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### Physiological Conditions of Ameba Proteus at Varying Hydrogen Ion Concentrations.

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An effort was made to determine observationally the various interrelationships of physiological manifestations of ameba at varying high pH concentrations in celery infusions. All the cultures were kept under the same temperature and light conditions, the temperatures varying between 21 and 25°C. The same temperature and light conditions prevailed throughout the period of observation. Efforts were made to maintain uniform concentration by the use of like containers and by the addition of 5 cc. of distilled water and infusion every three or four days to each of the cultures. All detailed microscopic observations were made with Zeiss Apchromat Lens 3 mm. n.a. 1.4.

In determining pH, the colorimetric drop method of LaMotte with Clark and Lubs' standards was employed.

Quantitative studies on population density at different pH were attempted for the first time. The limits of pH tolerance as stated by Hopkins<sup>1</sup> were extended. 15 of the 22 cultures had a maximum population density at pH 7.8-8.4. A maximum population density occurred in one culture at pH 8.4. Only cultures in which 10 adult ameba in 0.05 cc. of medium occurred were used in the averages. The average density at different pH was as follows:

pH	Average Number of Ameba
7.6	23.41
7.8	15.95
8.0	31.21
8.2	64.21
8.3	103.32

Hopkins has reported a relationship between pH and size, decrease in size occurring at pH 7.8 and higher. In our cultures maximum average size occurred at pH 8.0 and 8.2 (330-405 microns). Larger sizes (600 microns frequently and occasionally 700-900 microns) were found under these alkaline conditions when food supply was abundant.

<sup>1</sup> Hopkins, D. W., *J. Morph. and Physiol.*, 1928, xlv, 1:118.

The average rate of movement of *A. proteus* from reports of other workers is 0.1400 mm. per minute. Edwards<sup>2</sup> found a maximum of 0.2880 mm. per minute. In our cultures movements between 0.2-0.4 mm. were found in 50.5% of 2,300 records. It should be noted that 45.2% of these lay between velocities of 0.3-0.4 mm. per minute, thus giving the highest rate thus far recorded for *A. proteus*. 52.5% of 400 readings at pH 8.0 and 53.3% of 390 readings at pH 8.2 gave these high rates of locomotion.

The physiological condition described as "clear" is largely determined by the number of crystals present. At pH 7.4-7.6 the condition described as "dark" was found in 61.85% of 970 observations; at pH 7.8-8.4 only 45.26% of 1,960 observations were found with similar physiological conditions.

Monopodal conditions are reported "almost exclusively" at pH 8.0 and above. In our observations only 45.41% of 2,370 observations were monopodal at pH 8.0, while in 60.34% of 2,774 observations proteus-form occurred at pH 8.2 and 71.35% of 350 readings at pH 8.3 showed a bi- or tripodal condition.

An important by-product of these observations is that crystals have been found on the food mass within the vacuole. During observations on single vacuoles, the crystals were seen merging into the food mass. The evacuation of undigested food material and massed crystals was observed several times, and is also reported for the first time. Further study may settle the question concerning the excretory, secretory or nutritive character of the crystals.

To summarize, excellent and in some cases optimal conditions for various physiological conditions in the ameba have been observed in pH regions 8.0-8.4. The question is still open whether one may conclude that conditions in ameba are relatively independent of pH or whether a series of multi-modal curves with peaks at successive pH levels may possibly be found.

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<sup>2</sup> Edwards, J. G., and Fosgrave, H. S., *Johns Hopkins Hosp. Bull.*, 1923, i, 104.