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Yekaterina Kleydman
Pace University

Nigel Yarlett
Pace University

Thomas Gorrell
Pace University

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

Yekaterina, Kleydman^{*} ; Nigel Yarlett^{*,} ; Thomas Gorrell^{*,***}**

*Haskins Laboratories at Pace University NY, NY, ** Department of Chemistry and Physical Sciences, and ***Department of Biology at Pace University, NY, NY 10038

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Corresponding author: Thomas E. Gorrell
tgorrell@pace.edu
Phone 212 346-1246
Fax: 212 346-1586

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This paper is dedicated to the memory of Dr. Seymour H. Hutner (October 31, 1911-June 1, 2003) whose inspiration in the field of protozoology will be deeply missed.

Summary

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 amitochondriate zooflagellates that thrive under microaerophilic and anaerobic conditions. We were able to detect accumulation of ammonium ions and ammonia in cultures of *Tritrichomonas foetus* and *Trichomonas vaginalis* including those resistant to metronidazole. Ammonium ions and ammonia were detected using the indophenol colorimetric method. Aerobic overnight cultures had 0.9 mM of soluble ammonium (NH_4^+ and NH_3) or a 20% greater concentration of ammonium relative to sterile tryptose, yeast extract maltose medium with heat inactivated horse serum that was incubated similarly. Production of ammonia itself was confirmed by analysis of a wick that was moistened with sulfuric acid (40 mM) and placed above the liquid in sealed cultures of a strain of *T. vaginalis*. The wicks from these cultures captured the equivalent of 0.048mM of volatile ammonia (NH_3) from the liquid as compared to 0.021mM volatile ammonia from sterile medium after overnight incubation. Intact cells of trichomonads (0.1mg protein) incubated in Doran's buffer and with or without (1 mM) L-arginine produced significant amounts of soluble ammonium (0.07 mM, 0.035 mM respectively) during 60 minutes. These amounts are similar to those reported for the metabolism of carbohydrates by trichomonads. The results indicate that ammonium ions and the more irritating ammonia are significant metabolites of trichomonads.

Introduction

Trichomonads (Diamond 1957; Petrin, 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 H₂, CO₂ and organic acids. Production of H₂ occurs in a redox organelle, the hydrogenosome. Hydrogenosomes appear to be metabolically analogous to mitochondria for the fermentation of pyruvate to H₂ and CO₂. Metronidazole (Flagyl) is used to treat infections caused by anaerobic microorganisms. For trichomonads its selective mode of action results from reductive activation by the metabolism of pyruvate in the hydrogenosomal pathway. *Tritrichomonas foetus* infects the reproductive tract of cattle, and *Trichomonas vaginalis* is a major cause of vaginitis in humans. Human trichomoniasis has a characteristic odor and elevated vaginal pH. Metronidazole resistant strains of *Trichomonads* have been produced in the laboratory by growth in the presence of increasing metronidazole (Tachezy et al., 1993). *T. vaginalis* isolated from clinical specimens (Müller et al., 1980) of patients that had refractory trichomoniasis had decreased sensitivity to metronidazole *in vitro* (Müller et al., 1988).

Studies indicate that trichomonads require arginine for growth (Kidder, 1951). *Trichomonads* have an arginine dihydrolase pathway that may supplement the cells ability to form ATP (Linstead and Cranshaw, 1983; Yarlett et al, 1996). 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 *T. vaginalis*. Studies of the metabolism of ammonium and the more basic and toxic ammonia are difficult to find in the literature dealing with the metabolism of amino acids by protists (Gutteridge & Coombs, 1977; Honigberg, 1967; Knodler *et al.*, 1994; Marr, 1979; Cazullo *et al.*, 1985). In this study we are able to show the accumulation 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 there is a greater metabolic flux through the arginine dihydrolase pathway than previously suggested for *T. foetus* and most likely *T. vaginalis*.

Methods

A. Organisms and Growth Conditions

The strains of *Tritrichomonas foetus* and *Trichomonas vaginalis* used in this study are shown in Table 1. *T. foetus* KV1/M-100 (ATCC 50151), and *T. vaginalis* RU393 (ATCC 50142) and C1 NIH were obtained from the American Type Culture Collection (Manassas, Virginia). *T. vaginalis* strains TV10-02 and MR100 were generously provided by Dr J Tachezy (Kulda *et al.*, 1993). The properties of these strains are summarized in Table 1. Values for metronidazole susceptibility (Meingassner *et al.*, 1978; Muller *et al.*, 1988) were compiled from the values in the references. *T. foetus* KV1/M-100 and *T. 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 that was refractory to treatment with metronidazole and has much less 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 *in vitro* 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 provided basis for metabolic and molecular studies of hydrogenosomal proteins and is not pathogenic.

Cells grew to about 3×10^6 cells ml after overnight incubation at 37°C in Diamond's TYM medium (Diamond, 1957) containing (% wt/vol) 2% tryptose (Difco, Detroit, MI); 1% yeast-extract (Difco, Detroit, MI); 0.5% maltose (Difco, Detroit, MI), 0.1% L-cysteine monohydrate; 0.02% ascorbic acid, with the following modifications 0.8% potassium dihydrogen phosphate (anhydrous); 0.8% dipotassium hydrogen phosphate-3H₂O, pH 6.4 and supplemented with 10% heat inactivated horse serum,

(Müller et al., 1988). The strains of *T. vaginalis* 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 $\text{H}_2:\text{CO}_2$ atmosphere. For comparison to a zooflagellate that grows on amino acids, ammonium accumulation in cultures of *Trypanosoma brucei brucei* lab 110 EATRO procyclic insect forms (*Glossina* sp midgut) was also measured. Cells were grown in T2 medium Levandowsky and Katz (cited in Bacchi et al., 1989) for 72-96 hours at 27°C . This medium contained a defined mix of amino acids, vitamins, hemin, along with heat-treated (56°C , 30 minutes) fetal calf serum and had an initial pH of 7.4.

Cells of trichomonads and *T. b. brucei* were counted using a Neubauer haemocytometer. *T. vaginalis* grown in this study, vaginal exudates, and in vivo mouse (intra peritoneal) model have an ovoid shape as compared to the amoeboid forms seen during growth on agar plates, attached to the vaginal epithelial mucosa, an *in vivo* mouse subcutaneous model (Nielsen & Nielsen, 1975; Warton & Honigberg, 1979; Yarlett, 2000). Cultures were also microscopically examined for microbial contaminants. Heat fixed slides stained with 4',6-diamidino-2-phenylindole (DAPI) had defined spherical nuclear DNA but no extranuclear DNA that would be indicative of contamination with mycoplasma.

B. Metabolic Studies and Analytical methods

For studies of the metabolism of arginine, cells were harvested by centrifugation, washed in Doran's buffered solution (74mM NaCl, 1.6mM KCl, 0.6mM CaCl_2 , 30 mM sodium phosphate NaH_2PO_4 ; pH 6.4) and resuspended to 10^7 cells per ml. In This buffer cells remain motile and are metabolically active (Müller & Gorrell, 1983; Yarlett et al, 1996). Cells were incubated with or without 1mM L-arginine, for 60 minutes at

37°C. Cells were cooled to 4°C and removed by centrifugation before analysis of soluble ammonium in the supernatant fluid. Protein was determined by the method of Bradford (1976).

C. Soluble and Volatile Ammonium

The indophenol colorimetric method of Bertholet (1859) as 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 ($\epsilon = 0.0124 A_{625}/\text{nmol NH}_4\text{Cl/ml}$). Reaction mixtures contained 1 ml of phenate reagent consisting of 1.1% (v/v) phenol and 5mg% (w/v) sodium nitroprusside and 1 ml of alkaline hypochlorite [0.8%(v/v) hypochlorous acid (Chlorox) and 0.6%(wt/v) sodium hydroxide].

- 1) Soluble ammonium (NH_4^+ and NH_3) was measured by placing an aliquot of the liquid from overnight cultures in a sealed glass scintillation vial that contained a wick moistened with (20mM sulfuric acid) to trap ammonium released by the addition of 2.5mM boric acid pH 9.5 and subsequently incubated overnight at room temperature. The wick 0.5cm x1cm 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 aliquot of cultures to the reagents for the formation of indophenol. Trichomonads for these experiments were grown in 5ml of TYM media using screw capped culture tubes, or in 1ml of TYM media 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 multiwell plates. The direct indophenol method was used in preliminary studies to determine the concentration of urea in the medium by incubating (37°C, 20 minutes) the medium (4ul) in 0.2ml of 100 mM sodium phosphate, 26 mM EDTA buffer pH 7.1 with or without commercially available urease (0.08mg:Sigma).

- 2) Volatile ammonia (NH_3) production was confirmed by analysis of a wick that was moistened with sulfuric acid and placed above the liquid during the growth of the cells (5ml) in sealed glass scintillation vials (20ml). The wick was then assayed in the indophenol method for volatile ammonia. *T. b. brucei* was grown in 10 ml of medium using 50 ml plastic tissue culture flasks.

Results and Discussion

Results shown in Table 2 indicate that quantitatively significant amounts of soluble ammonium accumulate during the growth of *T. foetus* and *T. vaginalis*. Cultures had on average 20% greater amounts of ammonium than the sterile controls that were similarly incubated. *T. vaginalis* is regarded as microaerophilic and grows under strict anaerobic conditions. Sterile controls of anaerobic medium showed less soluble ammonium than the aerobic medium. Thus it appears that anaerobic cultures accumulated more soluble ammonium than aerobic controls. The reason for the differences seen in the controls was not further studied. 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 *T. vaginalis* RU393 was grown under aerobic and anaerobic conditions with or without additional iron (2mM) (data not shown). Iron increases the metabolic flux of pyruvate through the hydrogenosomes of trichomonads.

From the standard deviations calculated for each strain the difference in soluble ammonium does not appear to be statistically significant. The combined average 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 the direct indophenol method. Cultures of RU393 accumulated an average of 1.61 mM (18% error) soluble ammonium, as compared to 1.26 mM (38% error) for strain C1-NIH and 1.01 (64% error) mM for sterile culture medium, based upon duplicate analysis of each of two cultures. Inter-experimental errors were much less with RU393 cultures having the most ammonium whereas C1-NIH was more variable as was the sterile

medium. RU 393 used for these experiments had been maintained for several weeks in agar free medium where it grew to a lower cell density, 0.5×10^6 and 6×10^6 cells ml^{-1} compared to agar containing media and C1-NIH, respectively. In this medium strain RU393 formed a pellet at the bottom of the tube as compared to strain C1-NIH that is dispersed throughout the tube. The significance of these differences not known. Addition of urease to the sterile culture gave increased soluble ammonium 1.74 mM (9%) 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. Lindstead and 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 trichomonads produce soluble ammonium. This was further demonstrated by capturing volatile ammonia during growth of cells. (Table 3).

Cultures of *T. vaginalis* accumulated increased amounts of soluble (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 *T. b. 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 the tsetse fly (Diptera:Glossinidae) relies on an endosymbiont for its fertility and nutrition.

Based upon the *Escherichia coli* gene array analysis (Akman & Aksoy, 2001) one of the endosymbionts (*Wigglesworthia*) was suggested to utilize ammonia in the absence of trypanosomes. Procyclic forms of *T. b. 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 *T. b. brucei* (Cross et al, 1975; 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 *T. vaginalis* and *T. b. brucei* were comparable when corrected for the amount seen in sterile controls. The amounts of soluble ammonium was greater for these cells grown in sealed vials than culture tubes and tissue culture plates (Table 1). The reason for this difference was not further studied. The amounts of soluble ammonium were comparable to soluble ammonium seen for other trypanosomatids (Yoshida & Camargo, 1978; Cazzulo et al, 1985). We have not found other studies that measured production of volatile ammonia by protists. Few studies have measured soluble ammonium by capturing it in the gas phase after treatment of cultures with alkali (Mah & Hungate, 1965). We have not seen any reports that captured ammonia during growth of protozoa or biochemical studies of them. The amounts of volatile ammonia detected were greater than expected from the acidic initial pH of the medium e.g. pH 6.4, which decrease upon growth presumably due to production of organic acids. Whether the nitrogen is produced by trichomonads as ammonium ion or ammonia remains to be determined since buffers used in this study favor the accumulation of ammonium ions.

The amounts of soluble ammonium detected in trichomonad cultures were greater than expected from Knodler et al. (1994) but comparable to values that can be calculated from studies of amino acids metabolism by trichomonads (Coombs *et al*, 1995; Knodler *et al.*, 1985; Rowe & Lowe, 1986). Knodler *et al.*, (1985) detected less soluble ammonium in spent media for *T. vaginalis* compared to fresh media, whereas their studies showed that spent medium from cultures of *Crithidia* and *Giardia*, another amitochondriate protozoan, had increased soluble ammonium. Studies show greatest loss of the basic amino acids, arginine and lysine (Coombs *et al*, 1995; Knodler *et al.*, 1985) from medium, and the most increase for alanine and

proline that seem to be dependent on the strain (Coombs *et al.*, 1995; Knodler *et al.*, 1985). Putrescine also accumulates in the medium (Yarlett, 1988). There may be strain dependent variations among *T. vaginalis* for the production of amino acids. Strain C1-NIH did not produce alanine (Steinbuchel and Müller, 1986; ter Kuile, 1986). Glutamate is removed from the growth medium in the studies of Coombs *et al.* (1995) whereas Knodler *et al.* (1985) 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 deamidation reaction had a pH optimum of 8.0 and a Km for glutamate about equal to that of the intracellular concentration of glutamate. Cells of *T. vaginalis* have a methionine gamma lyase (Coombs & Mottram, 2001) that may explain the decreased amounts of methionine seen in spent medium (Knodler *et al.*, 1985). This enzyme produces ammonia and methanethiol and is distinct from other methionine gamma lyases. Marr (1979) has suggested that protozoa such as *Leishmania* fix ammonia into amino acid to detoxify it.

The loss of arginine from the medium (Coombs *et al.*, 1995; Knodler *et al.*, 1985) and increased putrescine (Yarlett, 1988) indicates that ammonium was produced through the arginine dihydrolase pathway. *T. vaginalis* lacks arginase, urease (Lindstead & Cranshaw, 1983) and an arginine aminotransferase. *T. vaginalis* does, however have an ornithine/lysine amino transferase (among other amino transferases) (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). *T. 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 *T. vaginalis* volatile ammonia.

Production of ammonium was further detected by studies of cell suspensions of *T. vaginalis* and *T. foetus* in a buffered salt solution.

Catabolism of arginine was determined by the detection of soluble ammonium in incubations of *T. foetus* and *T. 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 (¹⁴C-guanidino) arginine (Linstead and Cranshaw, 1983) or (¹⁴C-U) arginine (Yarlett *et al.*, 1996). *T. vaginalis* and *T. foetus* (Linstead & Cranshaw, 1983; Yarlett *et al.*, 1996) have an active arginine dihydrolase pathway (Fig 1). Cells of *T. vaginalis* (Knodler *et al.*, 1994) have a two fold greater concentration of the intermediates (arginine, citrulline, and ornithine) than *T. foetus* (Maroulis *et al.*, 2003). The metabolic flux for soluble ammonium is less than the specific activity of carbamate kinase that 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.*, 1994) is localized in membrane bound particle but sedimented at a lower density than hydrogenosomes. The remaining enzyme activities of the arginine dihydrolase pathway and the carbamate kinase which would remove a second nitrogen as ammonium or ammonia were found in the non sedimentable fraction of *T. vaginalis*.

The amount of ammonium produced with arginine was similar to carbohydrate fermentation to organic acids, glycerol, H₂ and CO₂ by trichomonads (Chapman *et al.*, 1985; Steinbuchel and Müller, 1986; Müller & Gorrell, 1983).

T. vaginalis and *T. foetus* have sufficient concentrations of the other amino acids (1-2 μmols 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. No

decrease was seen in cell numbers (data not shown). Cells retained the ovoid motile forms as seen in culture. At least for *T. foetus* no decrease in protein was detected at 60 minutes (0.114mg protein ml⁻¹) versus zero time (0.09mg protein ml⁻¹).

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 flux 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 detected decreased levels of alanine, putrescine, cadaverine and gamma aminobutyric 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 have been more extensively studied (Cosiano-Colon & Marquis, 1988; Verdu *et al.*, 1988). 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.

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Table 1 Strains of Trichomonads used in this study

<u>Organism</u>	<u>Strain</u>	<u>Metronidazole Resistance*</u>	
		<u>Aerobic</u>	<u>Anaerobic</u>
<i>T. foetus</i>	KV1 / M-100	Yes	No
<i>T. foetus</i>	KV1 / M-100 -17	Yes	No
<i>T. vaginalis</i>	RU 393	Yes	No
“	MR100	Yes	Yes
“	TV1002	No	No

*Compiled from (Meingassner *et al*, 1978, and Kulda *et al.*, 1993) except for strain RU 393. Strain Kv1/M-100-17 was derived from Kv1/M-100 after its subcutaneous passage in an *in vivo* mouse model. Resistance defined as Minimum Lethal Concentration >100 µg/ml tryptose-yeast extract–maltose medium .

Table 2. Accumulation of Soluble Ammonium in cultures of Trichomonads

<u>Organism</u>	<u>Atmosphere</u>	<u>Soluble Ammonium(mM)*</u>	
		Total	Produced
<i>T. foetus</i>			
Kv1/M100	Air	0.95 +/- 0.60 (4)	0.2
	H ₂ CO ₂	0.86 +/- 0.1 (4)	0.36
Kv1/M100-17	Air	1.00 +/- 0.75 (4)	0.25
	H ₂ CO ₂	0.95 +/- 0.06 (4)	0.45
<i>T. vaginalis</i>			
RU393	Air	0.95 +/- 0.40 (4)	0.2
	H ₂ CO ₂	0.94 +/- 0.09 (4)	0.44
None	Air	0.75 +/- 0.32 (4)	
	H ₂ CO ₂	0.50 +/- 0.07 (4)	

*results are expressed as the mean +/- SD for the number of experiments shown in parenthesis.

Table 3. Capture of Volatile Ammonia during aerobic growth of *Trichomonas vaginalis* and *Trypanosoma brucei brucei* procyclic insect forms.

Organism	Soluble		Volatile	
	Ammonium		Ammonia	
	Total	Produced	Total	Produced
<i>T. vaginalis</i> RU39	2.8	1.5	0.048	0.027
None (TYM medium)	1.3		0.021	
<i>T. b. brucei</i>	5.1	1.2	0.125	0.06
None (T2 medium)	3.9		0.063	

*Data expressed as mM soluble ammonium (NH_4^+ and NH_3) and mM volatile

ammonia(NH_3). The values are the average of two analysis with less than 10 percent error.

Table 4 Production of soluble ammonium by Trichomonads from arginine in Doran's buffered salt solution.

Organism	Arginine (mM)	Soluble Ammonium (μ M)	
		0 min	60min
<i>T. foetus</i> Kv1/M100	1	26	75
	0	18	48
<i>T. vaginalis</i> MR-100	1	nd*	159
	0	nd	90
<i>T. vaginalis</i> TV 10-02	1	nd	175
	0	nd	52

*Not determined

Figure legends

Figure 1: Arginine dihydrolase pathway in *Trichomonads*. 1. arginine deiminase, 2. catabolic ornithine carbamyl transferase, 3. anabolic ornithine carbamyl transferase, 4. ornithine decarboxylase, 5. carbamate kinase. For clarity subcellular localization of arginine deiminase is not shown.



