The Effects of *in vitro* Culture on *Tritrichomonas suis* in Chick Chorio-allantoic fluid

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Abstract

Culturing of *Tritrichomonas suis* in chick chorio-allantoic fluid resulted in lower numbers and smaller individuals than when cultured in the commercial laboratory medium, C.P.L.M. A primary cause for this difference may be based on carbohydrate utilization.

Introduction

Culturing of protozoa in the laboratory is an essential part of many types of protozoological research done today. Among the protozoa, trichomonads in culture have been used widely throughout the world. Following the initial report by Nelson (10) of culturing *Trichomonas foetus* beneath the chorio-allantoic (C.A.) membrane of the developing chick embryo, a number of workers including Hogue (4), Trussell and Plass (15), and Florent (3), have used embryonic fluids *in situ* for culturing various trichomonads. Literature concerning the use of extra-embryonic fluids of developing chicken embryos for the culture of trichomonads was reviewed by Marr (8). Very little information is available concerning the effectiveness of this substrate for trichomonad culture.

This investigation was designed to determine the population growth characteristics and to determine the morphological characteristics of *Tritrichomonas suis* under *in vitro* C.A. fluid culture.

Materials and Methods

A long-term C.A. fluid *in vitro* growth experiment was initiated using 8 cultures incubated at 30°C and 7 tubes at 36°C. The population of protozoa was determined every 12 hours with the aid of a hemocytometer until the cultures died out. Three sets of slides from each temperature were prepared. These sets consisted of smear preparations of the inoculum from C.P.L.M. medium of Johnson and Trussell (14) and smears made after 4 and 6 days of C.A. fluid culturing.

Stained specimens were prepared for morphological studies using Heidenhain's iron hematoxylin following fixation with Schaudinn's fluid and with a modified protein silver stain of Bodian (1) and Moskowitz (9) following Bouin's fixation. Hematoxylin was used primarily to show gross body dimensions and nuclear detail while protein silver was used to demonstrate the argyrophilic mastigont structures such as flagella, undulating membrane, axostyle, and parabasal body.

Chorio-allantoic fluid for this experiment was prepared by using the following procedure. The C.A. fluid of eggs incubated 13 days was drawn by mouth aspiration into a length of 3/16-inch rubber tubing and pooled in a rubber stoppered Erlenmeyer flask. The tube was equipped

with a 2-inch, 19-gauge needle and a cotton stoppered mouth piece. Sterilization of the fluid was accomplished by passing it through a 0.1 μ Seitz filter under vacuum into a sterile Erlenmeyer flask. This sterile C.A. fluid was dispensed with a 5-ml Leur syringe equipped with a 3.5-inch, 15-gauge needle in 3.9 ml amounts into screw cap test tubes (16 x 150 mm) for culture. The inoculum for each tube consisted of 1.5 x 10^5 trichomonads suspended in 0.1 ml of C.P.L.M. medium.

Results

Peak populations of 3.94 x 10^6 and 4.08 x 10^6 organisms were reached after 4 days of C.P.L.M. culturing at 30°C and 36°C. Measurable populations were recorded from all 10 control culture tubes for 8 and 7 days at the respective temperatures (Figure 1). Minimum generation

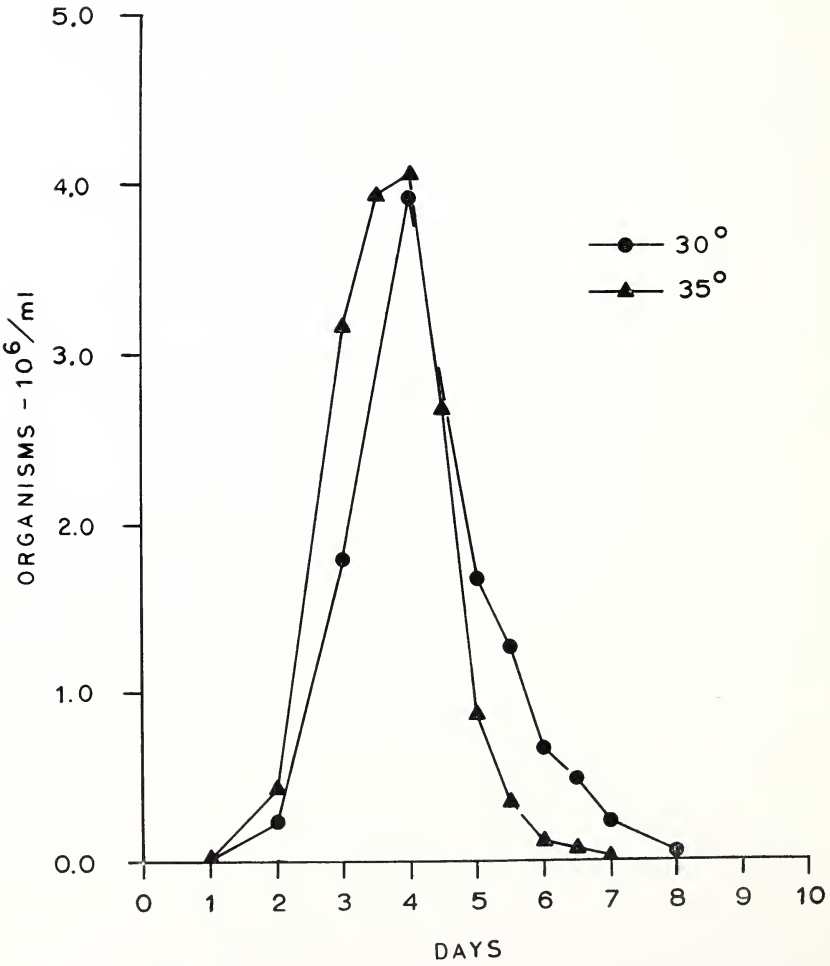

FIGURE 1. *Population growth curves of* T. suis *in C.P.L.M. medium.*

times of 6.6 and 6.1 hours were calculated for the control temperatures. Following the population peaks a logarithmic decrease occurred at both temperatures.

The maximum number of trichomonads reached after 6 days of 30°C C.A. fluid *in vitro* culturing was approximately 900,000 per ml, while a population of 1.15 x 10^6 per ml was reached in 4 days in those cultures incubated at 36°C (Figure 2). Those cultures incubated at 30°C reached a second peak which approached 900,000 organisms per ml in 8.5 days. The cultures incubated at 36°C also gave a slight indication of a second population peak after 5.5 days with 4 of the 7 cultures used exhibiting an increase; however, the overall population mean of the 7 cultures was lower than the previous reading. The duration of time in which measurable populations were found in each tube was 13 days for the 30°C cultures and 7 days for the 36°C cultures.

The generation time as determined from the straight line curve of the semilog plot of the populations during the logarithmic phase of growth was 26.2 hours in the 30°C cultures and 16.1 hours in the 36°C cultures. From the peak population, the rate and nature of decrease of the 36°C cultures followed very closely a logarithmic decrease as compared to the more gradual decline of the 30°C cultures.

Those organisms cultured at 30°C were longer and wider (10.74 ± 0.16 μ by 5.06 ± 0.12 μ) than those cultured at 36°C (9.46 ± 0.16 μ by 4.67 ± 0.12 μ) after 4 days incubation in C.A. fluid. After 6 days of culturing the same relationship was seen in regard to length, 9.83 ± 0.16 μ compared to 9.13 ± 0.16 μ; however, the widths were

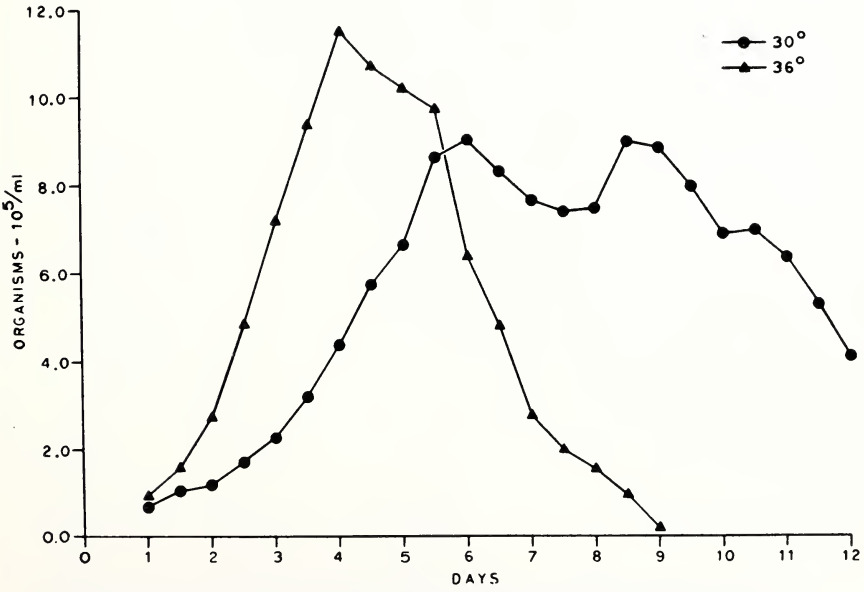

FIGURE 2. *Population growth curves of* T. suis *in chorio-allantoic fluid* in vitro.

essentially equal, being 4.81 ± 0.12 μ and 4.80 ± 0.12 μ respectively. In both cases there were decreases in length of the organisms compared to the length of 12.55 ± 0.16 μ of the organisms of the inoculum. Conversely, the widths of those organisms cultured at 30°C (5.06 ± 0.12 μ) and 36°C (4.67 ± 0.12 μ) were larger than the width (4.31 ± 0.10 μ) of the organisms of the inoculum (Tables 1 and 2).

Nuclear measurements taken from organisms of the experimental culture at 30°C (3.17 ± 0.10 μ by 1.94 ± 0.07 μ) and 36°C (2.59 ± 0.10 μ by 1.87 ± 0.07 μ) after 4 days culturing were slightly smaller as were those measurements taken after 6 days; 30°C (2.88 ± 0.10 μ by 1.85 ± 0.07 μ) and 36°C (2.76 ± 0.10 μ by 1.99 ± 0.07 μ) than those of C.P.L.M. cultured organisms of the inoculum type (Tables 1 and 2).

TABLE 1. *Measurements*[a] *of* T. suis *cultured in chorio-allantoic fluid* in vitro *at 30 C.*

Characteristic	0 days	4 days *in vitro*	6 days *in vitro*
Length	12.55 ± 0.16 (55)	10.74 ± 0.16 (40)	9.83 ± 0.16 (40)
Width	4.31 ± 0.10 (55)	5.06 ± 0.12 (40)	4.81 ± 0.12 (40)
Nuclear length	3.28 ± 0.06 (69)	3.17 ± 0.10 (20)	2.88 ± 0.10 (20)
Nuclear width	2.14 ± 0.04 (69)	1.94 ± 0.07 (20)	1.85 ± 0.07 (20)
Parabasal body length	3.71 ± 0.15 (25)	3.36 ± 0.27 (1)	2.78 ± 0.13 (20)
Parabasal body width	0.52 ± 0.02 (25)	0.48 ± 0.03 (1)	0.48 ± 0.01 (20)
Axostylar projection	1.94 ± 0.17 (25)	2.40 ± 0.18 (19)	2.17 ± 0.18 (19)

[a] Mean (μ) ± standard error; observations in ( ).

TABLE 2. *Measurements*[a] *of* T. suis *cultured in chorio-allantoic fluid* in vitro *at 36 C.*

Characteristic	0 days	4 days *in vitro*	6 days *in vitro*
Length	12.55 ± 0.16 (55)	9.46 ± 0.16 (40)	9.13 ± 0.16 (40)
Width	4.31 ± 0.10 (55)	4.67 ± 0.12 (40)	4.80 ± 0.12 (40)
Nuclear length	3.28 ± 0.06 (69)	2.59 ± 0.10 (20)	2.76 ± 0.10 (20)
Nuclear width	2.14 ± 0.04 (69)	1.87 ± 0.07 (20)	1.99 ± 0.07 (20)
Parabasal body length	3.71 ± 0.15 (25)	2.88 ± 0.60 (5)	2.57 ± 0.14 (17)
Parabasal body width	0.52 ± 0.02 (25)	0.48 ± 0.06 (5)	0.51 ± 0.01 (17)
Axostylar projection	1.94 ± 0.17 (25)	1.24 ± 0.18 (19)	2.13 ± 0.21 (14)

[a] Mean (μ) ± standard error; observations in ( ).

The parabasal body was progressively shortened at both experimental temperatures (Tables 1 and 2).

In slides from the 36°C culture, the trunk of the axostyle had assumed in about 25% of the population a sigmoid configuration (Plate I-1, 2, 3) in contrast to the rather straight-to-slightly-curved trunk normally seen (Plate I- 4, 5, 6). This configuration might have been caused by excessive shrinkage of the cell causing this axial rod

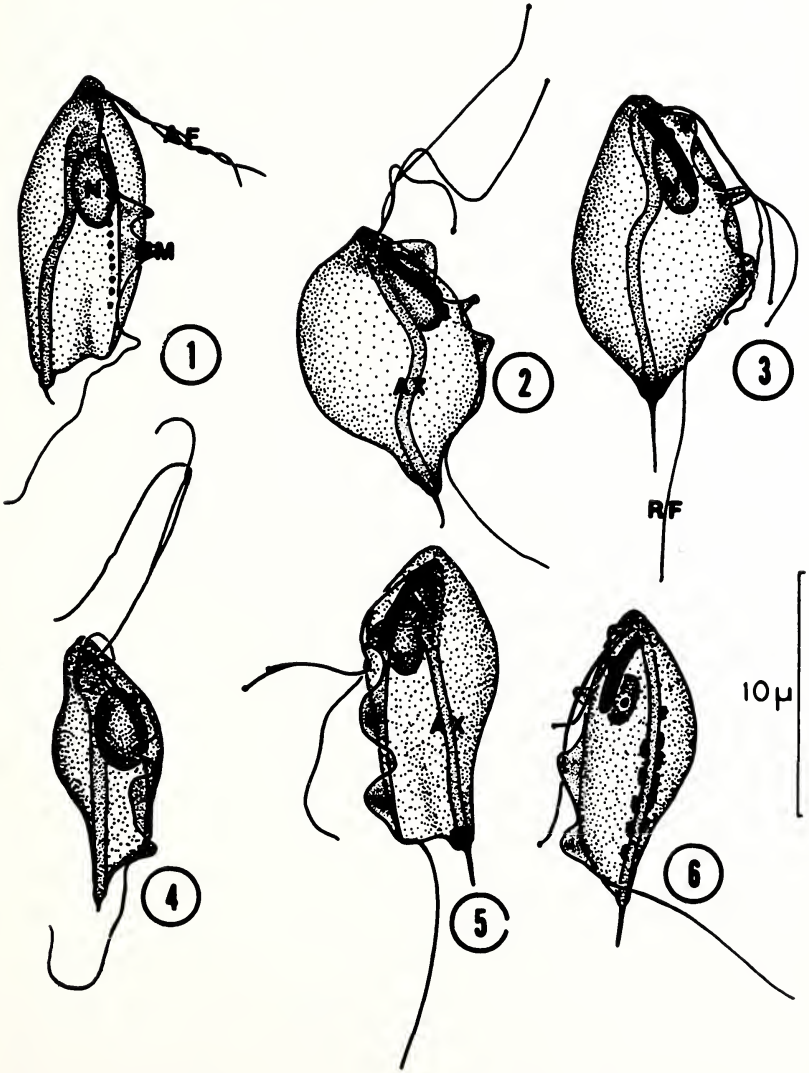

PLATE I. *1, 2, 3, 4, 5, 6.* Tritrichomonas suis, *nucleus (N), undulating membrane (UM), parabasal body (P), anterior flagellum (AF), recurrent flagellum (RF), axostyle (AX).*

to partially fold on itself; however, measurements taken at this point in the experiment did not reveal a noticeable difference in size of these organisms from those that had a more conventional appearing axostyle.

No apparent variability in the anterior or recurrent flagella or undulating membrane was noted between organisms cultured in C.A. fluid and those cultured in C.P.L.M. medium.

Discussion and Conclusions

These data indicate a pattern which has not been seen in trichomonad studies previous to this time. The second peak in population established during the culture cycle can be compared to the diauxic pattern of growth described by Thimann (13) in bacterial populations and by Daly (2) in a strain "X" of *T. gallinae*. However, the lag phase and subsequent reacceleration of growth here does not occur midway during the log phase of the cycle. The normal pattern of diauxic growth is based on the preferential metabolism of available carbohydrates in the medium in which all of the preferred energy source is utilized before the second is attacked. According to Romanoff (12), approximately 90% of the carbohydrate of chicken allantoic fluid is glucose. This large amount of one sugar might explain why the second increase occurred during the stationary phase of the cycle rather than the log phase.

This carbohydrate difference could also have been responsible for the lower peak population attained as compared to C.P.L.M. cultured controls. The major carbohydrate of C.P.L.M. medium is maltose although there is also an undefined amount of carbohydrate present due to the addition of liver extract to the medium.

The comparison of various laboratory media with these two sugars has been conducted using several species of trichomonads and in each case the maltose supplemented media supported better protozoan populations (14, 11, 7, 5).

The general decrease in body dimensions and organelle size in C.A. fluid cultured *T. suis* is also probably due to this carboyhydrate difference. Read (11) and Daly (2) also noted that laboratory media supplemented with maltose supported trichomonads that were larger and heavier than those cultured in glucose supplemented media.

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