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20. Abstract (continued)

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Stability of T-2 Mycotoxin in Aqueous Media

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Abstract

Radiolabeled [^3H] T-2 mycotoxin was dissolved in various aqueous media and stored for up to 3 weeks at 4, 22 and 37°C. At periods ranging from 1 to 21 days, aliquots were assayed by thin layer chromatography. Breakdown products were identified based on their co-migration with known standards. Results indicated that T-2 toxin was most stable in tissue culture medium with or without serum, compared to HBSS, at all temperatures. The metabolites HT-2, T-2 triol, and T-2 tetraol were detected in decreasing concentrations, as early as 1 day (HBSS, 37°C) and as late as 3 weeks (HBSS, 4°C). Stability of the toxin in aqueous media decreased with increasing temperature.

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Many investigators who utilize mycotoxins in their work find it necessary to dilute stock toxin solutions for various experimental protocols. While toxins generally come lyophilized from the manufacturer, they are usually reconstituted in methanol or ethanol for storage at 4° C. Studies conducted in vitro often call for adding toxins to aqueous media for extended time periods. Since such protocols are prevalent in our laboratory, we were interested in examining the stability of T-2 mycotoxin in several aqueous media.

Radiolabeled [³H] T-2 mycotoxin (0.1 ug/ml). (New England Nuclear, Boston, MA) was dissolved in sterile solutions of Hanks balanced salt solution (HBSS), and tissue culture medium with Hanks salts (H-199) (GIBCO, Grand Island, NY) with and without 10% fetal calf serum. All samples were then stored for up to 21 days at 4, 22 and 37° C. At each of the following time periods (i.e. 1,3,5,7,14 and 28 days) aliquots of each sample were spotted on thin layer chromatography (TLC) plates with known standards. The TLC plates were developed and scanned for radioactivity with the Bioscan BID100 (Bioscan Inc., Washington, D.C.). Breakdown products were identified based on their co-migration with known standards which included the trichothecene mycotoxins; T-2, HT-2, T-2 triol, and T-2 tetraol.

Results are totally summarized in Table 1

Table 1. T-2 Mycotoxin Stability in Aqueous Media

<u>Temperature</u>	<u>Aqueous Medium</u>	<u>Metabolite</u>	<u>First Appearance</u>
37°	HBSS	HT-2	1 Day
		T-2 Triol	1 Week
		T-2 Tetraol	2 Weeks
		Unknown	2 weeks
	Media + Serum	HT-2	1 Week
	Media - Serum	None	3 Weeks

22° C	HBSS	HT-2	1 Week
		T-2 Triol	1 Week
		T-2 Tetraol	3 Weeks
	Media + Serum	HT-2	2 Weeks
		Unknown	3 Weeks
	Media - Serum	HT-2	2 Weeks
4° C	HBSS	HT-2	3 Weeks
	Media + Serum	None	3 Weeks
	Media - Serum	None	3 Weeks

We chose to examine HBSS and tissue culture medium since the majority of our experiments are conducted in one of these aqueous solutions. Since most of our experiments use tissue culture media which contain fetal calf serum, we examined the effect of its deletion from one of our experiments. This is important because serum is the least defined component of tissue culture media.

We found T-2 toxin to be most most stable at 4° C and least stable at 37° C. This was not surprising since all metabolic reactions are slowed considerably at low temperature. T-2 toxin was as stable in medium with serum as it was without serum at both 4 and 22° C. At 37° C, the presence of serum did speed up breakdown of the toxin. In this sample, HT-2 was present after 1 week with serum but was not evident after 3 weeks without serum (Table 1). This indicates that unknown components of fetal calf serum may play a role in T-2 breakdown at physiologic temperatures. Since most experiments are not run for 7 days at physiologic temperatures, this should not have an adverse effect.

What was not expected was the greater stability of T-2 toxin in tissue culture medium compared to HBSS. Both H-199 and HBSS contain 8 inorganic salts plus D-glucose. Formulations for each are based on Hanks formula of inorganic salts with slight differences in the specific salt or the amount of the salt that is employed. The major difference lies in many additional ingredients found in H-199 compared to HBSS. These include 23 amino acids or their derivatives, 17 vitamins, and other added components (GIBCO Laboratories, Technical Brochure, Grand Island, NY, p. 27,50).

The data in Table I illustrate that breakdown of T-2 occurs at least 1 week sooner in HBSS compared to H-199 at all temperatures. Therefore, we conclude that the additional ingredients in H-199, not found in HBSS, increase stability of toxin in aqueous solutions.

These results should be of interest to investigators who utilize T-2 mycotoxin in in vitro studies at physiologic temperatures. If studies conducted over several days involve dilution of toxin in aqueous solutions, metabolic breakdown products may appear. This may be checked by the use of pilot studies, under experimental conditions, using TLC to monitor toxin stability. Therefore, T-2 toxin stored in ethanol or methanol should be freshly diluted just prior to use.

Stock solutions of non-radiolabeled T-2 toxin (ethanol or methanol) stored at 4° C for extended time periods may also degrade. We recently tested by TLC a stock solution of T-2 toxin (methanol) which had been stored for 2 years at 4° C, and discovered the presence of HT-2, T-2 triol, and one unknown metabolite (data not shown). Therefore, it may be necessary to periodically check the purity of stock toxin solutions when stored under supposedly ideal conditions. Other mycotoxins may be similarly effected.

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