mg/kg. These levels as well as those of precursors of the marker compound, *N*-methyl-1,3-propanediamine, which constitute 38% of total residues, decline in parallel with a half-life of 1 day.

Registry No. $MeNH(CH_2)_2CHTNH_2$, 107082-64-4; $MeNH(CH_2)_2CN$, 693-05-0; MeOH, 67-56-1; $MeNH(CH_2)_3NH_2$, 6291-84-5; 3-methyl-2-thiopheneacrylonitrile (imino ether), 107082-63-3; 3-methyl-2-thiopheneacrylonitrile, 20527-86-0; tartaric acid, 87-69-4; morantel, 20574-50-9; [*4,4-pyrimidyl*-$^3H_2$]morantel (tartrate salt), 107098-03-3; [$^3H_2$]morantel, 107082-65-5.

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Acetylation/Deacetylation Reactions of T-2, Acetyl T-2, HT-2, and Acetyl HT-2 Toxins in Bovine Rumen Fluid in Vitro[1]

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A tritiated preparation of the trichothecene mycotoxin, T-2 toxin, underwent both acetylation and deacetylation reactions when incubated with bovine rumen fluid in vitro. Products from incubations of T-2 in rumen fluid included acetyl T-2, HT-2, and acetyl HT-2. Direct studies with tritiated samples of each of these metabolites confirmed their relatively facile interconversion in the rumen. Studies with [^{3}H]HT-2 under conditions of inhibited esterase activity (added diisopropyl fluorophosphate) showed that acetylation is preferred at C-3 vs. C-4. Studies with [^{3}H]acetyl T-2 indicated that deacetylation similarly occurs with greater rapidity at C-3. There were no indications that ester hydrolysis of these trichothecenes occurred at C-8 or C-15 or that they were subjected to epoxide reduction reactions. These data suggest that acetylation of T-2 and other trichothecenes in the rumen in situ may ultimately result in the absorption of more lipophilic metabolites whose toxicological and residual properties are at present unknown.

The trichothecenes, as presently known, are a group of some 50 biologically active secondary fungal metabolites. Certain of these compounds have been implicated as causal agents in both human and animal poisonings (Ueno, 1983). One of the most widely studied trichothecenes is T-2 toxin [4β,15-diacetoxy-8α-[(3-methylbutyryl)oxy]-3α-hydroxy-12,13-epoxytrichothec-9-ene; Figure 1], a compound known to be of high acute toxicity to a number of vertebrate species (Marasas et al., 1969; Sato and Ueno, 1977; Chi et al., 1978). T-2 has been found in certain grains infected with *Fusarium sp.* (Hsu et al., 1972; Mirocha et al., 1976; Puls and Greenway, 1976), and the ingestion of T-2-contaminated grain has resulted in livestock poisonings (Ueno, 1983).

To better evaluate the toxicological significance of T-2, it is important to define the nature of its interactions with livestock species that may be exposed to T-2 through the diet. The present study was designed to evaluate the nature of T-2 biotransformations that occur in bovine rumen fluid.

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MATERIALS AND METHODS

Chemicals. Tritiated T-2 toxin was synthesized by Amersham Corp. (Arlington Heights, IL) and provided to us by the U.S. Department of Defense (Fort Detrick, MD). The label was incorporated at C-3, and the specific activity

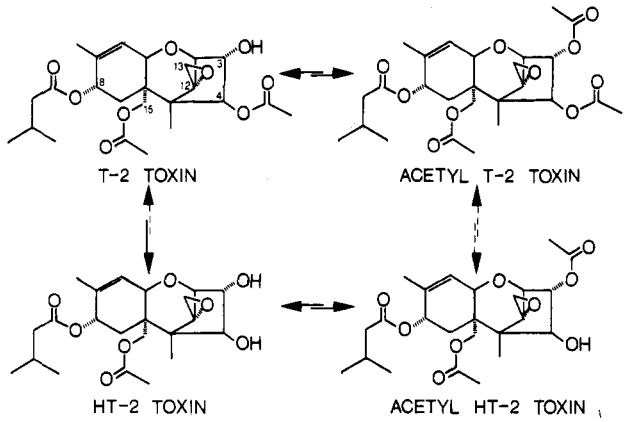

Figure 1. Acetylation/deacetylation reactions of T-2 toxin and related trichothecenes in bovine rumen fluid in vitro.

of the preparation as received was 14.0 Ci/mM. The [^{3}H]T-2 was checked for radiochemical purity by thin-layer chromatography (TLC) in six solvent systems: (A) ethyl acetate–toluene (3:1); (B) chloroform–acetone (3:2); (C) chloroform–methanol (97:3); (D) ethanol–ethyl acetate–acetone (4:4:1); (E) ethanol–ethyl acetate–acetone (1:4:4); (F) ethyl acetate. Radioautography and subsequent liquid scintillation studies (vide infra) showed that the [^{3}H]T-2 was of >98% radiochemical purity in each solvent system. Unlabeled T-2 toxin (>98% purity) was obtained from Sigma Chemical Co. (St. Louis, MO), as was HT-2 toxin (Figure 1), T-2 triol, T-2 tetrol, and acetyl T-2 toxin. Acetyl HT-2 toxin (Figure 1) was synthesized by acetylation of authentic HT-2 according to procedures reported by Yoshizawa et al. (1980). [Note: The trichothecene mycotoxins are highly toxic compounds, with acute oral LD_{50} values generally <10 mg/kg and should be handled with extreme care.] Diisopropyl fluorophosphate (Sigma) was utilized in certain studies as an inhibitor of esterase enzymes.

Animals. Two crossbred fistulated steers (≥500 kg each) were utilized in these studies. Initially, one of the animals was allowed to graze on native grass pasture, and the other was held in a pen and fed approximately 4 kg daily of a 13% protein commercial grain concentrate, as well as coastal Bermuda grass hay ad libitum. Both animals were provided water ad libitum.

Sample Incubation, Extraction, and Analysis. When active rumen fluid was required, the animals were placed in a stanchion and rumen fluid was collected through the fistula and filtered through layered cheesecloth into a prewarmed (~40 °C) themos. Carbon dioxide was slowly bubbled through the rumen fluid in the thermos during the collection procedure to ensure maintenance of an anaerobic environment. After collection, the thermos was tightly capped and brought into the laboratory.

To each of several 50-mL glass screw cap culture tubes (Kimax) was added 20 μL of a methanol solution of [^{3}H]-T-2 (diluted with unlabeled T-2 to a specific activity of 10000 dpm/μg). Aliquots (10 mL) of the fresh rumen fluid were pipetted into the CO_2-flushed tubes and then tightly capped with Teflon-lined screw caps. Enzyme-deactivated controls were run with rumen fluid that had been autoclaved at 115 °C for 0.5 h. To most samples, [^{3}H]T-2 was added to obtain a concentration of 2.5 or 5.0 ppm in the rumen fluid—samples run at toxin concentrations above 10 ppm showed greatly reduced rates of T-2 biotransformation. In some experiments, DFP was added to the incubations (at a concentration in the rumen fluid equivalent to 2×10^{-3} M) to inhibit esterase activity. In quantitative studies, duplicate samples were incubated (in a shaking water bath in the dark at 39 °C) for time intervals of 1, 2, and 4 h, and each experiment was repeated at least twice on different days using different collections of rumen fluid.

After the appropriate incubation periods, samples were immediately extracted by shaking vigorously with benzene (20 mL). The resultant suspension was centrifuged, the solvent was aspirated off, and the extractions were repeated two more times. Radioactivity in the aqueous and combined organic phases was quantitated by LSC (appropriate corrections were made for quench and instrument efficiency); then the organic extracts were concentrated under reduced pressure and finally a gentle stream of nitrogen to ~0.2 mL prior to TLC analysis.

Initial resolution of T-2 and its metabolites from rumen fluid was accomplished by TLC using precoated silica gel chromatoplates (0.25- or 0.5-mm gel thickness, 20 × 20 cm; Merck, Darmstadt, Germany). Plates were developed in one dimension with solvent system A or F (vide supra). The resolved tritiated components were visualized by first spraying the plates with ScintiPrep 2 (Fisher Scientific, Fair Lawn, NJ) and then exposing them to X-ray film (X-Omat; Eastman Kodak Co., Rochester, NY) for, usually, 1 week. The visualized compounds were quantitated by scraping the appropriate gel regions and subjecting them to LSC analysis.

Metabolite Generation on a Milligram Scale. Scaled-up incubations of [^{3}H]T-2 (10000 dpm/μg) were performed in order to obtain sufficient quantities of T-2 metabolites for structural identification and further study. Preliminary studies indicated that 10 ppm T-2 in rumen fluid was the highest toxin concentration that could be utilized while maintaining an appreciable degree of biotransformation of the parent T-2. Thus, rumen fluid was collected as above and incubated as 100-mL samples (in 500-mL Erlenmeyer flasks) with [^{3}H]T-2 at either 5 or 10 ppm. During the incubations (shaking water bath at 39 °C), the CO_2-flushed flasks were covered with rubber balloons to maintain an anaerobic environment while accommodating the slight positive pressure that generally developed during the incubation procedure. After appropriate incubation times (with or without 2×10^{-3} M DFP added and utilizing incubation times up to 24 h depending on the metabolite under emphasis), the samples were extracted with benzene, which was concentrated and subjected to TLC (solvent system A or F). To recover the metabolites from these scaled-up studies, bands of radioactivity were located by sequentially scraping small-width, 0.5-cm, bands from origin to solvent front and then subjecting them to LSC. After the positions of individual metabolites were determined, the appropriate gel region was scraped, eluted with ethyl acetate, and subjected to a further TLC purification using a solvent system of ether–acetone (1:1) (solvent system G). The silica gel containing the metabolite from this final TLC purification was first eluted with hexane to remove possible nonpolar contaminants and then with ethyl acetate to recover the metabolite. The ethyl acetate extract was filtered and then concentrated to dryness. Final isolation was effected by precipitation from hexane or ether–hexane to yield the apparently pure metabolites, which were subsequently analyzed by proton NMR or mass spectroscopy and/or by TLC chromatographic comparison with standards of known structure.

Metabolite Interconversions. The metabolites of [^{3}H]T-2, as isolated from rumen fluid incubations for studies of their chemical nature, were also subjected to direct studies of their further fate in rumen fluid. These

Table I. Metabolic Transformations of [^{3}H]T-2 Toxin in Rumen Fluid of Steers Fed Different Diets[a,b]

	% indicated compd or fraction after 2-h incubn[c]	
	grass fed	grain fed
T-2 toxin	40.3 ± 1.6	54.8 ± 4.5
acetyl T-2	26.2 ± 2.7	15.9 ± 3.0
HT-2	22.0 ± 1.7	17.2 ± 1.1
acetyl HT-2	6.7 ± 1.5	8.4 ± 1.9
unextractable[d]	4.8 ± 1.4	3.7 ± 1.6

[a] All incubations with 2.5 ppm [^{3}H]T-2 at 39 °C. [b] Diisopropyl fluorophosphate not added to the incubation mixtures. [c] $\bar{X}$ ± SD of at least four replicates involving at least two separate experiments. No significant radioactivity was found on other regions of the TLC plates in this or subsequent studies. [d] Tritium remaining in the rumen fluid after benzene extraction.

incubations were at 5.0 ppm toxin concentration, utilizing 10-mL rumen fluid samples. Incubation parameters, replication, extraction, and TLC analyses were in every case the same as those reported above for quantitative studies with [^{3}H]T-2.

Instrumentation. Liquid scintillation studies were done with a Beckman LS 6800 scintillation system and Beckman HP/b scintillation cocktail. Proton NMR studies were done in deuteriochloroform on a Jeol FX-90Q Fourier transform spectrometer. Chemical shifts were recorded (ppm) from tetramethylsilane, with the chloroform signal (7.28 ppm) as an internal standard. Mass spectral studies were performed by direct-insertion probe analysis using a VG 70-250 instrument with electron-impact ionization at 70 eV.

RESULTS

Nature of T-2 Rumen Metabolites. Preliminary studies indicated that [^{3}H]T-2 toxin is converted to three metabolites upon incubation with bovine rumen fluid under the conditions of our study. There was no detectable loss of tritium in these samples, indicating that transformations to volatile products had not occurred to any significant extent. Radioactivity not extracted from the aqueous phases of these samples generally amounted to ≤5% of total tritium added.

Large-scale incubations of [^{3}H]T-2 with rumen fluid in the absence of DFP resulted in the accumulation of a polar metabolite (R_f ~0.15, solvent system A) that was successfully isolated (≥1 mg as a yellow oil) from ether–hexane. This metabolite was identified as HT-2 toxin (Figure 1) on the basis of TLC, mass spectral, and NMR data that were identical with those of authentic HT-2.

The metabolite of least polarity (TLC R_f ~0.69, solvent system A) was found to accumulate in the large-scale incubations only when DFP was added as an esterase inhibitor. This metabolite was successfully isolated (≥1 mg as a yellow oil) from hexane, and its proton NMR spectrum clearly indicated the presence of three apparent acetyl methyl groups in the region of δ 2.1–2.2. The metabolite was suspected to be acetyl T-2 (Figure 1); the authentic compound was obtained commercially and subsequent TLC, mass spectral, and NMR studies showed that the metabolite and authentic acetyl T-2 were indeed identical.

The third T-2 metabolite (R_f ~0.59, solvent system A) did not accumulate to any appreciable extent in any of the large-scale incubation experiments, which were varied with respect to both the presence or absence of DFP and the time of incubation. This metabolite was, however, identified with near certainty as acetyl HT-2 (Figure 1) on the basis of TLC behavior (solvent systems A-F) that was identical with that of synthetic acetyl-HT-2, and on the basis of data from the metabolite interconversion studies (vide infra).

The unreacted parent T-2 from the large-scale incubations (R_f ~0.47, solvent system A) was confirmed as T-2 by TLC cochromatography studies (solvent systems A and B) and mass spectral data.

Dietary Effects on T-2 Rumen Biotransformations. The metabolism of [^{3}H]T-2 by rumen fluid of steers maintained on dissimilar diets did not differ qualitatively (Table I). After 2 h of incubation, however, T-2 levels were lower and acetyl T-2 and HT-2 levels higher in rumen fluid obtained from the pasture-grazed steer. That the differences shown in Table I were not simply normal biological variations between the two animals was evidenced by data from all preliminary dietary effect experiments that showed metabolite distribution trends similar to the data in Table I, including data from the same animal in which diets were switched over periods of several weeks. Given the fact that subsequent studies were to be conducted over a period of several months (in which the pasture composition would inevitably change dramatically), all further quantitative work was done with rumen fluid collected from animals fed exclusively the grain/hay diet.

Effects of Diisopropyl Fluorophosphate. The addition of the esterase inhibitor DFP clearly inhibited acetyl hydrolysis of T-2 to HT-2 (Table II). Without DFP, ~31% of the tritium was as HT-2 after 4 h, levels that were almost 10-fold higher than those observed in the DFP-inhibited samples. DFP greatly enhanced the accumulation of acetyl T-2 but reduced the buildup of acetyl HT-2 (Table II).

The presence of detectable HT-2 in the rumen fluid samples that had been heat deactivated (Table I) indicated that T-2 underwent limited spontaneous hydrolysis of the 4-acetyl moiety under the conditions of study.

Metabolite Interconversions. On the basis of the data in Table II, it seemed clear that T-2 metabolite interconversions were occurring to some appreciable extent during the course of the incubations. The fact that T-2 levels per se did not change to any major extent as incubation time increased (Table II) suggested that T-2 not only was being

Table II. Metabolic Transformations of [^{3}H]T-2 Toxin in Bovine Rumen Fluid Both with and without Added Diisopropyl Fluorophosphate (2×10^{-3} M)[a]

	% indicated compd or fraction at various incubn times[b]						
	T-2 with DFP			T-2 without DFP			
	1 h	2 h	4 h	1 h	2 h	4 h	4 h (deactivated)
T-2 toxin	29.7 ± 16.3	23.4 ± 11.7	23.6 ± 12.5	28.7 ± 9.7	36.0 ± 5.7	34.0 ± 2.3	90.7 ± 2.9
acetyl T-2	59.7 ± 18.5	66.0 ± 12.9	63.7 ± 15.4	49.4 ± 13.5	25.7 ± 11.9	15.2 ± 0.9	0
HT-2	4.3 ± 2.7	4.0 ± 1.4	3.7 ± 2.8	14.0 ± 3.4	24.9 ± 10.3	31.3 ± 5.1	5.2 ± 2.5
acetyl HT-2	2.1 ± 0.3	3.0 ± 0.4	4.5 ± 0.5	5.2 ± 0.4	6.6 ± 4.2	14.2 ± 3.4	0
unextractable[c]	4.2 ± 1.3	3.6 ± 2.8	4.5 ± 2.1	2.7 ± 1.1	6.8 ± 2.9	5.3 ± 3.1	4.1 ± 1.7

[a] All samples incubated with 5.0 ppm [^{3}H]T-2. [b] $\bar{X}$ ± SD of at least four replicates involving at least two separate experiments. [c] Tritium remaining in the rumen fluid after benzene extraction.

Table III. Metabolic Transformations of [^{3}H]HT-2 Toxin in Bovine Rumen Fluid with Added Diisopropyl Fluorophosphate (2×10^{-3} M)[a]

	% indicated compd or fraction at incubn time[b]			
	1 h	2 h	4 h	4 h (deactivated)
T-2 toxin	1.8 ± 0.7	2.0 ± 0.6	4.6 ± 0.8	0
acetyl T-2	1.1 ± 0.3	1.8 ± 0.9	2.1 ± 1.3	0
HT-2	69.8 ± 2.7	64.4 ± 7.3	50.6 ± 14.8	94.5 ± 0.2
acetyl HT-2	17.9 ± 3.1	23.7 ± 6.9	35.4 ± 13.3	0
unextractable[c]	9.4 ± 4.1	8.1 ± 3.7	7.3 ± 2.1	5.5 ± 2.1

[a] All samples incubated with 5.0 ppm [^{3}H]HT-2. [b] $\bar{X}$ ± SD of at least four replicates involving at least two separate experiments. [c] Tritium remaining in the rumen fluid after benzene extraction.

metabolized but was being regenerated as well (either by 3-acetyl hydrolysis of acetyl T-2 or by 4-acetylation of HT-2). Acetyl T-2 can arise by acetylation of either T-2 or acetyl HT-2, while acetyl HT-2 can be formed either by acetylation of HT-2 or by 4-acetyl hydrolysis of acetyl T-2. The data in Table II are, however, equivocal as to which might be the major pathways involved in the formation of these products.

Subsequent studies were undertaken in which radiochemically pure (>98%) preparations of [^{3}H]HT-2, [^{3}H]acetyl HT-2, and [^{3}H]acetyl T-2 (each at ~10 000 dpm/μg) were incubated with rumen fluid under conditions exactly the same as those reported in Table II for [^{3}H]T-2. Results from these studies (Tables III–V) confirmed the facile interconversion of these products in bovine rumen fluid in vitro.

Because preliminary experiments, including incubations of up to 24 h in duration, had indicated that HT-2 itself does not undergo additional degradation reactions in rumen fluid, [^{3}H]HT-2 incubations for quantitation were done only with added DFP. The data (Table III) indicate that acetylation of HT-2 is greatly favored at C-3 (to acetyl HT-2) but also that some acetylation occurs at C-4 (to T-2), as well as lesser amounts of diacetylation to acetyl T-2.

Studies with [^{3}H]acetyl HT-2 in the absence of DFP indicate that acetyl hydrolysis to HT-2 is the major pathway, although acetylation reactions also occur. The appreciable amounts of T-2 generated in these incubations must have arisen through either HT-2 or acetyl T-2 (or both) as intermediates. In the [^{3}H]acetyl HT-2 incubations with added DFP (Table IV), there was a surprising, but replicable, inefficiency in the inhibition by DFP of the hydrolysis of acetyl HT-2 to HT-2. The data in Table IV do, however, strongly suggest that acetyl HT-2 is subjected to both acetylation and deacetylation reactions in rumen fluid.

Data from incubations of [^{3}H]acetyl T-2 with heat-deactivated rumen fluid show that this compound is quite susceptible to nonenzymatic hydrolysis, primarily at C-3 to generate T-2 (Table V). Incubations with active rumen fluid do, however, suggest that enzymatic hydrolysis of acetyl T-2 occurs at both C-3 and C-4, with initial hydrolysis greatly favored at C-3.

DISCUSSION

In an earlier study not utilizing radioisotope methodology, Kiessling et al. (1984) detected only HT-2 as a T-2 metabolite generated by bovine and ovine rumen fluid in vitro. These earlier experiments studied T-2 concentrations in rumen fluid of 20 ppm, levels somewhat higher than those studied here. Our preliminary data confirmed that HT-2 is, in fact, the only T-2 metabolite seen in non-esterase-inhibited rumen fluid when T-2 concentrations are increased above 10 ppm.

The present work has established that, at concentrations in rumen fluid of 5 ppm or below, T-2 is subjected to rapid acetylation/deacetylation reactions that lead to the generation of acetyl T-2, HT-2, and acetyl HT-2. Studies with tritiated preparations of each of these T-2 metabolites show them to be readily interconvertible such that the fate of these compounds in rumen fluid can best be depicted as shown in Figure 1.

The data in Tables II–V allow several conclusions to be drawn regarding the actions of rumen fluid enzymes on these trichothecenes in vitro, although overinterpretation of these data is risky since multiple and competing reactions were occurring: (1) Acetylation is greatly favored at C-3 vs. C-4 as evidenced by studies with HT-2 (Table III). Yoshizawa et al. (1980) have similarly found that certain *Fusarium sp.* efficiently acetylate C-3 but not C-4 of trichothecenes. (2) Deacetylation is likewise favored at C-3 vs. C-4, as evidenced by studies with acetyl T-2 (Table V). (3) The C-3 ester linkage of acetyl T-2 is susceptible to appreciable nonenzymatic hydrolysis, whereas T-2, HT-2, and acetyl HT-2 are relatively stable to nonenzymatic breakdown (Tables II–V). (4) The ester linkages at C-8 and C-15 are apparently stable in rumen fluid, as no evidence was seen to indicate the presence of T-2 triol or T-2 tetrol. (5) The occurrence of comparable levels of unextractable radioactivity in both active and heat-deactivated samples (Table II–V) suggests that this radioactivity may be primarily attributable to incomplete extractions of the identified compounds (or, less likely, to tritium exchange) rather than to metabolism of these trichothecenes to polar, unidentified products.

We saw no evidence that any of these trichothecenes were subjected to reduction of the 12,13-epoxide moiety to an olefin. Epoxide reduction is a major reaction catalyzed by rumen microbes for the related trichothecenes deoxynivalenol and 3-acetyldeoxynivalenol (King et al., 1984), and epoxide reduction of deoxynivalenol occurs to a significant extent in orally treated rats (Yoshizawa et al., 1983). Yoshizawa et al. (1985) have reported that reduction of the 12,13-epoxide of 3′-hydroxy HT-2 toxin and of T-2 tetrol does in fact occur in orally treated rats. It may be that substituent effects (presumably C-4 substitution) sterically or otherwise render the epoxide moiety of certain

Table IV. Metabolic Transformations of [^{3}H]Acetyl HT-2 Toxin in Bovine Rumen Fluid Both with and without Added Diisopropyl Fluorophosphate[a]

	% indicated compd or fraction at various incubn times[b]						
	with DFP			without DFP			
	1 h	2 h	4 h	1 h	2 h	4 h	4 h (deactivated)
T-2 toxin	4.1 ± 1.1	4.7 ± 0.6	5.7 ± 2.0	3.8 ± 1.4	5.1 ± 1.4	8.3 ± 2.2	0
acetyl T-2	2.6 ± 0.8	4.3 ± 2.7	5.1 ± 4.2	1.6 ± 0.5	2.0 ± 0.4	2.5 ± 1.6	0
HT-2	13.2 ± 8.5	21.0 ± 9.5	26.7 ± 5.3	26.3 ± 6.6	42.2 ± 7.0	52.3 ± 11.1	3.0 ± 1.2
acetyl HT-2	77.0 ± 8.3	67.2 ± 7.2	59.3 ± 13.3	64.8 ± 8.2	46.5 ± 9.2	33.0 ± 7.3	94.9 ± 1.4
unextractable[c]	3.1 ± 1.6	2.8 ± 2.1	3.2 ± 2.6	3.5 ± 1.9	4.2 ± 2.3	3.9 ± 1.1	2.1 ± 0.9

[a] All samples incubated with 5.0 ppm [^{3}H] acetyl HT-2. [b] $\bar{X}$ ± SD of at least four replicates involving at least two separate experiments. [c] Tritium remaining in the rumen fluid after benzene extraction.

Table V. Metabolic Transformations of [^{3}H]Acetyl T-2 Toxin in Bovine Rumen Fluid[a,b]

	% indicated compd or fraction at incubn time[c]			
	1 h	2 h	4 h	4 h (deactivated)
T-2 toxin	20.5 ± 4.3	28.5 ± 3.5	38.4 ± 4.5	24.8 ± 0.6
acetyl T-2	67.7 ± 6.9	53.6 ± 7.4	27.0 ± 2.2	67.2 ± 1.0
HT-2	4.4 ± 2.4	10.2 ± 4.4	22.9 ± 4.5	0.6 ± 0.2
acetyl HT-2	2.2 ± 0.6	3.9 ± 0.6	8.3 ± 1.3	3.6 ± 0.5
unextractable[d]	5.2 ± 0.7	3.8 ± 3.3	3.4 ± 2.8	3.8 ± 1.2

[a] All samples incubated with 5.0 ppm [^{3}H]acetyl T-2. [b] Diisopropyl fluorophosphate not added to the incubation mixtures. [c] $\bar{X}$ ± SD of at least four replicates involving at least two separate experiments. [d] Tritium remaining in the rumen fluid after benzene extraction.

of these compounds immune to epoxide reductase attack. Alternatively, epoxide reduction of trichothecenes such as T-2 may indeed occur in the rumen but went undetected under the conditions of our in vitro studies.

The toxicological implications of the acetylation/deacetylation reactions observed with T-2 and related compounds are not clear. T-2 is a potent vertebrate toxin, and its hydrolysis to HT-2 apparently results in only a slight reduction of toxicity (Chi et al., 197; Sato and Ueno, 1977). The toxicological properties of acetyl T-2 and acetyl HT-2 are apparently unknown. Both of these compounds seem to be of greater lipophilicity than T-2, as evidenced by their TLC behavior. Acetyl T-2 in particular is quite lipophilic—we found it to be highly soluble in hexane. The limited available literature suggests that stepwise acetylation of trichothecenes may lead to products of either enhanced or diminished toxicological significance but that the effects are not predictable (Grove and Mortimer, 1969; Ueno, 1977).

The data in Tables II, IV, and V seemingly indicate that HT-2 is the end product of uninhibited rumen metabolism of these trichothecenes, because HT-2 accumulates as incubation time increases. However, we believe that data from our in vitro studies may not be quantitatively representative of the fate of these chemicals in the rumen in situ. With T-2 after 1 h of incubation, acetyl T-2 levels are more than twice those of HT-2 (Table II), indicating that 3-acetylation of T-2 predominates over 4-deacetylation, at least over the short term. Although we have no data on the stability, cofactor requirements, etc., of the rumen fluid acetylation enzymes in vitro, we did observe incidentally that the esterase enzymes are quite stable. Rumen fluid held under refrigeration for several days was found to retain some activity in converting T-2 to HT-2, but it retained no detectable acetylation capacity. Thus, it may well be that, under steady-state conditions in the rumen in situ, acetylation of T-2 (and presumably of other trichothecenes as well) is the predominant reaction, leading to the ultimate absorption of derivatives of considerably enhanced lipophilicity and thus far undetermined toxicity. It would be prudent to extend the data base on the fate of trichothecenes in ruminant systems, to evaluate the toxicity and residual behavior of partially or fully acetylated derivatives of naturally occurring trichothecenes to which ruminants may be exposed, and perhaps also to determine the extent to which identical or similar metabolic reactions may occur in the digestive tracts of other organisms.

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Registry No. T-2, 21259-20-1; HT-2, 26934-87-2; acetyl T-2, 21259-21-2; acetyl HT-2, 34084-03-2; esterase, 9013-79-0.

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