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Abstract. The growth and morphology of a caecal porcine trichomonad (probably *Tritrichomonas rotunda*) were studied from both the trichomonad's natural environment, the pig's caecum, and in culture. Growth of the organism was studied in C.P.L.M. medium at 37° C. After an initial inoculum of 100 organisms per ml. the population rose to 2,900,000 per ml. on day 4 and then decreased to near extinction on day 7. In comparison to the organisms on stained smears made directly from the pig's caecum, the organisms after extensive subculturing (100 subcultures over a period of 20 months) were more elongate and often almost tubular; possessed longer undulating membranes and costas approaching full body length instead of only ½-¾ body length; had axostyles with a more bulbous capitulum; and had club-shaped parabasal bodies instead of V-shaped structures. No significant variations were found in the morphology of the trichomonads during the various days of the culture cycle. Because of these morphological changes during extended subculturing, the interpretation of the morphological structures based upon trichomonads after extended periods of subculturing must be viewed with caution.

In an earlier paper the author (1956) described a *Tritrichomonas* from the nasal cavity of swine and reported the presence of two additional trichomonad species, both different from the nasal form, in the caecum. Hibler et al. (1960) confirmed the presence of three species of trichomonads in pigs: *Tritrichomonas suis* (Gruby and Delafond, 1843) and two new species which they named *Trichomonas buttreyi* and *Tritrichomonas rotunda.* The taxonomic separation of these three species was based primarily on morphological data.

The true significance of morphological differences and their interpretation regarding taxonomy is often questioned. Often the morphological studies are based on organisms from culture and the effects from subculturing are not fully understood. In culturing various microorganisms, i.e., bacteria, *Tetrahymena,* and other protozoa, many investigators have reported morphological variations between strains of the same species grown under standardized conditions and even significant differences in the average dimensions of the same strain in different phases of the growth cycle. Thus, some authorities believe that for any conclusions on morphological studies on protozoa from culture to have meaning, the results must be compared with similar studies made on organisms taken directly from the normal host.

To further study this caecal porcine trichomonad and to test the taxonomic significance of morphology in the interpretation of morphological differences in the trichomonads of pigs, the present study was

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undertaken. The experiments using a strain of trichomonad (Buttrey's strain 1/C) from the caecum were designed to investigate the following: (1) determination of the growth curve throughout the culture cycle; (2) comparison of the normal morphology of the trichomonads in their normal caecal environment with that of organisms in culture; and (3) determination if the population varies in morphology during the complete culture cycle.

Materials and Methods

The morphology of this trichomonad in its normal host was determined by studying sets of slides from the caecal contents of the normally infected pig. The slides were stained with Heidenhain's iron-hematoxylin, following fixation in Schaudinn's fixative, and the Bodian (1937) protargol technique as modified by Moskowitz (1950), following fixation in Bouins and Nissenbaum's (1953) fixatives.

The culture strain used in the study, originally isolated from the caecum of a pig sacrificed 20 months prior to the study, was in its 100th subculture. A bacteria-, yeast-, and mold-free population was obtained by using a U-shaped migration tube and aseptic laboratory procedures were subsequently followed. The stock culture had been maintained at 20° C. ± 1° and had been subcultured every three to seven days.

C.P.L.M. medium (Johnson, 1947) was used for growing the organisms. One ml. sterile inactivated bovine serum, 25,000 units of penicillin and 25 mg. of dihydrostreptomycin were added to each tube containing 8 ml. C.P.L.M. medium.

The inoculum for the growth studies was prepared from the stock culture which had been stimulated into active growth by subculturing twice at 37° C. A 60-hour culture containing approximately 1,500,000 active protozoa per ml. was diluted to approximately 1,000 organisms per ml. One ml. of this dilution (1,000 organisms) was inoculated into 9 ml. of culture medium, making 100 organisms per ml. of medium. A series of eight "identical" subcultures were run on all experiments and an average was taken. 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.

Morphological changes occurring throughout the culture cycle were based upon stained smears made at each 24-hour interval on days 1-7, inclusive. During the lower populations, centrifugation was used to increase the number of organisms for smearing. A total of 12 smears were fixed at each 24-hour period. Eight in each set of 12 smears were fixed in Nissenbaum's fixative (1953), four of which were stained in

Bodian's protargol technique (1937) as modified by Moskowitz (1950) and four with Heidenhain's iron-hematoxylin. The remaining four smears were fixed in Schaudinn's fluid with 5 percent (v/v) acetic acid and stained with Heidenhain's iron-hematoxylin. On the third and sixth days, an additional set of 12 smears were made prior to centrifugation, and these were used for a comparative study of the effects of centrifugation on the protozoa.

The generation time (g), figured on an increasing population up to day 4, was calculated from the equation $g = \frac{t}{3.3 \log b/B}$ by Johnson et al. (1944) where t is the interval time in hours, b is the populations in organisms per ml. at the end of the time interval, and B is the population in organisms per ml. at the beginning of the time interval.

To check the possibility of contamination during the culturing experiments, samples at the close of each experimental series were inoculated into Fluid Thioglycollate Medium and Brain Heart Infusion Medium prepared with 1.5 percent (w/v) agar and adjusted to pH 7.4. Both media were incubated for four days at 37° C.

Results and Discussion

Normal Growth Curve Throughout the Culture Cycle. Throughout the culture cycle the population level increased from the initial inoculum of approximately 100 organisms per ml. to 2,900,000 per ml. after four days of incubation at 37° C., and then decreased at approximately the same rate. Examination of the mean growth polygon reveals that the increase of the population in absolute numbers conforms to a normal distribution with days 1 and 2 (actual populations of 3,500 and 55,000 per ml.) showing a slow increase which becomes greatly accelerated during days 3 and 4 (865,000 and 2,900,000 per ml.) and day 5 (885,000 per ml.) showing an accelerated decrease which becomes less during days 6 and 7 (75,000 and 25,000 per ml.). The result is a normal growth curve over a period of seven days. During days 8 to 10, living organisms gradually disappear from the populations, but signs of life were observed for as long as days 19 and 20.

The generation time in hours, computed on each day of the increasing population up through day 4, is as follows: 4.7 (day 1), 6.1 (day 2), 6.1 (day 3) and 13.7 (day 4).

Comparison of the Morphology of Trichomonads from the Normal Hosts and from Cultures. On smears made directly from the pig's caecum followed by Heidenhain's iron-hematoxylin and protargol stains, the over-all shape and gross form of this trichomonad appears to be of a spherical robust nature, often approaching a true spherical condition, with graduations to a slightly pyriform condition. In over-all size the length and width measurements are 6.4-11.2 μ (average 8.5 μ) by 4.8-8.8 μ (average 6.8 μ) on protargol stained organisms

and 8.0-11.2 μ (average 9.5 μ) by 4.8-8.8 μ (average 6.8 μ) on iron-hematoxylin stained organisms. A single blepharoplast, 0.8-1.0 μ in diameter and appearing as the typical complex of granules (especially in iron-hematoxylin stain), is located in the anterior dorsal portion of the animal, and from it arise the mastigont structures—anterior flagella, undulating membrane, costa, axostyle and parabasal body. Three anterior flagella ending in terminal knob-like enlargements are present. In length, two of these flagella are approximately 1½ times the length of the entire body; the shorter one is approximately the length of the body. An undulating membrance arises at the blepharoplast and extends posteriorly ½-¾ the body length. Throughout its course this structure is thrown into 3-5 indistinct folds of low magnitude. A distinct marginal filament is present along the border of the undulating membrane and extends beyond the undulating membrane as a trailing flagellum for a distance of 6.4-12.8 μ. The costa, or chromatic basal rod, arises from the blepharoplastic complex and extends posteriorly along the basal portion of the undulating membrane. Its length is usually equal to that of the undulating membrane, but it may extend beyond for a distance of up to 2.5 μ. Subcostal granules are noted in close association throughout the anterior half of the costa. A slender solid rod-like axostyle arises in juxtaposition with the blepharoplast, extends posteriorly throughout the body, and continues beyond the posterior limits of the body as a conical tip for a distance of 1.0-3.2 μ. At the point of emergence no chromatic ring was observed. Throughout most of its length, the axostyle measures 0.7-1.2 μ in width, but throughout the anterior ⅕ to ¼ it enlarges into a ladle-shaped capitulum measuring 1.2-1.9 μ at its greatest width. No endoaxostylar granules were observed. A well developed V-shaped parabasal body, 3.2-4.8 μ long and 0.7-1.9 μ wide, consisting of a thicker body and a slender parabasal fibril, which extends beyond the main body for a distance of 0.6-1.5 μ, originates in or near the blepharoplastic region and extends posteriorly. The two rami of the parabasal body are approximately of equal size. The nucleus varies in shape from round to oval, in size from 2.4-4.8 μ in length to 2.4-4.0 μ in width, and in position from one of close proximity with the blepharoplast to approximately ¼ its body length from the anterior end. An endosome, 0.7-1.2 μ in diameter, is located toward one end or in the center of the nucleus. A small area of clear cytoplasm ventral to the capitulum is apparent in some specimens, and this clear area is interpreted as being the cytostome. The cytoplasm appears granular in nature and a few very large granules, 0.7-1.9 μ in diameter, were noted.

In comparing the trichomonads on smears made directly from the pig's caecum with those after an extended period of subculturing (100th subculture), significant morphological differences were observed. In over-all shape and gross form the organisms changed from

a spherical robust form to one that was more elongate, with many appearing almost tubular. In actual measurements the culture organisms in iron-hematoxylin stain averaged 11.0 μ (length) by 5.0 μ (width) in comparison with 9.5 μ by 6.8 μ on orgnisms in the caecal smears. During the culturing period, other changes were also noted. These changes, and in parentheses the condition of the organisms from the caecal smears, follow: The longest anterior flagellum of the organism is shorter, averaging 9.4 μ (13.8 μ); trailing flagellum is shorter, averaging 8.5 μ (9.0 μ); undulating membrance and costa lengthen in most cases to full length of the body (½-¾ body length); axostyle is wider and capitulum appears bulbous (axostyle slender with only a slight enlargment in width at the anterior end); parabasal body is club-shaped, consisting of a single ramus 2.3-4.8 μ long with no continuing parabasal fibril (V-shaped parabasal body with parabasal fibrils); and the nucleus is more oval in shape, averaging 4.0 μ by 2.7 μ in length and width (nucleus averaged 4.0 μ by 3.3 μ).

Variation in Morphology During the Complete Culture Cycle. No significant variations were found in the morphology of the trichomonads for each day of the culture cycle. Except for the last two days of the 7-day culture cycle, the shape remained typically pyriform, elongate, and sometime tubular. During the later part of the culture cycle (days 6 and 7) many abnormally shaped individuals appeared, including giant spherical forms, very slender organisms appearing as "flagellated axostyles" devoid of most of the cytoplasm, and "teardrop" shaped organisms. In actual measurements there was little variation in length and width of the organisms as indicated by the averages on consecutive days as follows: 11.1 μ by 5.8 μ, 11.2 μ by 5.1 μ, 11.5 μ by 5.1 μ, 10.8 μ by 5.0 μ, 11.8 μ by 5.3 μ, and 10.1 μ by 4.0 μ. Also, in studying other morphological characteristics such as length and width of the nucleus, number of undulations in the undulating membrane, length of anterior flagella, length of posterior flagellum, and length of parabasal body, there was no significant difference in morphology noted throughout the culture cycle.

Discussion

The interpretation of the morphological structures based upon trichomonads after extended periods of culturing must be viewed with caution. The organisms in this investigation lost many of the diagnostic morphological characteristics of the caecal trichomonad after it went through 100 subcultures. The change in morphology of porcine trichomonads has previously been reported by Hammond and Fitzgerald (1953) who reported that "trichomonads grown in culture underwent a modification involving elongation of the body, some change in the undulating membrane and rate of movement." The apparent change in morphology of this caecal trichomonad may be attributed to the possible isolation of a particular morphological

strain when starting the original stock culture. A particular morphological strain, possessing physiological requirements compatible with the physical and chemical properties of the culture medium, may also have been inadvertently selected.

While great morphological change took place during the extended period of subculturing, the morphology seemed to be very stable during the various days of a single culture cycle. This finding agrees favorably with the results of McCashland and Johnson (1957) who reported that one strain of *Tetrahymena pyriformis* and one of *T. vorax* remained about the same size during the culture cycle. However, Ormsbee (1942) found that *T. pyriformis* became shorter during the early part of the culture cycle and then later increased in length. Obviously, more studies need to be done on changes in morphology of protozoa throughout their culture cycle.

Summary and Conclusions

The population level of a caecal porcine trichomonad (probably *Titrichomonas rotunda*) varied during the culture cycle from an inoculum of 100 organisms per ml. to 2,900,000 per ml. on day 4 and then decreased to near extinction on day 7. The morhphology of the trichomonad directly from the pig's caecum is described and compared with the morphology of the organism in culture and throughout its life cycle. Significant morphological differences were observed in the organisms from culture. The more important changes in the morphology of organisms in culture include the following: over-all shape was more elongate and often almost tubular; actual length increased from 9.5 μ to 11.0 μ and decreased in width from 6.8 μ to 5.0 μ; undulating membrane and costa length from ½-¾ body length to the full length of the body; axostyle develops a more bulbous capitulum at the anterior end; and the parabasal body changes from V-shaped to a club-shaped structure. No significant variations were found in the morphology of the trichomonads during the various days of the culture cycle.

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