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.

[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.

BENJAMIN KRAMER AND M. J. SHEAR.

From the Harry Caplin Pediatric Research Laboratory, The Jewish Hospital of Brooklyn, New York City.

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

obtained were: 2.05 for the shafts; 2.10 for the epiphyses and diaphyses; and 2.38 for the test lines.

A similar study using irradiated yeast instead of cod-liver oil concentrate gave similar results. In this case care was taken to remove only fresh calcification from the metaphyses. The values obtained were: 2.05 for the shafts; 2.25 for the metaphyses; and 2.16 for the test lines. Test lines in which the healing was induced by feeding irradiated cholesterol to rachitic rats gave a ratio of 2.24.

Another series of rats was fed 1% butter in addition to the rickets producing diet for 4 weeks. Fresh calcification was obtained in the metaphysis, but not in the provisional zone. The ratios obtained were 1.94 for the shafts and 2.14 for the metaphyses As a further control the shafts of a normal rat of the same age as the test rats were analyzed and gave a value of 2.03.

The carbonate calcium constituted 15 and 16% of the total calcium in the shafts of the test rats. In these same rats, however, the fresh calcification contained only from 10 to 12% carbonate calcium. Thus the proportion of carbonate calcium in the older bones is 30 to 50% higher than in the fresh deposit. This is similar to the difference between the composition of the bones of older rats as compared with very young rats.

The values for the ratio resid. Ca/P in the specimens of fresh calcification were calculated from mean values of Ca and P. These mean values were obtained by averaging the results of repeated analyses. In one case, for example, 10 determinations of calcium and 12 determinations of phosphorus were made. The high values of the ratio obtained, therefore, do not appear to be ascribable to experimental error, since a sufficient number of determinations were made to rule out accidental high values of calcium and low values of phosphorus.

The results are interpreted as showing that primary calcification has a different empirical composition from older bone in that it contains a larger proportion of calcium than the latter. In the light of the mechanism discussed in the preceding communication[4] it seems probable that this excess calcium is present as calcium hydroxide and that the phosphate is present as $CaHPO_4$.

---

[1] Shear, M. J., and Kramer, B., *J. Biol. Chem.*, 1927, lxxiv, ix.

[2] Kramer, B., and Shear, M. J., PROC. SOC. EXP. BIOL. AND MED., 1927, xxv, 141.

[3] Howland, J., Marriott, W. McK., and Kramer, B., *J. Biol. Chem.*, 1926, lxviii, 721.

[4] Shear, M. J., and Kramer, B., PROC. SOC. EXP. BIOL. AND MED., 1928, xxv, 283.