THE SOLUBILITY OF CALCIUM PHOSPHATE

1. THE EFFECT OF pH AND OF AMOUNT OF SOLID PHASE

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In 1937, Logan and Taylor (1) came to the conclusion "that the bone salts cannot precipitate spontaneously from the bone plasma unless the ion product is increased. Once formed, the bone salts cannot dissolve unless the concentrations of the ions composing them are decreased below the concentrations found in the blood plasma." This conclusion was based upon some observations which seemed to Logan and Taylor to prove that the apparent solubility product, \([\text{Ca}^{++}]_3[\text{PO}_4^{-2}]_2\), depended on the amount of the solid phase, being greatest with small amounts and decreasing with larger amounts. The solid phases they employed were precipitated calcium phosphate and powdered bone. Such a change of ion product, at equilibrium, with the amount of solid phase is, of course, quite remarkable and, in order to explain their results, Logan and Taylor resorted to as remarkable an explanation. We will discuss their experiments and their explanation later.

A recent paper by Klement and Weber (2) leads to a quite different conclusion from that of Logan and Taylor. Klement and Weber prepared a hydroxyapatite by mixing dilute solutions of \(\text{Ca(OH)}_2 \) and of \(\text{H}_3\text{PO}_4 \) in the equivalent proportions. Their preparation was, as they stated, assuredly free of foreign ions, but there is no evidence that it was a single chemical individual. No such claim was made for it. The precipitate was filtered and dried. Weighed quantities of from 100 to 1200 mg. were added to 250 ml. of conductivity water in paraffined flasks and equilibrated for 24 hours at 25°. The amounts of Ca dissolved varied from 3.6 to 11.0 mg. per liter and those of P from 8.6 to 32.2 mg., increasing regularly with the amount of solid phase. Upon the basis of this experiment, Klement and Weber deny the significance of the solubility product \([\text{Ca}^{++}]_3[\text{PO}_4^{-2}]_2\).

However, it may well be objected that 24 hours may not have been sufficient for the attainment of equilibrium.

We have been engaged in a study of the influence of various substances upon the solubility of calcium phosphate. In the course of this work we found that this solubility, whether it be expressed as \([\text{Ca}^{++}]_3[\text{PO}_4^{-2}]_2\) or, as we prefer, as \([\text{Ca}^{++}]_3[\text{HPO}_4^{-2}]_2/[\text{H}^+]^3\), is not constant, but varies with the

\(^1\) In the present instance, calculations of the product \([\text{Ca}^{++}]_3[\text{PO}_4^{-2}]_2\) are impossible because no good values for the third dissociation constant of \(\text{H}_3\text{PO}_4 \) at \(\mu = 0.2\)
amount of the solid phase, not, however, in the manner reported by Logan and Taylor, but in precisely the opposite fashion; viz., the solubility increases with the amount of solid phase.

We also found that the apparent solubility product varies with the pH, increasing as the latter increases. This effect is not appreciable with small amounts of solid phase, but becomes considerable as the latter increases.

EXPERIMENTAL

In order to enable determinations of calcium and phosphorus of the order of $10^{-5}$ M to be made in the presence of salts in a concentration of 0.2 M, the Ca and P were added in the form of a suspension of known content and the amount remaining undissolved was determined.

A suspension of calcium phosphate was prepared by mixing 2.5 liters of 0.04 M Ca(NO$_3$)$_2$, 2.5 liters of 0.04 M Na$_2$HPO$_4$, and 200 ml. of 0.1 N NaOH. After the mixture had stood, with frequent stirrings, for a week, the precipitate was washed by decantation until a test of a portion dissolved in dilute acetic acid gave no precipitate with nitron, and for several times thereafter. The precipitate was then kept in suspension for at least a year before use. As required, more dilute suspensions were prepared and analyzed. The figures in Table I show slightly varying values for the ratio of Ca:P. These are for different dilutions of the stock suspension, each of which was separately analyzed at widely separated intervals with different standard solutions.

Measured volumes of these suspensions were added to 1 or 2 liter volumetric flasks containing HzO, KCl to give a final concentration of 0.2 M, 10 ml. of 0.04 per cent chlorophenol red or phenol red solution per liter,$^2$ and small quantities of 0.1 N HCl or 0.1 N NaOH. After the solutions were diluted to the mark, the stoppered flasks were allowed to stand for the number of days indicated in Table I; they were shaken once almost every day. The precipitate settled very slowly, separation being quite incomplete even after 6 or 8 or even 24 hours.

appear to be available. Other reasons for preferring $[\text{Ca}^{+++}]^3[\text{HPO}_4^{-}]^2/[\text{H}^+]^3$ are presented in Paper II. The values for $-\log [\text{Ca}^{+++}]^3[\text{HPO}_4^{-}]^2/[\text{H}^+]^3$ given in Table I can be converted into values for $pK'_s$ $[\text{Ca}^{+++}]^3[\text{PO}_4^{-}]^3$ by adding 2pK'$_s$ whenever values for the latter become available. However, the use of neither product is theoretically valid at pH > 6. The solid phase, at equilibrium, does not have the composition indicated by Ca$_3$(PO$_4$)$_2$. The use of the third power of the calcium concentration and the second power of the phosphate concentration in this paper is due only to the fact that such a product has been used by others, particularly by Logan and Taylor.

$^2$ These indicators were added in order that the pH of these mixtures, which were to serve as controls, should be nearly the same as those of other mixtures containing other substances.
When analyses were to be begun, the mixtures were again shaken thoroughly and the pH was determined with the Coleman glass electrode. The cell was flushed with the mixture until no change in pH was observed. Four or five rinsings sufficed. Measured portions of the mixture were then filtered, with suction, through sintered glass filters covered with a thin layer of infusorial earth. This was prepared by washing commercial acid-washed infusorial earth with concentrated HCl and then with H₂O until free of acid. As much as 400 ml. was taken for the determination of Ca and 75 ml. for the determination of P.

The precipitates were not washed, but were sucked dry and were then

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The precipitates were not washed, but were sucked dry and were then

\[ \text{If the amounts of Ca and of P contained in the liquid adhering to the precipitate} \]

\[ \text{and filter were appreciable, their effects should have been most marked in those} \]
dissolved in approximately 0.02 N HCl, which was allowed to run through by gravity. In these filtrates, evaporated if necessary, phosphorus was then determined by the method of Fiske and Subbarow (3) and calcium by precipitation as the oxalate, by centrifuging, washing, and titrating with KMnO₄.

In a number of instances, the original filtrate contained enough Ca and P to permit of direct determination. Quite good agreement with that calculated by difference was obtained. Repeatedly, the filtrates obtained in two or three determinations were combined and passed through other filters. The latter were then extracted in the usual manner. No trace of Ca or of P was obtained, indicating that the previous removal of Ca and P was due to filtration and not to adsorption.

For the calculation of $-\log [\text{Ca}^{++}]^3 [\text{HPO}_4^{-}]^2/[\text{H}^+]^2$ we used the value $pK'_2 = 6.85$, calculated from the formula of Cohn (4). Those who prefer to use $[\text{Ca}^{++}]^3 [\text{PO}_4^{3-}]^2$ may add $2pK'_4$.

DISCUSSION

In all but one of our experiments, "equilibration" was continued for at least 7 days. As may be seen from the results of Experiments 11 and 12, or from those of Experiments 3, 4, and 5, prolongation up to 20 days increased the solubility comparatively little, if at all (Table I).

The increase in the amount of calcium and phosphate in solution with the increase in the amount of solid phase is not surprising. It has long been known (see Eisenberger, Lehrman, and Turner (5) for a review of the literature) that calcium phosphate, in contact with aqueous solutions, rarely, if ever, has the composition Ca₃(PO₄)₂. Usually, at pH 6.0 or more, it contains an excess of calcium. If it is not a pure substance, but a mixture, increasing the amount added to a liquid may increase the amount dissolved up to a certain maximum. Apparently, that maximum, at pH 6.3, was approached in Experiment 18, for a 25 per cent increase in the amount of solid phase added (Experiment 20) produced a comparatively small change in the ion product.

It is remarkable that, in spite of all that has appeared in biological literature regarding the solubility product $[\text{Ca}^{++}]^3 [\text{PO}_4^{3-}]^2$, the validity of the use of this formulation has never been tested save over a very limited range of pH. With one exception, to be mentioned presently, all determinations have been made between pH 7.10 and 7.60. In spite of that,
the variations even with large excess of solid phase are considerable—at least 10-fold (see the data of Sendroy and Hastings (6)).

The one exception to this restriction of measurements to a limited pH is that furnished by some observations of Holt, La Mer, and Chown (7). They approached equilibrium from supersaturation and determined the solubility of calcium phosphate in the presence of the precipitate in NaCl or KCl solution at pH 5.2 to 5.5 and in “serum salt” solutions at pH 6.9 to 7.4. At comparable ionic strengths the ion product was about 100 times as great in “serum salt” solutions as in KCl or NaCl solutions. Even if the ion product is recalculated by the method of Sendroy and Hastings and also after allowance is made for the presence of undissociated CaHPO₄, CaCO₃, and CaHCO₃ (8, 9), the difference remains nearly as great. It is interesting to observe, therefore, that our Experiments 8 and 13 show a difference of 1.77 in the logarithms of the ion products between pH 6.30 and 7.28 and that other experiments show smaller variations over smaller variations in pH.⁴

The effect of pH on the apparent solubility product cannot be due to an error in the value of pK₉, for so extreme a change of the latter from 6.85 to 6.50 still leaves a difference in the value of \(-\log[Ca^{++}]^{3} [HPO_{4}^{2-}]/[H^{+}]^{3}\) amounting to 0.62 between Experiments 8 and 13, to 0.45 between Experiments 17 and 18, and to 1.08 between Experiments 19 and 20.

There remain to be discussed the reasons for the results obtained by Logan and Taylor. We believe these to have been due to insufficient equilibration. They approached equilibrium from supersaturation and their sole test for completeness of equilibrium appears to consist of one experiment in which they brought together an unstated excess of Ca²⁺ and Na₂HPO₄, centrifuged to remove the precipitate, added NaOH to adjust the pH for the acidification due to the precipitation, and repeated the process until no further obvious precipitation was produced. The three solutions were then shaken with a small quantity of the precipitate for 4 and 8 days. In their paper, Logan and Taylor did not report the actual concentrations nor the amount of solid phase added, but Dr. Logan kindly made the former of these available to me. They are given in Table II, together with the values for pH and for \(-\log[Ca^{++}]^{3} [PO_{4}^{3-}]^{3}\) reported by Logan and Taylor in their paper. The amount of the solid phase added is not known but a comparison of the values for \(-\log[Ca^{++}]^{3} [PO_{4}^{3-}]^{3}\) with those reported by Logan and Taylor in other experiments (their Table I) would indicate that it was less than 2.5 mg. and probably less than 1 mg. per liter. 1 mg. per liter is the equivalent of 0.01 mM of Ca and of 0.0065

⁴ It is also possible that the differences in the calculated ion products in the two series of experiments of Holt, La Mer, and Chown were due to the formation of mixed complexes of Ca²⁺, HCO₃⁻, and HPO₄²⁻.
mm of P. Actually, the difference between the concentrations observed at 4 and at 8 days far exceeds these quantities. In the second experiment, \([P]\) is said to have changed from 4.60 to 5.40 mM, an increase of 0.8 mM, while the \([\text{Ca}]\) decreased by 0.02 mM. These changes in the solution would make a very great difference in the composition of the solid phase. With even so large a quantity as 310 mg. of \(\text{Ca}_3(\text{PO}_4)_2\), the ratio of \(\text{Ca}:\text{P}\)

### Table II

**Composition of Fluid after 4 and 8 Days (Experiments of Logan and Taylor)**

<table>
<thead>
<tr>
<th></th>
<th>4 days</th>
<th>8 days</th>
<th>Reported by Logan and Taylor (1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>Ca</td>
<td>P</td>
<td>pH</td>
</tr>
<tr>
<td>7.12</td>
<td>0.75</td>
<td>5.25</td>
<td>7.15</td>
</tr>
<tr>
<td>7.44</td>
<td>0.74</td>
<td>4.60</td>
<td>7.37</td>
</tr>
<tr>
<td>8.10</td>
<td>0.57</td>
<td>1.06</td>
<td>7.93</td>
</tr>
</tbody>
</table>

*Personal communication from Dr. Logan to the author.

### Table III

**Data of Logan and Taylor***

Composition of the solution at the start, \(\text{CaCl}_2 1.06 \text{ mM per liter, Na}_2\text{HPO}_4 3.06 \text{ mM per liter.**}

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Solid added Bone</th>
<th>Calculated as (\text{Ca}_3(\text{PO}_4)_2)</th>
<th>Composition of solutions after equilibration</th>
<th>(\left[\text{Ca}^{++}\right] \times \left[\text{PO}_4^{3-}\right]) in solid phases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg. per l.</td>
<td>mg. per l.</td>
<td>pH</td>
<td>Ca</td>
</tr>
<tr>
<td>61</td>
<td>1.3</td>
<td>0.74</td>
<td>7.43</td>
<td>1.06</td>
</tr>
<tr>
<td>62</td>
<td>5.0</td>
<td>2.87</td>
<td>7.38</td>
<td>0.929</td>
</tr>
<tr>
<td>63</td>
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<td>14.3</td>
<td>7.34</td>
<td>0.619</td>
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<td>28.1</td>
<td>7.29</td>
<td>0.467</td>
</tr>
<tr>
<td>65</td>
<td>100.0</td>
<td>58.0</td>
<td>7.22</td>
<td>0.365</td>
</tr>
</tbody>
</table>

*The first seven columns are taken from Logan and Taylor ((1) p. 299).

would change from 1.5 to 2.5. Such experiments cannot be accepted as evidence of "equilibrium." If this was not attained in these experiments, there can be no reason for believing that it was attained in the later experiments. The lower values for \([\text{Ca}^{++}]^2 \left[\text{PO}_4^{3-}\right]^2 \) with increasing amounts of solid phase are, obviously, only an indication of a more nearly complete approach to true equilibrium, which is only to be expected.

Logan and Taylor explain the lowered solubility with increases in solid phase as being due to adsorption of \(\text{Ca}^{++}, \text{PO}_4^{3-}\), and other ions upon the
true precipitate or upon the solid phase introduced. We may test this assumption by calculations, the results of which are summarized in Table III in which all but the last column is copied from Table II of Logan and Taylor. From the changes in [Ca] and [P] reported by them, we have calculated the amount of Ca₃(PO₄)₂ precipitated in each experiment. Adding this value to the amount originally present, we have the figures given in the last column. According to the hypothesis of Logan and Taylor, the 2.13 mg. of additional Ca₃(PO₄)₂ added in Experiment 62, as compared with Experiment 61, adsorbed 12 mg. of Ca₃(PO₄)₂. This cannot be "adsorption" as ordinarily understood. It must be crystal growth. It is also obvious from an examination of Table II that, at the time of analysis, the amount of solid phases present in Experiment 62 was greater than was originally added in Experiment 63. The same relation holds between Experiments 63 and 64 and between Experiments 64 and 65. It is, we believe, impossible to interpret the increases in the value of pK', Ca₃(PO₄)₂ with increases in the amount of bone introduced except as evidence of continued supersaturation in Experiments 61 to 64.

We believe that the evidence that the results of Logan and Taylor were due to incomplete equilibration is overwhelming. We freely admit that our results may, similarly, be due to incomplete equilibration. A true equilibration can be demonstrated only if the same conditions are attained from both undersaturation and oversaturation. Quite evidently, this can be attained, in a reasonable length of time, only with considerable amounts of precipitate. With small amounts, equilibrium is, apparently, not to be attained in any feasible period, for a continuation of the experiment much beyond 8 days at 38° involves serious risk of reaction with the walls of the vessel. The results reported by Logan and Taylor and in the present paper can be reconciled if one accepts the view that the composition of the precipitate, approximately Ca₃(PO₄)₂, depends upon the composition of the liquid and that changes in this composition, involving a change from one insoluble substance into another, take place only very slowly. An apparently true equilibrium constant, pK', w = approximately 27 at 38°, can be attained with large amounts of precipitate because, with the large surface offered, equilibrium at the surface is more readily attained. It is this surface and not the bulk of the precipitate that is believed to be in equilibrium with the solution.

**SUMMARY**

The claim of Logan and Taylor (1) that "The ion product [Ca⁺⁺][PO₄⁻²] increases as the amount of bone or tricalcium phosphate, equilibrated with solutions of their ions, decreases below 150 mg. per liter" has been examined. It is believed that the data presented by them are more in
accord with the view that the mixtures containing the smaller amounts of precipitate were supersaturated. Other experiments are reported which indicate that the reverse relation obtains; viz., that the ion product increases with the amount of solid phase. Attention is also directed to the apparent effect of increasing pH on the ion product. It is believed that the composition of the precipitate depends upon the composition of the liquid but that changes in the former are brought about only very slowly and incompletely and, probably, only at the surface, within any period of time thus far employed.

BIBLIOGRAPHY

THE SOLUBILITY OF CALCIUM PHOSPHATE: I. THE EFFECT OF pH AND OF AMOUNT OF SOLID PHASE
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