Subacute Toxicity of Dietary T-2 Toxin in Mice: Morphological and Hematological Effects

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ABSTRACT

Changes in hematopoietic and lymphoid tissues of young Swiss mice fed a balanced semipurified diet containing T-2 toxin (20 ppm) were examined after one, two, three, four or six weeks. During the first three weeks of exposure to T-2 toxin, lymphoid tissues, bone marrow and splenic red pulp became hypoplastic, resulting in anemia, lymphopenia and eosinopenia. Subsequently, during continued exposure to T-2 toxin, hematopoietic cells regenerated in bone marrow and splenic red pulp and became hyperplastic by six weeks. Granulopoiesis and thrombopoiesis resumed in advance of erythropoiesis. All lymphoid tissues remained atrophic throughout the six week trial. Mice exposed to T-2 toxin also developed perioral dermatitis and hyperkeratosis with ulceration of the mucosa of the esophageal region of the stomach. These results indicated that young mice were susceptible to both the irritant and the hematopoietic-suppressive toxic effects of dietary T-2 toxin. However, suppression of hematopoiesis was transient and did not lead to hematopoietic failure.

RÉSUMÉ

Cette expérience consistait à vérifier, au bout d'une, deux, trois, quatre ou six semaines, la présence de changements dans les organes hémopoïétiques et lymphoïdes de jeunes souris suisses qui recevaient une ration balancée, semi-purifiée et contaminée par 20 ppm de toxine T-2. Durant les trois premières semaines de l'expérience, les organes lymphoïdes, la moelle osseuse et la pulpe rouge splénique subirent une hypoplasie qui se traduisit par de l'anémie, de la lymphopénie et de l'éosinopénie. Au cours des semaines subséquentes, les cellules hémopoïétiques régénérèrent dans la moelle osseuse et la pulpe rouge splénique; elles devinrent hyperplasiques, au bout de six semaines. La granulopoïèse et la thrombopoïèse reprirent avant l'érythropoïèse. Tous les organes lymphoïdes demeurèrent atrophiques, tout au long de cette expérience de six semaines. Les souris qui recevaient de la toxine T-2 développèrent aussi une dermatite péri-buccale et une hyperkératose ulcéreuse de la muqueuse gastrique avoisinant le cardia. Les résultats de cette expérience révélèrent que les jeunes souris sont susceptibles aux effets irritants et suppresseurs de l'hémopoïèse de la toxine T-2. La suppression de l'hémopoïèse s'avéra cependant transitoire et n'entraîna pas de défaillance hémopoïétique.

INTRODUCTION

T-2 toxin [3α-hydroxy-4β ,15-diacetoxy-8α-(3-methylbutyryloxy)-12,13-epoxytrichothec-9-ene] is a trichothecene mycotoxin produced by some *Fusarium* fungi, especially under cold moist conditions (1). Trichothecene mycotoxins, including T-2 toxin, have been implicated in several mycotoxic diseases of man and livestock in which potentially lethal suppression of lymphopoiesis and hematopoiesis occurs (1, 4, 9, 12, 32). T-2 toxin has also been associated with an uncharacterized hemorrhagic syndrome of cattle fed *F. tricinctum*-contaminated corn (8). However,

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TABLE I. Experimental Design Used for Examination of Sequential Changes During Subacute Dietary T–2 Toxicosis of Young Swiss Mice

Group	Diet Treatment	Number of mice examined					
		Day 0	Day 7	Day 14	Day 21	Day 28	Day 41
A	Semipurified diet[a] containing 20 ppm of T–2 toxin, *ad lib*		4[b]	4	4	4	4
B	Semipurified control diet at restricted intake		4	4	4	4	4
C	As for group A for three weeks then as for group B					4	
D	Semipurified diet, *ad lib*					8	
E	Pelleted natural-ingredient regular mouse diet[c]	12				8	

[a] The semipurified diet contained 16% protein and was formulated as described elsewhere (7)
[b] Different groups of four mice were examined at each time
[c] Department of Animal Science, University of Saskatchewan

the causative role of T-2 toxin in these mycotoxicoses has not been established.

Although T-2 toxin and other trichothecenes are potently toxic to hematopoietic and lymphoid tissues of many species given acutely toxic single doses (23, 24, 31, 33) or repeated parenteral (21, 22, 24, 29) or oral doses (12), these tissues appear to be much less sensitive to T-2 toxin given by the dietary route. The harmful consequences of ingestion of dietary T-2 toxin have been examined in rats (13, 16), mice (16), poultry (3, 19, 35, 36) and swine (33). In these species, T-2 toxin consistently caused reduced food consumption, reduced growth rates and usually caused inflammatory lesions around the mouth and in the upper alimentary tract. Mild leukopenias have been reported in laying hens fed dietary T-2 toxin (20 ppm) for three weeks (36) and in mice fed T-2 toxin (20 ppm) for 24 days (16). Otherwise, abnormalities in hematopoiesis have not been recognized during subacute or chronic dietary T-2 toxicosis.

Several reasons may explain this difference between natural and experimental mycotoxicoses. These include: 1) some species may be resistant to the suppressive effects of dietary T-2 toxin, 2) other toxins may be more important than T-2 toxin in the naturally occurring pancytopenic mycotoxicoses and 3) dietary or other factors including different mycotoxins may increase the toxicity of T-2 toxin to hematopoietic tissues.

We have studied the effects of subacute dietary T-2 toxicosis in young Swiss mice because mice are suitable experimental animals for morphological and functional examination of the hematopoietic and immune systems. This report describes the hematological and morphological changes in mice fed T-2 toxin (20 ppm) for up to six weeks in a nutritionally adequate, semipurified diet containing 16% protein. The influence of variations in the dietary protein level on these effects were also examined and are described elsewhere (7).

MATERIALS AND METHODS

Seventy-two male weanling outbred Swiss mice[1] weighing 16.0 ± 1.5 g and in randomly selected groups of four per cage were housed in stainless steel, shoebox cages on soft-wood shavings. Tap water was supplied *al libitum*. The room was maintained at 21°C with 12 hours of fluorescent light per day and all animal care procedures conformed to the guidelines of the Canadian Council on Animal Care. A semipurified moist diet containing 16% protein (7) was supplied each day in overhead feeding racks.

The mice were arranged into experimental groups according to the design in Table I. Twenty mice in group A were

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

supplied *ad libitum* with the semipurified diet containing crystalline purified T-2 toxin[2] at a level of 20 μg/g of dry diet (20 ppm). A matching group (B) of 20 mice was supplied with the same diet without T-2 toxin such that the diet consumption rate, per gram of body weight, was similar for groups A and B. A third group (C) of four mice was supplied with the toxic diet for 21 days and then transferred to the control diet for seven days, during which time the control diet was supplied at the rate at which the toxic diet had been consumed during the week prior to transfer. Group D consisted of eight mice which were fed the semipurified diet *ad lib*. A further group of 20 mice (group E), supplied *ad lib* with a pelleted, natural-ingredient rat and mouse diet containing 18.6% crude protein[3], served as a control for the semipurified diet and also provided pretreatment control values.

All mice were observed daily, and each mouse was weighed on day 0 and every seven days thereafter. Weekly consumption rates for all diets, with the exception of the pelleted ration, were monitored. Each cage of mice in group B was given a quantity of control diet matched with the amount consumed during the previous 24 hours by mice on T-2 toxin in a corresponding cage. At various times, according to the schedule on Table I, mice were selected and bled from the tail into heparinized volumetric tubes for determination of 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)[4]. Blood smears were stained with Wright's-Giemsa for differential leukocyte counts and morphological evaluation and samples of blood were stained with methylene blue for determination of reticulocyte percentage counts. Platelet counts were not attempted so that maximum quantities of serum could be collected for subsequent electrophoretic studies. After blood collection, mice were killed by decapitation for necropsy. Fresh weights of liver, stomach, spleen, thymus and testes were determined. Bone marrow imprint smears from the right tibial shaft were stained with Wright's-Giemsa. Samples of these tissues, and also of duodenum, jejunum, ileum, cecum, colon, pancreas, mesenteric lymph node, cervical lymph node, salivary gland, muzzle skin, heart, lung, kidney, bladder, adrenal gland and skeletal muscle were collected in Heidenhain's Susa fixative. Tissues were embedded in paraffin sectioned at 6 μm and stained with hematoxylin and eosin.

Toxin and time effects on the continuous variables from groups A and B were evaluated in a two-way analysis of variance. Means of groups A, B, C, D and E from day 28 were compared by one-way analyses of variance and Student-Newman-Keuls' multiple range tests. Means from toxin-treated groups on days 7, 14, 21, 28 and 41 were compared with the corresponding means from group B by Student's t-tests. The 95% probability level was used to determine significant factor effects and differences between means. All analyses were computed using a package of computer programs described by Nie *et al* (15).

RESULTS

GENERAL FINDINGS

Dietary T-2 toxin substantially reduced growth and food consumption, as described elsewhere (7). The rates of voluntary food consumption by mice fed the control diet *ad lib* (group D) were approximately double those by mice in groups A and B, and were associated with much greater weight gains similar to the weight gains of mice consuming the natural-ingredient diet (group E).

Mice fed T-2 toxin appeared small and lethargic with dry ruffled fur, in contrast to the control mice in group B which were active with normal fur gloss. Several mice developed mild, moist exudative dermatitis of the perioral skin between day 14 and day 28. By day 21, most mice on T-2 toxin had scaley skin on their tails and exhibited pallor of the muzzle, ears and eyes. Skin pallor became more noticeable between

[2]Makor Chemicals, Jerusalem, Israel.

[3]Department of Animal Science, University of Saskatchewan.

[4]Coulter-S, Coulter Electronics Inc., Hialeah Florida.

TABLE II. Sequential Changes in Erythrocyte Values[a] in Peripheral Blood of Mice Consuming a Semipurified Diet Containing T-2 Toxin (20 ppm)

	Group[b]	Day 0	Day 7	Day 14	Day 21	Day 28	Day 41
Hemoglobin concentration, g/dL	A (T-2 toxin)		15.0 ± 0.4	13.7 ± 0.4[c]	13.1 ± 0.9[c]	9.8 ± 0.7[d]	6.0 ± 2.2[e]
	B (restricted)		15.5 ± 0.1	15.7 ± 0.5	17.0 ± 0.8	16.4 ± 0.8[e]	17.1 ± 0.6
	C (T-2 withdrawn)					15.0 ± 1.1[e]	
	D (ad lib)					16.0 ± 0.3[e]	
	E (regular diet)	13.8 ± 0.3					
Erythrocyte count, x10⁶/μL	A (T-2 toxin)		9.1 ± 0.4	8.2 ± 0.5	7.8 ± 0.5	5.5 ± 0.5[d]	3.3 ± 1.2[e]
	B (restricted)		8.6 ± 0.1	8.8 ± 0.3	9.7 ± 0.8	8.7 ± 0.5[e]	9.1 ± 0.3
	C (T-2 withdrawn)					8.4 ± 0.6[e]	
	D (ad lib)					8.7 ± 0.2[e]	
	E (regular diet)	7.8 ± 0.2				8.6 ± 0.5[e]	
Packed cell volume (PCV) percent	A (T-2 toxin)		46.3 ± 1.0	40.2 ± 1.0[c]	37.7 ± 2.7[c]	25.7 ± 2.2[d]	16.7 ± 7.0[e]
	B (restricted)		44.8 ± 0.6	45.0 ± 1.1	48.4 ± 3.4	42.4 ± 2.2[e]	44.8 ± 1.2
	C (T-2 withdrawn)					41.5 ± 3.3[e]	
	D (ad lib)					41.2 ± 1.0[e]	
	E (regular diet)	41.5 ± 1.9				42.7 ± 0.4[e]	

[a] All values are means (± SEM) of separate groups of four mice
[b] Group A: Semipurified diet containing 20 ppm T-2 toxin
Group B: Semipurified control diet (restricted)
Group C: As for Group A for 21 days, then as for Group B for seven days
Group D: Semipurified control diet (*ad lib*)
Group E: Natural-ingredient mouse diet
[c] Different from control group B ($p < 0.05$)
[d, e] Means followed by the same letter do not differ at $p = 0.05$ by Student-Newman-Keuls' multiple range test

days 21 and 41, while perioral dermatitis was no longer evident in mice remaining on T-2 toxin on days 28 and 41. No deaths occurred in any group, but on day 41 when the trial was terminated, one extremely anemic mouse seemed unlikely to survive much longer. Withdrawal of T-2 toxin from the diet of mice in group C was associated with improvement in physical condition. All mice consuming the control diets remained active and physically normal with the exception that mice in group B were smaller because of the diet restriction.

HEMATOLOGICAL FINDINGS

Mice consuming T-2 toxin in the diet progressively developed normochromic, normocytic anemia during the six week period (Table II). Initially, the anemia was not regenerative, with circulating reticulocyte counts consistently less than 0.1%, but by day 41, reticulocytes returned into circulation (Fig. 1). Withdrawal of toxin from the diet resulted in a marked regenerative response, with restoration of erythrocyte values to near normal levels (Table II), and with release of many reticulocytes into circulation (Fig. 1). Reticulocyte counts declined in mice on the restricted control diet (Fig. 1) but none of these mice became anemic (Table II).

The MCV progressively decreased in both groups A and B during the six week trial, but on day 28 mice on T-2 toxin had a significantly lower MCV than did all control groups (Table III). Values for MCH and MCHC increased slightly in all mice during the trial, but no significant differences due to T-2 toxin were evident.

Total counts of peripheral blood leukocyte did not vary consistently among the different groups, but marked reductions in lymphocyte counts occurred in mice consuming T-2 toxin (Table IV). Restriction of food intake in control group B did not deplete numbers of circulating lymphocytes, but in the *ad lib*-fed control mice (group D) lymphocyte counts had increased normally with age to be significantly higher than values for group B on day 28 (Table IV). Mice in group C had slightly higher lymphocyte counts than did mice remaining on T-2 toxin (group A) (Table IV).

T-2 toxin caused eosinopenia and neutrophilia of mice in group A, in comparison with mice in the restricted intake group

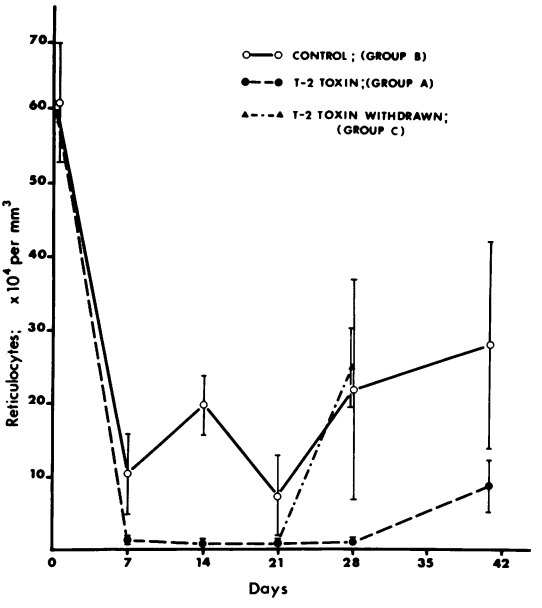

Fig. 1. Reticulocyte counts of Swiss mice after various periods on dietary T-2 toxin (20 ppm) (group A) in comparison with counts in control mice fed toxin-free diet at the same rate (group B), and with mice removed from the toxic diet on day 21 (group C). All points are means (± SEM) of separate groups of four mice.

B and the *ad lib* fed group D (Table IV). Platelets were not counted, but they were not appreciably depleted on smears of peripheral blood from any of the mice fed T-2 toxin. However, because platelets were clumped, this interpretation could be inaccurate.

ORGAN WEIGHT CHANGES

Absolute and relative weights of the liver and stomach increased in mice consuming T-2 toxin (Table V). The spleens from these mice were initially smaller compared to those of control group B, but subsequently they increased in size such that by day 41, they were much larger than in the controls (Fig. 2). The organ to body weight ratios for liver (Table V) and spleen of mice in the restricted-intake control group B did not differ significantly from the corresponding relative weights of the *ad lib*-fed control groups D and E.

The ratio of thymic weight to body weight decreased soon after mice were supplied with dietary T-2 toxin and remained low throughout the 41-day trial period. Progressive atrophy of the thymus

TABLE III. Sequential Changes in Erythrocyte Indices in Mice Consuming a Semipurified Diet Containing T-2 Toxin (20 ppm)

	Group[b]	Day 0	Day 7	Day 14	Day 21	Day 28	Day 41
Mean corpuscular volume (MCV) μ^3	A (T-2 toxin)		51.3 ± 1.0	49.8 ± 0.5	48.3 ± 0.5	46.5 ± 0.6[d]	48.0 ± 2.1
	B (restricted)		52.3 ± 0.8	50.8 ± 0.8	49.8 ± 0.9	48.7 ± 0.3[e]	49.3 ± 0.6
	C (T-2 withdrawn)					48.7 ± 0.8[e]	
	D (ad lib)					50.0 ± 0.4[e]	
	E (regular diet)	53.3 ± 0.6				49.8 ± 0.3[e]	
Mean corpuscular hemoglobin (MCH) pg	A (T-2 toxin)		17.1 ± 0.3	17.0 ± 0.3	16.8 ± 0.3	17.9 ± 0.4	18.3 ± 0.1[c]
	B (restricted)		18.1 ± 0.2	17.7 ± 0.3	17.7 ± 0.8	18.9 ± 0.5	19.1 ± 0.2
	C (T-2 withdrawn)					17.7 ± 0.3	
	D (ad lib)					19.3 ± 0.7	
	E (regular diet)	17.8 ± 0.1				18.3 ± 0.3	
Mean corpuscular hemoglobin concentration percent	A (T-2 toxin)		34.3 ± 0.3	34.9 ± 0.2	35.5 ± 1.0	39.1 ± 0.6	39.0 ± 1.6
	B (restricted)		35.6 ± 0.5	35.8 ± 0.6	36.4 ± 1.0	39.9 ± 1.1	39.6 ± 0.3
	C (T-2 withdrawn)					37.1 ± 0.3	
	D (ad lib)					39.7 ± 1.2	
	E (regular diet)	33.4 ± 0.6				37.5 ± 0.6	

[a] All values are means (± SEM) of separate groups of four mice
[b] Group A: Semipurified diet containing 20 ppm T-2 toxin
Group B: Semipurified control diet (restricted)
Group C: As for Group A for 21 days, then as for Group B for seven days
Group D: Semipurified control diet (*ad lib*)
Group E: Natural-ingredient mouse diet
[c] Different from control group B ($p < 0.05$)
[d, e] Means followed by the same letter do not differ at $p = 0.05$ by Student-Newman-Keuls' multiple range test

TABLE IV. Sequential Changes in Leukocyte Values[a] in Peripheral Blood of Mice Consuming a Semipurified Diet Containing T-2 Toxin (20 ppm)

	Group[d]	Day 0	Day 7	Day 14	Day 21	Day 28	Day 41
Lymphocyte count, /mL	A (T-2 toxin)		730 ± 50[e]	1400 ± 360[e]	570 ± 180[e]	990 ± 450[d]	1200 ± 690[e]
	B (restricted)		6100 ± 1300	4900 ± 1400	5100 ± 1200	4200 ± 1300[e]	5100 ± 1900
	C (T-2 withdrawn)					1500 ± 220[d]	
	D (ad lib)					10100 ± 730[f]	
	E (regular diet)	7100 ± 980				9500 ± 760[f]	
Neutrophil count, /μL	A (T-2 toxin)		2000 ± 260[e]	3400 ± 940[e]	3500 ± 700[e]	4900 ± 700[d]	5200 ± 1600
	B (restricted)		930 ± 260	650 ± 310	690 ± 60	2000 ± 260[e,f]	3000 ± 1200
	C (T-2 withdrawn)					3000 ± 490[e]	
	D (ad lib)					940 ± 250[f]	
	E (regular diet)	1600 ± 270				1400 ± 170[f]	
Eosinophil count, /μL	A (T-2 toxin)		60 ± 40	0[e]	0[e]	10 ± 10[d]	0[e]
	B (restricted)		260 ± 110	160 ± 60	210 ± 90	90 ± 60[d]	480 ± 280
	C (T-2 withdrawn)					40 ± 30[d]	
	D (ad lib)					110 ± 50[d]	
	E (regular diet)	110 ± 40				110 ± 40[d]	

[a] All values are means (± SEM) of separate groups of four mice
[b] Group A: Semipurified diet containing 20 ppm T-2 toxin
Group B: Semipurified control diet (restricted)
Group C: As for Group A for 21 days, then as for Group B for seven days
Group D: Semipurified control diet (*ad lib*)
Group E: Natural-ingredient mouse diet
[c] Different from control group B ($p < 0.05$)
[d,e,f] Means followed by the same letter do not differ at $p = 0.05$ by Student-Newman-Keuls' multiple range test

TABLE V. Sequential Changes in Organ Weight:Body Weight Ratios[a] in Mice Consuming a Semipurified Diet Containing T-2 Toxin (20 ppm)

	Group[b]	Day 0	Day 7	Day 14	Day 21	Day 28	Day 41
Liver weight, percent of body weight	A (T-2 toxin)		5.27 ± 0.20	4.75 ± 0.45	5.74 ± 0.14[c]	6.25 ± 0.59[d]	7.61 ± 1.69
	B (restricted)		5.08 ± 0.49	4.01 ± 0.11	4.62 ± 0.19	4.08 ± 0.10[e]	4.47 ± 0.13
	C (T-2 withdrawn)					4.31 ± 0.40[e]	
	D (ad lib)					3.84 ± 0.26[e]	
	E (regular diet)	5.90 ± 0.18				5.00 ± 0.15[e]	
Stomach weight, percent of body weight	A (T-2 toxin)		1.38 ± 0.05[c]	1.58 ± 0.22	2.21 ± 0.38[c]	1.72 ± 0.20[d]	3.07 ± 0.50[c]
	B (restricted)		1.31 ± 0.06	1.07 ± 0.05	1.20 ± 0.07	1.26 ± 0.08[e]	1.31 ± 0.05
	C (T-2 withdrawn)					1.77 ± 0.11[d]	
	D (ad lib)					0.65 ± 0.02[f]	
	E (regular diet)	1.20 ± 0.11				0.69 ± 0.04[f]	
Thymic weight, percent of body weight	A (T-2 toxin)		0.06 ± 0.03[c]	0.03 ± 0.01[c]	0.05 ± 0.02	0.05 ± 0.01[d]	0.07 ± 0.01[c]
	B (restricted)		0.34 ± 0.06	0.19 ± 0.03	0.14 ± 0.04	0.03 ± 0.01[d]	0.21 ± 0.03
	C (T-2 withdrawn)					0.09 ± 0.03[d]	
	D (ad lib)					0.20 ± 0.02[e]	
	E (regular diet)	0.22 ± 0.02				0.17 ± 0.02[e]	

[a] All values are means (± SEM) of separate groups of four mice
[b] Group A: Semipurified diet containing 20 ppm T-2 toxin
Group B: Semipurified control diet (restricted)
Group C: As for Group A for 21 days, then as for Group B for seven days
Group D: Semipurified control diet (*ad lib*)
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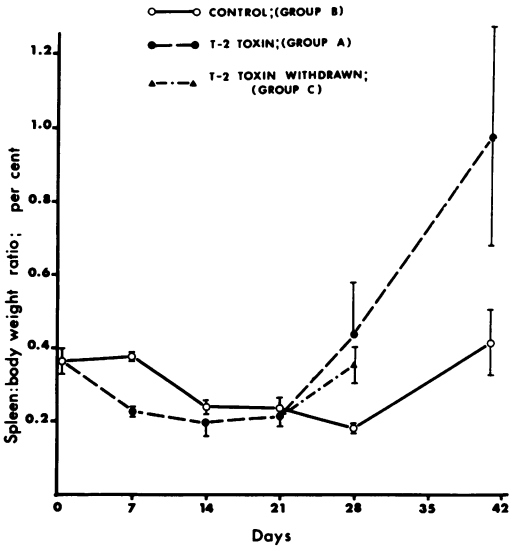

Fig. 2. Spleen-body weight ratios of Swiss mice after various periods on dietary T-2 toxin (20 ppm) (group A), in comparison with control mice fed toxin-free diet at the same rate (group B), and with mice removed from the toxin on day 21 (group C). All points are means (± SEM) of separate groups of four mice.

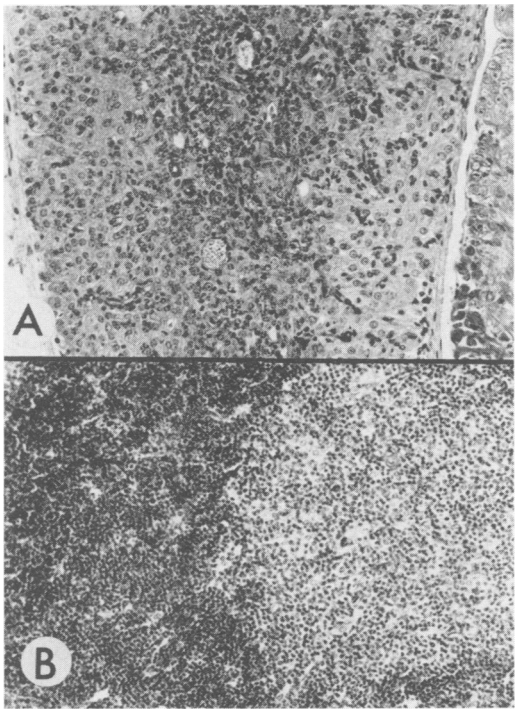

Fig. 3. A. Atrophic thymic cortex of a mouse after 14 days on dietary T-2 toxin (20 ppm). H & E. X120. B. Normal appearance of thymic cortex of a mouse after 14 days on the semi-purified control diet fed at the restricted rate. H & E. X75.

also occurred at a more gradual rate in some mice in the restricted-intake control group, especially in those examined on day 28 (Table V). By comparison, thymic weights in the *ad lib*-fed control groups (D and E) increased during the trial period. No consistent intergroup differences in relative testicular weights occurred.

MACROSCOPIC OBSERVATIONS

Mice consuming the diet containing T-2 toxin developed marked atrophy of the thymus and Peyer's patches. By day 7, and throughout the trial, these organs were noticeably smaller than in all control groups. The lymphoid component of the spleen (white pulp) diminished over the first 21 days and subsequently was not visible. The red pulp atrophied during the first seven days, such that on days 7 and 14, it was pale, tan and greatly reduced in size. By day 28, two mice exhibited splenomegaly, due to proliferation of greyish-red, homogeneous tissue throughout the red pulp, and by day 41, splenomegaly occurred in three of the four mice examined. At this stage, the red pulp had returned to the normal dark red colour.

Bone marrow of mice fed T-2 toxin was dark red on days 7 and 14, but as mice became visibly anemic, the marrow cavities appeared pale along with all tissues. The liver became enlarged and pale tan-yellow. Hyperplasia of the squamous mucosa of the esophageal region of the stomach occurred in all mice that had consumed T-2 toxin for more than 14 days. Small ulcerated regions, 0.5-1 mm in diameter, were sometimes present on the hyperplastic mucosa.

Mice in group C, after withdrawal of the T-2 toxin from the diet, had a larger thymus and spleen and redder bone marrow than mice still on T-2 toxin (group A).

MICROSCOPIC OBSERVATIONS

From day 7 onwards, all lymphoid tissues in mice consuming T-2 toxin were hypocellular. Lymphocytes disappeared from the

thymic cortex within seven days (Fig. 3), at which stage neutrophils and eosinophils had infiltrated the medulla and some of the depleted regions of the cortex. Thymic cortical atrophy without granulocytic infiltration occurred in mice on the restricted control diet (group B), but it was more gradual and less severe than in mice on T-2 toxin (group A). The thymus of mice in the *ad lib*-fed control group (D) contained normal dense populations of lymphocytes.

Follicular activity in lymph nodes and spleen was not affected in the restricted-intake control group (B), but it virtually disappeared in mice consuming T-2 toxin. Thymic-dependent peripheral lymphoid populations, including periarteriolar sheaths in the spleen (Fig. 4), paracortical regions of lymph nodes and intraepithelial lymphocytes of the small intestine (theliolymphocytes) (Fig. 5), were depleted. B-cell-dependent populations of lymphocytes and plasma cells in the intestinal lamina propria (Fig. 5), in medullary cords of lymph

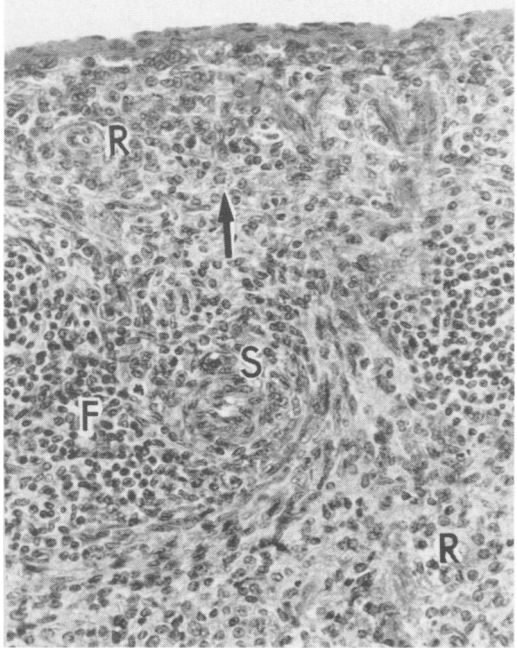

Fig. 4. Microscopic appearance of atrophic spleen of a mouse after 21 days on dietary T-2 toxin (20 ppm). Lymphoid populations of periarteriolar sheaths (S) and follicles (F) are extremely depleted. The red pulp (R) contains mostly condensed reticular tissue and macrophages containing hemosiderin (arrow). Hematopoietic activity is absent. H & E. X300.

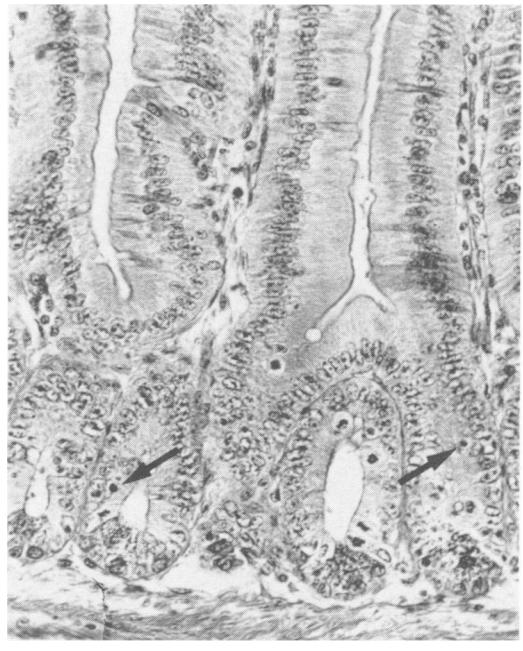

Fig. 5. Jejunal mucosa of a mouse after four weeks on dietary T-2 toxin (20 ppm). Note the thickened villus epithelium and virtual absence of intraepithelial lymphocytes. The lamina propria of the villi contains reduced numbers of mononuclear cells. Several phagolysosomes are visible in crypt epithelial cells (arrows). H & E. X300.

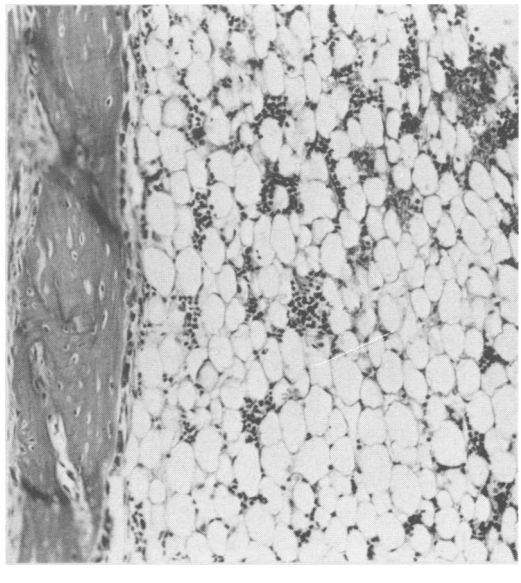

Fig. 6. Atrophic tibial bone marrow of a mouse after three weeks on dietary T-2 toxin (20 ppm). H & E. X120.

nodes and in splenic cords were also decreased in all mice consuming T-2 toxin. After withdrawal of T-2 toxin from the diet (group C), lymphoblastic proliferation was evident in the thymic cortex and medulla, lymphoid follicles, medullary regions of the lymph nodes and the intestinal lamina propria.

During exposure to T-2 toxin, the bone marrow became hypocellular during the first three weeks (Fig. 6), but subsequently regenerated to be cellular by day 41 (Fig. 7). Islands of developing erythrocytes in the splenic red pulp and bone marrow had disappeared in mice killed on days 14 and 21, but on days 28 and 41, erythropoietic activity was again evident in sections of bone marrow and spleen of some mice. On smears of tibial bone marrow, various patterns of abnormal erythropoiesis were observed in anemic mice. On days 14 and 21, red cell precursors were almost completely absent in three mice, whereas in three others, prorubricytes predominated and later stages were infrequent (Fig. 8). Development of rubricytes and metarubricytes was evident in one mouse at day 21, in three at day 28, and in all four at day 41. In these mice abnormalities of erythroid maturation included stippling of the cytoplasm of rubricytes and metarubricytes, fragmentation of the nucleus in

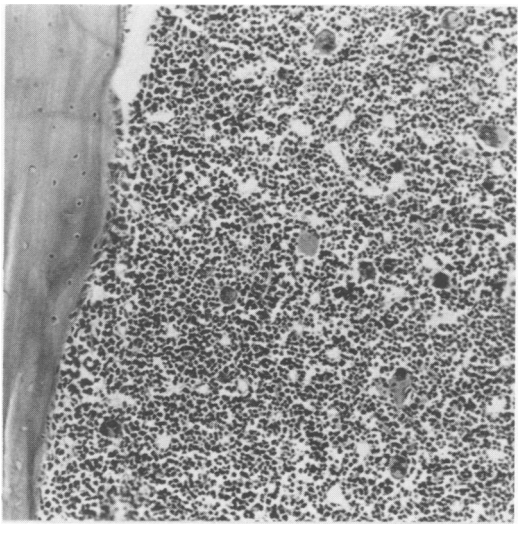

Fig. 7. Tibial bone marrow in an anemic mouse after six weeks on dietary T-2 toxin (20 ppm). Marrow is densely cellular, with both erythropoietic and myelopoietic activity. H & E. X120.

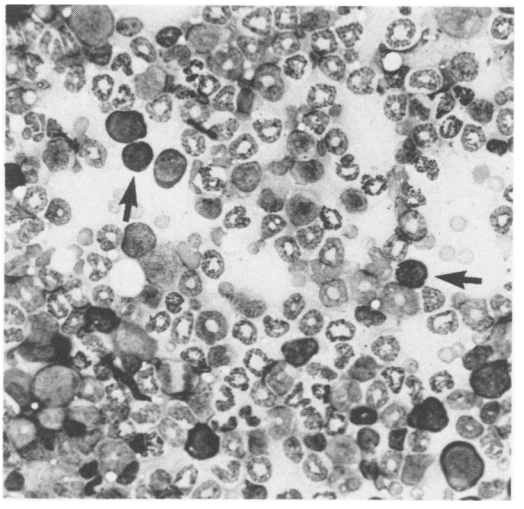

Fig. 8. Impression smear of bone marrow from an anemic mouse after 28 days on dietary T-2 toxin (20 ppm). Note the predominance of myeloid cells. Many prorubricytes (arrows) are present, but later stages of the erythrocytic series are absent. Wright's-Giemsa. X300.

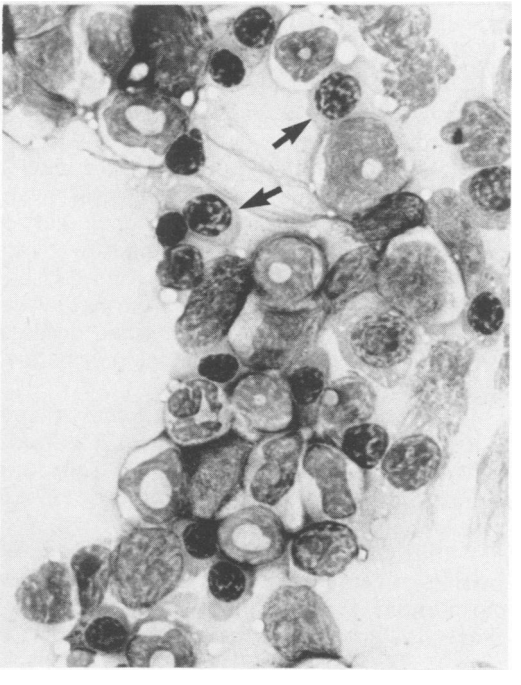

Fig. 9. Impression smear of bone marrow from an anemic mouse after six weeks on dietary T-2 toxin (20 ppm). Erythropiesis has resumed, but is abnormal because some rubricytes have an abnormal amount of hemoglobinized cytoplasm (arrows). Wright's-Giemsa. X750.

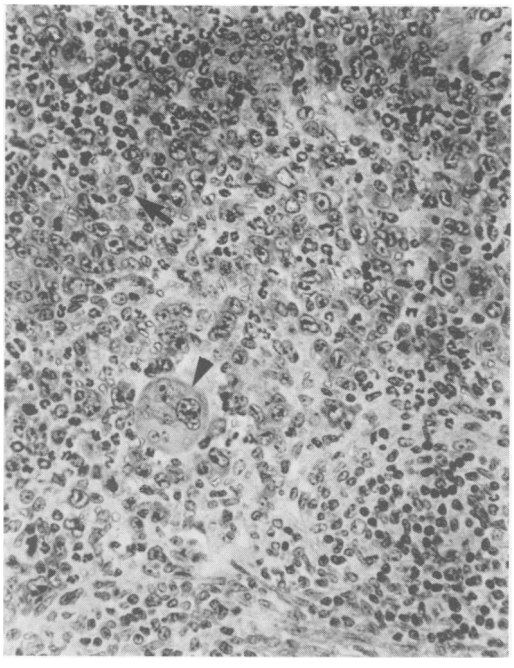

Fig. 10. Regenerating hematopoietic cells in the splenic red pulp of a mouse after four weeks on dietary T-2 toxin (20 ppm). Both granulopoiesis (arrow) and thrombopoiesis (arrowhead) are evident, but erythropoietic activity is absent. H & E. X300.

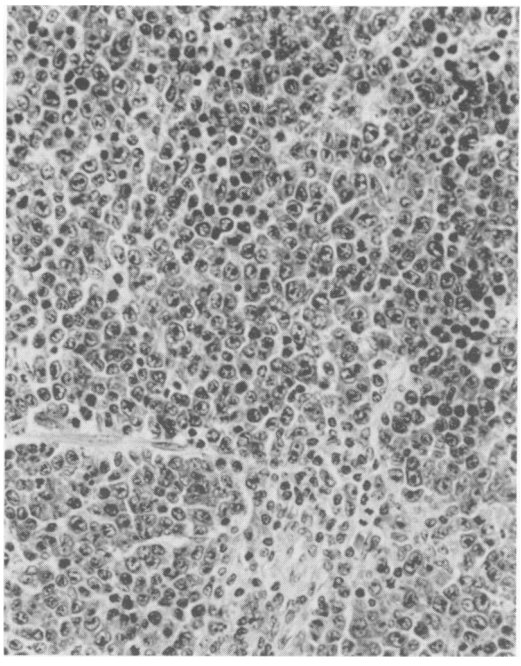

Fig. 11. Appearance of regenerated erythroid precursors in the red pulp of the enlarged spleen of an anemic mouse after six weeks on dietary T-2 toxin (20 ppm). Undifferentiated cells predominate, indicating that erythropoiesis is ineffective. H & E. X300.

metarubricytes and an increased ratio of cytoplasm to nucleus in rubricytes were evident (Fig. 9). Myeloid and megakaryocytic populations of bone marrow were moderately reduced on days 7 and 14, but subsequently returned to near normal levels, although in several mice examined on days 21 and 28, neutrophilic myelopoiesis appeared hyperplastic.

During the first 14 days, the splenic red pulp in mice consuming T-2 toxin became devoid of hematopoietic cells and hemosiderin had accumulated in splenic macrophages (Fig. 4). From day 14, foci of undifferentiated cells were observed initially in subcapsular locations, but later throughout the red pulp, resulting in the marked splenomegaly evident by days 28 and 41. The first undifferentiated cells to appear were generally large with pale vesicular nuclei and with pale-staining poorly demarcated cytoplasm. In these regenerating populations, a progressive sequence of nuclear indentation and elongation, indicating myelocytic differentiation was observed (Fig. 10). Megakaryocytes were also present in these foci. In mice with splenomegaly on days 28 and 41, undifferentiated cells were densely packed throughout the entire red pulp, and, in contrast to the undifferentiated cells observed at the earlier stages, most of these cells appeared to be erythroid, having denser, somewhat eosinophilic cytoplasm and round nuclei (Fig. 11).

After T-2 toxin was withdrawn from the diet of mice in group C, many active erythropoietic islands with normal erythroid maturation were evident, both in the bone marrow and in the splenic red pulp. Such foci of normal erythropoiesis were observed among undifferentiated cells similar to those which had proliferated in the splenic red pulp in mice still on T-2 toxin.

Perioral dermatitis of mice fed T-2 toxin was associated with epidermal hyperkeratosis and neutrophilic exudation. In the esophageal region of the stomach, the squamous mucosa was hypertrophic and hyperkeratotic, with numerous villous projections some of which were ulcerated and

infiltrated by many neutrophils. Histological changes in the lower intestinal tract were minimal except for the depletion of lymphoid cells in the lamina propria and intestinal epithelium (Fig. 5). Villi of the small intestine were of normal length and conformation, but epithelial cells in intestinal crypts appeared moderately hyperplastic. A few, round, intracytoplasmic bodies resembling phagolysosomes were observed in the epithelial cells at the base of the crypts of some mice consuming T-2 toxin (Fig. 5), whereas these were rarely observed in control mice.

DISCUSSION

In this subacute toxicity study in young mice, dietary T-2 toxin at 20 ppm caused reduced growth, reduced food consumption, hypoplasia of bone marrow and splenic red pulp, hypoplasia of lymphoid tissues, gastritis with hyperkeratosis and perioral dermatitis. Because T-2 toxin inhibits voluntary food consumption (3, 10, 19, 32, 33), malnourishment must often occur in animals exposed to T-2 toxin by the natural dietary route. In mice, reduced food intake was largely responsible for the reductions in growth rates (7). Other observed effects such as hypoplasia of hematopoietic and lymphoid tissues, and inflammation of the perioral skin and gastric mucosa can be considered to be toxic effects of T-2 toxin because these effects did not occur in mice on restricted diet intake (group B), and because they began to resolve rapidly after withdrawal of T-2 toxin from the diet fed at the same rate (group C).

Perioral dermatitis and gastritis in mice fed T-2 toxin may have been due to the well-recognized irritant toxicity of T-2 toxin to the skin and mucous membranes (1, 31). Perioral dermatitis has been observed in experimentally induced T-2 toxicosis of rats (13, 16) and poultry (3, 19, 35), and in naturally occurring mycotoxicoses of pigs and poultry exposed to feeds containing T-2 toxin or other trichothecenes (6, 17, 34). Stomatitis and dermatitis occur in the early stages of alimentary toxic aleukia of man (9, 14), and also in stachybotryotoxicosis of horses (20), each of which is thought to be caused by trichothecenes. Hyperkeratosis and ulceration of the esophageal region of the stomach of mice and rats fed T-2 toxin (15 ppm) has been reported by Ohtsubo and Saito (16). Similar lesions have also been produced in rats by intragastric administration of crude extracts of *Fusarium poae* and *F. sporotrichioides* (26). Esophagitis and hyperkeratosis of the ventriculus occurred in geese after consumption of T-2 toxin (17) or food contaminated by T-2 toxin (6).

All mice fed T-2 toxin developed severe lymphopenia and depletion of all lymphoid tissues, especially of the thymus and thymus-dependent tissues. Thymic atrophy developed rapidly and completely in mice consuming T-2 toxin, but less severe thymic hypoplasia gradually occurred in mice consuming the control diet at the restricted rate. Malnutrition is a well-recognized cause of thymic atrophy in young animals (27). The marked reduction of intake of feed containing T-2 toxin must therefore have contributed to the degree of atrophy observed in mice fed dietary T-2 toxin but, because proliferation of lymphoblasts in the thymic cortex, lymph nodes, spleen, and intestinal mucosa occurred soon after withdrawal of T-2 toxin from the diet supplied at the same restricted intake, T-2 toxin must have been partly responsible for the severe atrophy observed in treated mice. Thymic atrophy has been reported in turkey poults after consumption of T-2 toxin (19) and in mice given repeated intraperitoneal doses of T-2 toxin (21). Accordingly, concerns that food-borne trichothecenes may be immunosuppressive and may thereby predispose to various infectious diseases (19, 21, 31) appear justified.

Dietary T-2 toxin at 20 ppm suppressed hematopoiesis in the bone marrow and splenic red pulp of young mice, thereby supporting the implication that ingestion of T-2 toxin alone could lead to hematopoietic failure in some species (12, 24). The effects of T-2 toxin on hematopoiesis of mice in this study were different from effects attributed to T-2 toxin in other species. Mice developed hypoplastic anemia without granulocytopenia or thrombocytopenia, whereas in putative trichothecene mycotoxicoses such as alimentary toxic aleukia of man (9) and stachybotryotoxicosis of horses (20), affected individuals are neutropenic and susceptible to bacterial sepsis, or thrombocytopenic with hemorrhagic diathesis. In these diseases, erythro-

poiesis is also impaired but affected individuals do not often survive long enough to become severely anemic. Patterns of pancytopenia resembling the naturally occurring diseases have been experimentlly reproduced in cats by repeated subcutaneous (24) or intragastric (12) doses of T-2 toxin, and in dogs, rats and monkeys by repeated parenteral doses of a closely-related trichothecene, diacetoxyscirpenol (29). The different pattern of response observed in mice consuming T-2 toxin can be explained partly by the greater tendency of this species to become anemic during toxic suppression of hematopoiesis. Mice have a short erythrocyte lifespan in the order of 20 to 65 days, whereas in man, horses and cattle, erythrocytes generally survive for more than 100 days so anemia develops only after prolonged erythroid hypoplasia in these species (25).

Mice with extremely hypocellular bone marrow after three weeks on dietary T-2 toxin had persistence of reduced numbers of myeloid and megakaryocytic cells at a stage when erythropoiesis was totally suppressed. This suggests that erythropoiesis of mice is more susceptible to inhibition by T-2 toxin than is myelopoiesis or thrombopoiesis. Furthermore, as mice overcame the hematopoietic suppression, neutrophilic myelopoiesis and thrombopoiesis resumed in splenic red pulp and bone marrow in advance of erythroid regeneration. This resulted in neutrophilia while mice were still anemic. The relative susceptibilities of the different populations of marrow cells to T-2 toxin or other trichothecenes have not been examined in other species, so it is not known whether erythropoietic cells are generally more susceptibilities of the different populations hematopoietic precursors.

The mechanisms by which T-2 toxin and other trichothecenes inhibit hematopoiesis *in vivo* have not been determined. *In vitro*, trichothecenes are potent inhibitors of eukaryotic protein synthesis (30), but this activity is not necessarily responsible for the *in vivo* effects on proliferating cells. In mice fed dietary T-2 toxin, erythroid hypoplasia, with ineffective erythropoiesis during the early regenerative phase, resembled effects on erythropoiesis induced by various anti-cancer drugs that inhibit DNA synthesis, either by interfering with folate metabolism (e.g. methotrexate), or by inhibiting enzymes involved in the synthesis of deoxyribonucleotides (e.g. analogues of purines, pyrimidines and pyrimidine nucleosides) (2). Moreover, proliferation of hematopoietic precursors in splenic red pulp, with predominance of undifferentiated cells similar to those observed during subacute exposure to T-2 toxin in our study has been observed in mice exposed to repeated doses of triethylenemelamine, azathioprine (a purine analogue), or methotrexate, each of which interferes with DNA synthesis (11).

Transition from erythroid hypoplasia during the initial three weeks of dietary exposure to T-2 toxin, to erythroid regeneration between four to six weeks, suggests that mice became less susceptible to the toxic effects on hematopoiesis during continuous exposure. Further evidence for such resistance was provided by the observation of reduction and, subsequently, hyperplasia of myelopoiesis in splenic red pulp and bone marrow. Transient suppression of hematopoiesis was unlikely due to undernutrition because anemia did not develop in control animals fed at the same rate, and because extreme nutritional deprivations are needed before the observed degree of suppression would occur (5).

Not all rapidly proliferating tissues of mice were inhibited by dietary T-2 toxin. Intestinal crypts remained active and neither crypt necrosis nor atrophy of the villi was observed. Furthermore, the squamous mucosa of the stomach became hyperplastic while in close contact with T-2 toxin in the diet. These observations suggest that the proliferating epithelial cells of the alimentary tract are less susceptible than lymphoid and hematopoietic precursors to suppression of proliferation by T-2 toxin. This interpretation is consistent with the apparent absence of crypt necrosis and atrophy of the villi in naturally occurring trichothecene mycotoxicoses (6, 9, 14, 28, 34) and during experimental administration of dietary T-2 toxin (3, 16, 19, 33, 35, 36). However, during acute toxicosis, T-2 toxin and other trichothecenes destroy crypt epithelial cells, in addition to proliferating cells in lymphoid tissues and hematopoietic tissues (23, 31, 33). Crypt epithelial necrosis associated with enteritis has been produced in cats by repeated intragastric administration of T-2 toxin (12), and intestinal hemorrhage of undetermined pathogenesis has been observed in calves given repeated intraruminal doses of T-2 toxin (18). Peak body levels after such administrations

might be higher than would occur during consumption of T-2 toxin in the diet. Alternatively, there may be differences among species in the susceptibility of intestinal crypt cells to T-2 toxin.

The mechanism by which mice overcame the suppressive effects of dietary T-2 toxin on hematopoiesis is not known. Recovery could be explained by an increase in hepatic biotransformation of T-2 toxin into a metabolite which did not impair hematopoiesis. Evidence for this interpretation was provided by an observed association between regeneration of hematopoietic tissues and development of hepatomegaly under conditions of adequate protein nutrition (7)

The present study in mice indicates that under some conditions, T-2 toxin alone in the diet may inhibit lymphopoiesis and hematopoiesis. The observation that hematopoietic suppression may be transient during continuous exposure to dietary T-2 toxin may explain the sporadic occurrence of hematopoietic failure in experimental and natural trichothecene mycotoxicoses. Various conditions, including nutritional composition of the diet or the presence of other trichothecene or nontricothecene mycotoxins might influence either the susceptibility of hematopoietic cells to T-2 toxin or the ability of the animal to become resistant to the hematopoietic suppression. Determination of such factors influencing the development of such resistance may clarify our understanding of the role of these toxins in mycotoxic diseases.

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