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Ureotelism and Ammonotelism in Trypanosomatids

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According to their genera, trypanosomatids excrete urea, ammonia, or both. Species of *Herpetomonas* and *Trypanosoma* are ammonotelic. Species of *Leishmania, Leptomonas, Crithidia,* and *Blastocrithidia* can be ureotelic, ammonotelic, or both, depending on growth media composition.

Protozoa are generally regarded as ammonotelic organisms (5). Ureotelism among them has been disclaimed on the basis of the proved absence of urea excretion or urea-cycle enzymes in a few species examined (4, 8). Recently, however, urea-cycle enzymes plus other enzymes for interconversion of arginine, citrulline, and ornithine were found in trypanosomatids, a conspicuous group of parasitic flagellates (E. N. Figueiredo, N. Yoshida, C. Roitman, and E. P. Camargo, J. Protozool., in press; N. Yoshida, J. V. Jankevicius, I. Roitman, and E. P. Camargo, J. Protozool., in press). Although the urea cycle of trypanosomatids was seldom found to be complete, the presence of arginase in many species made it reasonable to anticipate urea excretion by these flagellates. On the other hand, the basic ammonotelic character of protozoa could be retained by some other species which, for arginine metabolism, possess ammonia-producing enzymes (arginine deiminase and citrulline hydrolase) instead of arginase. Thus ureotelism and ammonotelism could coexist among trypanosomatids.

To verify the validity of these considerations we examined cultures of representatives of various genera of *Trypanosomatidae* for the presence of urea and ammonia. Urea was determined in the supernatant of cultures by diacetylmonoxime reaction, according to Archibald (1). Ammonia was determined by the phenol-hypochlorite method, as modified by Searcy et al. (7). Organisms were cultured in complex or defined media, as indicated in Table 1, according to their growth requirements. Production of urea and ammonia was also comparatively studied in defined media containing arginine, citrulline, or ornithine. Some species cannot be indefinitely subcultured in these media, so, for those species, only first-passage cultures were examined.

Our results (Table 1) have shown that both ammonotelism and ureotelism can be found in trypanosomatids. The excretion of urea or ammonia in many cases is brought about or enhanced by the addition of arginine or citrulline to the media. In every case, however, the nature of the nitrogenous compound excreted is in accordance with the known enzyme constitution of the organism studied. Thus, *Herpetomonas* spp., which possess arginine deiminase and citrulline hydrolase, excrete ammonia only. Species that do not possess enzymes for the ornithine-arginine metabolism, such as *Trypanosoma* spp., also excrete ammonia, probably originated in other metabolic areas. Species of *Leptomonas, Leishmania, Crithidia,* and *Blastocrithidia,* which possess arginase, may excrete urea as well as ammonia. Species that possess arginase and citrulline hydrolase simultaneously, such as *Crithidia* spp., preferentially excrete urea or ammonia, depending on the presence of arginine or citrulline in the growth medium (Fig. 1).

The presently disclosed ureotelism of trypanosomatids, although widespread, seems not to have the same physiological significance as in higher organisms, where the excretion of urea represents the last step in the elimination of catabolic ammonia. In trypanosomatids lacking a complete urea cycle, the production of urea represents a somewhat isolated activity of arginase, probably geared into the production of ornithine (S. Galinari and E. P. Camargo, Exp. Parasitol., in press). Only in *Crithidia deanei,* a species harboring endosymbionts, could urea serve as a vehicle for the excretion of catabolic nitrogen, since this organism has a complete urea cycle (3) and apparently does not excrete ammonia.

The detection of urea and ammonia in cultures of trypanosomatids may be of taxonomical use since the production of either one of these compounds reflects the presence of specific enzymes of arginine-ornithine metabolism, whose distribution within the family *Trypanosomati-*

TABLE 1. *Excretion of urea and ammonia in Trypanosomatidae*

Organism	Media[a]	Excretory product[b]	
		Urea	Ammonia
Crithidia deanei (ATCC 30255)	RDM	0	0
	RDM-Arg	1,520	0
	RDM-Cit	+[c]	0
	RDM-Orn	0	0
Crithidia fasciculata (ATCC 11745)	FYTS	70	0
	RDM-Arg	480	0
	RDM-Cit	+[c]	250
Crithidia acanthocephali (ATCC 30251)	FYTS	30	0
	RDM-Arg	350	0
	RDM-Cit	+[c]	700
Leptomonas seymouri (ATCC 30220)	FYTS	50	0
	RDM-Arg	740	0
	RDM-Cit[d]	0	0
Leptomonas collosoma (ATCC 30261)	LIT	0	670
	LIT-Arg	620	590
Blastocrithidia culicis (ATCC 30257)	LIT	0	1,130
	LIT-Arg	130	1,300
	RDM-Arg[d]	310	1,280
Leishmania braziliensis	LIT	0	460
	LIT-Arg	470	560
Herpetomonas megaseliae (ATCC 30209)	FYTS	0	0
	RDM-Arg	0	720
	RDM-Cit	0	440
	RDM-Orn	0	0
Herpetomonas samuelpessoai (ATCC 30252)	FYTS	0	0
	RDM-Arg	0	220
	RDM-Cit	0	420
	RDM-Orn	0	200
Herpetomonas muscarum subsp. *muscarum* (ATCC 30260)	FYTS	0	0
	RDM-Arg	0	320
	RDM-Cit	0	370
	RDM-Orn	0	0
Trypanosoma cruzi (Y strain)	LIT	0	920
	RDM-Arg[d]	0	450
Trypanosoma mega (ATCC 30038)	LIT	0	Trace
	RDM-Arg[d]	0	Trace
Trypanosoma conorhini	LIT	0	630

[a] RDM, Roitman defined medium (6) without arginine. RDM-Arg, RDM-Cit, and RDM-Orn contain, respectively, 2.3 mM L-arginine, L-citrulline, or L-ornithine. FYTS, Trypticase-sucrose medium (6); LIT, liver infusion-tryptose medium (2).

[b] Results are expressed in nanomoles of urea or ammonia per milliliter of medium. Production of urea or ammonia varied according to age of culture, size of inoculum, temperature of growth, and concentration of nutrients in the culture medium. Under similar conditions, however, results are reproducible within a 10% variation range. Data were obtained from cultures at the beginning of the stationary growth phase.

[c] Urea estimation is inaccurate in media containing citrulline, since this amino acid also reacts with diacetyl-monoxime. In these experiments urea was evaluated qualitatively after treatment of the samples with urease.

[d] This medium does not allow subcultures. Results presented are from first-passage cultures.

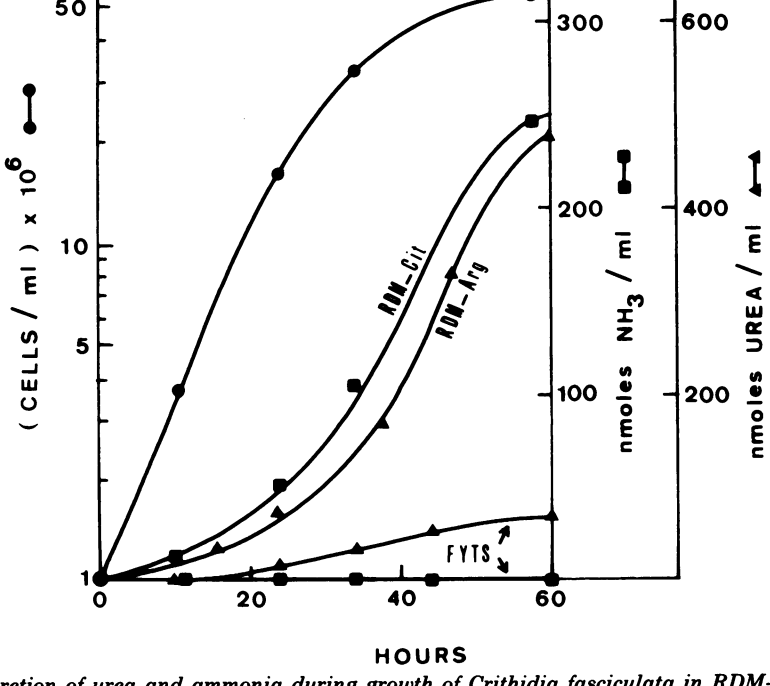

FIG. 1. *Excretion of urea and ammonia during growth of Crithidia fasciculata in RDM-Arg, RDM-Cit, and FYTS media (see Table 1). Results are expressed in nanomoles of urea or ammonia per milliliter of medium. Urea is produced in RDM-Cit, but was not quantitatively estimated due to citrulline interference (see Table 1). The growth curve represented corresponds to that obtained in RDM-Arg. Curves for the other two media were similar.*

dae is thought to be genus specific (E. P. Camargo, J. A. Coelho, G. Moraes, and E. N. Figueiredo, Exp. Parasitol., in press).

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