

a complete new growth may shortly be obtained for reinoculation.

It has been shown that the amount of oxygen necessary for just visible luminescence in a suspension of luminous bacteria in seawater is only of .0053 mm. of mercury pressure.⁵ The amount of oxygen in tubes sealed in pure hydrogen with nutrient medium out of which dissolved gases have been driven by boiling, must be very minute indeed.

It has been demonstrated that oxygen is necessary for the growth of this species of luminous bacterium, (*B. fisheri*) as well as for the maintenance of luminescence. The very fact that the glow reappeared in the experimental tubes in each case only after several hours of exposure to an atmosphere containing a partial pressure of oxygen, indicated that it was necessary for growth to occur and permit luminescence over a larger area of medium before it became visible to the observer.

The above method is also suggested as a means of storage and transport of the living luminous bacteria where one is especially anxious that luminescence reappear quickly. The inoculation itself while in pure hydrogen will lie dormant on the nutrient medium until again exposed to an atmosphere containing a partial pressure of oxygen sufficient for growth and luminescence, whereupon both occur, and a vigorous culture of the organisms will cover the surface of the medium.

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The Presence of an Unknown Factor in Serum Which Influences Calcification.

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It has been shown by Shipley¹ and the observation has been confirmed by Shipley, Howland and Kramer² that calcification of slices of cartilage and bone from a rickety animal takes place *in vitro* within 48 hours when the slices are immersed in normal blood serum at 37° C.

In the course of a series of experiments upon calcification from

⁵ Harvey and Morrison, *J. Gen. Physiol.*, 1923, vi, 13.

¹ Shipley, P. G., *Johns Hopkins Hosp. Bull.*, 1924, xxxv, 304.

² Shipley, Kramer and Howland, *Trans. Am. Ped. Soc.*, 1925, xxxvii, 36; *Am. J. Dis. Child.*, 1925, xxx, 37; *Biochem. J.*, 1926, xx, 379.

solutions of inorganic salts, we have repeatedly observed that calcification takes place only when the concentrations of blood electrolytes are considerably reduced below the concentrations in the blood serum. When a salt mixture is used approximating as closely as possible the inorganic composition of blood serum,³ no calcification whatever occurs, even in preparations which have been incubated as long as 10 days. A sample experiment is given in the table. It would therefore seem necessary to postulate the existence in the blood serum of some factor which enables calcification to take place in spite of the high concentration of electrolytes.

TABLE I.

Medium	Bone	Number of Preparations	Time	Calcification
Normal Serum	Rat	6	48 hours	Positive
<i>Salt Solution:</i>				
Na 120.0 mM.	Rat	15	48 hours	Negative
K 0.3 mM.				
Ca 2.2 mM.				
Mg 0.8 mM.				
Cl 90.0 mM.				
CO ₃ 32.0 mM.				
P 1.33 mM.				
SO ₄ 0.8 mM.	Rat	3	2 weeks	Negative

It seems altogether improbable that the serum proteins could be concerned in producing this effect, since proteins in general, owing to their ionic nature, would be expected to inhibit rather than to promote calcification. Moreover, the experiments of Shipley, Howland and Kramer have shown that egg albumen and gelatin actually do inhibit the process.

Experiments to determine the nature of this unknown factor are now in progress.

³ Shipley and Holt, *Johns Hopkins Hosp. Bull.*, 1927, **x**l, 1.