

of phosphorus, or of both, in all solutions with $\text{Ca} \times \text{P}$ 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 $\text{Ca} \times \text{P}$ 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 $[\text{Ca}^{++}] \times [\text{HPO}_4^{--}]$.

Howland and Kramer⁵ showed that in active rickets the serum $\text{Ca} \times \text{P}$ 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 $[\text{Ca}^{++}] \times [\text{HPO}_4^{--}]$ 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 $\text{Ca} \times \text{P}$ 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 HCN⁷ 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 disintegrates 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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