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3815

The Composition of Bone. III. Physico-Chemical Mechanism.

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There have recently been two notable investigations of the physico-chemical mechanism of bone salt formation. Holt, La Mer and Chown[1] concluded that blood serum is normally supersaturated with $Ca_3(PO_4)_2$ to the extent of more than 200%. Sendroy and Hastings[2] also made careful determinations of $K'_{sp}Ca_3(PO_4)_2$ and of the solubility of this salt in serum, and obtained similar experimental results. They interpreted the results differently however, and concluded regarding supersaturation that "the evidence in every direction seems to be strongly opposed to this point of view," that "the data indicate that the theory of supersaturation is inadequate," that "the theory of complete dissociation of electrolytes . . . seems to fail as an adequate explanation for the solubility of calcium in serum." These investigations have thus demonstrated that the behavior of calcium and inorganic phosphorus in serum and in solutions is inexplicable when $K'_{sp}Ca_3(PO_4)_2$ is applied as the criterion for the precipitation of calcium phosphate.

When their data are studied from another viewpoint, more plausible explanations result. The empirical Ca×P product of Howland and Kramer[3] has proved so valuable that it seemed to us that it might have theoretical validity. This empirical product would have theoretical validity if the calcium phosphate were deposited as a result of a second order reaction such as $Ca^{++} + HPO_4^{--} \rightarrow \downarrow Ca\,HPO_4$.

If $Ca\,HPO_4$ is the substance first deposited then its solubility product is likely to be the quantity which determines whether or not precipitation shall occur. From data in the literature we have calculated that $pK_{sp}Ca\,HPO_4 = 6.4 \pm 0.1$ and $pK'_{sp}Ca\,HPO_4 = 6.4 - 2.3\sqrt{\mu}$ at 38° C.

The ion product $[Ca^{++}] \times [HPO_4'']$ has been calculated for inorganic serum solutions and serum on the basis of the complete dissociation of electrolytes. For such solutions containing 10 mg. Ca per 100 cc. and from 2.0 to 7.0 mg. inorganic P per 100 cc., the results range as follows: $[Ca^{++}] \times [HPO_4''] = 1.4 \times 10^{-6}$ to 4.8×10^{-6}, while $K'_{SP}CAHPO_4 = (3.2 \pm 0.8) \times 10^{-6}$.

Thus the ion products for $CaHPO_4$ in such solutions are of the same order of magnitude as $K'_{SP}CaHPO_4$. Moreover, for solutions with empirical Ca × P products less than 35 the ion products are definitely less than $K'_{SP}CaHPO_4$.

For the same solutions calculation gives $[Ca^{++}]^3 \times [PO_4]^2 = 0.7 \times 10^{-23}$ to 8.3×10^{-23}, while $K'_{SP}Ca_3(PO_4)_2 = 10^{-27}$ for solutions and $K'_{SP}Ca_3(PO_4)_2 = 2 \times 10^{-26}$ for serum.

Thus with a Ca × P product ranging from 20 to 70 the ion product for $Ca_3(PO_4)_2$ is about 1000 times greater than its K'_{SP} in serum and about 10,000 times as great as its K'_{SP} in inorganic solutions of the same ionic strength.

These otherwise inexplicable values may be accounted for if we consider that $K_{SP}Ca_3(PO_4)_2$ does not give the conditions necessary for the formation of $Ca_3(PO_4)_2$. This would be the case if $Ca_3(PO_4)_2$ could not be prepared by precipitation, but was formed only as a result of secondary reactions in the solid phase. A search of the literature of the calcium phosphates revealed not a single instance in which the precipitation of pure $Ca_3(PO_4)_2$ has been described. Bassett[4, 5] stated that it is a peculiar fact that this compound, discussed in all textbooks, has been known in neither the natural nor artificial condition, and that laboratory preparations vary considerably from the correct Ca/P ratio. The evidence is such as to arouse the suspicion that there may be no such chemical entity as $Ca_3(PO_4)_2$. But whether or not such a substance can be prepared in other ways, no one has succeeded in preparing it by direct precipitation. It has been repeatedly found that in acid solution the precipitate was always $Ca\,HPO_4$, and that in alkaline solution the precipitate always contained a larger proportion of lime than is required for the formula $Ca_3(PO_4)_2$. It has also been repeatedly demonstrated that the precipitated $Ca\,HPO_4$ reacts with the supernatant solution. This reaction is sometimes written $4\,CaHPO_4 \rightarrow Ca(H_2PO_4)_2 + Ca_3(PO_4)_2$ in which the primary phosphate formed goes into solution, leaving as the final solid phase $Ca_3(PO_4)_2$. This latter, however, is not a precipitate—the precipitate is the substance which is deposited from solution. The evidence which we have presented here indicates strongly that the initial solid phase is

Ca HPO_4 and that $K'_{SP}CaHPO_4$ is the quantity which governs the formation of the precipitate. If the solution is alkaline, $Ca(OH)_2$ may be deposited simultaneously with the Ca HPO_4.

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[1] Holt, L. E., Jr., La Mer, V. K., and Chown, H. B., *J. Biol. Chem.*, 1925, lxiv, 509.

[2] Sendroy, J., Jr., and Hastings, A. B., *J. Biol. Chem.*, 1927, lxxi, 783.

[3] Howland, J., and Kramer, B., *Trans. Am. Ped. Soc.*, 1922, xxxiv 204.

[4] Bassett, H., Jr., *Z. anorg. Chem.*, 1908, lix, 1.

[5] Bassett, H., Jr., *J. Chem. Soc.*, 1917, cxi, 620.

3816

The Composition of Bone. IV. Primary Calcification.

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Sixteen specimens of normal rat bone were analyzed using the micro-technique described in previous communications.[1, 2] The age of the rats ranged from 1 day to maturity. A mean value of 1.99 ± 0.05 was obtained for the ratio residual Ca/P for these normal bones. In the adult rats, carbonate calcium constituted 15 to 16% of the total calcium; in the very young rats this value was only 8 to 10%. Transition values of 12 and 13% were obtained in rats 6 and 9 weeks old respectively. Since the proportion of calcium carbonate to calcium phosphate varies with age, it is incorrect to assign a definite constant formula to bone such as $3\,Ca_3(PO_4)_2\,CaCO_3$. Bone apparently consists of at least two compounds whose proportions may vary independently. This is also borne out by the results reported by Howland, Marriott and Kramer.[3] They found that the bones of rachitic rats contain a smaller proportion of calcium phosphate to $CaCO_3$ than do the bones of normal rats.

Twenty-five rachitic rats were fed cod-liver oil concentrate for 8 days. The rats were then autopsied and the "lines" of freshly deposited lime salts were removed and analyzed. The adjacent epiphyses and diaphyses were also removed and analyzed. Although the ratio for normal rats with this method had been established as described above, the shafts of the leg bones of the same animal were analyzed as an additional control. The values for residual Ca/P