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Physiology of Pure Culture of *Trichomonas vaginalis*.
II. Cell Size in Relation to pH.*

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Several workers have found large variations in the size of *Trichomonas vaginalis* in fresh preparations from vaginal discharges. Powell¹ reported lengths of 10 to 30 μ and Wenrich² in 2 sets of preparations obtained lengths of 18 to 36 and 12.6 to 27 μ . There has been some question concerning the cause of these variations,¹ and it was with the hope of clarifying this situation that this experiment was undertaken.

The materials and culture-methods used in this experiment are the same as those employed by Johnson.³

T. vaginalis was grown in media at various pH values and the organisms were measured. Observations were made on the living organisms sealed with vaseline under a microslip supported by glass fragments. The organisms were thoroughly distributed throughout the media before samples were taken. Measurements of length and width were made on the first 35 motile organisms encountered in the field. No measurements were taken of the amoeboid forms which are considered degenerative.²

Two-day cultures—The minimal mean size of the organisms ($12.4 \pm 0.13\mu$ in length and $9.4 \pm 0.11\mu$ in width) appeared at approximately pH 5.40. The maximal mean size ($15 \pm 0.23\mu$ in length and $11.6 \pm 0.18\mu$ in width) occurred at pH 7.60. Between these extremes there was a progressive although somewhat irregular increase in size with increase in pH. The extreme range was from 6.4 to 24 μ in length and from 4.8 to 19.2 μ in width.

Four-day cultures—After 4 days the media which exhibited a pH of 6.64 to 5.15 after 2 days had become more acid (pH 6.44 and

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¹ Powell, W. N., *Am. J. Hyg.*, 1936, **24**, 145.

² Wenrich, D. H., *The Morphology of Trichomonas vaginalis*. Volumen Jubilare Pro Prof. Sadao Yoshida, Vol. II, 1939.

³ Johnson, Garth, in press.

4.93), while the less acid media became slightly more alkaline (about 0.1 pH unit).

The minimal mean size of protozoa (11.4 ± 0.12 by $9.0 \pm 0.10 \mu$) occurred at about pH 5.10 to 5.17. In more acid cultures the mean size increased slightly to 12.0 ± 0.12 by $10.6 \pm 0.11 \mu$ at pH 4.93, and in more alkaline cultures (pH values of 6.46 to 7.60) the mean length increased considerably to about 15.0μ . There were slight variations (14.2 to 16.0μ) of the means within this range, but the sizes were significantly greater than those at pH 5.10 to 5.20. The mean width increased more or less progressively from 9.0 ± 0.05 at pH 5.3 to a maximum of about 12.8μ . The extreme range in 4-day cultures was from 6.4 to 25.6μ in length and from 3.2 to 17.6μ in width.

Six-day cultures—After 6 days cultures in the pH range 4.93 to 5.45 had shifted to 4.58 to 5.34. Cultures more alkaline than pH 5.50 did not change significantly. The minimal size (11.6 ± 0.17 by $9.20 \pm 0.08 \mu$) occurred at about pH 4.9 to 5.0. The organisms in the more acid cultures were larger (12.5 ± 0.12 by $10.6 \pm 0.14 \mu$), and with increasing alkalinity there was a regular and progressive increase in size to a maximum at pH 6.40 to 7.54. Throughout this latter pH range the mean length of the organisms was 15.2μ , and variations of the mean values were very small (15.0 to 15.4μ). The extremes ranged from 6.4 to 27.2μ in length and from 4.8 to 22.4μ in width. The width increased more or less progressively from the minimum of 9.3μ at pH 4.95 to a maximum of 13.0 at pH 7.54.

Johnson³ found that the maximal growth in 2-day cultures of *Trichomonas vaginalis* (bacteria-free) occurred at pH 5.45 to 5.55, in 4-day cultures at pH 5.50 to 5.80, and in 6-day cultures at pH 5.15.

The present results indicate that at the optimal pH for growth the organisms are smaller than at any other pH. Johnson, in his 4- and 6-day cultures, found that a decided decrease in numbers of the protozoa occurred at suboptimal hydrogen-ion concentrations. Under such conditions the organisms have apparently ceased to carry on their reproductive and normal metabolic processes, and thus have retained approximately the same size as when in an optimal environment after 2 days. The data show that large variations in size comparable to those previously reported from vaginal smears may also occur in bacteria-free cultures under relatively constant environmental conditions.