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The Isoelectric Point and the Median Minimum of Growth.

CLIFFORD H. FARR. (Introduced by Leo Loeb.)

From the Department of Botany, Washington University, St. Louis.

The typical curve which has been secured for growth of organisms in varying hydrogen ion concentrations is platycurtic and bimodal. Jacques Loeb[1] secured similar curves for the viscosity, osmotic pressure, potential difference, and swellability of gelatin and other proteins. He explained the bimodality of these graphs upon the basis of the Donnan's equilibrium, and arrived at the conclusion that the median minimum lying between the acid and the alkaline optima respectively is located at the isoelectric point of the protein in each case. In physico-chemical investigations, then, one seems quite warranted in regarding the median minimum of curves plotted along the pH scale as a sound criterion of the isoelectric point of the protein involved. A number of biologists, including Robbins[2] and Pearsall,[3] have undertaken to utilize this relationship in the interpretation of similar graphs obtained in the study of plants and animals, that is, concluding, for instance, that the median minimum for growth curves is an index of the isoelectric point of the constituent proteins.

Michaelis[4] has pointed out that "the isoelectric point is not deflected by true salt formation" (p. 145). If the median minimum of biological graphs plotted along the pH scale is at the isoelectric point of the proteins composing the tissue concerned, then it would seem that this medium minimum should be located at approximately the same hydrogen ion concentration irrespective of the molar concentration of the salt solution bathing the tissue. If, on the other hand, the median minimum is found to shift toward the acid or the alkaline side with alteration of the salt concentration, there would be definite evidence that the median minimum of these biological curves is determined by some other factor than the isoelectric point of the organism.

Some experiments, which are to be reported in full elsewhere,[5] throw some light upon this question. The rate of elongation of the root hairs of Georgia collards, a variety of *Brassica oleracea*, growing in solutions of calcium chloride of various hydrogen ion and molar concentrations, has been measured. The increase in size of these root hairs can be determined very accurately, inasmuch as they are single cells, which enlarge in only one dimension, and have very definite termini. Furthermore, they will grow in calcium hydroxide alone, and in all concentrations of calcium chloride below a certain maximum, approximately 0.185 M. Data has been obtained upon the rate of growth of about 700 of these root hairs in a total of 49 solutions of different concentration, over a period of three hours in each case. Curves for variations in rate in different hydrogen ion concentrations were plotted for calcium hydroxide, and for calcium chloride at 0.008, 0.020, 0.028, 0.060, and 0.120 mols. respectively. In all cases except the last-named concentration the curves were bimodal.

The data thus obtained may be plotted in a graph of three dimensions: the ordinates, being rate of growth; the two abscissae, being hydrogen ion concentration and molar concentration of the salt respectively. When this is done, it becomes apparent that the median minimum, which begins at pH 9 in calcium hydroxide, shifts with increasing salt concentration to pH 8 in 0.020, pH 7.4 in 0.028, pH 6.4 in 0.060. No median minimum occurs in 0.120 M. inasmuch as it, by projection, is found to lie outside of the concentrations in which the root hairs will grow, that is, in too acid a solution for the amount of calcium present. Not only does the median minimum shift toward the acid side with increased salt concentration, but the acid optimum, the alkaline optimum, the alkaline limit, and, at low concentrations, acid limit do likewise.

From this study it may, therefore, be concluded that the median minimum of some growth curves, at least, are determined by some other factor than the isoelectric point of the constituent proteins alone. The latter may be one of the factors involved, but it can hardly be the sole consideration. It is apparent that the median minimum in shifting toward the acid side, will in all probability intersect the hydrogen ion concentration of the isoelectric point; so that, at one salt concentration the two will be identical. The finding that they are identical in a given solution is not, however, to be taken as evidence that they are necessarily related to each other.

This is a preliminary report.

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1 Loeb, J., Proteins and the theory of colloidal behavior. N. Y. 1922.

2 Robbins, W. J., The isoelectric point for plant tissues and its importance in absorption and toxicity. *Univ. Mo. Studies* 1926, i, 3-60.

3 Pearsall, W. H., *Sci. Prog.*, 1925, xx, 58-67.

4 Michaelis, L., (tr. Perlzweig) Hydrogen ion concentration, Balt. 1926.

5 Farr, C. H., *Am. J. Bot.*, 1927, xiv, Nos. 8, 9, 10; xv, Nos. 1, 2, 3, in press.