Altered Tissue Amino Acid Metabolism in Acute T-2 Toxicosis (43947)

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Abstract. **T-2 toxin is a Fusarium trichothecene mycotoxin that has been shown to alter brain neurochemistry and eating behavior in animals eating contaminated diets. Experiments were conducted to determine the role of altered tissue amino acid metabolism in the etiology of acute T-2 toxicosis. Fasted weanling rats were orally dosed with 0 or 2.0 mg T-2 toxin/kg body weight. Blood, brain, liver, and muscle tissue were excised 4 and 8 hr after dosing, and amino acid concentrations were determined. Hepatic enlargement coupled with reduced liver concentrations of free small neutral, large neutral, and basic amino acids were seen 4 hr after dosing. Brain and muscle amino acid concentrations were largely refractory to treatment, while the plasma concentrations of tyrosine and lysine, and the sum of the basic amino acids fell. Hepatic amino acid concentrations returned to control levels 8 hr after dosing at which time aminoacidemia was seen. This was due partially to an increase in plasma concentrations of large neutral amino acids including particularly the branched-chain amino acids. A subsequent experiment was conducted to determine the effect of T-2 toxin on ^{14}C-leucine uptake and incorporation into protein in liver slices 4 hr after dosing. Exposure to T-2 toxin reduced total (free + protein-bound) uptake of leucine due primarily to reduced incorporation of leucine into newly-synthesized hepatic protein. It was concluded that reduced amino acid uptake by liver preceded aminoacidemia in acute T-2 toxicosis, although it is not clear how this might influence subsequent changes in brain neurochemistry and behavior.** [P.S.E.B.M. 1995, Vol 210]

Acute doses of the Fusarium trichothecene mycotoxin T-2 toxin have been shown to alter rat brain neurochemistry by sequentially increasing concentrations of tryptophan, serotonin (5-HT) and 5-hydroxy-3-indole acetic acid (HIAA) (1). Such responses are less obvious in chickens (2, 3), although acute doses of deoxynivalenol, also a Fusarium trichothecene, result in similar effects in both rats (4) and swine (5). This increased availability of tryptophan to the brain and subsequent increase in neurotransmitter availability may be the cause of loss of appetite seen in animals suffering from trichothecene mycotoxicoses. Increases in brain tryptophan availability have been suggested as a common pathogenic mechanism for anorexia associated with a variety of diseases (6).

The biochemical mechanism by which T-2 toxin alters brain tryptophan concentration remains to be determined. Oral administration of T-2 toxin can cause extensive pathology in the gastrointestinal tract (7) resulting in decreased absorption of sugars (8) and tryptophan (9). T-2 toxin infused into swine produced a shock-like syndrome resulting in reduced blood flow to the stomach and small intestine (10).

Liver pathology resulting from an inhibition of protein synthesis is also common in T-2 toxicosis. Hepatic drug-metabolizing capacity is reduced (11), while lipid peroxidation increases (12). This can be accompanied by elevated serum glutamate oxaloacetate transaminase and glutamate pyruvate transaminase indicative of hepatic injury (13). Hepatic free leucine concentrations can increase (14), resulting in aminoacidemia (15) and elevated blood concentrations of large neutral amino acids (16).

Experiments were conducted, therefore, to deter-

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mine the effects of acute doses of T-2 toxin on amino acid concentrations and metabolism in tissues that might be important in indirectly influencing brain neurochemistry and subsequent eating behavior.

Material and Methods

Experimental Design. Male weanling Wistar rats (Charles River, Montreal, Canada) housed in individual stainless steel cages with wire floors were used in both experiments. In experiment 1, a total of 40 rats was fed a casein and gelatin-based, semi-purified diet (16) for 4 days before dosing. All animals were fasted 2 hr before 20 were dosed orally with a diet slurry (eight parts water + one part diet) containing 2.0 mg T-2 toxin/kg body wt (Myco-Lab Co., Chesterfield, MO) and 20 were dosed with diet slurry alone. Twenty rats (10 per treatment) were sacrificed under diethylether anaesthesia 4 and 8 hr after dosing. Samples were taken of blood, brain, liver, and muscle for determination of concentrations of free amino acids.

In a second experiment, rats were sacrificed by cervical dislocation 4 hr after dosing, and livers were excised and placed immediately in HEPES-Ringer buffer at 2°C. The right medial lobe of each liver was cut to give slices 1 mm thick. Slices from each liver were immediately incubated in HEPES-Ringer buffer containing amino acids, glucose, and pyruvate (17) at 37°C and flushed with a mixture of 95% O_2 and 5% CO_2.

Amino Acid Analysis. Tissue free amino acid concentrations were determined by high-performance liquid chromatography (18) (Waters Pico-Tag; Waters Scientific, Mississauga, ON).

Determination Of Liver Slice Membrane Integrity. A total of 12 rats were dosed with diet slurry with and without T-2 toxin (6 rats per treatment) and sacrificed 4 hr after dosing. Livers were excised and slices prepared and incubated. After 10, 20, 25, and 30 min of incubation, 200 μl aliquots of incubation medium were removed and measured for lactate dehydrogenase activity (19).

Liver Leucine Uptake Studies. A total of 18 rats were used, with nine controls and nine dosed with T-2 toxin, and six slices were taken from each liver. Slices were incubated for 0 (background), 5, 10, 15, 20, and 30 min in 4 ml of medium containing 1.13 μCi [1-^{14}C]-leucine (50 mCi/mmol; ICN Biochemicals, Inc., Irvine, CA). Following incubation each slice was washed four times with ice-cold HEPES-Ringer buffer, homogenized, sampled and radioactivity was quantified in the sample by liquid scintillation spectrophotometry. A separate sample of homogenate was blended with 5% tricholoracetic acid to precipitate protein and the supernate was collected and radioactivity quantified (20).

Liver Leucine Efflux Studies. Slices prepared in the manner described above were incubated in the presence of radioisotope for 5 min. After this time they were placed in fresh incubation medium without radioisotope and processed as described for uptake studies.

Statistical Analyses. Treatment effects on tissue amino acid concentrations were compared using Student's *t* test (21). In the uptake and efflux studies, differences in the rates of change in radioactivity comparing samples from control and T-2 toxin treated rats were determined by orthogonal polynomial comparisons using regression analysis (22).

Results

Clinical Signs. In the first experiment, rats dosed with T-2 toxin showed some clinical signs of toxicity 4 hr after exposure including arching of the back, piloerection, watery feces, and gastric and intestinal distention. There was no effect of T-2 toxin on brain or spleen weights although hepatic enlargement was seen 4 and 8 hr after dosing (Table I). At the shorter interval, this was accompanied by reduced liver water and protein content.

Tissue Free Amino Acid Concentrations 4 Hr after Dosing. There were decreased concentrations of asparagine, serine, tryptophan, methionine, tyrosine, phenylalanine, histidine, threonine, valine, leucine, ornithine and lysine in livers of rats dosed with T-2 toxin when compared to controls 4 hr after dosing (Table II). This included the sum of the large neutral amino acids (tryptophan, methionine, tyrosine, phenylalanine, isoleucine, histidine, threonine, valine, and leucine [23]) and basic amino acids (ornithine, arginine and lysine [24]) (Fig. 1). The same overall trend was noted even when amounts of amino acids were expressed on a whole liver basis to account for hepatic enlargement in rats dosed with T-2 toxin. Amino acid concentrations in other tissues were less affected by treatment. Dosing with T-2 toxin reduced the sum of the basic amino acids in plasma and also reduced concentrations of tyrosine and lysine. Although dosing did not influence concentrations of individual amino acids in brain, the ratio of tryptophan to the sum of the large neutral amino acids was reduced. Concentrations of amino acids in muscle were refractory to treatment.

Tissue Free Amino Acid Concentrations 8 Hr after Dosing. Exposure to T-2 toxin had no effect on amino acid concentrations in liver or muscle 8 hr after dosing (Table III). Brain concentrations of leucine increased and the ratio of tryptophan to the sum of the large neutral amino acids was no longer reduced. The largest changes 8 hr after dosing were seen in plasma (Fig. 2). There were increases in the concentration of histidine, aspartic acid, asparagine, proline, methionine, isoleucine, histidine, threonine, valine, and leucine, and in the sum of the large neutral amino acids

Table I. Effect of Acute T-2 Toxicosis on Rat Tissue Size and Composition

	Control ± SEM	T-2 Toxin + SEM
Liver weight/body weight (%)		
4 hr after dosing	3.67 ± 0.17 (10)[a]	4.45 ± 0.11[b] (10)
8 hr after dosing	3.58 ± 0.11 (10)	4.10 ± 0.17[c] (10)
Liver protein content (mg protein/g liver)		
4 hr after dosing	152.32 ± 10.53 (10)	121.06 ± 9.60[d] (9)
8 hr after dosing	151.02 ± 11.24 (9)	151.12 ± 12.06 (10)
Liver water content (%)		
4 hr after dosing	71.20 ± 0.57 (10)	68.80 ± 0.49[c] (10)
8 hr after dosing	71.50 ± 0.40 (10)	71.00 ± 0.33 (10)
Spleen weight/body weight (%)		
4 hr after dosing	0.43 ± 0.02 (10)	0.41 ± 0.02 (10)
8 hr after dosing	0.40 ± 0.03 (10)	0.37 ± 0.03 (10)
Brain weight/body weight (%)		
4 hr after dosing	1.25 ± 0.03 (10)	1.29 ± 0.04 (10)
8 hr after dosing	1.26 ± 0.03 (10)	1.25 ± 0.04 (10)

[a] Numbers in parentheses indicate *n*.
[b] $P < 0.001$ compared with controls.
[c] $P < 0.01$ compared with controls.
[d] $P < 0.05$ compared with controls.

Table II. Effect of Acute Doses of T-2 Toxin on Tissue Amino Acid Concentrations 4 hr after Dosing

Amino acid	Plasma (μmol/ml)		Liver (μmole/g)		Brain (μmol/g)		Muscle (μmole/g)	
	Control	T-2	Control	T-2	Control	T-2	Control	T-2
Glutamic acid	0.401	0.305	4.311	4.840	6.500	6.160	2.935	2.147
Aspartic acid	0.063	0.047	1.175	1.064	1.245	1.114	0.194	0.121
Serine	0.633	0.483	4.586	2.950[a]	0.746	0.762	0.989	1.209
Asparagine	0.117	0.130	1.528	0.975[a]	0.135	0.122	0.313	0.241
Proline	0.312	0.342	2.755	1.664	0.077	0.082	0.392	0.404
Tyrosine	0.190	0.080[b]	1.439	0.596[b]	0.134	0.113	0.229	0.286
Histidine	0.069	0.076	1.792	1.012[a]	0.048	0.039	0.913	0.807
Threonine	0.241	0.304	2.406	1.552[a]	0.230	0.282	0.474	0.343
Valine	0.261	0.324	2.903	1.762[a]	0.146	0.160	0.364	0.378
Methionine	0.085	0.089	1.380	0.875[a]	0.046	0.048	0.264	0.206
Isoleucine	0.227	0.212	1.609	1.143	0.045	0.042	0.492	0.403
Leucine	0.255	0.284	3.523	2.259[a]	0.038	0.042	0.340	0.353
Phenylalanine	0.105	0.085	1.541	0.968[a]	0.050	0.043	0.258	0.272
Tryptophan	0.025	0.022	0.369	0.216[a]	0.011	0.009	0.063	0.054
Ornithine	0.062	0.065	2.109	1.093[a]	0.020	0.021	0.065	0.051
Lysine	0.620	0.279[b]	4.106	2.186[b]	0.245	0.310	0.848	1.266
Arginine	0.161	0.136	0.272	0.115	0.106	0.109	0.343	0.343
Glycine	1.029	0.758	8.022	6.069	0.990	0.951	5.411	7.000
Glutamine	1.372	1.568	10.920	9.280	0.088	0.084	3.693	3.070
Alanine	0.980	0.868	13.768	9.796	0.614	0.633	3.084	3.234
SEM	0.052	0.030	0.478	0.278	0.010	0.011	0.058	0.084

Note. Values are means ($n = 10$).
[a] Significantly different from control, $P < 0.05$.
[b] Significantly different from control, $P < 0.01$.

and the sum of the branched-chain amino acids. The concentrations of plasma tryptophan and the ratio of tryptophan to the sum of the large neutral amino acids, however, were not affected by treatment.

Effect of Acute T-2 Toxicosis on Liver Slice Membrane Integrity. There was no effect of treatment on hepatic lactate dehydrogenase activity when measured in liver homogenates obtained 4 hr after dosing. As the duration of slice incubation increased there was a linear increase in lactate dehydrogenase activity in the incubation medium with no significant difference in slopes comparing livers from control animals (0.427) to those from animals dosed with T-2 toxin (0.486).

Effect of Acute T-2 Toxicosis on Leucine Uptake by Liver Slices. There was linear uptake of ^{14}C-leucine by liver slices regardless of treatment (Fig. 3). The rate of uptake, however, was depressed in slices from animals exposed to T-2 toxin ($P < 0.05$). This was not due to differences in the rate of accumulation

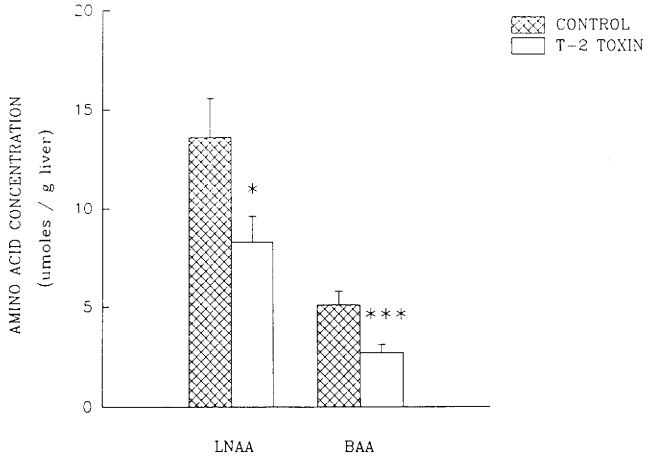

Figure 1. Concentrations of large neutral amino acids (LNAA) and basic amino acids (BAA) in livers of rats dosed with and without T-2 toxin. Values are means (n = 10) ± SEM and were compared with controls (*$P < 0.05$, ***$P < 0.001$).

of free ^{14}C-leucine ($P > 0.05$) but was caused by T-2 toxin depressing the incorporation of ^{14}C-leucine into liver protein ($P < 0.01$).

Effect of Acute T-2 Toxicosis on Leucine Efflux from Liver Slices. The efflux of ^{14}C-leucine from liver slices followed a quadratic curve regardless of treatment. Exposure to T-2 toxin had no effect of the rate of efflux (Fig. 4).

Discussion

The clinical signs seen 4 hr after dosing are typical of acute T-2 toxicosis especially gastric and intestinal distention (25). The lack of effect of an acute dose of T-2 toxin on brain weight has previously been reported (1). Although there was a trend towards splenic atrophy 4 and 8 hr after dosing, this was not as significant as that reported in the literature in experiments with mice (26) and chickens (27). Hepatic enlargement and reduced hepatic protein content following T-2 toxin administration to rats were also noted by Suneja *et al.* (28). Tissue water content is often positively correlated with protein content as was seen in the current study and negatively correlated with lipid content. Hepatic lipid content of rats has been seen to increase in T-2 toxicosis (29), and this is likely due to reduced lipid mobilization resulting from impaired lipoprotein synthesis. Such an effect has been reported in aflatoxicosis (30).

Amino acid concentrations were altered mainly in the liver in the immediate postdosing period (4 hr). One possibility for the decline in hepatic amino acid concentrations is a decreased amino acid uptake from both the portal vein and arterial blood, although the period of fasting before dosing should minimize the effect of dietary amino acids. The effect seen was not due to dilution of amino acid pools arising from hepatic enlargement, as the effect was still present even when values were corrected for liver weight.

The results of the second experiment support this hypothesis, since total leucine uptake was reduced by liver slices from rats dosed with T-2 toxin 4 hr previously. The lack of effect of T-2 toxicosis on hepatic slice lactate dehydrogenase activity indicates that slice

Table III. Effect of Acute Doses of T-2 Toxin on Tissue Amino Acid Concentrations 8 hr after Dosing

Amino acid	Plasma (μmol/ml)		Liver (μmole/g)		Brain (μmol/g)		Muscle (μmole/g)	
	Control	T-2	Control	T-2	Control	T-2	Control	T-2
Glutamic acid	0.199	0.233	6.179	4.755	7.265	8.530	2.148	1.905
Aspartic acid	0.022	0.046[a]	1.386	0.978	1.302	1.592	0.111	0.116
Serine	0.465	0.584	4.156	5.217	0.957	1.197	1.002	1.000
Asparagine	0.125	0.190[b]	1.150	1.892	0.157	0.169	0.302	0.210
Proline	0.215	0.281[b]	2.458	2.990	0.086	0.091	0.336	0.289
Tyrosine	0.131	0.131	0.954	1.507	0.120	0.162	0.224	0.241
Histidine	0.052	0.065[b]	1.401	1.537	0.072	0.118	0.600	0.727
Threonine	0.201	0.386[b]	2.463	2.717	0.406	0.393	0.476	0.344
Valine	0.252	0.443[a]	2.882	3.378	0.187	0.263	0.392	0.327
Methionine	0.071	0.096[a]	1.328	1.522	0.052	0.067	0.180	0.174
Isoleucine	0.137	0.262[a]	1.808	2.251	0.070	0.078	0.363	0.437
Leucine	0.206	0.353[a]	3.479	4.253	0.113	0.070[b]	0.342	0.308
Phenylalanine	0.062	0.072	1.452	1.853	0.069	0.089	0.219	0.233
Tryptophan	0.017	0.013	0.297	0.396	0.014	0.019	0.058	0.046
Ornithine	0.052	0.076	1.883	1.991	0.029	0.036	0.075	0.037[b]
Lysine	0.367	0.320	3.439	3.992	0.334	0.514[b]	1.221	0.910
Arginine	0.099	0.120	0.065	0.085	0.136	0.153	0.471	0.363
Glycine	0.660	0.720	7.749	8.062	1.092	1.328	5.687	4.747
Glutamine	0.989	1.250[b]	9.266	9.728	0.098	0.108	5.687	5.275
Alanine	0.673	0.804	11.476	14.315	0.756	0.913	3.178	2.368
SEM	0.025	0.028	0.470	0.522	0.082	0.124	0.108	0.071

Note. Values are means (n = 10).
[a] Significantly different from control, $P < 0.01$.
[b] Significantly different from control, $P < 0.05$.

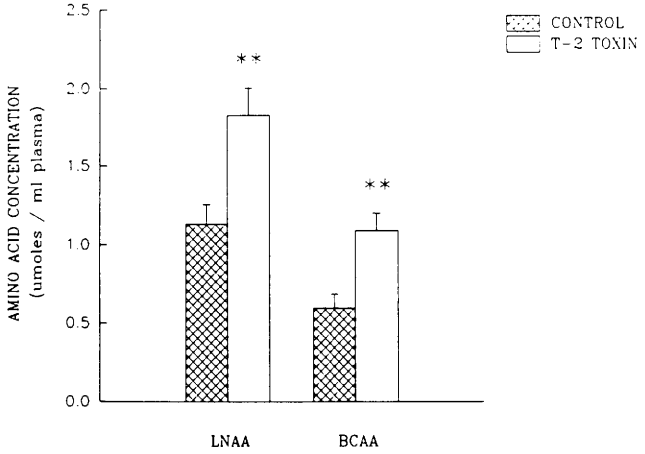

Figure 2. Concentrations of large neutral amino acids (LNAA) and branched-chain amino acids (BCAA) in plasma of rats dosed with and without T-2 toxin. Values are means (n = 10) ± SEM and were compared with controls (**$P < 0.01$).

membrane integrity was not influenced by treatment. The slice studies also clearly show that the reduced uptake was correlated with reduced incorporation of leucine in protein. There was almost no ^{14}C found in protein of slices from animals dosed with T-2 toxin. This could result in reduced synthesis of amino acid transport proteins contributing to reduced uptake. Increased breakdown of transport proteins could also occur. There was very little selectivity in the reduction in amino acid concentrations, moreover, indicating that the inhibition of transport was a general phenomenon.

The reduced hepatic amino acid concentrations 4 hr after dosing were partially reflected in plasma amino acid concentrations in which the sum of the concentrations of the basic amino acids was reduced. This may have altered brain amino acid uptake leading to an increase in brain leucine concentration which was enough to reduce the brain ratio of tryptophan to large neutral amino acids. The lack of effect of treatment on amino acid concentrations in muscle, the largest body reserve of protein, further suggests that the liver is likely the organ with the greatest influence on blood amino acid concentrations in T-2 toxicosis.

Eight hours after dosing, however, hepatic amino acid concentrations had largely recovered and aminoacidemia was seen. This may have been facilitated by increased blood flow as the shock-like syndrome caused by T-2 toxin (10) ebbed and hepatic amino acid concentrations and protein synthesis returned to normal.

The aminoacidemia seen 8 hr after dosing was mainly due to increased concentrations of large neutral amino acids including all of the branched-chain amino acids. This is in agreement with an earlier report (16). Plasma tryptophan concentrations changed little, however, resulting in a decrease in the ratio of tryptophan to large neutral amino acids. It is not obvious how

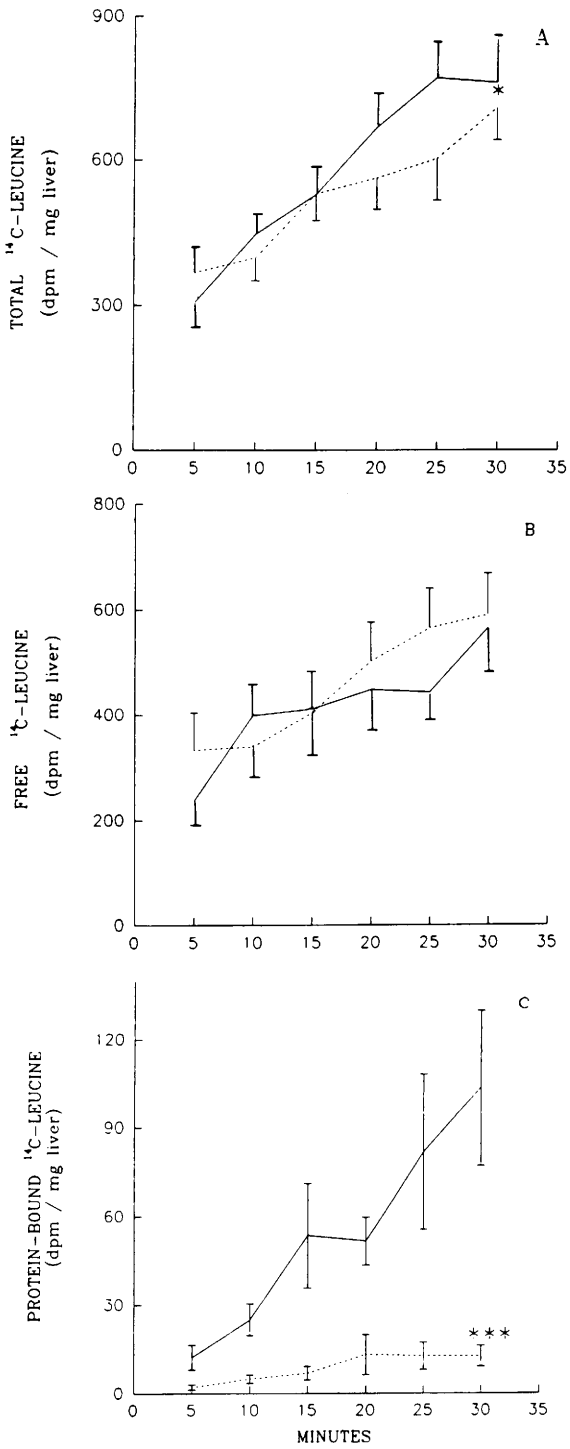

Figure 3. Uptake of total ^{14}C-leucine (A), free ^{14}C-leucine (B), and protein-bound ^{14}C-leucine (C) by liver slices of rats dosed with (· · · ·) and without (—) T-2 toxin. Values are means (n = 9) ± SEM, and rates were compared with controls (*$P < 0.05$, ***$P < 0.001$).

such changes in blood amino acid concentrations could have resulted in the increase in the brain ratio of tryptophan to large neutral amino acids when compared to values 4 hr after dosing. This is particularly so in light of the competition between tryptophan and the large neutral amino acids for transport across the

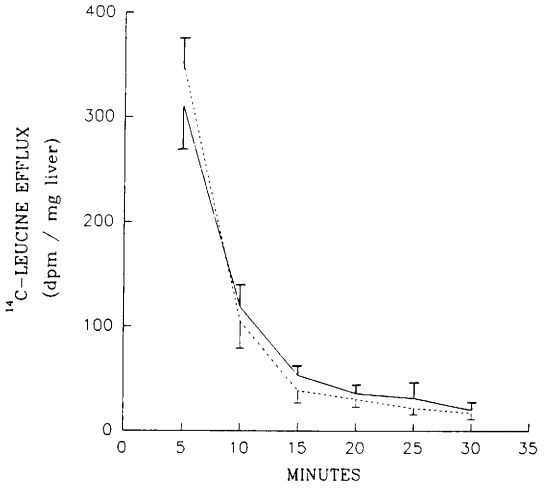

Figure 4. Efflux of ^{14}C-leucine from liver slices of rats dosed with (· · · ·) and without (—) T-2 toxin. Values are means (n = 9) ± SEM, and rates were compared with controls ($P > 0.05$).

blood-brain barrier (23). A possibility, however, is that metabolism of tryptophan to serotonin was reduced during this phase of T-2 toxicosis.

It was concluded that in the early phase after acute exposure to T-2 toxin, amino acid metabolism, in the organs tested, was most affected in the liver. This was due to a marked decrease in hepatic amino acid uptake caused by reduced protein synthesis. As the animals recovered, hepatic amino acid metabolism returned to normal resulting in aminoacidemia. Brain concentrations of tryptophan, however, were not obviously influenced by blood amino acid concentrations and the effect of altered hepatic amino acid metabolism on subsequent eating behavior is not clear.

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