usually have many efferent branches to adjacent tissues which are short as compared with the total length of the tracheal tree.

If the above view of the function of the air sacs is correct there would be an enormous advantage in having such a great number of small sacs scattered throughout the body, rather than a few large ones. Each sac would serve to ventilate the section of tracheal tube between it and the nearest open spiracle. The agitation given to the column of air in the tortuous tracheae would be very effective in aiding the diffusion of gases. Krogh[5] and Winterstein[6] have expressed doubts as to the possibility of diffusion alone through the long, narrow tracheal tubes being able to supply an adequate amount of oxygen to the tissues, especially during activity. Krogh has further shown experimentally in a series of ingenious experiments that diffusion alone is sufficient for an adequate gas exchange in the great majority of larvae, all pupae, and in the smaller and more lethargic adult insects. In the larger and more active forms with well-developed powers of flight, however, diffusion alone must be inadequate. If the function of the air sacs outlined above is correct, the difficulty disappears. For the comparatively short distances between the air sacs and the tissues the process of diffusion would supply a sufficient amount of oxygen, even to the organs of the head which are farthest removed from any spiracles. The parallel development of the air-sac system with the powers of flight would indicate the efficiency of the mechanism for supplying an adequate gas exchange for such an oxygen-consuming activity.

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COMPOSITION OF BONE, VII. EQUILIBRATION OF SERUM SOLUTIONS WITH $CaHPO_4$

IT is generally taken for granted that bone consists chiefly of $Ca_3(PO_4)_2$ and that when calcium phosphate is deposited in the bones it is in the form of this compound, tricalcium phosphate. There have been several notable attempts to account for the deposition of tricalcium phosphate on the basis of solubility product considerations. Holt, La Mer and Chown[1] interpreted their experiments as showing that "serum is normally supersaturated with tertiary calcium phosphate to the extent of more than 200 per cent." Not only normal serum is supersaturated but also "Even in active rickets this ion product is greater than that required to precipitate $Ca_3(PO_4)_2$," according to Holt.[2] Sendroy and Hastings[3] obtained similar experimental data with $Ca_3(PO_4)_2$, but concluded that supersaturation can not be the sole explanation "for the apparently abnormal amounts of calcium in serum," and that the data indicate "that calcium exists in serum in abnormal amounts bound to some substance or substances which hold it in solution in unionized form."

In a previous communication[4] we presented evidence which indicated that serum does not contain abnormal amounts of calcium and that serum and inorganic serum solutions are not supersaturated but are undersaturated. Furthermore, the important substance appears to be $CaHPO_4$ and not $Ca_3(PO_4)_2$. Our calculations showed that in inorganic serum solutions with $Ca \times P$ products less than 30, $[Ca^{++}] \times [HPO_4'']$ is less than the solubility product of $CaHPO_4$, and that in solutions with $Ca \times P$ products ranging from 40 to 60, $[Ca^{++}] \times [HPO_4'']$ is very nearly equal to the solubility product of $CaHPO_4$.

This value for $K'_{s.p.}CaHPO_4$, the solubility product of $CaHPO_4$, was obtained by extrapolation from data in the literature. The present paper is a preliminary communication giving the value of $K'_{s.p.}CaHPO_4$ obtained experimentally at the ionic strength of serum.

An inorganic serum solution was made up containing 8 mg per cent. calcium and 3 mg per cent. phosphorus. Aliquots of this solution were equilibrated at room temperature with an excess of crystalline $CaHPO_4$. It was found that equilibrium was obtained after shaking for only one hour, and that the equilibrium was independent of the amount of the solid phase. The concentrations both of calcium and of phosphorus were *greater* at equilibrium than in the initial solution. Fifteen experiments were performed at room temperature; the mean value obtained for $K'_{s.p.}CaHPO_4$ was 3.2×10^{-6}. Twenty similar experiments were performed at 38°; the mean value obtained for $K'_{s.p.}CaHPO_4$ at 38° was 3.4×10^{-6}.

In making these inorganic serum solutions, the initial $Ca \times P$ product was varied from zero to 60; some contained high calcium and low phosphorus concentrations; others contained the reverse. After equilibration, the ion product obtained in all these solutions was identical. Equilibration with $CaHPO_4$ caused an *increase* in the concentration of calcium or

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[2] L. Emmett Holt, Jr., *Jour. Biol. Chem.*, 64: 579, 1925.

[3] Julius Sendroy, Jr., and A. Baird Hastings, *Jour. Biol. Chem.*, 71: 783, 797, 1927.

[4] M. J. Shear and Benjamin Kramer, *Jour. Biol. Chem.*, 79: 125, 1928.

[5] *Pfl. Arch. f. d. ges. Physiol.*, 179: 113, 1920.

[6] "Hdb. d. vergl. Physiol.," 1, p. 111, Jena, 1921.

of phosphorus, or of both, in all solutions with Ca × P products less than 45. In all these solutions, the ion product after equilibration was greater than the initial ion product; such solutions are therefore *undersaturated.* Inorganic serum solutions with Ca × P products in the neighborhood of 50 are saturated with respect to $CaHPO_4$ at a pH of 7.4, since equilibration with $CaHPO_4$ causes no change in the ion product $[Ca^{++}]\times[HPO_4'']$.

Howland and Kramer[5] showed that in active rickets the serum Ca × P product was always less than 35, whether the calcium was high and the phosphorus was low, or whether the calcium was low and the phosphorus was high. The ion product $[Ca^{++}]\times[HPO_4'']$ in a serum with a given calcium and phosphorous content can not be greater than the ion product in an inorganic serum solution with the same calcium and phosphorus content. If it is at all different, it must be less in serum since part of the calcium appears to be bound to protein. Inorganic serum solutions with Ca × P products of 35 or less are definitely undersaturated with respect to $CaHPO_4$; ricketic serum must therefore also be undersaturated with respect to this substance.

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ACTION OF HYDROGEN SULFIDE ON THE PROTOPLASM OF AMOEBA PROTEUS

IT has been reported by various investigators [1, 2, 3, 4, 5] that certain weak acids, namely, CO_2, HCN and H_2S, enter living cells largely in the molecular form and probably exert their toxic effect by intracellular ionization. It has recently been reported, however, that microinjections of CO_2[6] and HCN[7] into amoebae do not irreversibly injure the cell unless the dosage is so large that it destroys the surface membrane. On the other hand, amoebae immersed in the same solutions die very quickly. Therefore, it appears that the physiological action of CO_2 and HCN is largely a surface one. Since the toxicity of HCN and CO_2 seems to be due to their effect on the cell membrane, it was thought desirable to ascertain if H_2S exerts its effect on the cell membrane. The solutions of H_2S used were saturated, three quarters, one half and one quarter saturated. The pH values of the solutions varied from 5.0 to 5.4, depending upon the concentration of sulfide. Some amoebae were immersed in the sulfide solutions and others were injected with the same solutions by means of Chambers' micromanipulator. The organisms were studied under direct and indirect illuminated microscopes. The rate of Brownian movement of the microparticles was used as an index of the viscosity of the protoplasm.

Immersion Experiments: When amoebae are immersed in aqueous solutions of hydrogen sulfide, the viscosity of the protoplasm is increased. The animals retract their pseudopodia and assume a spherical form. The granular portion of the protoplasm collects into a semi-gelated mass near one end of the cell, and the remainder of the organism is composed of a hyaline material. Finally, the cell membrane ruptures in one or more places and the hyaline fluid escapes into but does not mix with the surrounding solution. The granuloplasm distintegrates and the individual particles scatter in the sulfide solution.

Injection Experiments: Aqueous solutions of H_2S were injected into amoebae in amounts equal to nearly one half the volume of the cell. The sulfide quickly diffuses throughout the protoplasm, producing a reversible increase in viscosity. The organisms completely recover within one to two hours.

When amoebae are injected with H_2S and the animals immediately immersed in the sulfide solution, the animals react in a similar manner to the uninjected individuals in the same sulfide solution, *i.e.,* a slight swelling of the protoplasm and disintegration of the cell. The injected amoebae do not die any sooner after immersion than the uninjected animals.

Tearing the Cell Membrane: If amoebae are immersed in solutions of hydrogen sulfide and the cell membrane torn with microdissection needles, a new membrane is formed over the injured surface which indicates that the internal protoplasm has not been greatly injured.

Summary: Experiments on immersion and injection indicate that for amoebae the toxicity of hydrogen sulfide is largely due to its action on the surface membrane and that the internal protoplasm is not irreversibly injured.

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