Production of ammonia by *Trichomonas foetus* and *Trichomonas vaginalis*

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Production of ammonia is difficult to find among the various studies of amino acid metabolism in protozoa. Several studies suggest that catabolism of arginine to ammonium is important for the growth of trichomonads. Trichomonads are amitochondrial zooflagellates that thrive under microaerophilic and anaerobic conditions. The authors were able to detect accumulation of ammonium ions and ammonia in cultures of *Trichomonas foetus* and *Trichomonas vaginalis*, including those resistant to metronidazole. Ammonium ions and ammonia were detected using the indophenol colorimetric method. Cells incubated overnight under an ambient oxygen gas phase had 0.9 mM soluble ammonium (NH$_4^+$ and NH$_3$) or a 20% greater concentration of ammonium relative to sterile growth medium that had been incubated similarly. Production of ammonia itself was confirmed by analysis of a wick that was moistened with sulfuric acid (20 mM) and placed above the liquid in sealed cultures of a strain of *Trichomonas vaginalis*. The wicks from these cultures captured the equivalent of 0.048 mM volatile ammonia (NH$_3$) from the liquid as compared to 0.021 mM volatile ammonia from sterile medium after overnight incubation. Intact trichomonads, 0.7 x 10$^6$ cells ml$^{-1}$ equivalent to 0.7 mg protein ml$^{-1}$, incubated in Doran’s buffer with or without (1 mM) L-arginine produced significant amounts of soluble ammonium (0.07 mM and 0.04 mM, respectively) during 60 min. The results indicate that ammonium ions and the more irritating ammonia are significant metabolites of trichomonads. In addition, based upon end-product amounts, it appears that the rate of arginine metabolism is of the same order of magnitude as that for carbohydrate metabolism by trichomonads.

**INTRODUCTION**

Trichomonads (Diamond, 1957; Petrin *et al*., 1998; Yarlett, 2000) thrive in the nitrogenous milieu of their host’s digestive, reproductive and respiratory tracts. They are characterized by flagella, undulating membrane and an axostyle (Warton & Honigberg, 1979). Trichomonads survive under aerobic conditions, but require microaerophilic or anaerobic conditions for growth, relying mainly on fermentation of carbohydrates and amino acid metabolism for energy. Fermentation of carbohydrates produces H$_2$, CO$_2$ and organic acids. Production of H$_2$ occurs in a redox organelle, the hydrogenosome. Hydrogenosomes appear to be metabolically analogous to mitochondria for the fermentation of pyruvate to H$_2$ and CO$_2$. Metronidazole (Flagyl®) is used to treat infections caused by anaerobic micro-organisms. For trichomonads, the selective mode of action of metronidazole results from reductive activation by the metabolism of pyruvate in the hydrogenosomal pathway. *Trichomonas* (*Tri.*) *foetus* infects the reproductive tract of cattle, and *Trichomonas* (*Tri.*) *vaginalis* is a major cause of vaginitis in humans. Human trichomoniasis has a characteristic odour and elevated vaginal pH (Rein, 1989). Trichomonad strains exhibiting both anaerobic and aerobic metronidazole resistance have been developed in the laboratory by growth in the presence of increasing concentrations of metronidazole [1–100 µg (ml growth medium)$^{-1}$] (Kulda *et al*., 1993; Tachezy *et al*., 1993). *Tri. vaginalis* organisms isolated from clinical specimens (Müller *et al*., 1980) of patients that had refractory trichomoniasis exhibit decreased sensitivity to metronidazole *in vitro* (Müller *et al*., 1988).

Studies indicate that trichomonads require arginine for growth (Kidder, 1951). Trichonomads have an arginine dihydrolase pathway that may supplement the cells’ ability to form ATP (Linstead & Cranshaw, 1983; Yarlett *et al*., 1996a). Protozoa are regarded, along with bacteria and metazoa, as primarily ammonotelic (Kidder, 1967; Yoshida & Camargo, 1978). Maroulis *et al.* (2003) have suggested that trichomonads rely upon the transport of inorganic ions (e.g. potassium ions) during hyperosmotic stress to maintain the cell volume; presence of ammonium was not mentioned. Knodler *et al.* (1994) did not see changes in the ammonium content of spent medium after growth of *Tri.*
vaginalis. Studies of the metabolism of ammonium and the more-basic and toxic compound ammonia are difficult to find in the literature describing the metabolism of amino acids by protists (Cazzulo, 2003; Cazzulo et al., 1985; Gutteridge & Coombs, 1977; Honigberg, 1967; Knodler et al., 1994; Marr, 1979). In this study, we were able to show the accumulation of significant amounts of soluble ammonium (NH$_4^+$) and volatile ammonia (NH$_3$) by cultures of trichomonads after growth in complex media. Production of soluble ammonium from L-arginine indicated that there is a greater metabolic rate through the arginine dihydrolase pathway than suggested previously for *Try. foetus* and most likely *Tri. vaginalis*.

**METHODS**

**Organisms and growth conditions.** Strains of *Try. foetus* and *Tri. vaginalis* used in this study are listed in Table 1. *Try. foetus* KV1-1MR-100 (ATCC 50151), and *Tri. vaginalis* RU393 (ATCC 50142) and C1-NIH (ATCC 30001) were obtained from the American Type Culture Collection (Manassas, VA). *Tri. vaginalis* strains TV10-02 and MR-100 were generously provided by Dr J. Tachezy (Kulda et al., 1993). Values for metronidazole susceptibility (Meingassner et al., 1978; Müller et al., 1988) were compiled from the values in the references. *Try. foetus* KV1-1MR-100 and *Tri. vaginalis* MR-100 were derived in *vitro* in the presence of increased concentrations of metronidazole. Strain RU393 was a clinical isolate from a patient with trichomoniasis; the strain was refractory to treatment with metronidazole (Müller et al., 1988) and exhibited lowered susceptibility to metronidazole under aerobic conditions. Other such strains have been isolated from additional cases of refractory trichomoniasis. These metronidazole refractory strains have decreased susceptibility to metronidazole in an *in vivo* mouse (intraperitoneal and subcutaneous) susceptibility test. These refractory isolates and those derived in *vitro* that have decreased susceptibility under aerobic conditions have intact hydrogenosomal pathways (Müller et al., 1980, 1988). The anaerobic resistant strains lack the enzyme activities for metabolism of hydrogen. No clinical isolates with anaerobic resistance to metronidazole have been obtained. Strain C1-NIH is one of the original axenic isolates of *Tri. vaginalis* (isolated in axenic culture in 1956 by L. Diamond) and, although this isolate is no longer pathogenic in animal studies, it has been extensively used for biochemical and molecular studies and is mycoplasma-free. The more-recent clinical isolates TV10-02, MR-100 and RU393 are pathogenic in animal models.

Cells grew to about 3 x 10$^6$ cells ml$^{-1}$ after overnight incubation at 37°C in Diamond’s TYM medium (Diamond, 1957) containing (w/v) 2% tryptose (Difco), 1% yeast extract (Difco), 0.5% maltose (Difco), 0.1% L-cysteine monohydrate, 0.02% ascorbic acid, with the following modifications: 0.8% potassium dihydrogen phosphate (anhydrous), 0.8% dipotassium hydrogen phosphate trihydrate pH 6-4 and supplemented with 10% heat-inactivated horse serum (Müller et al., 1988). *Tri. vaginalis* strains were maintained in a medium containing 0.05% agar but grown overnight without agar for studies of ammonia accumulation in cultures. Sterile medium was stored frozen without serum at –20°C. Medium was warmed to 37°C and 10% serum (Invitrogen/Gibco) was added just before inoculation. For anaerobically grown cells, the cultures were incubated in a GasPak jar with a H$_2$/CO$_2$ atmosphere. For comparison to azooleagelate that grows on amino acids, ammonium accumulation in cultures of *Trypanosoma* (Try.) *brucei* brucei lab. 110 EATRO procyclic insect forms (Glossina sp. midgut) was also measured. Cells were grown in T2 medium Levandowsky & Katz (cited in Bacchi et al., 1989) for 72–96 h at 27°C. This medium contained a defined mixture of amino acids, vitamins and haematin, along with heat-treated (56°C, 30 min) fetal calf serum, and had an initial pH of 7-4.

Cells of trichomonads and *Try. brucei* were counted using a Neubauer haemocytometer. *Tri. vaginalis* grown in this study, from vaginal exudates and from an *in vitro* mouse (intraperitoneal) model, have an ovoid shape as compared to the ameboid forms seen during growth on agar plates, attached to the vaginal epithelial mucosa, and in an *in vivo* mouse subcutaneous model (Nielsen & Nielsen, 1975; Warton & Honigberg, 1979; Yarlett, 2000). It is not clear if there are metabolic differences between these forms. Cultures of *Try. foetus* KV1-1MR-100 and RU393 were examined microscopically using a 40 x objective for microbial contaminants. Heat-fixed slides stained with 4,6-diamidino-2-phenylindole dihydrochloride (DAPI) had defined spherical nuclear DNA but no extranuclear DNA that would be indicative of contamination with mycoplasma.

**Metabolic studies and analytical methods.** For studies of the metabolism of arginine, cells were harvested by centrifugation, washed in Doran’s buffered solution (74 mM NaCl, 1.6 mM KCl, 0.6 mM CaCl$_2$, 30 mM NaH$_2$PO$_4$; pH 6.4) and resuspended to 10$^7$ cells (equivalent to 1 mg protein) (ml buffer) based upon protein determinations for strain C1-NIH. In this buffer, cells remain motile and are metabolically active (Müller & Gorrell, 1983; Yarlett et al., 1996a). Cells were incubated with or without 1 mM L-arginine, for 60 min at 37°C. Cells were cooled to 4°C and removed by centrifugation before analysis of soluble ammonium in the supernatant fluid. Protein content was determined by the Bradford method.

**Soluble and volatile ammonium.** The indophenol colorimetric method of Berthelot (1859) cited in Chaney & Marbach (1962) and modified by Weatherburn (1967) was used to determine the amounts of ammonium ions (NH$_4^+$) and ammonia (NH$_3$). Absorbance was measured using a Beckman DU-640 spectrophotometer at 625 nmol–1 (ml NH$_4$Cl)–1. Reaction mixtures contained 1 ml of phenate reagent consisting of 1:1% (w/v) phenol, 0.005% (w/v) sodium nitroprusside and 1 ml alkaline hypochlorite [0.8% (w/v) sodium hypochlorite (Clorox) and 0.6% (w/v) sodium hydroxide].

Soluble ammonium (NH$_4^+$ and NH$_3$) was measured by placing a 5 ml sample of the liquid from overnight cultures in a sealed glass scintillation vial that contained a wick moistened with (20 mM) sulfuric acid to trap ammonium released by the addition of 2.5 mM boric acid pH 9.5 and subsequently incubated overnight at room temperature. Values for metronidazole susceptibility (Meingassner et al., 1978; Müller et al., 1993) were compiled from the values in the references. *Trichomonas vaginalis* (isolated in axenic culture in 1956 by L. Diamond) and, although this isolate is no longer pathogenic in animal studies, it has been extensively used for biochemical and molecular studies and is mycoplasma-free. The more-recent clinical isolates TV10-02, MR-100 and RU393 are pathogenic in animal models.

**Table 1. Strains of trichomonads used in this study**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Strain</th>
<th>Metronidazole resistance*</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Try. foetus</em></td>
<td>KV1-1MR-100</td>
<td>Yes</td>
</tr>
<tr>
<td><em>Try. foetus</em></td>
<td>KV1-1MR-100-17†</td>
<td>No</td>
</tr>
<tr>
<td><em>Tri. vaginalis</em></td>
<td>RU393</td>
<td>Yes</td>
</tr>
<tr>
<td><em>Tri. vaginalis</em></td>
<td>MR-100</td>
<td>Yes</td>
</tr>
<tr>
<td><em>Tri. vaginalis</em></td>
<td>TV10-02</td>
<td>No</td>
</tr>
</tbody>
</table>

*Compiled from Meingassner et al. (1978) and Kulda et al. (1993), except for strain RU393. Resistance is defined as minimum lethal concentration, >100 μg metronidazole (ml TYM medium)–1.†Strain KV1-1MR-100-17 was derived from KV1-1MR-100 after its subcutaneous passage in an *in vivo* mouse model.
temperature. The wick (0.5 cm × 1 cm) was cut from a piece of Whatman filter paper. The wick was then assayed with the indophenol method for soluble ammonium. Similar results were obtained by directly adding the reagents for the formation of indophenol to the method for soluble ammonium. Similar results were obtained by Whatman filter paper. The wick was then assayed with the indophenol method.

Volatile ammonia (NH₃) production was confirmed by analysis of a sample of culture (Weatherburn, 1967). Trichomonads for these experiments were grown in 5 ml of TYM medium using screw-capped culture tubes, or in 1 ml of TYM medium using 24-well tissue culture plates. For aerobic incubation, tubes and plates were incubated in an ambient atmosphere. Cysteine in the medium would maintain the cultures in the tubes under lower redox condition than those incubated in the multi-well plates. The direct indophenol method was used in preliminary studies to determine the concentration of urea in the medium by incubating (37 °C, 20 min) the medium (4 ml) in 0.2 ml of 100 mM sodium phosphate, 26 mM EDTA buffer pH 7.1 with or without commercially available urease (0.08 mg; urea amidohydrolase EC 3.5.1.5; Sigma).

RESULTS AND DISCUSSION

Results shown in Table 2 indicate that quantitatively significant amounts of soluble ammonium accumulate during the growth of Trt. foetus and Tri. vaginalis. Cultures had on average 20 % greater amounts of ammonium than the sterile controls that were similarly incubated. Tri. vaginalis is regarded as microaerophilic and grows under a range of oxygen concentrations from 10 % air to strict anaerobic conditions. To determine if ammonium production by trichomonads was affected by the atmospheric oxygen content, ammonium production by cells grown under ambient oxygen tensions was compared to cells grown under anaerobic conditions (under H₂ plus CO₂ gas phase). Both Trt. foetus and Tri. vaginalis produced 80 % and 120 %, respectively, more ammonia under anaerobic conditions than ambient oxygen concentrations. In all cases sterile controls of anaerobic medium showed less soluble ammonium than the aerobic medium. The reason for the differences seen in the controls was not studied further. Air is inhibitory to the hydrogenosomal metabolism of CO₂, H₂ and protects cells from the complete metabolism of metronidazole to yield toxic products (Yarlett, 2000). A free-living flagellate, Hexamita inflata, has been shown to have increased rates of arginine metabolism under anaerobic conditions but it is not clear if there was increased production of soluble ammonium (Biagini et al., 2003). No differences were detected among the various genera or strains examined (Table 2) nor when Tri. vaginalis RU393 was grown under aerobic and anaerobic conditions with or without additional iron (2 mM) (data not shown). Iron increases the metabolic rate of pyruvate through the hydrogenosomes of trichomonads (Gorrell, 1985).

From the standard deviations calculated for each strain, the difference in soluble ammonium does not appear to be statistically significant. The combined mean, however, shows a significant increase of 20 %. Increased accumulation of soluble ammonium was also seen in two additional experiments for RU393 and C1-NIH during initial studies to measure urea in cultures. For these studies, samples of cultures were incubated with and without urease before analysis by a direct indophenol method (Weatherburn, 1967). Cultures of RU393 accumulated an average of 1.61 mM (18 % error) soluble ammonium (determined by the direct method), as compared to 1.26 mM (38 % error) for strain C1-NIH and 1.01 mM (64 % error) for sterile culture medium, based upon duplicate analyses of each of two cultures. Intraexperimental errors were much less, with RU393 cultures having the most ammonium, whereas C1-NIH cultures were more variable, as was the sterile medium. RU393 used for these experiments had been maintained for several weeks in agar-free medium, in which it grew to a lower cell density, 0.5 × 10⁶ cells ml⁻¹, compared to when it was maintained in 0.05 % agar-containing medium (3 × 10⁶ cells ml⁻¹). For these studies of urea in cultures of trichomonads, C1-NIH had more growth (6 × 10⁶ cells ml⁻¹) than RU393. In the agar-containing medium, strain RU393 formed a pellet at the bottom of the tube as compared to strain C1-NIH which was dispersed throughout the tube. The significance of these differences is not known. Addition of urease to the sterile culture increased soluble ammonium to a concentration of 1.74 mM (9 % error), indicating the presence of urea. This method included the controls that had either ammonium or urea added as positive controls. Results of urea analysis using cultures of RU393 and C1-NIH were more variable than that for the sterile medium, and it is not clear if there is any difference relative to the sterile medium. Linstead & Cranshaw (1983) did not detect any urease activity by cell suspensions that had been washed in a buffered salt solution. The amount of soluble ammonium detected without added urease does support the idea that

<table>
<thead>
<tr>
<th>Organism</th>
<th>Atmosphere</th>
<th>Soluble ammonium (mM)</th>
<th>Total*</th>
<th>Produced</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trt. foetus</td>
<td>Air</td>
<td>0.95 ± 0.60 (4)</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>H₂ + CO₂</td>
<td>0.86 ± 0.1 (4)</td>
<td>0.36</td>
<td></td>
</tr>
<tr>
<td>KV1-1MR-100</td>
<td>Air</td>
<td>1.00 ± 0.75 (4)</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td></td>
<td>H₂ + CO₂</td>
<td>0.95 ± 0.06 (4)</td>
<td>0.45</td>
<td></td>
</tr>
<tr>
<td>Tri. vaginalis</td>
<td>Air</td>
<td>0.95 ± 0.40 (4)</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>H₂ + CO₂</td>
<td>0.94 ± 0.09 (4)</td>
<td>0.44</td>
<td></td>
</tr>
<tr>
<td>RU393</td>
<td>Air</td>
<td>0.75 ± 0.32 (4)</td>
<td>0.44</td>
<td></td>
</tr>
<tr>
<td></td>
<td>H₂ + CO₂</td>
<td>0.50 ± 0.07 (4)</td>
<td>0.44</td>
<td></td>
</tr>
</tbody>
</table>

*Results are expressed as the mean ± SD for the number of experiments shown in parentheses.
trichomonads produce soluble ammonium. This was further demonstrated by capturing volatile ammonia during growth of cells (Table 3).

Cultures of *Try. vaginalis* accumulated increased amounts of soluble ammonium (NH$_4^+$ and NH$_3$) and volatile ammonia (NH$_3$) relative to the sterile control (Table 3). Growth medium was used for these experiments to permit the detection of the much smaller amounts of volatile ammonia that would accumulate at the acidic pH. We compared these results to the production of soluble ammonium and volatile ammonia by procyclic forms of *Try. brucei brucei* since this insect form grows in a medium that lacks carbohydrate other than what is present in the serum. Production of ammonia by trypanosomes was of further interest since it has been suggested that the tsetse fly (Diptera: Glossinidae) relies on an endosymbiotic bacterium for its fertility and nutrition (Akman & Aksoy, 2001). Based upon *Escherichia coli* gene array analysis, one of the endosymbionts (*Wigglesworthia*) was suggested to utilize ammonia in the absence of trypanosomes. Procyclic forms of *Try. brucei brucei* develop in the ‘midgut’ of the tsetse fly vector (*Glossina* sp.) after ingesting a blood meal from a mammalian host that has nagana (cattle) or African sleeping sickness (humans). Catabolism of amino acids occurs in the single mitochondrion (the kinetoplast) in the cell but it is not clear that ammonia is produced by *Try. brucei brucei* (Cross et al., 1973; Gutteridge & Coombs, 1977; Honigberg, 1967; Kidder, 1967; van Weelden et al., 2003). The total amount of soluble ammonium and volatile ammonia that accumulated in cultures of *Tri. vaginalis* and *Try. brucei brucei* was comparable when corrected for the amount seen in sterile controls. The amount of soluble ammonium was greater for these cells grown in sealed vials than for cells grown in culture tubes and tissue culture plates (Table 3). The reason for this difference was not studied further. The amounts of soluble ammonium were comparable to soluble ammonium seen for other trypanosomatids (Cazzulo et al., 1985; Yoshida & Camargo, 1978).

We have not found other studies that measured production of volatile ammonia by protists. There are published values for soluble ammonium determined by capturing it in the gas phase after treatment of cultures with alkali (Mah & Hungate, 1965). However, there are no reported values for ammonia captured during growth of protozoa or biochemical studies of ammonia production. The amounts of volatile ammonia detected were greater than expected from Knodler et al. (1994) but comparable to values that can be calculated from studies of amino acid metabolism by trichomonads (Zuo et al., 1995; Knodler et al., 1994; Rowe & Lowe, 1986). Knodler et al. (1994) detected less soluble ammonium in spent media for *Tri. vaginalis* compared to fresh media, whereas their studies showed that spent medium from cultures of *Crithidia* and *Giardia*, another amitochondriate protozoan, had increased levels of soluble ammonium. Studies show greatest loss of the basic amino acids arginine and lysine (Zuo et al., 1995; Knodler et al., 1994) from the medium and the greatest increase for alanine and proline; these changes seem to be dependent on the protozoan strain (Zuo et al., 1995; Knodler et al., 1994). Putrescine also accumulates in the medium (Yarlett, 1988). There may be strain-dependent variations among *Tri. vaginalis* isolates for the production of amino acids. Strain C1-NIH did not produce alanine (Steinbuchel & Müller, 1986; ter Kuile, 1996). Glutamate was removed from the growth medium in the studies of Zuo et al. (1995), whereas Knodler et al. (1994) detected increased amounts of glutamate. Chyle et al. (1971) detected several isoenzymes for glutamate dehydrogenase. Biochemical characterization (Turner & Lushbaugh, 1988) of the glutamate dehydrogenase activity indicated the deamination reaction had a pH optimum of 8.0 and a $K_m$ for glutamate about equal to that of the intracellular concentration of glutamate. Cells of *Tri. vaginalis* have a methionine gamma lyase (Coombs & Mottram, 2001) which may explain the decreased amounts of methionine seen in spent medium (Knodler et al., 1994). This enzyme produces ammonia and methanethiol and is distinct from other methionine gamma lyases. Marr (1979) suggested that protozoa such as *Leishmania* fix ammonia into amino acids for detoxification purposes.

The loss of arginine from the medium (Zuo et al., 1995; Knodler et al., 1994) and increased putrescine present (Yarlett, 1988) indicate that ammonium was produced through the arginine dihydrolase pathway. *Try. vaginalis* lacks arginase, urease (Linstead & Cranshaw, 1983) and an arginine aminotransferase. *Tri. vaginalis* does, however, have an ornithine/lysine aminotransferase (among other

| Table 3. Capture of volatile ammonia during aerobic growth of *Tri. vaginalis* and *Try. brucei brucei* procyclic insect forms |
|---|---|---|---|
| Organism | Soluble ammonia (mM) | Volatile ammonia (mM) |
| | Total | Produced | Total | Produced |
| *Tri. vaginalis* RU393 | 2.8 | 1.5 | 0.048 | 0.027 |
| None (TYM medium) | 1.3 | 1.0 | 0.021 |
| *Try. brucei brucei* | 3.1 | 1.2 | 0.125 | 0.06 |
| None (T2 medium) | 3.9 | 2.9 | 0.063 |
aminotransferases) (Lowe & Rowe, 1986). This enzyme activity may explain the production of proline in the growth medium via ornithine provided by the arginine dihydrolase pathway. Proline may also accumulate from metabolism of glutamate. Ornithine from the pathway can be metabolized to putrescine (Yarlett, 2000) as detected in vitro and indicated from in vivo studies (Chen et al., 1982). Tri. vaginalis does secrete proteases (Scott et al., 1995). Their influence on the available amino acids in cultures is not clear. Each of the strains of two genera of trichomonads used in this study showed increased amounts of soluble ammonia, and at least for Tri. vaginalis volatile ammonia. Production of ammonium was further detected by studies of cell suspensions of Tri. vaginalis and Trt. foetus in a buffered salt solution.

Catabolism of arginine was determined by the detection of soluble ammonium in incubations of Trt. foetus and Tri. vaginalis under aerobic conditions in a buffered salt solution. The results are shown in Table 4. The amount of soluble ammonium produced was greater than expected from previous studies that measured CO₂ production from [guanido-¹⁴C]-arginine (Linstead & Cranshaw, 1983) or [U-¹⁴C]arginine (Yarlett et al., 1996a). Tri. vaginalis and Trt. foetus (Linstead & Cranshaw, 1983; Yarlett et al., 1996b) have an active arginine dihydrolase pathway (Fig. 1). Cells of Tri. vaginalis (Knodler et al., 1994) have a twofold greater concentration of the intermediates (arginine, citrulline and ornithine) than Trt. foetus (Maroulis et al., 2003). The metabolic rate for soluble ammonium is less than the specific activity of carbamate kinase, which catalyzes the release of a second ammonium in cell extracts. Of these enzymes, the carbamate kinase has been characterized at the molecular level (Minotto et al., 2000). The arginine deiminase (Yarlett et al., 1996b) is localized in a membrane-bound particle but sedimented at a lower density than hydrogenosomes. The remaining enzyme activities of the arginine dihydrolase pathway including carbamate kinase, which would remove a second nitrogen as ammonium or ammonia, were found in the non-sedimentable fraction of Tri. vaginalis.

![Diagram of arginine dihydrolase pathway](image)

**Fig. 1.** Arginine dihydrolase pathway in trichomonads. 1, Arginine deiminase; 2, catabolic ornithine carbamoyltransferase; 3, anabolic ornithine carbamoyltransferase; 4, ornithine decarboxylase; 5, carbamate kinase. For clarity, subcellular localization of arginine deiminase is not shown.

Based upon the concentration of ammonia produced from arginine by Trt. foetus it can be calculated that the rate through arginine metabolism is of the same order of magnitude as that for carbohydrate fermentation to organic acids, glyceral H₂ and CO₂ (Chapman et al., 1985; Müller & Gorrell, 1983; Steibuchel & Müller, 1986). More-detailed kinetics studies of arginine utilization will be necessary to determine if there are significant differences in the flow through the arginine dihydrolase pathway among various trichomonad species.

Tri. vaginalis and Trt. foetus have sufficient concentrations of the other amino acids (1–2 μmol per 10⁸ cells; Knodler et al., 1994; Maroulis et al., 2003) to explain the accumulation of ammonium when arginine was not added to the cell suspension. Cells retained the ovoid motile forms as seen in culture. In addition, for Trt. foetus no decrease in protein was detected after 60 min (0·114 mg protein ml⁻¹) versus zero time (0·09 mg protein ml⁻¹) of incubation in the buffer.

The combined results indicate that trichomonads produce ammonium ions and the potentially more irritable ammonia during growth. Previous work provides the biochemical information to support the idea that the measurement of soluble ammonium from arginine by trichomonads provided a method to detect the metabolic rate of the arginine dihydrolase pathway by intact cells. Anaerobic rumen ciliates (Mah & Hungate, 1965) are thought to produce ammonium by the deamination of amino acids (Coleman, 1979). Chen et al. (1982) have

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**Table 4.** Production of soluble ammonium by trichomonads from arginine in Doran’s buffered salt solution

<table>
<thead>
<tr>
<th>Organism</th>
<th>Arginine (mM)</th>
<th>Soluble ammonium (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 min</td>
<td>60 min</td>
</tr>
<tr>
<td>Trt. foetus KV1-1MR-100</td>
<td>1</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>18</td>
</tr>
<tr>
<td>Tri. vaginalis MR-100</td>
<td>1</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>ND</td>
</tr>
<tr>
<td>Tri. vaginalis TV10-02</td>
<td>1</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND, Not determined.

http://mic.sgmjournals.org
detected decreased levels of alanine, putrescine, cadaverine and gamma aminobutyric acid in vaginal fluid after treatment of vaginitis patients with metronidazole. Amounts of ammonium in vaginal fluid were not mentioned (Chen et al., 1982; Petrin et al., 1998; Pybus & Onderdonk, 1997), whereas production of ammonia by bacteria growing on epithelial tissues of the human digestive tract has been studied more extensively (Casiano-Colon & Marquis, 1988; Verdu et al., 1998). Further studies of ammonia metabolism by protozoa will most likely reveal interesting variations on the theme of nitrogen and energy metabolism, along with understanding endosymbiotic origins of protozoa.

ACKNOWLEDGEMENTS

The authors want to thank Dr Seymour H. Hutner for helping to keep this project alive. The technical assistance of and helpful discussions with other members of Haskins Laboratories are gratefully appreciated. Dr J. Tachezy (Charles University, Prague) generously provided cultures of *Tri. vaginalis* (TV10-02 and MR-100). The results were presented in part by Y. K. at the Annual Meeting of the Society of the Dyson Society of Fellows (2001; Pleasantville, NY) and the 11th East Coast Protozoology Conference (2003; Catonsville, MD). Y. K. was funded in part by an undergraduate stipend from the Eugene Lang Foundation; N.Y. was funded by NIH-NIAID 49785.

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