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Studies on a *Tritrichomonas* from the Nasal Cavity of Swine

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Abstract. The growth and morphology of a nasal porcine trichomonad (probably *Tritrichomonas suis*) were studied on organisms grown in axenic cultures in C.P.L.M. medium under a variety of environmental conditions including temperature changes (20°C., 25°C., 30°C., 35°C., 40°C.) and ranges in pH (4.1, 4.6, 5.1, 5.6, 6.1, 6.6, 7.1, 7.6, 8.1, 8.6, and 9.1). Temperatures of 25°C., 30°C., and 35°C. supported active growth with maximal populations of 3,050,000, 5,150,000, and 5,200,000 per ml., respectively. The optimal pH for growth was found to be within the range of 5.1-7.6, but the organism can be successfully grown between a pH range of 4.6 to 8.6.

The morphology of the organism was studied in relation to the environmental temperatures and a change in size was noted. At cooler temperatures the organisms were wider, nucleus was smaller, and the axostyle tip was longer. Both temperature and hydrogen ion concentration influenced the size of the organisms. At both 30°C. and 35°C. the smallest organisms were in the pH range of 6.1-7.1; whereas, the organisms were longer in the pH range of 4.6-5.6 and also in the pH range of 7.6-8.6. At each temperature the organisms were wider in the acid range. Such morphological changes associated with changing environmental conditions must be taken into consideration when taxonomy is based upon morphological structures.

In an earlier paper Buttrey (1956) described a *Tritrichomonas* from the nasal cavity of swine. Hibler *et al.* (1960) confirmed the presence of this organism in the nasal cavity, considering it to be *Tritrichomonas suis* (Gruby & Delafond, 1843), a species also found in the stomach and caecum of swine. Their conclusions were based primarily on morphological data from stained organisms.

The true significance of morphological differences and their taxonomic interpretation is often questioned. To further interpret the porcine trichomonad taxonomy, Buttrey (1960) studied the normal pattern of growth of the porcine *Tritrichomonas* from the nose and later (1968) made a similar study on the form from the caecum.

To further study the nasal porcine trichomonad and to better understand its taxonomic position in relation to the trichomonads throughout the alimentary tract of swine, it was felt appropriate to reinvestigate certain aspects of its physiology and morphology and correlate the results with those of earlier studies. The experiments using a strain of trichomonad (Buttrey's strain 3/N) from the nose of swine were designed to: (1) determine the growth curves for the organism grown at different temperatures; (2) determine if the populations vary in morphology when grown at different temperatures; and (3) determine the relation of hydrogen ion concentration to growth and size of the organism.

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Materials and Methods

The culture strain used in the study, originally isolated from the nose of a pig sacrificed 9 years and 10 months prior to the study, was in its 449th subculture. The axenic population was first obtained by using a U-shaped migration tube and aseptic laboratory procedures were subsequently followed during the extended period of subculturing. The stock culture had been maintained at 20°C.±1° and had been subcultured every 3 to 7 days.

C.P.L.M. medium (Johnson, 1947) with 10% bovine serum was used for growing the organisms throughout the experiments. To each 10 ml. of the final C.P.L.M. medium mixture, 25,000 units of penicillin and 25 mg. of dihydrostreptomycin were added.

The organisms for inoculation were prepared from a two-day-old flourishing culture containing 1-2 million active trichomonads per ml. which was diluted to approximately 2,000 organisms per ml. One-half ml. of this dilution (1,000 organisms) was inoculated into 9½ ml. of culture medium, making 100 organisms per ml. of medium. The study consists of two experiments. In the first, the pH value (pH 6.1) was constant but the temperatures varied (20°C., 25°C., 30°C., 35°C., and 40°C.). This experiment consisted of a series of 12 "identical" subcultures at each temperature. Counts of living cells were made every 12 hours, but data to form the growth curve are recorded at 24-hour intervals except where significant changes occurred between these longer periods. The populations were determined with a brightline improved Neubauer haemacytometer after thoroughly shaking the culture.

The second experiment regarding the relationship between pH values and growth involved varying the pH values but keeping the temperature constant. The pH values of 4.1, 4.6, 5.1, 5.6, 6.1, 6.6, 7.1, 7.6, 8.1, 8.6, and 9.1 were used at temperatures of 20°C., 25°C., 30°C., 35°C., and 40°C. Six "identical" subcultures were used at each pH value and temperature.

Morphological studies were based upon stained slides fixed in Schaudinn's and Bouin's and stained with iron-hematoxylin and protein silver (Moskowitz, 1950), respectively. In order to compare the morphological changes during the growth cycle, smears were made from the experimental cultures at 24 hour intervals throughout the growth cycle. All other smears were made at or near the maximal population levels. All measurements are based upon the average of ten to twenty-five organisms.

Results and Discussion

Growth Curves for the Organism Grown at Different Temperatures. Of the five temperatures (20°C., 25°C., 30°C., 35°C.,

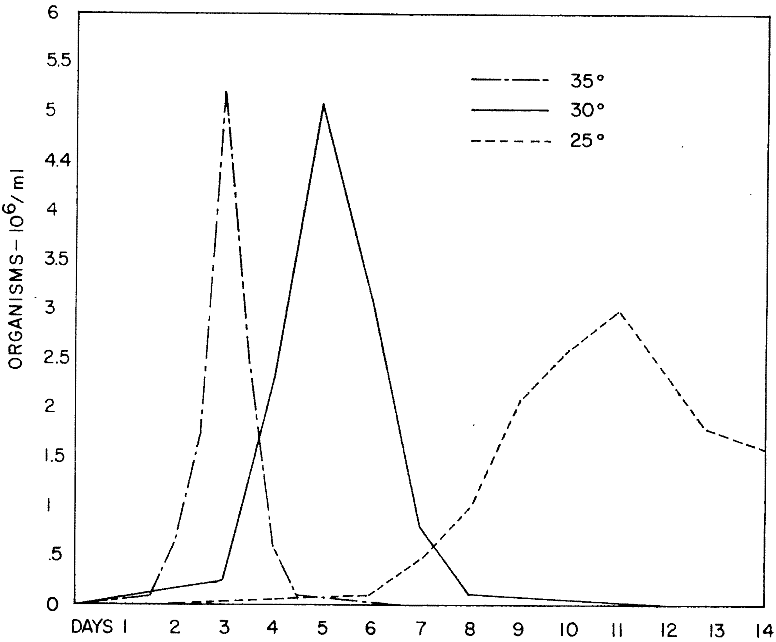

Fig. 1. Population growth curves of the nasal form of porcine trichomonad at different temperatures.

and 40°C.) only 25°C., 30°C., and 35°C. supported active growth. The population levels forming the growth curves at each of the temperatures which supported growth are shown in Fig. 1 where the number of organisms is plotted against time. An examination of the growth curves reveals that the increase of the population in absolute numbers conforms to a normal distribution with the maximal population being 5,200,000 per ml. on day 3 at 35°C., 5,100,000 per ml. on day 5 at 30°C., and 3,050,000 per ml. on day 11 at 25°C. (Fig. 1). At 20°C. there was no sustained increase in population and the maximal population obtained was 20,000 per ml. after 30 days of incubation. At 40°C. the maximal population obtained was 14,000 per ml. after 4 days of incubation. At all temperatures a few organisms remained alive for several days (7-18 days) beyond the time shown in Fig. 1, but these organisms were abnormal, often appearing as giant spherical forms or as "flagellated axostyles" devoid of most of the cytoplasm.

Variation in Morphology of Organisms Grown at Different Temperatures. Some variation was found in the morphology of the trichomonads when the organisms were grown at different temperatures. At 12 hours before the peak population the original stock

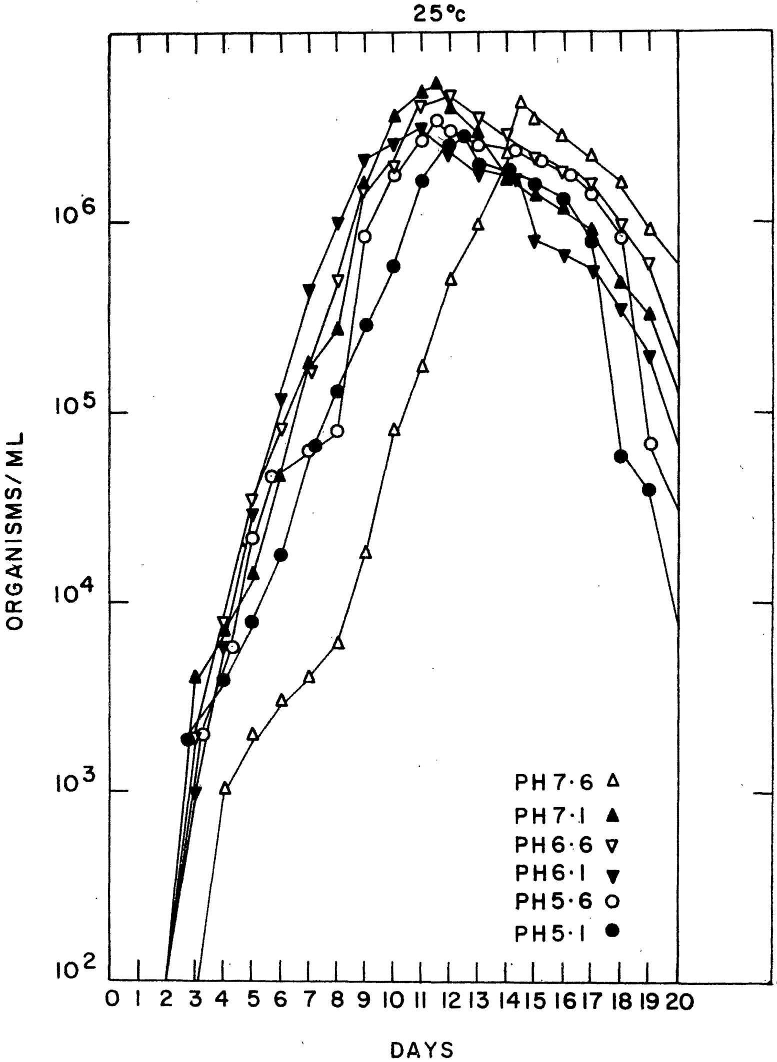

Fig. 2. Population growth curves of the nasal form of porcine trichomonad at different pH values at 25°C.

culture averaged 13.4 μ; whereas, at the same time in the culture cycle at 25°C., 30°C., and 35°C. the lengths were 12.6 μ, 10.6 μ and 13.6 μ respectively. After the peak populations on day 7½ for 30°C. and 3½ for 35°C., the average length was reduced to 9.3 μ and 10.5 μ, respectively.

The typical shape of this nasal form of *Tritrichomonas* was pyriform, but the flagellates grown at 25°C. were oval and much

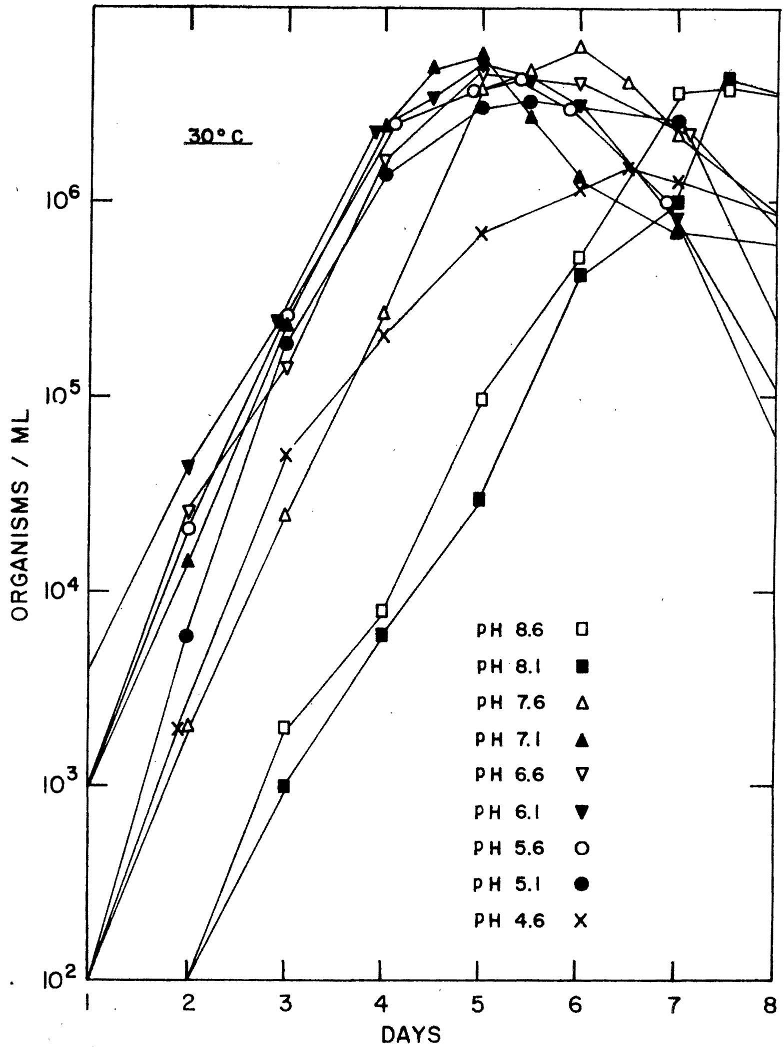

Fig. 3. Population growth curves of the nasal form of porcine trichomonad at different pH values at 30°C.

wider than those grown at the other temperatures. The width of the organisms of the stock culture averaged 4.4 μ; whereas, those at 25°C., 30°C., and 35°C. averaged 5.5 μ, 4.9 μ, and 4.7 μ, respectively.

When the morphology of the nucleus, parabasal body, axostyle, axostylar tip, flagella, and costa of the stock culture organisms was compared with the same structures of the organisms grown under

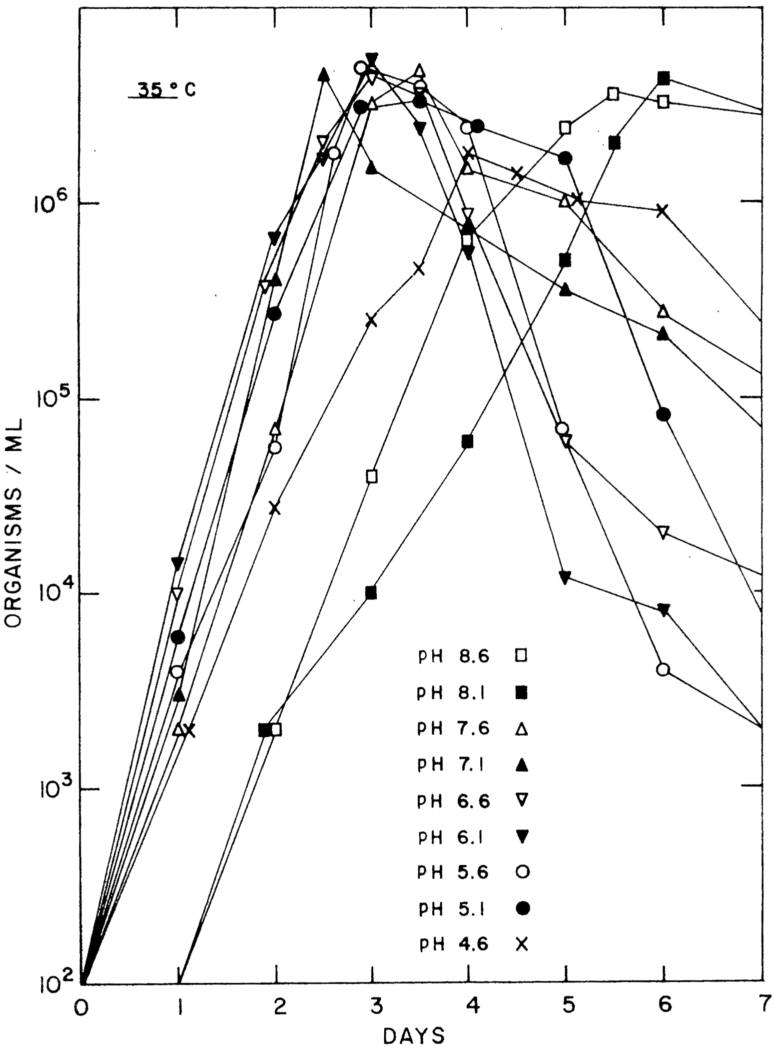

Fig. 4. Population growth curves of the nasal form of porcine trichomonad at different pH values at 35°C.

the experimental conditions, some changes were noticed. The organisms grown at 25°C. showed a slight decrease in size of the nucleus and parabasal body and the axostylar tip was longer; however, the other morphological structures at the various temperatures showed little change and were well within the limits of the earlier description of this organism by Buttrey (1956).

Relation of Hydrogen Ion Concentration to Growth and Size. The population growth curves of the nasal porcine trichomonad grown in media of different pH values (4.1, 4.6, 5.1, 5.6, 6.1, 6.6, 7.1, 7.6, 8.1, 8.6, and 9.1) at temperatures of 25°C., 30°C., and 35°C. are shown in Figs. 2, 3, and 4, respectively. Growth was observed between the pH values of 5.1-7.6 for 25°C. (Fig. 2) and 4.6-8.6 for 30°C. and 35°C. (Figs. 3 & 4), but the pH values beyond these ranges did not support growth. The maximal population ranging from 2,800,000 to 5,350,000 per ml. developed between the pH ranges of 5.1-7.6 at 25°C., 1,550,000 to 6,300,000 per ml. between the pH values of 4.6-8.6 at 30°C., and 1,800,000 to 5,200,000 per ml. between the pH values of 4.6-8.6 at 35°C. Figures 2, 3 and 4 reveal, also, that the time in days when the maximal population was reached for each pH value at each temperature varied considerably.

The stained organisms used for comparison to see if the hydrogen ion concentration affected the morphology were prepared from cultures at or near peak populations. Variations were found in both length and width among organisms grown at the different hydrogen ion concentrations. At 25°C. there was less variation with the organisms averaging 12.3 μ at pH 5.1 and 5.6 and 11.8 μ at pH 6.1-7.6. However, at both 30°C. and 35°C. the smallest organisms were in the pH range of 6.1-7.1 (average of 11.5 μ at 30°C. and 11.7 μ at 35°C.); whereas, the organisms were longer in the pH range of 4.6-5.6 (average of 12.4 μ at 30°C. and 12.5 μ at 35°C.) and also in the pH range of 7.6-8.6 (average of 12.3 μ at 30°C. and 12.4 μ at 35°C.). At each temperature the organisms were wider in the acid range. At 25°C. the organisms were 6.2 μ wide at pH 5.1 and 5.1 μ at pH 7.6; at 30°C. 6.4 μ at pH 4.6 and 4.3 μ at pH 8.6; and at 35°C. 5.2 μ at pH 4.6 and 3.9 μ at pH 8.6.

Discussion. The interpretation and taxonomic division of the species of the genus *Tritrichomonas* until recently have been based mainly on morphological data. More recently, however, many workers including Hammond and Fitzgerald (1953), Buttrey (1960), Palmquist and Buttrey (1960), and Buttrey (1968) have stressed that the physiology, especially the growth characteristics under various environmental conditions, must be thoroughly considered in working out any taxonomic scheme. Since the morphology of this nasal trichomonad was influenced by environmental changes, changing environmental conditions must be taken into consideration when taxonomy is based upon morphological structures.

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