Subacute Toxicity of Dietary T-2 Toxin in Mice: Influence of Protein Nutrition

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ABSTRACT

The subacute toxic effects of dietary T-2 toxin (20 ppm) incorporated in semi-purified diets of 8%, 12% or 16% protein, were examined in young Swiss mice after one, two, three and four weeks. Dietary T-2 toxin caused substantial reductions in growth and food consumption, the degrees of which were greatest in mice fed the diets of reduced protein content. T-2 toxin consistently caused similar degrees of nonregenerative anemia, lymphopenia, thymic atrophy and gastric hyperkeratosis irrespective of the dietary protein level. However, erythroid hypoplasia was temporary in mice fed T-2 toxin in the 16%-protein diet such that erythroid precursors regenerated in splenic and bone marrow and were hyperplastic after four weeks. Liver to body weight ratios of mice fed T-2 toxin in the 16%-and 12%-protein diets increased during the four week trial in comparison to control mice fed at a similar rate. These observations indicated that suppression of erythropoiesis in mice by dietary T-2 toxin was temporary and that the interval before regeneration was prolonged by diets of reduced protein content.

RÉSUMÉ

Cette expérience consistait à vérifier, chez des jeunes souris suisses, au bout d'une, deux, trois et quatre semaines, les effets toxiques subaigus de la présence de 20 ppm de toxine T-2 dans des diètes semi-purifiées qui contenaient respectivement 8%, 12% et 16% de protéines. Cette toxine entraîna des réductions substantielles de la croissance et de la consommation alimentaire, lesquelles s'avérèrent plus marquées chez les souris dont la diète contenait le plus faible pourcentage de protéines; elle provoqua aussi constamment un certain degré d'anémie non régénératrice, de lymphopénie, d'atrophie thymique et d'hyperkératose stomacale, indépendamment du pourcentage de protéines de la diète. L'hypoplasie érythroïde se révéla cependant transitoire chez les souris, lorsqu'on incorporait la toxine T-2 à la diète qui contenait 16% de protéines, de telle sorte que les précurseurs érythroïdes régénérèrent dans la pulpe splénique et la moelle osseuse, au bout de quatre semaines. Le rapport entre le poids du foie et le poids total des souris qui recevaient la toxine T-2 dans les diètes qui contenaient 12% et 16% de protéines, augmenta au cours des quatre semaines que dura l'expérience, comparativement aux souris témoins. Les résultats de cette expérience démontrèrent que la suppression de l'hémopoïèse, attribuable à l'ingestion de toxine T-2 par des souris, était temporaire et que l'intervalle qui précédait la régénération se trouva prolongé lorsque la diète contenait moins de protéines.

INTRODUCTION

Trichothecene mycotoxins have been considered to be one of the most important groups of mycotoxins in the temperate climatic zones of Asia, Europe and North America (24). This view has arisen from the knowledge that trichothecenes may be produced by *Fusarium* and other species of fungi growing on cereal products under cold, moist conditions (24). Trichothecenes, especially T-2 toxin, have been associated with lethal mycotoxicoses such as alimentary toxic aleukia of man in which suppression of hematopoiesis is a major toxic effect (9, 11, 24). More recently, T-2 toxin has been implicated in mycotoxicoses of poultry (5, 26) and swine (5) charac-

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Submitted May 30, 1979.

terized by poor growth, food refusal, vomition, perioral dermatitis and inflammation of the upper alimentary tract. In these mycotoxicoses, effects on hematopoiesis have not been reported (5, 26), nor have they been experimentally reproduced in animals fed T-2 toxin in the diet (3, 25). Repeated doses of T-2 toxin (11, 20, 24) or other trichothecenes (22) will suppress hematopoiesis in some species, especially cats (11, 20). However, because trichothecene mycotoxins inhibit voluntary food consumption (24), effects produced by nondietary exposure may not resemble effects produced under natural exposure.

Mice fed T-2 toxin for six weeks in a balanced, semipurified diet containing 16% protein developed hematopoietic suppression manifested as nonregenerative anemia, as described previously (7). During continuous exposure, hypoplastic hematopoietic tissues regenerated after several weeks of suppression, but lymphoid tissues remained hypoplastic. Because optimally formulated diets containing T-2 toxin were used in studies in which depression of hematopoiesis was not observed (3, 25), we also examined the influence of suboptimal protein nutrition on the subacute toxicity of dietary T-2 toxin in mice to determine if low protein diets would enhance the toxicity of T-2 toxin to the hematopoietic tissues. Outbreaks of alimentary toxic aleukia have been much less severe in human populations consuming moldy grain in balanced diets (9), and variation in nutritional composition and quality of the diet may modify biological detoxification of many toxicants (13) including mycotoxins such as aflatoxin (14). This report describes the influences of the dietary protein level on subacute dietary T-2 toxicosis of young Swiss mice.

MATERIALS AND METHODS

ANIMALS AND CARE

Male weanling outbred Swiss mice[1] were housed in screen-top cages on soft wood shavings in a room maintained at 21°C with 12 hours of fluorescent lighting per day and were managed according to the guidelines of the Canadian Council on Animal Care. Mice were supplied with tap water *ad lib* and with semipurified diets (Table I) at three protein levels (8%, 12% and 16% as casein and gelatin). The diets were prepared weekly as a moist gelatin bound cake, stored under refrigeration and freshly supplied each day in overhead feeding racks.

EXPERIMENTAL DESIGN

A total of 192 mice weighing 16.0 ± 1.2 g were randomly assigned to groups of four per cage. For each of the three dietary protein levels, mice were arranged in experimental groups according to the design in Table II. The principal groups (A), of 20 mice each, were supplied *ad lib* with the semipurified diets containing

TABLE I. Composition of Semipurified Mouse Diet[a]

Basic Diet Ingredients	Gram per kilogram
α-cellulose	50.0
gelatin	20.0
l-methionine-supplemented casein[b]	140.0[c]
carbohydrate mixture[d]	590.0[c]
fat mixture[e]	100.0
vitamin mixture[f]	50.0
mineral mixture[g]	50.0
Composition of prepared diet	**Proportions**
basic diet	100.0 g
water	70.0 mL
ethanol (± T-2 toxin 10 mg/mL)	0.2 mL

[a]Modified from John and Bell. Part of the protein content was provided as gelatin (2%). Ethoxyquin and tetracycline were deleted. Iron content was increased by 50% and menadione increased by 100%

[b]Vitamin-free casein, 96.9%; l-methionine, 3.1%

[c]Proportions shown are for a protein level of 16% (approx). This level is varied by interchanging l-methionine-supplemented casein with carbohydrate mixture

[d]Cornstarch, 50%; dextrose, 30%; sucrose, 20%

[e]Lard, 60%; sunflower oil, 40%

[f]Vitamin mixture components (g/kg): thiamin HCL, 1.0; riboflavin, 2.0; d-Ca pantothenate, 1.0; nicotinic acid, 0.5; α-biotin, 0.02; pyridoxine HCL, 0.1; folic acid, 0.05; vitamin B_{12}, 0.0005; meso-inositol, 0.04; choline chloride, 80; retinyl palmitate (Vitamin A, 500,000 IU/g), 0.2; cholecalciferol (Vitamin D_3, 4 x 10^7 IU/g) 0.0001; dl-α-tocopherol acetate (Vitamin E, 1,000 IU/g), 2.8; menadione, 8.0; sunflower oil, 110.0; cornstarch, 794.3

[g]Mineral mixture components (g/kg): NaCl (iodized), 40.0; $CaHPO_4.2H_2O$, 687.0; $KHCO_3$, 136.0; MgO, 18.0; $MnSO_4.H_2O$, 3.0; $FeSO_4H_2O$, 4.5; $CuSO_4.5H_2O$, 1.0; ZnO, 1.0; cornstarch, 109.5

[1]Canadian Breeding Farms and Laboratories, St. Constant-Laprairie, Québec.

TABLE II. Experimental Design Used for Examination of the Influence of Dietary Protein on Subacute Toxicity of Dietary T-2 Toxin (20 ppm) in Young Swiss Mice

Group[a]	Treatment	Number of mice examined				
		Day 0	Day 7	Day 14	Day 21	Day 28
A	Semipurified diets containing T-2 toxin (20 ppm)		4 x 3[b]	4 x 3	4 x 3	4 x 3
B	Semipurified control diets — restricted intake		4 x 3	4 x 3	4 x 3	4 x 3
C	As for group A for three weeks, then as for group B					4 x 3
D	Semipurified control diets — *ad lib*					8 x 3
E	Pelleted natural-ingredient mouse diet[c]	12				8

[a]Groups A, B, C and D were repeated for each of the three dietary protein levels, 8%, 12% and 16%
[b]Four mice from each of the three diets
[c]Department of Animal Science, University of Saskatchewan

purified crystalline T-2 toxin[2] at a rate of 20 μg/g of dry diet (20 ppm). Matching control groups (B) of 20 mice were supplied with toxin-free diets at a restricted rate such that cages of control mice were given an amount of diet similar to that consumed by a corresponding cage of mice fed the toxic diet.

The effect of withdrawal of the toxin was examined in groups of four mice that were removed from the respective toxic diets after three weeks and fed the corresponding control diet for one week at the rate at which the toxic diet had been consumed during the week before transfer. Groups D each contained eight mice which were supplied with the control diets *ad lib*. Group E consisted of 20 mice, 12 of which were examined on day 0, and eight which were fed *ad lib* for four weeks on a pelleted, natural-ingredient, balanced laboratory mouse ration containing 18.6% crude protein.

The experiment was conducted over a four week period, with mice being examined by necropsy according to the schedule in Table II. Mice remaining in groups A and B for the 8%- and 12%- protein diets after four weeks were discarded. Mice on the 16%-protein level in this design were also used for the six week morphological and hematological study described in the previous paper (10).

EXPERIMENTAL OBSERVATIONS

All mice in the experiment were inspected at least once daily and, at weekly intervals, were examined individually and weighed. The daily food consumption was measured for all groups (except E) and average weekly food consumption rates for mice in each cage were determined. At weekly intervals, according to the schedule in Table II, mice were selected for hematological and necropsy examination.

Packed cell volume (PCV), erythrocyte count (RBC), hemoglobin concentration (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and total leukocyte count (WBC) of peripheral blood were determined[3] from blood from the tail. Blood smears stained with Wright's-Giemsa were used for morphological evaluation and for differential leukocyte counts, and blood was stained with methylene blue for determination of reticulocyte counts. At necropsy, fresh weights of liver, stomach, spleen, thymus and testes were determined and imprint smears of tibial bone marrow were stained with Wright's-Giemsa. Various tissues were selected for histological examination, as described in the previous paper (7).

ANALYSIS OF DATA

Continuous variables from groups A and B in this four week study were analysed in a three-way analysis of variance with toxin, dietary protein and time as factors.

[2]Makor Chemicals, Jerusalem, Israel.

[3]Coulter S, Coulter Electronics Inc., Hialeah, Florida.

The influence of diet on significant toxic effects was assessed by a two-way analysis of variance of data from the toxin-treated groups. Intergroup comparisons between 28-day means of groups A, B, C and D, for each dietary protein level were made using one-way analyses of variance and Student-Newman-Keuls' multiple range tests. Selected comparisons between means of toxin-treated groups and the corresponding control groups were made by two-tailed Student's t tests. The 95%-probability level was used to determine significance of factor effects and differences between means. All analyses and computations were as described by Nie *et al* (15).

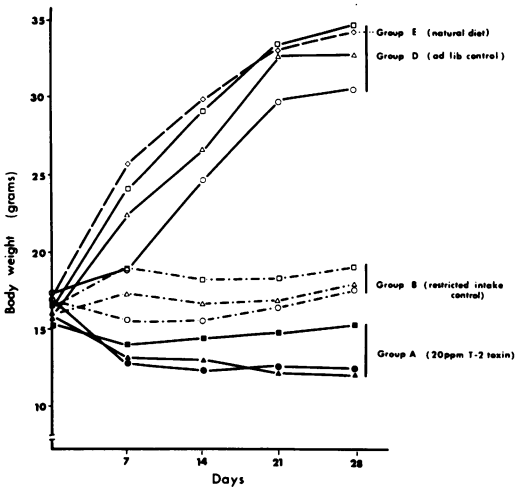

Fig. 1. Growth curves of mice fed semipurified diets of 16% protein (squares), 12% protein (triangles), 8% protein (circles) or a natural ingredient diet of 18.5% protein (diamonds). Points are means of all mice on the respective diets at each time.

RESULTS

Similar hematological and morphological changes were observed in all mice fed T-2 toxin. Hematological and morphological changes, including nonregenerative anemia, neutrophilia, generalized lymphoid depletion, atrophy of hematopoietic tissue in bone marrow and spleen, perioral dermatitis, hyperplasia with ulceration of the squamous gastric mucosa and hepatomegaly have been described in the previous paper (7). The present report deals with effects of T-2 toxin on growth and feed consumption and with the influence of dietary protein on the various toxic effects.

Significant diet-related differences in growth rates of mice consuming T-2 toxin occurred (Fig. 1), but a similar influence of dietary protein level on growth occurred in the restricted-intake (B) and in the *ad lib* fed control groups (D). Diet consumption rates were lower in mice fed T-2 toxin in the two low protein diets, but this difference was related to differences in body weight, because consumption of toxic diets per gram of body weight did not differ significantly ($p = 0.79$) among the three diet groups (Table III). T-2 toxin substantially reduced food consumption by mice; *ad lib* fed controls consumed about two to three times as much as mice on toxic diets (Table III). Mice discarded toxic diets into their bedding, especially during the first two weeks.

No deaths occurred in mice fed T-2 toxin in the 16%-protein diet, nor in any of the control groups, but four mice fed T-2 toxin in the diets of reduced protein content died during the experiment (Table IV). Of these, one on the 12%-protein diet died on day 7 with intestinal hemorrhage, perioral dermatitis and stomatitis. Two died (days 19 and 25) from the effects of severe perioral dermatitis, whereas one other which died was extremely anemic (day 24).

Mice fed T-2 toxin developed dry ruffled fur and some developed exudative dermatitis around the mouth, or less frequently on the feet. Many developed dry, scaley skin on the tail, and pallor of the ears, eyes and muzzle was evident in some during the later stages of the trial. These effects occurred less frequently in mice fed T-2 toxin in the 16%-protein diet (Table IV).

The dietary protein level did not significantly influence ($p<0.05$) the degree of anemia (Fig. 2), reticulocytopenia (Fig. 3), nor lymphopenia (Fig. 4). However, two mice on the 12%-protein diet became much more severely anemic than others in the same group (see Fig. 2). T-2 toxin also caused eosinopenia, neutrophilia, thymic atrophy and gastric hyperkeratosis in all groups. The severity and rate of development of these effects was not influenced by the dietary protein level and were similar to those described for the 16%-protein diet in the previous paper (7). Liver weights, both absolutely ($p =$

TABLE III. Mean Rates of Diet Consumption of Mice Fed Semipurified Diets Containing T-2 Toxin (20 ppm) and Different Levels of Protein

Diet	Group	Week 1	Week 2	Week 3	Week 4
8% protein	A (20ppm T-2)	11.3 ± 0.1[a] 1.4 ± 0.1[b]	8.3 ± 1.8 1.0 ± 0.3	9.1 ± 0.9 1.2 ± 0.2	13.1 ± 3.2 1.5 ± 0.3
	B (restricted control)	15.2 ± 0.5 2.4 ± 0.0	11.5 ± 0.6 1.9 ± 0.0	13.6 ± 0.8 2.0 ± 0.0	11.9 ± 0.7 1.9 ± 0.0
	D (*ad lib* control)	22.7 ± 1.7 4.2 ± 0.1	18.5 ± 0.3 4.5 ± 0.0	14.3 ± 0.1 4.2 ± 0.1	13.7 ± 0.4 4.1 ± 0.2
12% protein	A (20ppm T-2)	10.8 ± 1.4 1.4 ± 0.2	10.4 ± 1.1 1.5 ± 0.2	12.9 ± 0.6 1.5 ± 0.2	11.5 ± 3.7 1.3 ± 0.4
	B (restricted control)	12.3 ± 0.3 2.4 ± 0.1	12.2 ± 0.6 1.9 ± 0.0	11.9 ± 0.6 2.0 ± 0.0	11.7 ± 0.5 2.0 ± 0.0
	D (*ad lib* control)	18.0 ± 0.4 4.0 ± 0.0	15.7 ± 1.1 4.4 ± 0.1	12.0 ± 0.1 3.9 ± 0.0	12.2 ± 0.5 4.0 ± 0.1
16% protein	A (20ppm T-2)	13.4 ± 0.8 1.9 ± 0.1	10.1 ± 2.0 1.4 ± 0.3	13.9 ± 1.3 2.0 ± 0.2	12.4 ± 1.2 1.9 ± 0.3
	B (restricted control)	13.3 ± 0.3 2.5 ± 0.1	10.8 ± 0.2 2.0 ± 0.1	12.0 ± 1.1 2.0 ± 0.0	11.1 ± 0.1 2.0 ± 0.0
	D (*ad lib* control)	17.0 ± 0.1 4.1 ± 0.1	15.3 ± 0.8 4.4 ± 0.2	12.6 ± 1.0 4.2 ± 0.4	10.1 ± 0.6 3.5 ± 0.3

[a]Mean consumption (± SEM) expressed as g/100g body weight/day
[b]Mean consumption (± SEM) expressed as g/mouse/day
Over the entire experiment, diet consumption rates of groups A and B were not significantly different (P = 0.79)

TABLE IV. Toxic Effects Observed in Mice Fed T-2 Toxin (20 ppm) in Semipurified Diets of Different Protein Levels

Effects	Day	8% protein diet	12% protein diet	16% protein diet
Mortality		1/15[a] (day 19) 1/6 (day 25)	1/24 (day 7) 1/6 (day 24)	none
Perioral dermatitis	14	5/20	4/19	1/20
	21	9/14	11/15	4/16
Scaliness of tail skin	14	5/20	6/19	2/20
	21	11/14	10/15	10/16

[a]Values are ratios of number affected to number of mice remaining on the respective toxic diet

0.01) and as percentages of body weight (p = 0.04), were significantly greater in mice fed the two highest dietary protein levels (Fig. 5). Organ to body weight ratios of testes, thymus, stomach and spleen of mice consuming T-2 toxin did not differ significantly among groups consuming the different toxic diets.

Several quantitative and qualitative differences in the characteristic lesions induced by T-2 toxin were apparent among the groups fed different levels of protein. Mice fed T-2 toxin in each of the three diets initially developed atrophy of lymphoid, granulopoietic and erythropoietic populations in the spleen. By two weeks, myeloid and megakaryocyte precursors reappeared in the splenic red pulp and subsequently proliferated. The degree of proliferation was more pronounced in mice on the 16%-protein diet and resulted in splenomegaly by day 28 in three mice in this group (Fig. 6). Erythroid populations were evident amid proliferating undifferentiated cells in the splenic red pulp of one of these mice, whereas granulopoiesis was observed in all four.

Erythropoiesis in the spleen and bone marrow of mice fed T-2 toxin rapidly diminished during the first 14 days in each of the three diet groups. Numbers of metarubricytes and polychromatophilic

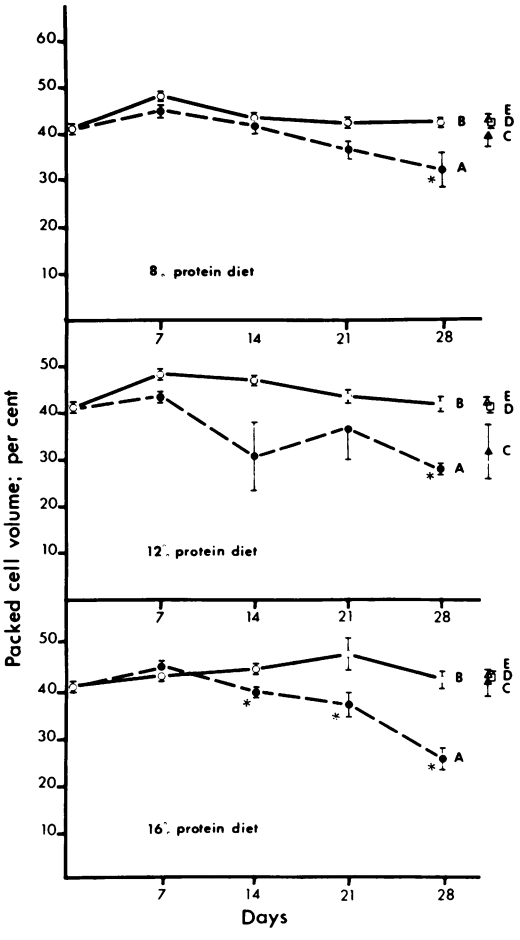

Fig. 2. Packed cell volumes of mice fed T-2 toxin (20 ppm) (A) in comparison with restricted-intake control groups (B). For comparison, 28-day values of mice after toxin withdrawal (C), of *ad lib*-fed controls (D) and of natural-ingredient diet-fed controls (E) are shown. Asterisks indicate means significantly different from control group B. All points are means (± SEM) of different groups of four mice. Wide deviations at 14 and 21 days for group A on the 12%-protein diet are due to severe blood loss by one mouse in each group.

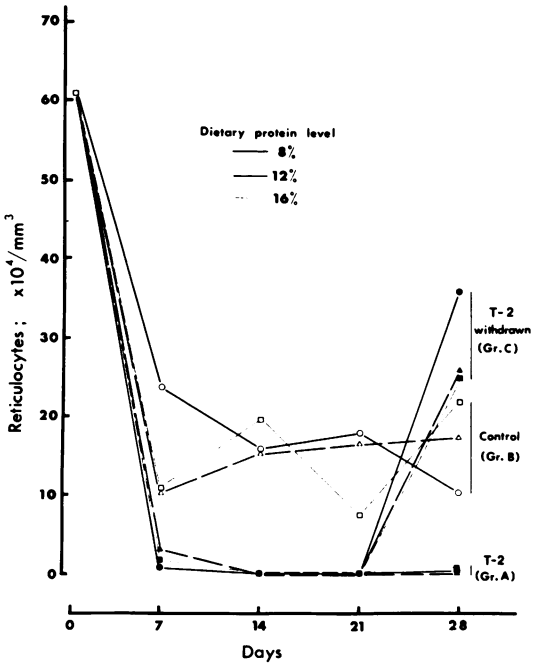

Fig. 3. Reticulocyte counts of young mice fed dietary T-2 toxin (20 ppm) (solid symbols; group A), in comparison with counts from mice fed the control diets at the same rate (open symbols; group B). The effect of withdrawal of the toxin from the diet on day 21 is shown (group C). All points are means of different groups of four mice.

erythrocytes in tibial bone marrow smears were almost totally depleted. Erythropoiesis resumed sooner and more frequently in mice consuming T-2 toxin in the 16%-protein diet (Table V).

The dietary protein level did not influence the pattern of recovery that occurred in mice transferred to the toxin-free diets (groups C). Erythropoiesis in spleen and bone marrow, and lymphopoiesis in the thymus, lymph nodes, spleen and intestinal lamina propria had resumed in all mice within seven days. No differences or abnormalities in regenerating erythropoiesis and lymphopoiesis were evident among mice on the different protein levels, but the degree of activity was variable among individuals in each of the three diet groups.

DISCUSSION

The severity of the characteristic toxic effects of dietary T-2 toxin at 20 ppm on the gastric mucosa, lymphoid organs and bone marrow, and the degree of the resulting hematological changes, were generally unrelated to the dietary protein level. Dietary T-2 toxin was slightly more harmful in diets containing 8% or 12% protein because low mortality occurred in mice fed T-2 toxin in these diets, but not in mice fed the toxic 16%-protein diet. Furthermore, mice fed T-2 toxin in the 16%-protein diet had higher growth rates than those consuming T-2 toxin in diets of

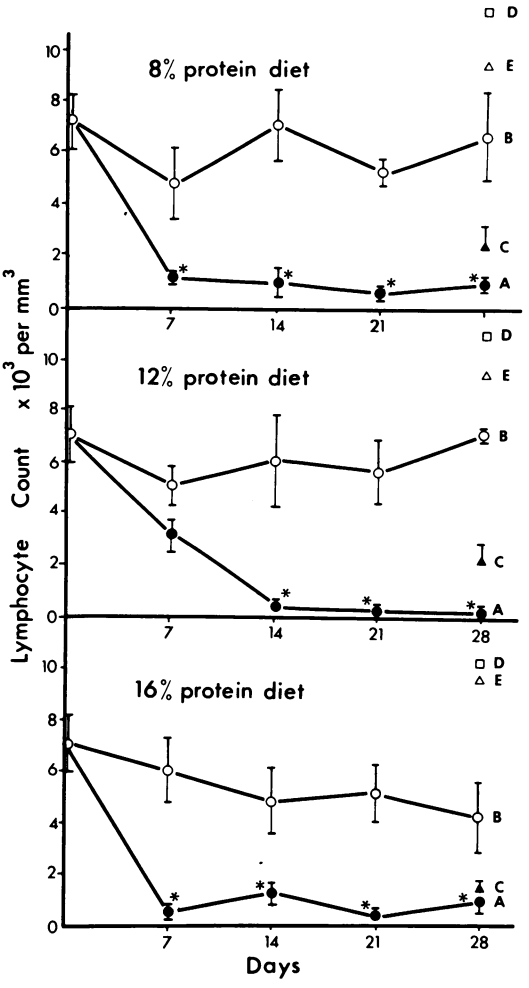

Fig. 4. Lymphocyte counts of young mice fed dietary T-2 toxin (20 ppm) (A), in comparison with restricted-intake control groups (B), *ad lib*-fed control groups (D) and mice on natural-ingredient diet (E). Mice in group C were removed from the toxic diets on day 21. Asterisks indicate means significantly different from control group B. All points are means (± SEM) of different groups of four mice.

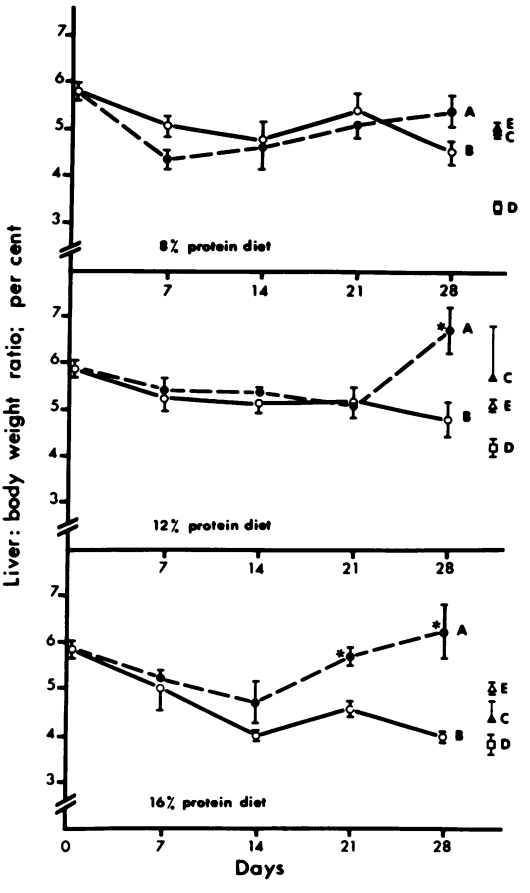

Fig. 5. Liver weight to body weight ratios of young mice fed dietary T-2 toxin (20 ppm) (A), in comparison with mice fed control diets at the same rate (B). Means at 28 days for groups C (toxin withdrawn on day 21), *ad lib*-fed controls (D) and natural-ingredient diet-fed controls (E) are shown. Asterisks indicate means significantly different from control group B. All points are means (±SEM) of different groups of four mice.

reduced protein content. However, these differences between the dietary groups were small compared with the overall effects.

One toxic effect observed only in mice fed T-2 toxin in diets of reduced protein content was intestinal hemorrhage. Fatal hemorrhage into the intestine was observed in only one mouse of the 60 fed toxic diets. In three others, hemorrhage probably occurred because erythrocyte counts rapidly decreased without hemosiderosis or icterus being evident, but in these mice neither the site of hemorrhage nor the pathogenesis was determined. Direct injury to the intestinal mucosa by T-2 toxin may have been involved. Alternatively, infectious enteritis may have developed due to depletion of lymphoid tissues by T-2 toxin, but there was no histological evidence that this may have occurred. Mice with evidence of previous hemorrhage had hypoplastic bone marrow and reduced numbers of platelets on smears, but many other mice that were similarly affected did not develop intestinal hemorrhage.

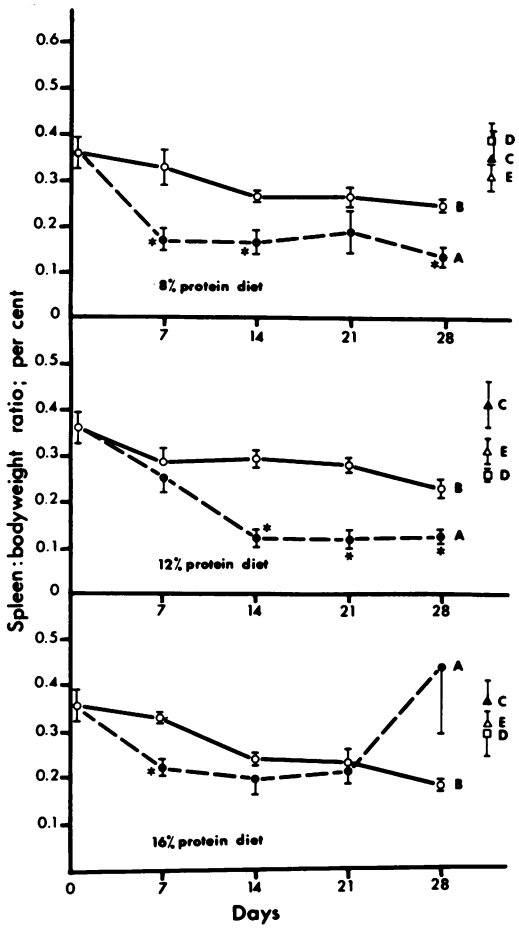

Fig. 6. Splenic weight to body weight ratios of young mice fed dietary T-2 toxin (20 ppm) (A), in comparison with mice fed control diets at the same rate (B). Values at 28 days for groups C (toxin withdrawn on day 21), D (*ad lib*-fed controls) and E (natural-ingredient diet-fed controls) are indicated. Asterisks indicate means significantly different from control group B. All points are means (± SEM) of different groups of four mice.

TABLE V. Occurrence of Erythroid Regeneration in Tibial Bone Marrow in Mice Fed T-2 Toxin (20 ppm) in Semipurified Diets of Different Levels of Protein

Period on T-2 toxin	Dietary protein level		
	8%	12%	16%
7 days	0/4[a]	0/4	0/4
14 days	0/4	0/4	1/4
21 days	0/4	1/4	1/4
28 days	1/4	0/4	3/4

[a]Values are ratios of number of mice exhibiting erythroid regeneration per group of four mice

Intestinal hemorrhage, associated with prolonged prothrombin times, has been reported in calves given 30 daily intrarumenal doses of T-2 toxin (18) and in one steer given 65 daily intramuscular doses of T-2 toxin (6). The pathogenesis of such hemorrhage is unknown. In some of the calves, hemorrhagic enteritis occurred before prothrombin times were increased, so direct injury to the alimentary mucosa by T-2 toxin may have been involved (18). Hemorrhagic syndromes of undetermined pathogenesis have been reported in cattle fed rations contaminated by T-2 toxin (8).

Several observations in this study indicated that the dietary protein level influenced the rate of spontaneous recovery from toxic inhibition of erythropoiesis and myelopoiesis. During the trial, all mice consuming T-2 toxin developed erythroid hypoplasia during the first 14 days, but subsequently immature myeloid and erythroid cells appeared in the spleen and bone marrow of many mice, as described previously (7) The onset of recovery of erythropoiesis, indicated by the appearance of maturing rubricytes, occurred most frequently in mice fed T-2 toxin in the 16%-protein diet (Table V). Furthermore, during exposure to dietary T-2 toxin, undifferentiated hematopoietic cells regenerated in the splenic red pulp in greatest numbers in mice fed the 16%-protein diet. Thus, splenomegaly occurred after four weeks in these mice, while the spleens of mice fed the other toxic diets remained small.

The mechanism by which mice overcame suppression of hematopoiesis has not been determined. Because hematopoiesis resumed in mice that were continuously consuming T-2 toxin in the diet, either the hematopoietic cells became less sensitive to inhibition of proliferation by T-2 toxin, or the amount of the active form of T-2 toxin in the susceptible hematopoietic tissues decreased. In this study, hepatic enlargement occurred in mice fed T-2 toxin in both the 12% and 16%-protein diets, and was correlated with the occurrence of regeneration of hematopoietic tissues. Hematopoiesis may have resumed because of an acquired competence of the liver to biotransform T-2 toxin into a metabolite that was less toxic to the hematopoietic precursors. Diets low in protein are known to reduce production of hepatic microsomal mixed function oxidases

(2) which are responsible for most biotransformations of xenobiotics (17). Many xenobiotics, such as DDT (23) and phenobarbitone (21) cause hepatomegaly during stimulation of microsomal enzyme activity, and the degree of DDT-induced hepatomegaly is much lower in rats consuming diets deficient in protein (1). Mice in the present study were housed on softwood shavings, which may contain agents capable of inducing hepatic microsomal enzymes (21) so the recovery may have been influenced by bedding. However, further experiments with mice housed in suspension cages have shown similar development of hepatomegaly and hematopoietic regeneration during exposure to dietary T-2 toxin (unpublished). The metabolic fate of T-2 toxin during subacute exposure is not known. Although T-2 toxin is deacetylated to HT-2 toxin by human, bovine and rat hepatic microsomal enzymes (4, 24), HT-2 toxin is only slightly less acutely toxic than T-2 toxin (4, 24). However, the metabolic fate and subacute toxic effects of HT-2 toxin have not been examined.

The degree of hyperplasia of the gastric mucosa, as determined by gastric weight, was not diet-related, and progressively increased throughout the trial period. This lesion is likely caused by direct irritation of the gastric mucosa by T-2 toxin, which is extremely irritant to the skin and mucous membranes (12). Such a direct effect is unlikely to be lessened by hepatic metabolism of T-2 toxin. Similar hyperplasia has been observed in mice and rats consuming diets containing 15 ppm of T-2 toxin for up to 52 weeks (16).

The ability of mice to overcome hematopoietic suppression by dietary T-2 toxin depended on the diet, suggesting that dietary T-2 toxin might also impair hematopoiesis of other animals under some nutritional conditions. Trichothecenes in adequate diets caused reduced growth, reduced food intake, and sometimes irritation of the upper alimentary tract in poultry (3, 26) and pigs (5, 25), but did not cause hematological abnormalities. The mechanisms by which these species avoid the suppressive effects of T-2 toxin, and the influences of suboptimal nutrition on such resistance, have not been investigated.

Concerns have been expressed that trichothecene mycotoxins may be hazardous to other livestock and man because these toxins have been associated with lethal mycotoxicoses such as alimentary toxic aleukia of man (9), stachybotryotoxicosis of horses and cattle (19) and moldy corn poisoning of cattle (8). In these diseases, toxic hematopoietic suppression is probably the underlying problem, leading to critical depletion of granulocytes or platelets, and resulting in either bacterial sepsis or hemorrhagic diathesis respectively. Outbreaks of alimentary toxic aleukia have been reported to be more severe when moldy diets were of inferior nutritional quality (9). Thus, evaluation of the hazards due to foodborne trichothecene mycotoxins appears dependent on knowledge of the influence of nutrition and other factors on the toxicity of these mycotoxins to hematopoietic cells. In addition to nutritional influences, other factors could affect toxicity, including age, and other mycotoxins, particularly any which might be capable of interfering with hepatic metabolism of trichothecenes.

Although it is as yet unknown whether the murine responses to T-2 toxin resemble those of other species, mice are useful experimental animals for evaluation of factors influencing the toxicity of trichothecenes. Dietary conditions that decrease or potentiate systemic toxicity of trichothecenes could be identified in mice, thereby minimizing the number of expensive and potentially hazardous feeding trials needed to determine the toxicity of trichothecenes to various livestock.

ACKNOWLEDGMENTS

We are grateful to Mrs. G. Green, Mr. E. Bueckert and Mr. D. Olexson for expert technical assistance, and to Mrs. Carol Kettles for typing the manuscript.

This work was supported by the Natural Sciences and Engineering Research Council of Canada, and is based upon a thesis submitted by the senior author in partial fulfillment for a Ph.D. degree, University of Saskatchewan.

During this study M.A. Hayes was a Fellow of the Medical Research Council of Canada.

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