reduce the positive charge on the crystal. This in turn will reduce the negative ions about the crystal surface by an equivalent amount. In Fig. 6 is illustrated this reduction in the % P_{ex} with increased sodium content of the solution.

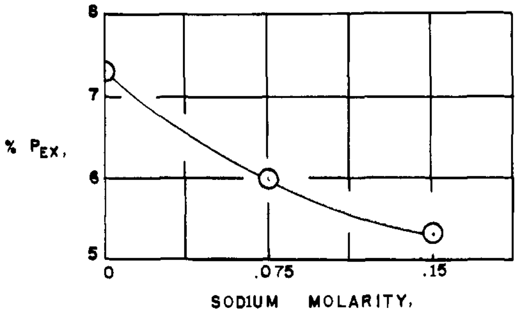

Fig. 6.—The effect of sodium ion concentration of % P_{ex}.

From the phosphate displaced (0.113 millimole/g. of solid) and the sodium taken up by the solid (0.196 millimole/g. of solid)[21] it is interesting to calculate the valence charge of the exchangeable phosphate groups in the hydration shell. Since each sodium ion must reduce the crystal's positive charge by one unit, 0.196/0.113 or 1.73 represents the average negative charge on the phosphate group displaced. This is in excellent agreement with 1.70, the value predicted from the ionization constant[12] of phosphoric acid in aqueous solution under comparable conditions of ionic strength and pH.

Calcium Concentration.—As has been shown, a univalent cation, sodium, may displace surface calcium ions. There already exists evidence that hydronium ion, too, may partake in such a displacement reaction.[9] In other words, the net positive charge on the crystal should vary with the ratio: $(Ca^{++})/(H_3O^+)$ in the absence of other competing cations.

The net positive charge and, therefore, also the quantity of exchangeable phosphate, can be expected to vary between the limits of substitution permitted by the surface of the apatite lattice.[9] Such a relationship is shown in Fig. 7 where the $(Ca^{++})/(H_3O^+)$ has been varied by additions of calcium while the pH was kept constant. Surface area determinations, 68 square meters per gram, were made of the apatite crystals before and after equilibration. There were no significant changes in the surface area resulting from the treatment. The variations in % P_{ex} were, therefore, from changes in the crystals charge, not changes in size.

The Effect of Phosphate Concentration.—Due to problems arising from the distribution of radioisotope, it is impractical to study the exchange process at high phosphate concentration. How-

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(19) I. Greenwald, *J. Biol. Chem.*, **143**, 703 (1942).

(20) H. C. Hodge, "Metabolic Interrelations," 3rd Josiah Macy Conf., 1951, p. 199.

(21) W. R. Stoll and W. F. Neuman, unpublished results.

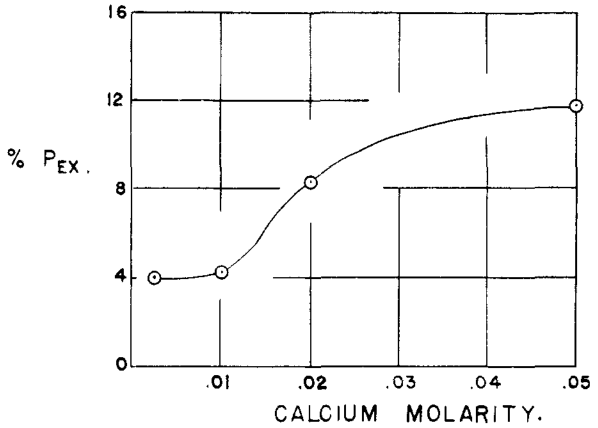

Fig. 7.—The effect of calcium ion concentration at constant *p*H on the % P_{ex}.

ever, as is illustrated in Table II, within the range of phosphate concentration which might be encountered in this study, the extent of exchange is independent of the concentrations of phosphate ion in the bulk solution.

TABLE II

THE CONSTANCY OF % P_{ex} WITH VARYING PHOSPHATE CONCENTRATION

Concn. of phosphorus, µg./ml.	P_{ex}, %	Concn. of phosphorus, µg./ml.	P_{ex}, %
1.8	4.40	9.9	4.52
2.1	4.70	14.2	4.29
8.2	4.57	50	5.00
9.0	4.32		

Effect of Temperature.—Most of the studies described above have arbitrarily been carried out at body temperatue (37°). Although the actual temperature at which the exchange occurs is not important *per se*, it is necessary to compare a series of exchanges which are performed at one temperature only. From the data in Fig. 8 it can be seen that increasing temperature markedly decreases the magnitude of the exchange, % P_{ex}. This effect, like the effect of varying solid to solution ratio cannot be interpreted with present, limited knowledge.

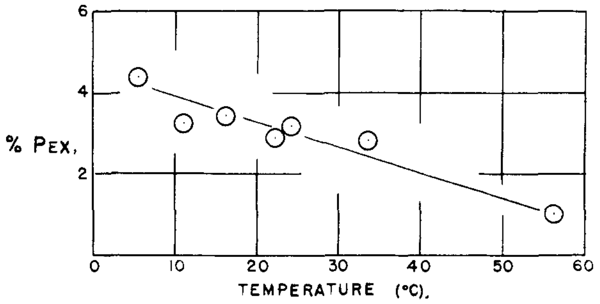

Fig. 8.—The effect of temperature on % P_{ex}.

Effect of Varying % P_{ex} on Rate of Exchange.—As noted above, a number of variables, temperature, *p*H, $[Ca^{++}]/[H_3O^+]$, μ, and solid/solution ratio affect the magnitude of % P_{ex}, the phosphate in the hydration shell. It is of interest that with increasing values of % P_{ex}, decreasing values of K for step II were obtained with only one exception: when small amounts of calcium were added to the buffer solution, K increased with *no change* in % P_{ex}.

Discussion

The apparatus of Schweitzer and Nehls[7] has provided a satisfactory means for obtaining kinetic data on an ionic-exchange reaction occurring between a solution and a finely dispersed solid. In this instance, the exchange of labeled phosphate ions in solution for phosphate ions associated with hydroxyapatite crystals, the data obtained were sufficient to permit a partial interpretation of the actual reaction mechanics. The analysis of step II is given most consideration in the following discussion for two reasons: (a) step III, the reaction occurring within the crystal, must represent a "thermal" or a "diffusion" process and (b) the experimental technique did not provide data on step I the initial, fast reaction.

Because these experiments were carried out at equilibrium, there is no justification *a priori* for assuming the rate-determining step to be the transfer of labeled ions from solution to solid. Just as likely, the transfer of non-isotopic ions from solid to solution might be rate determining. Indeed, the data are best explained by assuming the rate-determining step to be escape or self-diffusion of phosphate ions from the crystal surface to the hydration shell—a mechanism consistent with all of the facts: One such a process would be independent of the concentration or specific activity $(P^{32}O_4)/(P^{31}O_4)$ of the solution but would decrease in rate as the potential barrier increased. This barrier results from the net positive charge on the crystal and the surrounding field of anions in the hydration shell. Two, the effect of ionic strength is easily explained in these terms. With increasing ionic strength, % P_{ex} increases producing an increased potential barrier and, thus, a reduced rate. Were it necessary to interpret the effect of ionic strength in terms of a conventional collision mechanism, both the sign and the slope (−0.78) of the curve given by the Brønsted plot would be difficult to relate to the system under study.

If the rate of isotope uptake is indeed governed by the escape of surface ions, the assignment of the observed reaction steps to the proper location in the solid:solution interface can be made with greater assurance. Thus, step III, evincing a rate independent of ionic strength must represent a reaction originating within the crystal. Step II, dependent upon ionic strength must represent a reaction originating either on the surface or in the hydration layer. Since step I is a reaction much faster than step II, by elimination step II remains as the escape of surface ions to the hydration layer.

Long-term experiments utilizing Ca^{45} demonstrated, Fig. 9, that the course of the exchange process continues with ever-decreasing rate for at least 44 days, and, presumably, indefinitely. The observed quantity of calcium taking part in the exchange (17%) is much too large to attribute to surface reactions alone. A monolayer would correspond to an exchange of only 3 or 4%.[9] This process of exchange *within* the crystal interior has been described previously and has been variously termed "thermal aging"[8] and "recrystallization."[18] Because, as described above, the rate of step III is independent of ionic strength the term "thermal aging" seems preferable. Certainly the process does not involve a solution and redeposition of ions. While it is possible that certain ions may be able to "diffuse" throughout the lattice of certain crystals, in the present instance where the lattice is quite dense and where the diffusing ion, phosphate, is large and multicharged, it seems more probable that ion-migration is mediated throughout the presence of unoccupied lattice positions or "faults" in the crystals. This view is supported by the fact that high-temperature ashing markedly reduces the recrystallization or thermal equilibration of fresh bone.[18] Such heat treatment would be expected to render the crystals more perfect with fewer "faults" and vacant positions as attested by improved X-ray diffraction patterns.[9] The participation of "holes" which move as the result of thermal vibration of adjacent ions is also consistent with the ever-decreasing rate of reaction, Fig. 9, as the diffusing ion penetrates deeper layers of the crystals. With aged crystals, the reaction rate falls so rapidly that one must conclude that only a few "molecular layers" can take part in equilibria covering an observational period of a few weeks.

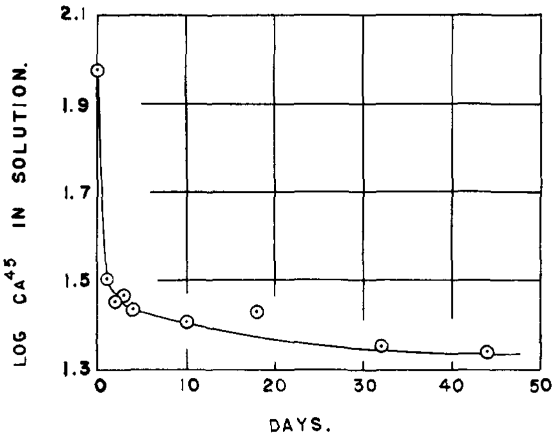

Fig. 9.—A long-term calcium exchange showing a continued but progressively slowed removal of Ca^{45} from solution by the solid phase. In this experiment eight identical flasks containing apatite and Ca^{45} were rotated at a constant temperature of 37°, and sampled serially for radioactivity assay and Ca concentration of the liquid.

Despite the complexity and variability of the hydroxy apatite lattice, its extremely small crystal size makes it an excellent material for the study of solid:solution interactions.

ROCHESTER, NEW YORK

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, POLYTECHNIC INSTITUTE OF BROOKLYN]

Electrokinetics of Hydrogen Evolution. IV. Isotopic Separation at Mercury Cathodes[1,2]

BY MARTIN ROME[3] AND C. F. HISKEY[4]

RECEIVED MARCH 16, 1954

Techniques have been developed and a cell designed for the determination of the electrolytic separation factor at a mercury cathode by means of a mass spectrometer. The separation factor has been determined over a range of temperatures from 0 to 96.5° and of current densities from 10^{-4} to 3×10^{-2} amp./cm.^{2}. Impurity effects of stopcock grease have been evaluated. The dual theory of Horiuti and Okamoto for the mechanism of electrode separation is applied for the case of a mercury cathode.

Recent studies of hydrogen and deuterium overvoltages[5,6] at a mercury cathode have stimulated interest in the related problem of the electrolytic separation factor of these isotopes. This factor is expressed as a ratio of the rates of electrolytic evolution of the two isotopes, *viz.*

$$S = \frac{dH/H}{dD/D} = \frac{(C^H/C^D)_g}{(C^H/C^D)_s} = \frac{i^H/i^D}{(C^H/C^D)_s} \qquad (1)$$

In this definition C^H and C^D are the concentrations of hydrogen and deuterium, and the subscripts g and s refer to the gas and solution phases, respectively. Values of the separation factor have been reported by Topley and Eyring,[7] Eucken and Bratzler,[8] Walton and Wolfenden,[9] and Horiuti and Okamoto[10] for mercury cathodes. A resumé of these reported separation factors is given in Table I and

TABLE I

RESUMÉ OF REPORTED SEPARATION FACTORS FOR MERCURY

Author	i, amp./cm.^{2}	T, °C.	% D_2O	Electrolyte	S	Reported error
Topley and	7×10^{-1}	20	8	H_2SO_4 0.5 N	2.8	0.15
Eyring			9	H_2SO_4 .5 N	2.9	
			10	H_2SO_4 0.75 N	2.7	
			15	H_2SO_4 1 N	2.8	
Walton and	5×10^{-4}	15	30	HCl 0.5 N	3.3	.2
Wolfenden	to	55	to	and	3.5	.1
	10^{-3}	95	50	KCl 0.5 N	3.3_5	.1
Eucken and	5×10^{-2}		15	H_2SO_4 1.5 N	4.3	.1
Bratzler	2×10^{-1}				4.8	.1
Horiuti and Okamoto			6	H_2SO_4 0.1 N	3.1	

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(1) The experimental data reported herein are part of the thesis submitted by Martin Rome to the faculty of the Polytechnic Institute of Brooklyn in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

(2) Presented, in part, before the Division of Physical and Inorganic Chemistry at the 122nd meeting of the American Chemical Society at Atlantic City, N. J., on September 18, 1952.

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(4) International Biochemical Corp., Brooklyn, N. Y.

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