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Effect of pH on Thickness of Surface Films Made with Insulin and Other Large Molecules.

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By using suitable adaptations of the methods developed by Langmuir, Schaefer and Blodgett[1, 2] films of salmine, insulin, thymus histone, thymus nucleic acid, trypsin, and casein have been built up on chrome plated iron or on stainless steel plates. These plates were first covered with a suitable number of layers of barium stearate by the method of Blodgett.[2] They were then immersed in a 10^{-3} M solution of uranyl acetate at pH 6.0 to condition the barium stearate surface, thus rendering it hydrophilic. Each conditioned plate with an adhering layer of water was then immersed in a 0.1% solution of the substance to be adsorbed and shaken in this solution for the period of time cited. The plate was then removed, washed, and dried in air. The insulin and other surface films described below were thus formed by adsorption of molecules or molecular aggregates directly from solution and not by transfer of films previously formed on the surface of a solution. The thickness of such films has been studied as a function of a number of factors of physiological interest, such as the nature of the adsorbing surface, the time of exposure of the plates to the solutions, and the concentration, salt content, and pH of the solutions to which the plates were exposed.

The purpose of the present paper is to point out, in a preliminary way, the great influence of pH in determining the thickness of certain of these films, using typical experiments with salmine, insulin, and casein as examples. The present experiments were made at room temperature, 25°-30°C, with the salt content of the solutions kept at the minimum level.

From the preliminary data given in Table I, it may be noted that the thickness of films of salmine, insulin, or casein deposited on a conditioned barium stearate surface varied markedly with the pH of the solution from which the adsorption was made and that, with each substance, an optimum thickness was attained in the pH region near the isoelectric point of the substance in question. The thickness of films of insulin deposited on an initial salmine layer was likewise highly dependent on the pH of the insulin solution.

[1] Langmuir, I., and Schaefer, V. J., *J. Am. Chem. Soc.*, 1937, **59**, 1406, 1762.
[2] Blodgett, K. B., *J. Phys. Chem.*, 1937, **41**, 975.

TABLE I.

Approximate thickness of salmine, insulin, and casein films deposited from solutions having various pH values. Salmine, 0.1% solution for 30 minutes. Insulin, 0.1% solution for 60 minutes. Casein, 0.1% solution for 60 minutes.

	Approximate thickness of film in Angstroms				
pH	Salmine on conditioned plate	Insulin on conditioned plate	Insulin on Salmine	Casein on conditioned plate	Casein on Salmine
4.0	15	30	10	5	25
5.0	25	70	75	400	60
6.0	35	200	500	100	50
6.5	40	400	300	75	40
7.0	50	40	150	40	25
8.0	65	20	50	—	—
9.0	75	20	—	—	—
11.*	100	—	—	—	—
12.*	75	—	—	—	—

*These values were only approximately determined.

The very thick films deposited on salmine from insulin solutions having pH values near the isoelectric point of insulin were obtained even when that fraction of the insulin which was precipitated in this pH range was removed prior to the exposure of the plates to the solution. The thickness of films of casein deposited on an initial layer of salmine varied with pH to a much smaller degree than the thickness of casein films on conditioned barium stearate.

The physiological significance of the insulin data of Table I will be briefly discussed in a forthcoming paper.[3] The extension of the present methods to the study of other cellular constituents will be more fully considered at a later date.

The authors wish to express their great indebtedness to Dr. Langmuir and his staff for the information which they have provided regarding the experimental methods of approach to the study of various types of films.

[3] Clowes, G. H. A., to be published in Symposium of Section C, Am. Assn. for the Advancement of Science, December 28, 1937, The Science Press, 1938.