MEDICAL PARASITOLOGY

Growth and survival of *Trichomonas vaginalis*

IOANNA KOSTARA, H. CARAGEORGIOU, D. VARONOS and S. TZANNETIS*

Departments of Experimental Pharmacology and *Microbiology, School of Medicine, University of Athens, Goudi, Athens 11527, Greece

This study confirmed that Feinberg and Whittington medium was suitable for the cultivation and detailed study of the growth cycle of two clinical strains of *Trichomonas vaginalis* under anaerobic conditions. Both strains showed a similar growth pattern characterised by early but slow growth, extended duration of the logarithmic phase and limited survival never exceeding 144 h. Duration of survival and growth rate were inversely proportional to the inoculum density. Growth rate was pH dependent; pH values in the range 6.9–6.5 delayed the initiation of growth of *T. vaginalis* for at least 48 h. On the other hand, pH values of 6.4–4.5 were indifferent or slightly favourable for growth during the logarithmic and survival in the early decline phase. Normal saline and Ringer's solution exerted an early and progressively lethal effect on trichomonads and led to the disappearance of protozoa suspended in them in 150 min. In general, these in-vitro results shed light on some aspects of the biology of *T. vaginalis* and contribute to a better understanding of the epidemiology and clinical manifestations of the infection.

Introduction

The flagellate protozoon *Trichomonas vaginalis* causes a mild urogenital infection [1–3]. It has a world-wide distribution and its prevalence in developed countries in women and men ranges from 5 to 20% and 2–10% respectively. The parasite is mainly transmitted by sexual intercourse and occasionally by direct contact with infected fomites [3]. For all these reasons and because of an association between chronic trichomoniasis and cancer of the cervix in females and infertility in males, the infection has long attracted scientific interest [3–5]. However, knowledge of the survival and growth of *T. vaginalis* under laboratory conditions is inadequate or uncertain because of the difficulties in culturing it.

The present work aimed to determine the dynamics of the growth cycle of two clinical strains of *T. vaginalis* in Feinberg and Whittington medium [6, 7] and its survival in two other common suspending media, as such results might contribute to a better understanding of the life-cycle and the infectivity of the parasite under conditions of natural infection.

Materials and methods

Strains

Two clinical strains of *T. vaginalis* isolated from two cases of acute vaginal trichomoniasis were used. Diagnosis of the infection was based on clinical signs and on the presence of motile parasites in wet preparations of vaginal exudate.

Microbiological methods

Samples were taken with sterile cotton swabs and were transported to the laboratory within 30 min of sampling. Swabs were vortex mixed for 30 s in 2 ml of Feinberg and Whittington nutrient medium at pH 6.4. Inoculated tubes were incubated anaerobically in an Oxoid Anaerobic System at 37°C for 72 h. These 72-h cultures were used to prepare the standard inocula for any further growth and survival testing, as preliminary experiments showed that viable counts were comparatively more stable at this phase than at any other. Tubes containing 2.7 ml of Feinberg and Whittington medium, Ringer solution or normal saline were inoculated separately with 0.3 ml of a standard inoculum of each strain and the tubes were incubated anaerobically as described above. Cultures in Feinberg and Whittington liquid medium were sampled at intervals of 0, 2, 4, 6, 8, 24, 48, 72, 96, 120 and 144 h, and growth curves of strains A and B were made by calculating the mean viable counts of 36 and 27 successive cultures, respectively. Suspensions of both strains in the two

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Corresponding author: Dr D. I. Kostara, 6 Diomidus St., N. Liossia, Athens 13122, Greece.

diluents adjusted to pH 6.4 with 0.1 M Na₂HPO₄/0.1 M KH₂PO₄ were sampled every 15 min and survival curves were made by calculating the mean viable counts in three separate suspensions of each strain.

The effect of pH on the growth and survival of trichomonads was tested only on strain A in Feinberg and Whittington liquid medium buffered at pH 6.9 with 0.1 M Na₂HPO₄/0.1 M KH₂PO₄ as well as in the same medium only initially adjusted to pH 6.9 by the addition of N/10 NaOH. Growth curves were made by calculating the mean viable counts of three successive cultures in each type of medium under the conditions used throughout this study. Populations of motile trichomonads of each strain in cultures and in suspensions were determined microscopically in a Neubauer counting chamber at any given time.

Results

Growth in Feinberg and Whittington medium

Table 1 and Fig. 1 show the mean viable counts and the corresponding growth curves of 36 and 27 successive cultures of *T. vaginalis* strains A and B, respectively. From these results it can be seen that both strains grew relatively well in Feinberg and Whittington medium. They both showed an equally early initiation of multiplication, but their growth rate was slow because it took 48 h to attain maximum growth with populations merely 10-fold higher than those at the beginning of the experiment.

Growth curves of the two strains were similar, with well distinguished logarithmic, stationary and decline phases. However, during the logarithmic phase the growth rate of strain A was regular and progressive while that of strain B was temporarily arrested before recommencing, so that the corresponding part of its growth curve showed a biphasic pattern. Stationary and decline phases lasted 24 h and 72 h, respectively. During the first 48 h of the decline phase the death rate of both strains was rather slow. After that time the death rate accelerated rapidly and no viable trichomonads were observed after incubation for 144 h.

Estimation of mean generation time (MGT) and decimal reduction time (DRT) of strains A and B determined at corresponding points in their growth curves revealed that the former grew slightly faster and died, during the first part of its decline phase, more slowly than the latter (Table 2).

Effect of inoculum size on growth and survival

Figs. 2 and 3 present mean viable counts and the corresponding growth curves of the strain A and B cultures obtained by grouping the results for each strain separately on the basis of inoculum size. These results show that cultures of both strains raised with low density inocula, i.e., < 5.2 × 10³/ml, had a faster rate and a longer duration of growth and survival than those raised with inocula containing (2.74 × 10⁴)–(5.21 × 10⁴) viable trichomonads. Inocula of (7.8 × 10³)–(2.35 × 10⁴)/ml gave intermediate figures for growth rate and duration of survival (data not shown). This inversely proportional relationship of inoculum density to growth rate and survival was more apparent with strain B. The estimated generation and decimal reduction times of both strains (Table 3) made clear the effect of inoculum size and the differences in growth characteristics between the two strains. Moreover, growth curves calculated on a smaller number of cultures of each strain, raised with low density inocula, were very similar to those calculated on the sum of all cultures.

Effect of pH on growth and survival

In the laboratory conditions used and in buffered and non-buffered media adjusted to pH 6.9 the initiation of growth of strain A was retarded for at least 48 h. After that time both cultures entered their logarithmic phase slowly, reached their peaks at 96 h and showed a similar pattern of decline; the pH of the buffered medium remained stable at pH 6.9 while that of the unbuffered medium dropped progressively from 6.5 to 4.5 pH units (Fig. 4a and b). However, pH values of 6.4–4.5 slightly favoured growth during the logarithmic and the early decline phases. These results showed that pH values in the range 6.0–6.5 inhibit the initiation of growth, prolonging the duration of the lag phase of *T. vaginalis* considerably.

Table 1. Growth and survival of *T. vaginalis* clinical strains A and B in Feinberg and Whittington medium

Strain	Number of growth studies	Log ₁₀ mean viable counts of <i>T. vaginalis</i> after incubation for (h)										
		0	2	4	6	8	24	48	72	96	120	144
A	36	4.20	4.30	4.47	5.00	4.73	4.89	5.29	5.09	4.48	3.62	–
(SD)		2.13	2.05	3.44	2.51	4.48	2.95	3.17	3.04	2.68	1.97	
(SE)		1.35	0.46	0.77	0.56	1.00	2.18	2.40	2.26	1.90	1.19	
B	27	4.30	4.70	4.72	4.98	4.96	4.98	5.30	5.20	4.20	3.17	–
(SD)		2.26	4.54	5.19	10.42	6.89	2.93	3.10	3.14	2.54	1.66	
(SE)		1.54	1.10	1.25	2.52	1.67	2.20	2.38	2.42	1.83	0.94

Fig. 1. Growth curves of (a) *T. vaginalis* strain A and (b). *T. vaginalis* strain B cultured anaerobically at 37°C in Feinberg and Whittington broth at pH 6.4.

Table 2. Growth and death rate of *T. vaginalis* clinical strains A and B in Feinberg and Whittington medium

Strain	MGT	DRT between log ₁₀		
		5-4	4-3	3-2
A	11.5	31.6	17.2	6.7
B	14.4	25.5	16.8	9.6

Mean generation time (MGT) and decimal reduction time (DRT) were estimated in hours.

Effect of diluent on survival

Normal saline and Ringer’s solution proved deleterious for both strains of *T. vaginalis*. The lethal effect on *T. vaginalis* 5.02 × 10⁴/ml, suspended in either of the diluents, was progressive and started early after incubation for 15–30 min (Fig. 5a and b). Further incubation for 135–150 min caused an acute accelera-

tion of death and the appearance of lysed protozoal cells in both diluents.

Discussion

This work revealed some important elements of the growth dynamics of the *T. vaginalis* strains tested and confirmed the relative suitability of Feinberg and Whittington medium for culturing and preservation purposes [6–9].

The early initiation of growth was unexpected and reflects the ability of the parasite to adjust to, and use without undue delay, a fresh nutritional environment devoid of toxic products of metabolism. The slow growth rate, the length of time needed for both strains to attain their rather low level of maximum growth

Fig. 2. Effect of inoculum density on viability of *T. vaginalis* strain A cultured anaerobically at 37°C in Feinberg and Whittington broth: ●, low density inoculum; ○, high density inoculum.

Fig. 3. Effect of inoculum density on viability of *T. vaginalis* strain B cultured anaerobically at 37°C in Feinberg and Whittington broth: ▲, low density inoculum; △, high density inoculum.

Table 3. Growth and death rate of *T. vaginalis* clinical strains A and B in Feinberg and Whittington medium

Strain	Low density inoculum				High density inoculum			
	MGT	DRT between log ₁₀			MGT	DRT between log ₁₀		
		5-4	4-3	3-2		5-4	4-3	3-2
A	9.4	25.4	11.0	5.3	...	20.2	13.4	8.6
B	9.4	23.5	14.4	7.7	...	19.7	7.5	3.8

Mean generation time (MGT) and decimal reduction time (DRT) were estimated in hours; ..., estimation not possible.

Fig. 4. (a) Effect of constant pH 6.9 on viability of *T. vaginalis* strain A in buffered Feinberg and Whittington broth. (b) Effect of changing from pH 6.9 to pH 4.5 on viability of *T. vaginalis* strain A in non-buffered Feinberg and Whittington broth. ●, mean viable count; ○, pH value.

Fig. 5. Survival of *T. vaginalis* strains A (a) and B (b) in Ringer's solution (●—●) and in normal saline (×—×), both kept at 37°C.

and the well-distinguished growth phases resemble the cultural features of slowly growing bacteria. On the other hand, the short duration of the decline phase and of survival are similar to those of highly exacting micro-organisms which are susceptible to the exhaustion of nutrients and the accumulation of toxic by-products in the medium. However, the low density of population during all phases of growth and consequently the lack of a population effect [6] may partly contribute to the limited survival of *T. vaginalis*. These cultural features are in keeping with the clinical features and the progress of natural infection.

The population densities during various growth phases in serially repeated cultures and the growth curves constructed on the basis of the corresponding mean viable counts revealed growth similarities between the two strains. However, differences such as the biphasic logarithmic phase of strain B that makes doubtful the estimation of a mean generation time and its faster death rate are likely to be related to the existence of heterogeneity among strains of *T. vaginalis*. The variability in counts of motile trichomonads observed in repeated cultures of each particular strain further suggests the existence of inter-strain and intra-strain

heterogeneity in multiplication rate. However, limitations of the counting method and the possible presence of more than one protozoal clone in each sample may also contribute to the variability of the viable counts.

The results of the effects of inoculum density on the growth of *T. vaginalis* in the present study are generally in agreement with earlier reports [10, 11]. The inversely proportional relationship of inoculum size to growth rate and to duration of survival is a very complex phenomenon. The quicker exhaustion of nutrients and of other growth factors, the production and accumulation of greater amounts of inhibitory by-products of metabolism by a denser growing population and the possible presence of more aged and consequently less active protozoa in larger inocula could partly explain these findings.

Results based on a smaller number of experiments involving high density inocula made the existing strain-dependent differences of the growth cycle between the two strains more apparent. Moreover, similarities between the growth curves of each strain based either on the sum of its cultures or on a smaller number of them, raised with low density inocula, confirm that the latter results are adequately reliable.

The effects of pH on the growth cycle of *T. vaginalis* are also very complex and it seems that a pH value of 6.4 is crucial for early initiation of growth whereas a pH of 6.9 retards it.

After the initiation of growth, pH values in the range 6.9–4.5 seem to have little influence because they affect the growth and the life-cycle of the parasite only slightly. The effects of pH on the growth of *T. vaginalis* *in vitro* are generally similar to the results of other studies and seem to be at least partly compatible with the clinical progress of the infection [5, 12]. The duration of survival of light suspensions of *T. vaginalis* in both salt solutions tested at 37°C was very limited. Similar results, although not under exactly the same conditions, have been published earlier [13, 14]. This initially progressive and finally destructive nature of the lethal effect of both diluents that is associated with lysis of the protozoal cells is difficult to explain. However, such findings might have been due to a progressive split of RNA [15, 16], degradation of rRNA [15, 17–19] and leakage of RNA-like material [15, 20] or proteins [20] that may occur in bacteria exposed to sublethal conditions in non-protective suspending media and they also give an indication of the possible anatomical or functional site of injury.

The in-vitro observations on the growth cycle of *T. vaginalis* in Feinberg and Whittington medium, on the effects of low density inocula and of pH and the lethal effect of the two diluents contribute to the in-vitro study of the life of the parasite. Moreover, they may prove useful in studies of the biology of this parasite and provide a growth model for other protozoa.

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