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Studies upon Calcification in Vitro: I. Effect of Reaction on Calcification.

BENJAMIN KRAMER, DAVID H. SHELLING* AND ELSA R. ORENT.

From the Harry Caplin Pediatric Research Laboratory, The Jewish Hospital of Brooklyn, N. Y.

Shipley, Kramer and Howland[1] have shown that when a section of a rachitic bone is immersed in an inorganic solution containing calcium and phosphorus, calcification occurs in the provisional zone of cartilage. This calcification resembles that occurring *in vivo* following the administration of an antirachitic agent to a rachitic animal. These authors have also demonstrated that calcification occurs only when the product of the concentration of the calcium and inorganic phosphorus exceeds 35. The significance of the Ca × P product in the serum of patients with infantile rickets, and animals suffering from experimental rickets, has been emphasized by Howland and Kramer[2] and confirmed by others.

The present investigation is an extension of the study of calcification *in vitro,* and deals with the effect of reaction (pH) on this process. The procedures employed were essentially the same as described by Shipley, Kramer and Howland, with the exception of a few technical variations. Whereas they used the McCollum No. 3143 diet in producing a rachitic condition in rats, we have employed the Steenbock diet No. 2965. The latter diet produces a very severe type of rickets within 17 days, and the sections obtained from these animals are very well adapted to an investigation of this kind, since the metaphysis is very wide and the cartilaginous zone very pronounced.

In order to study the effect of reaction, the calcium-phosphorus concentration was kept constant, and the pH varied from the acid to the alkaline sides of the physiological range. The results may be summarized briefly as follows:

1. The optimum pH for *in vitro* calcification, when the product of Ca × P is 50, was found to be 7.25 to 7.30.

2. Under these conditions calcification may appear after two hours' incubation.

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* Harry Caplin Research Fellow.

3. Calcification occurs only in places in the zone of provisional cartilage at a pH of 7.1 to 7.2.

4. Slight calcification may occur at a pH of 7.0 to 7.1.

5. No calcification occurs in solutions more acid than pH 7.0, and the sections appear swollen and tenacious.

6. In solutions more alkaline than pH 7.35, calcification occurs, but the initial pH usually changes to the optimum pH of 7.25 or 7.3 over night.

7. Sections left in acid solutions for from 2 to 6 hours and later transferred into solutions in which the concentrations of calcium, inorganic phosphorus and hydrogen ions are optimal, fail to calcify; whereas sections transferred from solutions of low Ca × P product into solutions of a higher product may calcify if the pH is optimal.

[1] Shipley, P. G., Kramer, B., and Howland, J., *Biochem. J.*, 1926, xx, No. 2, 379.

[2] Howland, J., and Kramer, B., *Monatsschr. f. Kinderheilk*, 1923, xxv, 279.

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The Gasometric Determination of Potassium.

BENJAMIN KRAMER AND ISAAC GITTLEMAN*.

From the Harry Caplin Pediatric Research Laboratory, The Jewish Hospital of Brooklyn, N. Y.

We have used the constant volume apparatus of Van Slyke and Neill in the estimation of potassium in solutions whose inorganic composition approximates that of serum. The potassium is precipitated as the potassium-sodium cobalti-nitrite compound, essentially as in the original method of Kramer and Tisdall.[1] The precipitate is decomposed with sodium hydroxide as suggested by Clausen,[2]* and the nitrogen of the sodium and potassium nitrite is liberated by adding a solution of urea and sulphuric acid.[3]† The amount of nitrogen is then determined in the constant volume apparatus. It is possible to determine from 0.07 to 0.45 mg. potassium, with an error not exceeding 0.01 mg.

* Nathan M. Ohrbach Research Fellow.

* $K_2NaCo(NO_2)_6 + 3\ NaOH = 2\ KNO_2 + 4\ NaNO_2 + Co(OH)_3$.

† $CO(NH_2)_2 + 2\ HNO_2 = 2\ N_2 + CO_2 + 3\ H_2O$