

MORPHOLOGICAL AND TOXICOLOGICAL STUDIES  
ON EXPERIMENTAL T-2 MYCOTOXICOSIS

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## ABSTRACT

### Morphological and toxicological studies on experimental T-2 mycotoxicosis

T-2 toxin, a trichothecene mycotoxin produced by some Fusarium fungi, is possibly the cause of several mycotoxicoses of humans and livestock. Several studies were conducted to characterize the "dermatitic" and the "radiomimetic" properties of T-2 toxin, and to determine their effects on animals fed T-2 toxin in the diet.

The dermatitic responses to T-2 toxin and a similar trichothecene, diacetoxyscirpenol (DAS), were examined by applying the toxins to the skin of rats. The resulting acute non-specific dermal inflammatory reactions were, at all stages of development, histologically similar to reactions produced by croton oil. The skin of rabbits treated with T-2 toxin reacted in the same way.

Variation in sensitivity among rats was responsible for poor correlation between dose and visible signs of cutaneous inflammation, measured either by subjective rating of the intensity of inflammation, or by frequency-response. However, variation in sensitivity among test rats had little influence on the dose-response relationship when reactions were rated in units of equivalent concentration of T-2 toxin by comparison with reactions to a graded series of standard solutions of T-2 toxin applied to the same rat. This novel method of evaluating reactions improved the skin-irritation bioassay for trichothecenes such that test solutions in the range of 5 to 60  $\mu\text{g/ml}$  were measured accurately and precisely to within 13% of actual concentrations.

Intragastric administration of a single sublethal dose of T-2 toxin

(2.0 or 2.5 mg/kg) to juvenile Swiss mice caused necrosis of lymphoblasts in lymphoid follicles, of lymphocytes in the intestinal mucosa, of lymphocytes in the thymic cortex, of epithelial cells in intestinal crypts, and of hematopoietic cells in splenic red pulp and bone marrow within 6 hours. The severity of injury in these tissues was dose-dependent. Ultrastructurally and histologically, lesions resembled those reported in response to various anti-neoplastic chemicals. Cells in damaged target tissues regenerated during a 96-hour period after treatment.

The subacute toxic effects of dietary T-2 toxin on the hematopoietic, lymphopoietic, and alimentary systems were examined in Swiss mice and Wistar rats. Attempts were made to identify factors that potentiated the toxicity of dietary T-2 toxin to the hematopoietic system. Dietary T-2 toxin at levels of 10 or 20 ppm, in either natural-ingredient diets or in semipurified diets of various protein levels, consistently caused dose-dependent thymic atrophy, lymphopenia, gastric hyperkeratosis and gastric ulceration in juvenile mice. Similar, but less severe effects occurred in adult mice and in young rats fed these levels of T-2 toxin in natural-ingredient diets. Juvenile mice fed 20 ppm of T-2 toxin also developed aplastic anemia due to suppression of erythropoiesis in splenic red pulp and in bone marrow. Hypoplastic hematopoietic and lymphopoietic tissues began to regenerate within 7 days in mice transferred from toxic diets to control diets.

Suppression of hematopoiesis in juvenile mice fed T-2 toxin was probably a subacute manifestation of the toxicity of T-2 toxin to rapidly dividing cells. This effect was transient during continuous dietary exposure because suppression was gradually overcome and hematopoietic cells

regenerated after several weeks. Such recovery may have been due to biotransformation of T-2 toxin into a non-toxic metabolite by the liver because recovery occurred more slowly in mice housed in suspension cages than in mice housed on softwood bedding, a known inducer of microsomal enzyme activity. Perioral dermatitis, gastritis, and hyperplasia of the gastric and duodenal mucosa, all of which were attributed to direct irritant toxicity of T-2 toxin to the upper alimentary tract, persisted in mice that overcame the suppression of hematopoiesis. Thus, mice eventually exhibited similar toxic effects as were observed in rats, and as have been reported in swine and poultry fed dietary T-2 toxin. Diets of low protein content (8%) potentiated the toxicity of dietary T-2 toxin to juvenile mice by prolonging the period of suppression of hematopoiesis and lymphopoiesis. These results supported the hypothesis that the normally low susceptibility of animals to the hematopoietic-suppressive or immunosuppressive effects of dietary T-2 toxin is increased under conditions of suboptimal protein nutrition.

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## 1.0 INTRODUCTION

Fungi of the genus Fusarium are frequent contaminants of plant products. In western Canada, many different strains and species of Fusarium have been isolated from various agricultural products, including cereal grain (Gordon, 1952), forage plants (Gordon, 1954a), and other non-cereal plants (Gordon, 1959). Some strains of Fusarium synthesize trichothecene mycotoxins under cold moist conditions (Bamburg and Strong, 1971), and might therefore be toxigenic under the climatic conditions that sometimes occur during harvest in the prairie region of Canada.

In the agricultural regions of the world which are climatically similar to the Canadian prairies, several health problems have been attributed to trichothecene mycotoxins in Fusarium-contaminated cereal products. Such problems have occurred in humans and livestock in the Soviet Union (Joffe, 1978), and in various species of livestock in the north-central regions of the United States (Mirocha et al., 1977). Accordingly, the prospect of contamination by trichothecenes of western Canadian cereals or other food and forage crops might be of concern to producers and users of these products.

The prevalence of trichothecenes in Canadian cereal food and feedstuffs is unknown because analytical methods suitable for their detection in most agricultural products are not available. However, the recent recognition of trichothecene mycotoxicosis in western Canadian livestock (Greenway and Puls, 1976) and the isolation of T-2 toxin from barley (Puls and Greenway, 1976) indicate that trichothecenes may occur in this region under natural conditions. Moreover, toxigenic strains of Fusarium poae isolated from hay in Saskatchewan (Davis and Smith, 1977) are potent

producers of T-2 toxin (Westcott, 1978, personal communication).

The studies outlined in this thesis were initiated because circumstantial and indirect evidence suggested that trichothecenes might frequently cause health problems in western Canadian livestock. The toxic effects of T-2 toxin, a naturally occurring trichothecene, on mammalian tissues were examined after topical exposure, during acute oral exposure and during subacute dietary exposure.

The irritancy of T-2 toxin to mammalian skin after topical application was examined because cutaneous irritation is an important means of detecting trichothecenes in feed extracts, and because irritation to skin and mucous membranes of the upper alimentary tract occurs in naturally occurring trichothecene mycotoxicosis of poultry and swine. The pathogenesis of cutaneous lesions induced by T-2 toxin was described in Section 3. In Section 4, the dose-response relationships of cutaneous inflammation under various conditions were examined. These studies enabled the development of a quantitative version of the skin-irritation bioassay for trichothecenes (Section 5).

Much of the concern that food-borne trichothecenes may be health hazards to man and livestock has been based on the knowledge that trichothecenes selectively injure rapidly proliferating germinal cells of lymphoid and hematopoietic tissues. The pathogenesis of changes in the intestinal mucosa, lymphoid tissues, and hematopoietic tissues of young mice after a single, intragastric, sublethal dose of T-2 toxin were examined histologically (Section 6) and ultrastructurally (Section 7).

The subacute toxic effects of dietary T-2 toxin were examined in

mice and rats (Sections 8-10). The main objective of these studies was the definition of conditions under which dietary T-2 toxin suppressed hematopoiesis and lymphopoiesis. Accordingly, the influences of the dietary protein level, the type of diet, the method of housing, the age, the species, the duration of exposure, and the level of T-2 toxin in the diet on the toxicity were evaluated.

## 2.0 REVIEW OF LITERATURE

Mycotoxins, which are toxic metabolites of fungi, may sometimes contaminate food of people or animals and thereby may cause diseases called mycotoxicoses. Several mycotoxicoses of humans and livestock have been recognized for many decades, or even centuries, but the role of mycotoxins in these diseases did not receive much investigative attention until the last 30 years. The discovery of aflatoxins as the cause of outbreaks of disease in various species of livestock in England dramatized the harmful role that mycotoxins may have on animal health (Allcroft et al., 1961). When the aflatoxins were recognized as carcinogens (Schoental, 1961; LeBreton et al., 1962), interest and research in the mycotoxin field increased in an exponential fashion, largely because of the concerns that mycotoxins in food might cause deleterious effects in the human population.

The enormous research activity on the mycotoxins, especially over the past 10 years, has generated many comprehensive reviews on the topic. During one year alone, ending in mid-1978, no less than eight books on mycotoxins were published (see Stoloff, 1979, for bibliography) to add to a similar number that were previously available. The rapid growth of activity in investigation of mycotoxins is further illustrated by the numbers of articles on mycotoxins that are presently being published in the literature. For example, in 1973, only 289 articles on mycotoxins were indexed in the Review of Medical and Veterinary Mycology, compared with 770 in 1978.

The scope of this review is narrow in relation to the entire field

of mycotoxicology. Comprehensive reviews of the mycotoxins are available in the works of Kadis et al. (1971), Purchase (1974), Rodricks et al. (1977), and in the encyclopaedic handbook edited by Wyllie and Morehouse (1977-1978).

Of the many different groups of mycotoxins (Table 1), only the trichothecenes are reviewed in this thesis. The main objective was to consider the available information on the toxic effects of trichothecenes so that a strategy for toxicological evaluation of the possible harmful effects of foodborne trichothecenes to human and animal health could be devised.

## 2.1 Trichothecene mycotoxins

The trichothecene mycotoxins are a large group of sesquiterpenoid metabolites of fungi (Bamburg and Strong, 1971). At least 47 trichothecene derivatives have been discovered; of these, 24 are metabolites of Fusarium spp. (Stoloff, 1979). T-2 toxin was the first of the group to be associated with mycotoxic diseases (Bamburg, 1969), and it remains the most important member of the group, although at least 12 other trichothecenes have been considered to be responsible for mycotoxicoses of man and livestock (see Section 2.1.2).

### 2.1.1 Background history

The majority of the 47 presently known trichothecene metabolites were discovered during the past 10 years as part of the search for mycotoxins that started when Bamburg et al. (1968b) discovered T-2 toxin as a mycotoxin produced by a toxigenic strain of Fusarium tricinctum. However, the first trichothecenes recognized were found long before, during the 1940s and 1950s in the search for antibiotic metabolites of fungi.

TABLE 1: List of some important mycotoxins

Fungi	Mycotoxins	Effects
<u>Aspergillus</u> spp.	Aflatoxins, Sterigmatocystin	Hepatotoxicity, Carcinogenesis
	Ochratoxins, citrinin	Nephrotoxicity
	Patulin	Carcinogenesis
<u>Penicillium</u> spp.	Ochratoxins, Citrinin	Nephrotoxicity
	Patulin, Penicillic acid	Carcinogenesis
	Luteoskyrin, Islanditoxin, Cyclochlorotine	Hepatotoxicity
	Citreoviridin	Neurotoxicity
	Rubratoxins	Hepatotoxicity
	Xanthomegnin, Viomellein	Hepatotoxicity
<u>Fusarium</u> spp.	Penitrems, Verruculogen	Neurotoxicity (tremors)
	Zearalenone	Estrogenism
	Trichothecenes	Dermatitis, Emesis, Food refusal, Cytotoxicity
	Moniliformin	Muscular weakness
<u>Stachybotrys atra</u> <u>Myrothecium</u> spp. <u>Trichothecium</u> sp. <u>Trichoderma</u> sp. <u>Dendroochium</u> sp. <u>Cephalosporium</u> sp.         ]	Trichothecenes	[ Dermatitis Emesis Food refusal Cytotoxicity
<u>Claviceps purpurea</u>	Ergot alkaloids	Ischemia
<u>Claviceps paspali</u>	Tremorgens	Neurotoxicity
<u>Sclerotinia sclerotiorum</u>	Psoralens	Photoactive dermatitis
<u>Phomopsis</u> sp.	Phomopsins	Hepatotoxicity
<u>Rhizoctonia</u> sp	Slaframine	Neurotoxicity (salivation)
<u>Pithomyces chartarum</u>	Sporidesmins	Hepatotoxicity
<u>Alternaria</u> <u>Cladosporium</u> Others       ]	Dicoumarol	Coagulopathy

Brian and McGowan (1946) identified a toxic product of Myrothecium roridum (Metarrhizium glutinosum) that they termed "glutinosin". This product had antifungal activity, but was not active against bacteria. These investigators subsequently recognized that a component of glutinosin was irritant to human skin, leading them to draw attention to the similarity between metabolites of M. roridum and the unidentified irritant metabolites of Stachybotrys alternans that were believed to cause stachybotryotoxicosis (Drobotko, 1945; Brian et al., 1947). Metabolites of Myrothecium verrucaria were also found by Bowden and Schantz (1955) to be irritant to the skin of rabbits. These irritant metabolites of Myrothecium were not isolated in pure form at that time, but later work by Tamm and coworkers isolated various antifungal metabolites called verrucarins and roridins (Nespiak et al., 1961; Harri et al., 1962). The structures of these metabolites were determined in Tamm's laboratory during the mid-1960s (Tamm, 1977; see Section 2.1.2), and Grove (1968) demonstrated that the irritant metabolites of M. verrucaria, detected originally by Brian et al. (1947), consisted of a mixture of verrucarins.

The first trichothecene to be isolated in pure form was an antifungal metabolite of Trichothecium roseum called trichothecin by Freeman and Morrison (1948). The strain of T. roseum used had originally been found by Brian et al. (1947) to produce antifungal metabolites. Trichothecin was also the first trichothecene to be structurally described, partially by Freeman et al. (1959) and completely by Godtfredsen and Vangedal (1965).

By the mid-1960s, over 10 members of the trichothecene family had been chemically identified, but none had been recognized as mycotoxins

(Bamburg et al., 1968b). During this period, seasonal conditions in the United States and Japan lead to widespread contamination of corn, rice and wheat with Fusarium moulds. In Japan in 1963, many people were affected by akakabi-byo (red mould disease) due to consumption of wheat contaminated with F. nivale (Miyaki, 1970). In the United States, during 1962, 1964 and 1965, corn crops became heavily contaminated with F. tricinatum and F. roseum, and caused toxicoses in various species of livestock, mainly cattle and swine (Smalley et al., 1970).

Gilgan et al. (1966) identified diacetoxyscirpenol (DAS) as a toxic metabolite of a strain of F. tricinatum originally isolated from mouldy corn that had been associated with an outbreak of a hemorrhagic syndrome of cattle (mouldy-corn poisoning; see Section 2.2.5). A more potent toxigenic strain (T-2) of F. tricinatum later isolated produced a trichothecene metabolite named T-2 toxin (Bamburg et al., 1968a; 1968b). Although many strains of F. tricinatum were toxigenic to yeasts in culture (Burmeister et al., 1972), attempts to identify trichothecene metabolites in toxic mouldy corn were unsuccessful until 1971 when Hsu et al. (1972) detected T-2 toxin in the feed of dairy cows which had died with a hemorrhagic syndrome.

In Japan, toxigenic strains of F. nivale isolated from mouldy rice and wheat also produced trichothecene mycotoxins; these were named nivalenol (Tatsuno, 1968; Ueno and Fukushima, 1968) and fusarenon-X (Ueno et al., 1969).

After the recognition of the mycotoxic role of some trichothecenes, retrospective considerations were used to implicate trichothecenes in severe mycotoxicoses that occurred in Russia during the 1930s and 1940s.

Bamburg (1969) drew attention to the similarity between the dermatitic activity of the trichothecene mycotoxins, T-2 toxin and DAS, which he had isolated from toxigenic F. tricinctum, and the dermatitic activity of the toxic extracts of F. sporotrichioides and F. poae, the mycotoxigenic fungi involved in alimentary toxic aleukia (ATA) of people in Russia during the 1940s (see Section 2.2.3). He suggested (Bamburg and Strong, 1971) that trichothecenes might be involved in ATA, and also in stachybotryotoxicosis (Section 2.2.4), a similar disease affecting horses and other livestock (Drobotko, 1945). These suggestions were supported by subsequent work on isolates of F. sporotrichioides and F. poae from outbreaks of ATA. Ueno et al. (1972c) found that these fungi could produce neosolaniol and T-2 toxin in culture, and Mirocha and Pathre (1973) detected T-2 toxin, neosolaniol and T-2 tetraol in an authentic sample of poaefusarin. Stachybotrys atra (S. alternans) was shown to produce macrocyclic trichothecenes by Eppley and Bailey (1973) and later by Szathmary et al. (1976) after examining an authentic toxigenic strain of S. alternans from eastern Europe.

#### 2.1.2 Chemistry

Trichothecene metabolites of fungi contain only carbon, hydrogen and oxygen, and are based on a tricyclic skeleton that was first described by Godtfredsen et al. (1965). These mycotoxins were previously called scirpenes after diacetoxyscirpenol, a metabolite of Fusarium scirpi found by Brian et al. (1961). However, Godtfredsen et al. (1965) proposed the name trichothecane for the parent tricyclic skeleton and this has become the accepted basis for nomenclature, although the scirpene trivial names are still in general use.

All naturally occurring mycotoxins in the trichothecane family possess an olefinic bond at the 9, 10 position, and an epoxy group at the 12, 13 position, so they are termed 12,13-epoxytrichothecenes (Fig. 1).

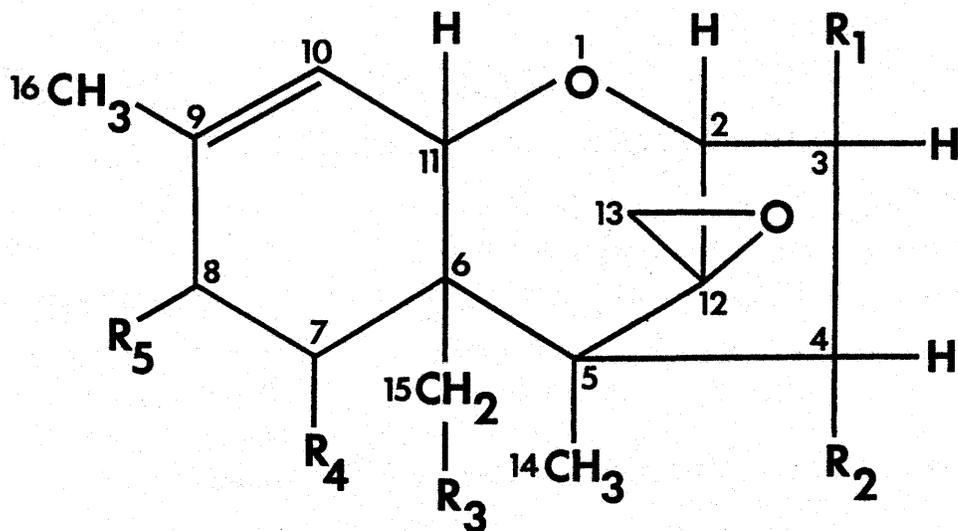


Figure 1: General structure of 12,13-epoxytrichothecene mycotoxins.

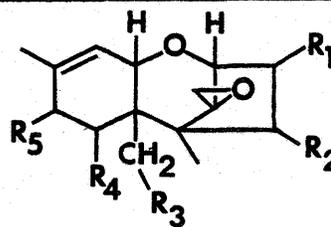
There are presently over 40 derivatives of 12,13-epoxytrichothec-9-ene that have been recognized as fungal metabolites (Stoloff, 1979). Detailed lists of 37 known derivatives have recently been published (Tamm, 1977; Mirocha et al., 1977). New derivatives have since been reported by Minato et al. (1975), Matsumoto et al. (1977), Ilus et al. (1977), Lansden et al. (1978), Steyn et al. (1978) and Ishii et al. (1978) (see Table 2).

The trichothecenes are divided into classes, depending on substitutions at positions 3, 7, 8 and 15. Ueno et al. (1972c)

classified trichothecenes into two classes; Group A consisted of derivatives with hydroxyl or acetoxy substituents at positions 3, 7, 8 and 15, whereas group B (Table 3) was characterized by a carbonyl group at C-8. Crotocin (Table 3) was classified separately into group C because of the existence of an additional epoxy group at the 7-8 position, whereas the macrocyclic trichothecenes (Table 4) were included in group D, due to the presence of a ring structure linking positions C-4 and C-15 (Ueno, 1977a). Some authors have classified the macrocyclic trichothecenes within group A because they lack the C-8 carbonyl group (Mirocha et al., 1977).

The known trichothecenes are alcohols or esters that exist as colourless crystalline stable compounds. They are soluble in moderately polar organic solvents, but are only slightly soluble in water (Bamburg and Strong, 1971). The polyalcohol members (e.g., trichothecin, deoxynivalenol and nivalenol) have less lipid solubility than the ester derivatives (Mirocha et al., 1977). Trichothecenes are chemically stable, remaining unchanged after cooking or after storage under laboratory conditions for long periods (Bamburg and Strong, 1971). However, under appropriate laboratory conditions, the esters and hydroxyl groups will undergo predictable chemical reactions (Bamburg and Strong, 1971). The chemical properties of the individual trichothecenes are generally recorded in the original descriptions of the first isolations listed for each toxin in Tables 2, 3 and 4. General reviews of their chemical properties are available in the recent discussions by Tamm (1977) or by Mirocha et al. (1977).

TABLE 2: Naturally occurring trichothecene mycotoxins. Group A.



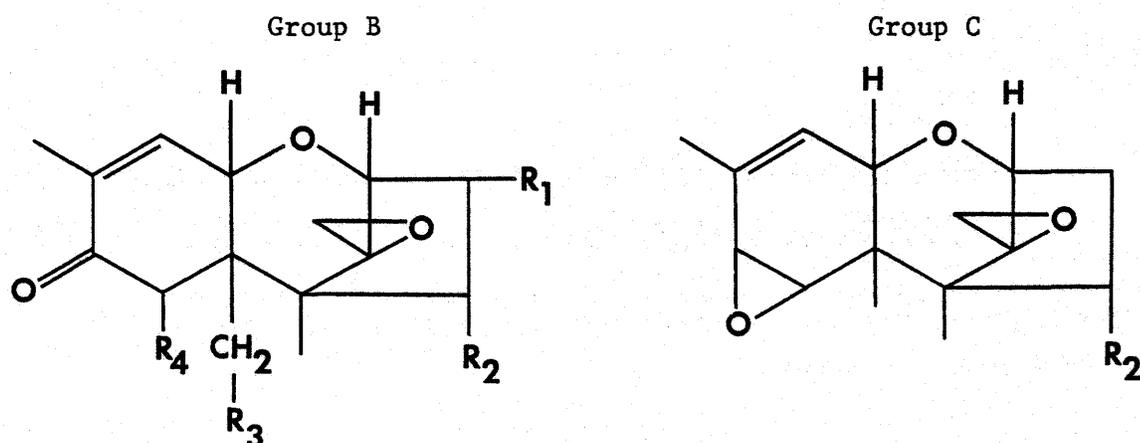
Group A toxins	C-3 (R <sub>1</sub> )	C-4 (R <sub>2</sub> )	C-15 (R <sub>3</sub> )	C-7 (R <sub>4</sub> )	C-8 (R <sub>5</sub> )	Reference
Trichodermol (Roridin C)	H	OH	H	H	H	4
Trichodermin	H	OAc	H	H	H	4
Verrucarol	H	OH	OH	H	H	*
Deacetylcalonecetrin	OAc	H	OH	H	H	3
Calonecetrin	OAc	H	OAc	H	H	3
Scirpentriol	OH	OH	OH	H	H	13
Monacetoxyscirpenol	OH	OH	OAc	H	H	13,14
Diacetoxyscirpenol	OH	OAc	OAc	H	H	2
4-acetoxyscirpendiol	OH	OAc	OH	H	H	9,14
Isoneosolaniol	OH	OAc	OAc	OH	H	8
T-2 tetraol	OH	OH	OH	H	OH	12
Neosolaniol	OH	OAc	OAc	H	OH	7
Monacetylneosolaniol	OH	OAc	OAc	H	OAc	9,11
7-hydroxydiacetoxyscirpenol	OH	OAc	OAc	OH	H	8
4,8-diacetoxyscirpenol	OH	OAc	OH	H	OAc	6
7,8-dihydroxydiacetoxyscirpenol	OH	OAc	OAc	OH	OH	8
Triacetoxyscirpendiol	OH	OAc	OAc	OH	OAc	5
HT-2 toxin	OH	OH	OAc	H	OCOCH <sub>2</sub> CH(CH <sub>3</sub> ) <sub>2</sub>	1
T-2 toxin	OH	OAc	OAc	H	OCOCH <sub>2</sub> CH(CH <sub>3</sub> ) <sub>2</sub>	1
Acetyl T-2 toxin	OAc	OAc	OAc	H	OCOCH <sub>2</sub> CH(CH <sub>3</sub> ) <sub>2</sub>	10
Triacetoxyscirpenol	OAc	OAc	OAc	H	H	14

References:

- Bamburg, *et al.*, 1968a
- Dawkins, 1966
- Gardner *et al.*, 1972
- Godtfredsen and Vangedal, 1965
- Grove, 1970b
- Ilus *et al.*, 1977
- Ishii *et al.*, 1971
- Ishii, 1975
- Ishii *et al.*, 1978
- Kotsonis *et al.*, 1975a
- Lansden *et al.*, 1978
- Mirocha and Pathre, 1973
- Pathre *et al.*, 1976
- Steyn *et al.*, 1978

\* Parent alcohol; natural production has not been demonstrated.

TABLE 3: Naturally occurring trichothecene mycotoxins. Groups B and C.

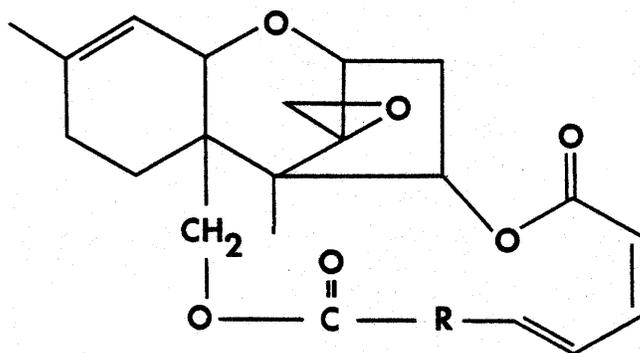


Group B toxins	C-3 (R1)	C-4 (R2)	C-15 (R3)	C-7 (R4)	References
Trichothecin	H	OCOCH=CHCH <sub>3</sub>	H	H	3
Deoxynivalenol	OH	H	OH	OH	8,9
Nivalenol	OH	OH	OH	OH	1,6
Monacetylnivalenol (Fusarenon-X)	OH	OAc	OH	OH	1,6,7
Diacetylnivalenol	OH	OAc	OAc	OH	1,4
Monacetyldeoxynivalenol	OAc	H	OH	OH	9
Group C toxin	C-3	C-4	C-15	C7-C8	
Crotocin	H	OCOCH=CHCH <sub>3</sub>	H	epoxy	2,5

References

1. Fujimoto *et al.*, 1972
2. Glaz *et al.*, 1959
3. Godtfredsen and Vangedal, 1965
4. Grove, 1970a
5. Gyimesi and Melara, 1967
6. Tatsuno *et al.*, 1969
7. Ueno *et al.*, 1969
8. Vesonder *et al.*, 1973
9. Yoshizawa and Morooka, 1973

TABLE 4: Important naturally occurring macrocyclic trichothecene mycotoxins



<u>Macrocyclic toxin</u>	<u>R group</u>	<u>References</u>
Verrucarin A	$\begin{array}{c} \text{OH} \quad \text{CH}_3 \\   \quad   \\ -\text{CH}-\text{CH}-(\text{CH}_2)_2-\text{O}-\overset{\text{O}}{\parallel}{\text{C}}- \\   \\ \text{CH}_3 \end{array}$	6,8
B	$\begin{array}{c} \text{O} \\ \diagup \quad \diagdown \\ -\text{CH}-\text{C}-(\text{CH}_2)_2-\text{O}-\overset{\text{O}}{\parallel}{\text{C}}- \\   \\ \text{CH}_3 \end{array}$	7
J	$\begin{array}{c} \text{O} \\ \parallel \\ -\text{CH}=\text{C}-(\text{CH}_2)_2-\text{O}-\text{C}- \\   \\ \text{CH}_3 \end{array}$	3,5
Roridin A	$\begin{array}{c} \text{OH} \quad \text{CH}_3 \quad \text{CHOHCH}_3 \\   \quad   \quad   \\ -\text{CH}-\text{CH}-(\text{CH}_2)_2-\text{O}-\text{CH}- \\   \\ \text{CH}_3 \end{array}$	1,8
D	$\begin{array}{c} \text{O} \\ \diagup \quad \diagdown \\ -\text{CH}-\text{C}-(\text{CH}_2)_2-\text{O}-\text{CH}- \\   \\ \text{CH}_3 \end{array}$	2,3
E	$\begin{array}{c} \text{CHOHCH}_3 \\   \\ -\text{CH}=\text{C}-(\text{CH}_2)_2-\text{O}-\text{CH}- \\   \\ \text{CH}_3 \end{array}$	3
H	$\begin{array}{c} \text{O}-\text{CHCH}_3 \\   \\ -\text{CH}=\text{C}-\text{CH}_2-\text{CH}-\text{O}-\text{CH}- \\   \\ \text{CH}_3 \end{array}$	9
Satratoxin H	$\begin{array}{c} \text{H} \quad \text{CHOHCH}_3 \\   \quad   \\ -\text{C}=\text{C}-\text{CH}_2-\text{CH}_2-\text{O}-\text{C}- \\   \quad   \\ \text{CHOH} \end{array}$	4

References

1. Böhner and Tamm, 1966a
2. Böhner and Tamm, 1966b
3. Böhner et al., 1965
4. Eppley et al., 1977
5. Fetz et al., 1965
6. Gutzwiller and Tamm, 1965a
7. Gutzwiller and Tamm, 1965b
8. Härrri et al., 1962
9. Traxler and Tamm, 1970

### 2.1.3 Fungi producing trichothecenes

Fungi belonging to the genera Fusarium, Stachybotrys, Myrothecium, Trichoderma, Trichothecium, and Cephalosporium (Acremonium) have been found to produce trichothecene metabolites in experimental cultures. Of the known trichothecenes, more than half are metabolites of Fusarium. All trichothecenes in Table 2 are metabolites of Fusarium, except for trichodermin and trichodermol (produced by Trichoderma lignorum and T. viride, Godtfredsen and Vangedal, 1965). All trichothecenes listed in group B in Table 3, with the exception of trichothecin, a metabolite of Trichothecium roseum (Godtfredsen and Vangedal, 1965), are also produced by members of the genus Fusarium. On the other hand, all of the macrocyclic trichothecenes, some of which are listed in Table 3, are metabolites of Myrothecium verrucaria, M. roridum, and Stachybotrys alternans (syn. S. atra). Crotochin is a product of Cephalosporium crotochinogenum (Glaz et al., 1959).

All fungi known to produce trichothecenes are members of the order Hyphomycetales in the Deuteromycotina (Fungi Imperfecti) (Smalley and Strong, 1974). Myrothecium and Stachybotrys, which belong to the family Dematiaceae, are mainly saprophytic soil fungi that grow on decaying plant matter, whereas the other genera of trichothecene-producing fungi belong to the family Moniliaceae. The species of Trichoderma (2 of 20), Trichothecium (1 of 4) and Cephalosporium (1 of 20-70) that have so far been recognized to produce trichothecenes are saprophytes that possess weakly parasitic capabilities (Smalley and Strong, 1974).

The genus Fusarium contains many species and strains of fungi that

are widely distributed in nature, either as saprophytes or as plant parasites. The diversity of hosts and substrates affected by fusaria, and the difficulties in defining species on morphological grounds have led to marked confusion in terminology and identification of isolates belonging to this genus. Snyder and Hansen (1940, 1941, 1945) proposed a simplified classification of 65 species of fusaria into only eight species, but this system has been widely criticized as being oversimplified, particularly in regard to F. tricinctum (Snyd. et Hans.) and F. roseum (Snyd. et Hans.), the two major producers of trichothecenes. Other more detailed systems have been recently devised (Booth, 1971; Joffe, 1974c).

The classification and the nomenclature of North American isolates of *Fusarium* involved in production of trichothecene mycotoxins has been largely based on the Snyder and Hansen system. Synonymy and problems associated with the various taxonomic systems have been discussed by many authors, including Booth (1975) and Tousson and Nelson (1975). Because of the confusion with the present systems, it might be better to identify mycotoxigenic fusaria according to the more discriminatory system used by Gordon (1952, 1954a, 1954b, 1959) in his extensive survey and taxonomy of fusaria isolated from Canadian cereals, grasses and other plants.

The major trichothecene-producing species of Fusarium and some of their synonyms are listed in Table 5. A list of all reports of production of trichothecene mycotoxins by various species of fungi has been recently compiled by Mirocha et al. (1977).

The list of known trichothecene metabolites of fungi is composed largely of reports arising from chemical analyses of extracts of cultured

TABLE 5: Fusarium species that produce trichothecenes in culture

Species		Trichothecenes produced in cultures	References
Booth (1971)	Snyder and Hansen (1945)		
<u>F. oxysporum</u>	<u>F. oxysporum</u>	Fusarenon-X, diacetylnivalenol	9
<u>F. solani</u> )	<u>F. solani</u>	HT-2, T-2, DAS, neosolaniol	9
<u>F. illudens</u> )			
<u>F. ventricosum</u> )			
<u>F. tumidium</u> )			
<u>F. decemcellulare</u>	<u>F. rigidiscula</u>	DAS, neosolaniol	9
<u>F. moniliforme</u>	<u>F. moniliforme</u>	--	
<u>F. lateritium</u> )	<u>F. lateritium</u>	DAS, 7-hydroxy-DAS 7,8-dihydroxy-DAS, neosolaniol diacetylnivalenol	10
<u>F. udum</u> )			
<u>F. xylarioides</u> )			
<u>F. stilboides</u> )			
<u>F. nivale</u>	<u>F. nivale</u>	Nivalenol, fusarenon-X, diacetylnivalenol	8
<u>F. tricinctum</u> <sup>a</sup> )	<u>F. tricinctum</u> <sup>d*</sup>	a. T-2 b. T-2, HT-2, T-2tetraol neosolaniol c. T-2, T-2tetraol, HT-2, neosolaniol d. T-2, HT-2, DAS, 4, 8-DAS, MAS	12 5,12 12 1,4
<u>F. poae</u> <sup>b</sup> )			
<u>F. sporotrichioides</u> <sup>c</sup> )			
<u>F. fusaroides</u> )			
<u>F. sambucinum</u>	<u>F. roseum</u> 'Sambucinum'	DAS	5
<u>F. culmorum</u>	<u>F. roseum</u> 'Culmorum'	DAS, neosolaniol, T-2, HT-2	9
<u>F. graminearum</u>	<u>F. roseum</u> 'Graminearum'	deoxynivalenol	5,11
<u>F. avenaceum</u>	<u>F. roseum</u> 'Avenaceum'	neosolaniol, T-2, DAS	9
<u>F. concolor</u>	<u>F. roseum</u> 'Concolor'	--	
<u>F. equiseti</u>	<u>F. roseum</u> 'Equiseti'	DAS, TAS, 7-hydroxy DAS	2
<u>F. acuminatum</u>	<u>F. roseum</u> 'Acuminatum'	--	
<u>F. gibbosum</u>	<u>F. roseum</u> 'Gibbosum'	scirpentriol, MAS 8-acetylneosolaniol 4-acetoxyscirpendiol	6 3
Various others incl. <u>F. sulphureum</u>	<u>F. roseum</u> 'Heterosporium'	DAS, T-2, 4-acetoxyscirpenol TAS	7
	<u>F. roseum</u> (Snyd. et Hans.) <sup>**</sup>	deoxynivalenol, 4-acetoxyscirpenol, nivalenol DAS, MAS	5

References:

1. Bamberg and Strong, 1971
2. Grove, 1970a; 1970b
3. Ishii et al., 1978
4. Lansden et al., 1978

Continued -

Table 5

References (continued)

- |                                 |                                   |
|---------------------------------|-----------------------------------|
| 5. Mirocha <u>et al.</u> , 1977 | 9. Ueno <u>et al.</u> , 1973c     |
| 6. Pathre <u>et al.</u> , 1976  | 10. Ueno <u>et al.</u> , 1977     |
| 7. Steyn <u>et al.</u> , 1978   | 11. Vesonder <u>et al.</u> , 1976 |
| 8. Ueno <u>et al.</u> , 1969    | 12. Yagen and Joffe, 1976         |

\* Letters indicate toxins produced according to the name of the isolates used. Joffe (1974b) handles this F. tricinctum section differently. His system is similar to Booth's, but he regards F. tricinctum as a subtype of F. sporotrichioides.

\*\* Many authors do not use the cultivar terminology of Snyder and Hansen and use the name F. roseum alone.

Abbreviations:           MAS = monacetoxyscirpenol  
                          DAS = diacetoxyscirpenol  
                          TAS = triacetoxyscirpenol

isolates of Fusarium. Many different substrates and culture media have been employed under various environmental conditions so the significance of the determined capabilities of the different species to produce or not produce trichothecene metabolites is of questionable relevance in assessing the toxigenicity of a particular species or isolate of Fusarium. Several studies have shown that production of toxins such as T-2 toxin (Bamburg et al., 1968a; Burmeister, 1971), diacetoxyscirpenol (Gilgan et al., 1966) or poaeufusarin (probably T-2 toxin) (Joffe, 1971, 1974a) is increased by low temperatures ( $< 8^{\circ}\text{C}$ ), although growth of the fungi is better at higher temperatures. The substrate and amount of light have also been found to influence toxigenicity of isolates of Fusarium (Joffe, 1974b). Under uniform culture conditions, a wide range of toxigenicity among isolates of Fusarium spp. has been observed in surveys by Burmeister et al. (1972), Ueno et al. (1973c), Kvashnina (1976), and Joffe and Yagen (1977). Thus, although species of Fusarium that are capable of producing trichothecenes are widely distributed, very little is known about the levels of the different toxins produced in cereals and forages during natural environmental conditions.

The biosynthetic pathways in which trichothecenes are formed have been thoroughly reviewed by Turner (1971) and Tamm (1977), and so will not be discussed here. Trichothecenes are considered to be secondary metabolites derived from acetate via mevalonate and farnesyl pyrophosphate, an acyclic sesquiterpene. After cyclization and minor modifications, the tricyclic trichothecene skeleton is formed which then undergoes hydroxylation and esterification to form the various 12,13-epoxy-

trichothecene metabolites. Most of the known trichothecenes are fungal metabolites, but baccharin, a triepoxytrichothecene, has recently been found in Baccharis megapotamica, a higher plant (Asteraceae) (Kupchan et al., 1976).

#### 2.1.4 Detection of trichothecenes

In spite of the large volume of literature on the chemical, biochemical and biological effects of trichothecenes, there are very few authentic reports of natural occurrence of trichothecenes in feedstuff (see Section 2.1.5). This is believed to be due to a lack of adequate practical methods for unambiguous detection of trichothecenes, rather than to infrequent occurrence of the toxins (Pathre and Mirocha, 1977). Although a large number of methods, both biological and physicochemical, suitable for detection of trichothecenes have been reported over the last 15 years, very few of them have been shown to be useful for routine detection of trichothecenes in feedstuffs.

The analytical difficulties are due to three problems not encountered in the analyses for other mycotoxins such as aflatoxins and ochratoxins. Firstly, the trichothecene compounds lack chemical groupings that either appreciably absorb ultraviolet light or exhibit fluorescence, so they are difficult to detect and quantify by thin layer chromatography (TLC) (Bamburg and Strong, 1971; Pathre and Mirocha, 1977). Secondly, the polarity and solubility characteristics are such that it is difficult to extract them from feedstuffs so that they are free of other contaminating plant or mould products. Thirdly, the complex methods needed to extract and detect trichothecenes at present cannot practically be used to detect more than three or four trichothecenes in any one sample (Stoloff, 1979). As a result of such problems, meaningful surveys for trichothecenes in feedstuffs have not been conducted, although recent developments in gas chromatographic-mass spectrometric methods by Mirocha et al. (1976a) have led to sensitive detection of T-2 toxin, diacetoxyscirpenol and

deoxynivalenol in mixed feedstuffs.

The physicochemical methods available for extracting, identifying and quantifying trichothecenes have been recently reviewed by Bamburg (1972), Eppley (1975), Pathre and Mirocha (1977) and Mirocha et al. (1977). The main methods with comparisons of their performance characteristics are presented in Table 6.

Due to the problems with or unavailability of physicochemical methods for detection and quantifying trichothecenes, many biological methods have been developed (Table 7). Such biological tests have the advantages that they are, in most instances, more sensitive than physicochemical methods, and they can be used to detect or measure a broad range of different trichothecenes, either alone or in mixtures. Furthermore, biologically inactive impurities cause less interference in biological methods. The major limitation of most of the biological methods is lack of specificity; most cannot be used to distinguish trichothecenes from other toxic compounds. Methods such as the rabbit reticulocyte method of Ueno et al. (1973c) and the various skin-irritation methods (Table 7) are more specific for trichothecenes as a group, but they cannot be used to distinguish among the different trichothecenes.

TABLE 6: Physiochemical methods for detection and quantification of trichothecenes in feed samples.

Method	Toxins detected	Detection limits	Performance and limitations	References
TLC,1-dimension	All <sup>a</sup>	1 µg/spot	Qualitative. Interference. Confirmation required.	Scott <u>et al.</u> (1970) Ueno <u>et al.</u> (1973c).
TLC,2-dimension	All <sup>a</sup>	0.1-1.0 µg /spot	Qualitative. Less interference. Confirmatory.	Mirocha <u>et al.</u> (1977)
GLC	Hydroxylated trichothecenes <sup>a</sup>	0.02-0.05 µg/µl	Quantitative. Equivocal identity. Interference from monoglycerides.	Bamburg (1969) Ikediobi <u>et al.</u> (1971), Mirocha <u>et al.</u> (1976b), Romer <u>et al.</u> (1978).
GC/MS	All <sup>b</sup>	0.02-0.05 µg/µl	Unequivocal. Semiquantitative. Some interference reduces sensitivity.	Mirocha <u>et al.</u> (1976a), Pathre and Mirocha (1977).
GC/MS with SIM	All <sup>b</sup>	0.007-0.02 µg/µl	Suitable for complex extracts. Unequivocal identification. May be quantitative.	Mirocha <u>et al.</u> (1976a) Pathre and Mirocha (1977).
NMR	All <sup>b</sup>	--	Confirmatory. Structure elucidation.	Bamburg and Strong (1971), Ellison and Kotsonis (1976).
RIA	T-2 toxin <sup>c</sup>	1-20 ng	Sensitive. Low interference.	Chu <u>et al.</u> (1979).

<sup>a</sup> Authentic standards required.

<sup>b</sup> Authentic standards or spectrograms required.

<sup>c</sup> Rabbit anti-T-2 toxin antibody and <sup>3</sup>H-T-2 toxin required.

Abbreviations: TLC, Thin-layer chromatography; GLC, Gas-liquid chromatography; GC/MS, Gas chromatography-mass spectrometry; SIM, Selected-ion monitoring; NMR, Nuclear-magnetic-resonance; RIA, Radioimmunoassay.

TABLE 7: Bioassay systems sensitive to trichothecenes

Bioassay system	End point	Use <sup>a</sup>	References
<b>1. Fungal cultures</b>			
<u>Candida albicans</u>	Fungistasis	S	Godtfredsen and Vangedal (1965)
		X	Glaz <u>et al.</u> (1959)
		X	Sorenson <u>et al.</u> (1975)
<u>Histoplasma capsulatum</u>	Fungistasis	X	Freeman (1955)
<u>Rhodotorula rubra</u>	Fungistasis	S,E	Burmeister <u>et al.</u> (1972)
<u>Saccharomyces cerevisiae</u>	Inhibition of fermentation	T	Reiss (1973)
<u>Penicillium digitatum</u>	Spore germination	X	Freeman (1955)
		S,E	Burmeister <u>et al.</u> (1972)
<b>2. Higher plants</b>			
Peas	Inhibition of germination	S	Burmeister and Hesseltine (1970)
Peas	Inhibition of growth	X	Brian <u>et al.</u> (1961)
		X	Marasas (1969)
Pollen grains	Inhibition of germination	E	Siriwardana and Lafont (1978)
<u>Kalanchoe daigremontiana</u>	Inhibition of growth of roots	T	Reiss (1977)
Peas, beans, barley	Inhibition of growth, Mortality of seedlings	S	Joffe (1974b)
<b>3. Protozoa</b>			
<u>Tetrahymena pyriformis</u>	Inhibition of multiplication	X	Ueno and Yamakawa (1970)
<b>4. Arthropods</b>			
<u>Tribolium confusum</u>	Inhibition of larval hatching	T	Wright <u>et al.</u> (1976)
<u>Aedes aegypti</u>	Larval mortality	T	Grove and Hosken (1975)
<u>Tenebrio molitor</u>	Larval growth inhibition	S	Davis <u>et al.</u> (1975)
	Larval mortality	S	Davis and Smith (1977)
<u>Corcyra cephalonica</u>	Growth inhibition	S	Parthasarathy <u>et al.</u> (1972)
<u>Artemia salina</u> (Brine shrimp)	Mortality	X,E,T	Harwig and Scott (1971)
		E,T	Eppley (1974)
		S	Reiss (1972)
<u>Drosophila melanogaster</u>	Larval mortality	T	Reiss (1975)

Table 7 - Continued

Bioassay system	End point	Use <sup>a</sup>	References
5. Birds			
Ducklings	Vomition	T	Ueno <u>et al.</u> (1971b)
Pigeons	Vomition	X	Ellison and Kotsonis (1973)
Chick embryos	Mortality	E	Dervish (1976)
Chickens	Toxicity, mortality	S	Kirksey and Cole (1974)
6. Mammals			
Cell cultures	Cytotoxicity	T	Grove and Mortimer (1969)
		T	Ohtsubo <u>et al.</u> (1968)
		S	Bodon and Zöldág (1974)
Rabbit reticulocytes	Inhibition of protein synthesis	T,S	Ueno <u>et al.</u> (1969)
		T,E	Ueno and Shimada (1974)
Topical application to skin of rabbits or rats	Dermatitis	X	Gilgan <u>et al.</u> (1966)
		T,E	Bamburg (1969)
		S	Joffe (1971)
		F	Wei <u>et al.</u> (1972)
		F	Chung <u>et al.</u> (1974)
Rats	Food refusal	X	Kotsonis <u>et al.</u> (1975b)
Mice	Lethality	F,T	Ueno <u>et al.</u> (1973c)
		S	Christensen <u>et al.</u> (1968)
Rats	Hepatic activity of glutathione-S-epoxide transferase	E	Foster <u>et al.</u> (1975)

<sup>a</sup> Code indicating use of each system as described:

- F Bioassay used to survey feed samples for trichothecenes.
- E Experimental method not fully developed for survey use.
- S Screening test for determining toxigenicity of fungal isolates.
- T Bioassay for examining comparative toxicity of pure trichothecenes.
- X Experimental method used to detect biological activity during identification of trichothecenes.

### 2.1.5 Occurrence of trichothecenes

Mycological surveys (Burmeister et al., 1972; Ueno et al., 1973c; Joffe and Palti, 1974; Szathmary et al., 1976; Joffe and Yagen, 1977; Pathre and Mirocha, 1978) have demonstrated that trichothecene-producing strains of Fusarium are widespread in the temperate climatic zones of eastern Europe, Japan and North America where cool, moist conditions conducive to production of trichothecenes often prevail during the harvesting of cereal crops. Since the detection of T-2 toxin in mouldy corn by Hsu et al. (1972), T-2 toxin has been detected in various grain products in USA, UK, Canada, India and France (see Table 8). Most of these reports have been published since 1976. Some of the reported identifications were based solely on TLC detection (Table 8) so are equivocal because of the possibility that interfering compounds may have produced spots with the same Rf value as T-2 toxin in the TLC systems used. Diacetoxyscirpenol has been found on only three occasions (Table 8). Deoxynivalenol (vomitoxin) has been frequently detected in samples of mouldy corn responsible for food refusal and emesis by swine (see Section 2.2.1) in various parts of the world since 1976 (Table 8). Deoxynivalenol is usually found together with zearalenone in corn contaminated by Fusarium roseum (Mirocha et al., 1976a; Mirocha, 1979). Deoxynivalenol has not yet been detected in Canadian corn because of the unavailability of analytical methods, but it seems likely that it is prevalent in southern Ontario where zearalenone is frequently found in corn that is refused by swine (Funnell, 1979).

The only large scale surveys for trichothecenes so far conducted have been done using dermal irritation bioassays. Two such surveys have

been reported (Eppley et al., 1974; Balzer et al., 1977) in which relatively high proportions of tested corn samples have contained extractable skin-irritant factors believed to be trichothecenes (Table 8). Analytical examination of some of the irritant samples previously examined by Eppley et al. (1974) has shown the presence of deoxynivalenol (Ciegler, 1978). These studies suggest that trichothecene contamination of corn samples in North America might be common in some years. However, meaningful analytical surveys are unlikely to be conducted for many years because of the present unavailability of suitable physicochemical methods for screening feed samples.

TABLE 8: Natural occurrence of trichothecene mycotoxins in feedstuffs.

Toxin	Country	Source	Levels (ppm)	Reasons for analysis	References
<u>T-2 toxin</u>					
	USA	Corn	2	Hemorrhagic syndrome in dairy cows	6
	USA	Mixed feed	0.08	Bloody feces in cows	11
	USA	Mixed feed	0.3	Emesis and diarrhea in swine	12
	Canada	Barley	25 <sup>a</sup>	Food refusal; alimentary irritation of swine and poultry	14
	UK	Brewers grains and silage	ND <sup>a</sup>	Hemorrhagic syndrome in cattle	13
	USA	Mixed feed	ND <sup>a</sup>	Hemorrhagic syndrome in cattle	5
	India	Safflower seed	ND <sup>a</sup>	Survey	4
	India	Sorghum	ND	Survey	14
	France	Corn	0.02 <sup>b</sup>	Feed refusal in swine, estrogenism	7
	UK	Mixed feed	100 <sup>a</sup>	Hemorrhagic disease in swine	10
<u>Diacetoxyscirpenol</u>					
	USA	Mixed feed	0.5 0.38	Hemorrhagic bowel syndrome in swine	11
	India	Safflower seed	ND <sup>a</sup>	Survey	4
	USA	Corn	ND	Feed refusal and emesis of swine	15
<u>Deoxynivalenol (vomitoxin)</u>					
	USA	Corn	ND	Feed refusal and emesis of swine	15
	USA	Corn	1.8 <sup>b</sup>	Feed refusal of swine	11
		"	1.0 <sup>b</sup>	" " " "	11
		"	0.1 <sup>b</sup>	" " " "	11
		"	0.04 <sup>b</sup>	" " " "	11
	USA	Mixed feed	1.0	Feed refusal of swine	11
	USA	Corn	1.0 <sup>b</sup>	Unthriftiness of cattle	12
		"	0.06 <sup>b</sup>	" " " "	12
	USA	Mixed feed	1.0	Vomiting of dogs	11
		" "	0.07 <sup>b</sup>	Death of dogs	12
	USA	Corn	Various	Survey	2
	France	Corn	0.6 <sup>b</sup>	Feed refusal of swine	8
	Japan	Barley	ND		7,17
	South Africa	Corn	2.5	Survey	9
	Zambia	Corn	7.4	Survey	9

Table 8 : Continued -

Toxin	Country	Source	Levels (ppm)	Reasons for analysis	References
<u>Skin irritant trichothecenes (undifferentiated)</u>					
	USA	Corn	93 positive of 173	Survey	3
	Yugoslavia	Corn	16 positive of 191	Survey	1

<sup>a</sup> Equivocal detection by TLC; identity not confirmed.

<sup>b</sup> Present with zearalenone.

ND = Level not determined.

References

1. Balzer et al. (1977)
2. Ciegler (1978)
3. Eppley et al. (1974)
4. Ghosal et al. (1977)
5. Hibbs et al. (1974)
6. Hsu et al. (1972)
7. Ishii et al. (1975)
8. Jemmali et al. (1978)
9. Marasas et al. (1977)
10. Miller (1976)
11. Mirocha et al. (1976a)
12. Mirocha (1979)
13. Petrie et al. (1977)
14. Puls and Greenway (1976)
15. Rukmini and Bhat (1978)
16. Vesonder et al. (1973)
17. Yoshizawa and Morooka (1973)

## 2.2 Trichothecene mycotoxicoses

Various diseases of animals and humans in various parts of the world have been attributed to the ingestion of trichothecene mycotoxins. However, much of the evidence used to incriminate the trichothecenes as the causative agents has been circumstantial, retrospective, or indirect; very few of the effects observed in the naturally occurring mycotoxicoses have been experimentally reproduced with pure trichothecene toxins. The various diseases described in this section were originally named according to the type of mouldy foodstuff (e.g., mouldy-corn poisoning), the type of fungus involved (e.g., fusariotoxicosis, stachybotryotoxicosis), or the clinical effects observed (e.g., alimentary toxic aleukia, hemorrhagic syndrome, emesis and refusal). These diseases have many similar features and should be considered as recognizable syndromes consistent with the range of effects caused by trichothecene mycotoxins; they should not be considered to be distinct disease entities. However, until the role of trichothecenes in the causation of these diseases is understood, it is better to consider them separately rather than collectively under the term trichothecene toxicosis.

### 2.2.1 Food refusal and emesis syndrome

After wet harvest seasons, corn or barley crops may become heavily contaminated by Fusarium graminearum (F. roseum, Gibberella zeae). In the U.S.A., the resulting "scabby" grain has long been recognized to either be refused by swine, or else cause vomiting soon after it has been eaten (Dickson et al., 1930). The same syndrome has also been recognized in swine fed mouldy grain in Korea (Cho, 1964), France (Jemmali et al., 1978), Romania (Mitroiu et al., 1976) and Canada

(Funnell, 1979), often in association with the estrogenic effects of zearalenone (see Section 2.2.2). Similar effects of Fusarium-contaminated grain have also occurred in people in Russia (Dounin, 1930) and Japan (Yoshizawa and Morooka, 1977).

A water-soluble agent that induced vomiting in swine and pigeons was extracted from corn contaminated by F. graminearum (F. roseum) by Prentice and Dickson (1968) and also by Curtin and Tuite (1966). Later work by Vesonder et al. (1973, 1976) and by Yoshizawa and Morooka (1977) identified deoxynivalenol (vomitoxin) as the extractable emetic agent, and this conclusion has been supported by the identification of deoxynivalenol in samples of feed that had been refused or vomited by pigs or dogs (Mirocha et al., 1976a; also see Table 8), and by experimental reproduction of the refusal and emesis syndrome with deoxynivalenol fed to swine (Forsyth et al., 1977). Some investigators have suggested that different toxins might be involved in causing emesis and refusal (Curtin and Tuite, 1966; Kotsonis et al., 1975). These latter investigators, using ethyl acetate, were not able to extract emetic factors from mouldy corn refused by swine, but it has since been shown that deoxynivalenol should be extracted with a more polar solvent such as methanol (Pathre and Mirocha, 1978). Although it appears now that deoxynivalenol is the main trichothecene responsible for most field occurrences of this syndrome (see Table 8), other trichothecenes such as T-2 toxin may cause the same food refusal and emetic effects (Ellison and Kotsonis, 1973; Ueno et al., 1974a; Szathmary and Rafai, 1978). Forsyth et al. (1977) found that the food refusal effects of naturally moulded corn were greater than those of

normal feed containing the same level of deoxynivalenol and suggested that other factors in addition to deoxynivalenol might be involved under natural conditions.

### 2.2.2 Fusariotoxicosis

Fusariotoxicosis is a term used to describe diseases due to the consumption of cereals contaminated by Fusarium fungi. The most common fusariotoxicoses are due to F. graminearum (F. roseum), and are associated with the food refusal and emesis syndrome described above (Section 2.2.1). Some authors use the term fusariotoxicosis to refer specifically to the effects of F. graminearum-infested corn in swine in which the predominant effect is due to zearalenone, the estrogenic mycotoxin of F. graminearum. Since F. graminearum produces toxic levels of zearalenone only on corn, wheat, and rice (Mirocha et al., 1971; Hesselatine, 1978), and because zearalenone does not cause recognizable estrogenic effects in ruminants and horses, the estrogenic syndrome of swine (zearalenone toxicosis) should be regarded as only one form of fusariotoxicosis.

Signs of irritation of the alimentary tract, such as gastroenteritis and diarrhoea, have also been reported in conjunction with estrogenic and emetic effects in swine with fusariotoxicosis due to F. graminearum (Moreau, 1974; Mitroiu et al., 1976). Similar clinical signs without estrogenism have occurred in swine consuming Fusarium-contaminated barley and oats (Greenway and Puls, 1976).

Fusariotoxicosis has been reported in chickens (Wyatt et al., 1972a), ducks and geese (Greenway and Puls, 1976) in which the main effects observed were weight loss, perioral dermatitis, and irritation of the

mucosa of the oral cavity and the upper alimentary tract. Similar lesions of caseous yellowish-white necrotic plaques in the oral cavity and at the angles of the mouth have been experimentally reproduced in chickens fed diets containing T-2 toxin (Wyatt et al., 1972b; Chi et al., 1977b; Speers et al., 1977; Joffe, 1977), diacetoxyscirpenol (Chi and Mirocha, 1978), or cultures of F. tricinctum (Christensen et al., 1972), or of F. sporotrichioides (Joffe, 1977). Wyatt et al. (1972b) suggested that the necrotic plaques, although sometimes infected with Staphylococcus aureus, Escherichia coli or Trichophyton gallinae, were probably initiated by the irritant effect of T-2 toxin present in the diet. Geese (Palyusik and Koplic-Kovács, 1975; Puls and Greenway, 1976) and turkey poults (Richard et al., 1978) appear to be more susceptible to dietary T-2 toxin than chickens under experimental conditions.

Fusariotoxicooses have long been recognized in various species of farm animals in the USSR (see Kurmanov, 1977, for review). The reported diseases have occurred in sheep, cattle, swine and poultry in association with the consumption of small grains or forages contaminated with Fusarium fungi, but the causative toxins have not been identified. Presently very little evidence indicates that trichothecenes are involved. Acute, subacute and chronic forms of naturally occurring fusariotoxycosis have been observed both in calves and sheep, and similar disease patterns have been reproduced experimentally in sheep and calves given cultures of F. sporotrichioides by stomach tube (Kurmanov, 1977). In acute experimental fusariotoxycosis, sheep become depressed, weak, ataxic and anorexic. The rumenal activity ceases and the feces become

loose and sometimes bloody after one or two days. Within three days, affected sheep become comatose and die. In the subacute form, sheep become depressed and anorexic and develop fissures and necrosis of the skin of the lips. They display signs of abdominal discomfort, muscle weakness, ataxia and unsteadiness on their feet and eventually develop blood-stained diarrhea and die after 5 to 8 days. During subacute toxicosis, sheep initially develop neutrophilic leukocytosis, but become leukopenic after 4-5 days. In the chronic form, the major signs are weakness, depression, anorexia, poor growth, poor mobility, and fissures in the skin of the mouth and nose. During chronic toxicosis, sheep develop leukopenia as in the subacute form, but subsequently develop a marked neutrophilia (Kurmanov, 1977).

The post mortem findings in sheep with fusariotoxicosis are regarded as being essentially similar in each of the acute, subacute and chronic forms. Consistent findings are hemorrhagic diathesis, catarrhal rhinitis, catarrhal to hemorrhagic gastritis, catarrhal colitis, enlarged lymph nodes, pulmonary edema, splenic and bone marrow atrophy. This spectrum of experimentally produced lesions and clinical signs is considered to be typical of naturally occurring fusariotoxicosis of calves and sheep (Kurmanov, 1977). In experimental trials with pure trichothecenes fed to various animal species, these patterns of clinical and post mortem changes have not been reproduced (see Sections 2.3.4; 2.3.5). Thus, the causative role of trichothecene mycotoxins in causing the effects of fusariotoxicosis in ruminants remains equivocal.

### 2.2.3. Alimentary toxic aleukia

Alimentary toxic aleukia (ATA) is a fusariotoxicosis of man caused by Fusarium sporotrichioides and F. poae that have contaminated millet, rye, wheat or other small grains left under snow in the fields over winter. Because many thousands of people died from ATA in the Orenburg district in the USSR during the 1930s and 1940s, this disease has been the subject of intensive investigations which have been reviewed by Gadjusek (1953a), Mayer (1953a, b), Forgacs and Carll (1962), Joffe (1971, 1978) and Leonov (1977).

The clinical course of ATA in people can be divided into four recognizable stages (see Mayer, 1953a, b; Joffe, 1978). The first stage, which occurs soon after eating toxic cereal foods, is typified by irritation and a sense of burning in the mouth, throat and stomach. Gastroenteritis, with vomiting and diarrhea, occurs within one or two days, but the affected people remain afebrile and temporarily recover in spite of continued consumption of the toxic cereal. The second stage is characterized as a relatively symptomless period during which leukopenia, thrombocytopenia and anemia develop. Some clinical signs of weakness, vertigo, headache, palpitations and slight asthma may occur. The third stage begins suddenly as an acute febrile disease and evolves into a hemorrhagic syndrome in which petechiae occur throughout the body, but mostly under the skin exposed to friction or trauma. Necrotic mucosal plaques develop in the oral cavity, pharynx, esophagus and sometimes in the stomach and lower alimentary tract, probably due to secondary bacterial invasion made possible by severe neutropenia. Focal necrosis may also develop in internal organs including liver and lung.

Regional lymph nodes, especially in the neck, become enlarged and edematous in response to the septic processes, and the swelling may lead to dyspnea or asphyxiation. Numbers of circulating neutrophils drop to less than 100/mm<sup>3</sup>. Lymphocytes, thrombocytes, and erythrocytes are also markedly reduced, but not to the same extent. Anemia develops due to the combined effects of impaired erythropoiesis and hemorrhagic diathesis.

If death does not occur during the third stage, it is followed by a fourth stage, which is a period of convalescence during which hemato-  
poiesis resumes and the septic necrotic lesions disappear, provided that toxic food is no longer consumed. The numbers of neutrophils and platelets return to normal first, and later erythropoiesis also recovers.

Russian investigators quickly established that ATA was a fusario-  
toxicosis by reproducing a similar pancytopenic disease in cats and monkeys fed food contaminated with F. sporotrichioides (Mayer, 1953b; Gadjusek, 1953a). The causative toxin originally isolated by Soviet workers was a steroid compound called poaefusarin that was irritant to the skin. However, after the development of methods for detection of trichothecenes, Mirocha and Pathre (1973) identified T-2 toxin, T-2 tetraol, and zearalenone in a sample of poaefusarin derived from toxigenic F. sporotrichioides and the quantity of T-2 toxin detected (2.5%) was sufficient to account for the dermal toxicity of poaefusarin. Further work by Szathmary et al. (1976) and by Yagen and Joffe (1977) has demonstrated that strains of F. sporotrichioides and F. poae involved in ATA were potent producers of T-2 toxin. By administration of multiple doses of T-2 toxin to cats, Lutsky et al. (1978) reproduced a

pancytopenic disease which they considered to be similar to ATA in man, and also to the effects of poaeufusarin in cats. Because cultures of F. sporotrichioides from which T-2 toxin had been extracted were harmless to cats, Lutsky et al. (1978) concluded that T-2 toxin is probably the toxic metabolite of F. sporotrichioides responsible for ATA.

At present, trichothecene mycotoxins, especially T-2 toxin, are considered by most authors to be the probable causative toxins in ATA. However, the possibility that other mycotoxins or dietary factors might be contributing causes needs further consideration because the pancytopenic effects of trichothecenes have not been reproduced in experimental animals fed diets containing trichothecenes (see Section 2.3.4.4).

#### 2.2.4 Stachybotryotoxicosis

Stachybotryotoxicosis is a mycotoxicosis of horses and other livestock caused by the saprophytic fungus Stachybotrys atra (syn. S. alternans) growing on cereal straw and other similar plant products. This disease was first recognized in horses in the Ukranian SSR in the 1930s and was later found to be caused by a toxic product of S. atra (Drobotko, 1945; Gadjusek, 1953b). Stachybotryotoxicosis has also been reported infrequently in Hungary (Danko, 1972), Romania (Mitroiu et al., 1973), India (Rajendran et al., 1975) and France (LeBars et al., 1977).

Descriptions of the clinical and pathological effects of Stachybotrys atra are available in the reviews of the Soviet literature by Drobotko (1945), Gadjusek (1953b), Forgacs and Carll (1962), Forgacs (1971), and Hintikka (1977). Stachybotryotoxicosis in horses is recognized in two forms, atypical and typical. The typical form is similar to ATA and occurs in three recognizable stages. During the first stage, which lasts

up to 12 days, horses develop stomatitis with necrosis of the skin at the angles of the mouth. This may be associated with secondary inflammatory edema of the lips and submandibular lymph nodes. With continued ingestion of toxic straw, the second stage develops with typical systemic effects of thrombocytopenia, coagulopathy, leukopenia, and agranulocytosis. This second stage may last from 15 to 20 days after which the third stage may suddenly occur as a febrile state due to sepsis arising from focal necrosis of the mucus membranes of the mouth and along the entire length of the alimentary tract. At this stage affected animals are severely leukopenic, and exhibit numerous hemorrhages throughout the body. Lymph nodes are usually edematous and hemorrhagic. Death occurs during the third stage from the effects of sepsis or hemorrhagic diathesis.

The atypical form of stachybotryotoxicosis is a fatal neurological disease that occurs after the ingestion of large quantities of toxic feed. Gastrointestinal or hematological systems are not affected. Horses become ataxic, areflexic, blind and stuporous. They avoid movement, either stand in a wide stance or lean against fixed objects, and become febrile, anorexic and thirsty, but have difficulty swallowing. They usually die within three days of consuming the toxic feed, and at post mortem, are reported to have generalized hemorrhagic diathesis and also focal necrosis of visceral organs.

The cause and pathogenesis of either of the two forms of stachybotryotoxicosis have not been determined. Stachybotryotoxin, the toxic extract of S. atra originally purified by Soviet investigators, was found to be strongly irritant to the skin of rabbits, but it was not chemically identified (Gadjusek, 1953b; Rodricks and Eppley, 1974). Recently,

Eppley and Bailey (1973) identified roridin E and several macrocyclic trichothecenes called satratoxins in extracts of cultured S. atra. Satratoxins were also found by Szathmary et al. (1976), who examined toxigenic strains of Stachybotrys obtained from Hungary. The toxicological properties of these recently identified satratoxins have not yet been examined.

#### 2.2.5 Mouldy-corn poisoning

In North America, sporadic outbreaks of poorly characterized lethal diseases called "mouldy-corn poisoning" have been recorded in horses, swine and cattle. Mouldy-corn poisoning in horses is the best characterized; affected horses develop leukoencephalomalacia (LEM) and hepatitis after several weeks of feeding on corn contaminated by Fusarium moniliforme (Wilson and Maronpot, 1971; Wilson, 1973; Marasas et al., 1976). The causative mycotoxin has not been identified, but since F. moniliforme is not recognized as a producer of trichothecenes (Mirocha et al., 1977), it is unlikely that trichothecenes are responsible.

Several diseases of swine have been termed mouldy-corn poisoning. The most important of these are the emesis and food refusal syndrome (Section 2.2.1) and the estrogenic syndrome (see Section 2.2.2) which are caused by Fusarium graminearum (F. roseum). An acute hemorrhagic disease of swine fed mouldy corn in Georgia was reported by Sippel et al. (1953). After chronic poisoning, hepatotoxic and nephrotoxic effects were observed. Further work by Burnside et al. (1957) identified toxigenic strains of Penicillium rubrum and Aspergillus flavus as the fungi responsible. P. rubrum is known to produce ochratoxins and rubratoxins, whereas A. flavus produces aflatoxins, but it was not determined if these

mycotoxins were originally involved in the field outbreaks. There is no evidence that trichothecenes were involved in this form of mouldy-corn poisoning.

Mouldy-corn poisoning of cattle refers to a mycotoxicosis that occurred sporadically in the mid-west of the U.S.A. during the 1960s (Smalley et al., 1970; Smalley, 1973). The disease was characterized by generalized hemorrhage, but the pathogenesis of the hemorrhagic tendency has not been clearly defined. Gilgan et al. (1966) found toxigenic Fusarium tricinctum to be the most common isolate from mouldy corn responsible for toxicosis in cattle in Wisconsin. One isolate of F. tricinctum produced diacetoxyscirpenol, whereas the most toxigenic isolate produced T-2 toxin (Bamburg et al., 1968b).

Although T-2 toxin has been proposed as the principal mycotoxin responsible for mouldy-corn poisoning in cattle (Bamburg and Strong, 1971), there is presently little evidence to support this theory. Hsu et al. (1972) reported the detection of 2 ppm of T-2 toxin in mouldy corn contaminated with F. tricinctum responsible for a fatal hemorrhagic syndrome in dairy cattle, but clinical and pathological aspects of the disease were not described. T-2 toxin has been tentatively detected, but not measured, in mixed feed that was considered responsible for an episode of fatal hemorrhagic diarrhea, epistaxis, and subcutaneous hemorrhage in adult cows (Hibbs et al., 1974). The prolonged prothrombin times, delayed clotting, and the pattern of hemorrhage observed were consistent with a coagulopathy. However, the implication of T-2 toxin as the cause is equivocal because detection was by TLC alone (see previous Section 2.1.5 and Table 8).

Hemorrhagic syndromes in dairy cows have also been recognized in England (Shreeve et al., 1975). In one such outbreak, described in detail by Dyson and Reed (1977), cows developed pancytopenia and dermatitis but had normal prothrombin times. The cause of this disease was not established, but neither Fusarium moulds nor T-2 toxin were detected in the barley-based ration. A similar hemorrhagic syndrome that was fatal in nine of 115 dairy cows fed mouldy brewer's grains and silage was reported by Petrie et al. (1977). T-2 toxin was detected by TLC in extracts of brewer's grains, but the level was not determined nor was its presence confirmed.

Although these preliminary reports of apparent mycotoxic disease in cattle suggest that T-2 toxin might be one causative factor, it is premature to consider mouldy-corn poisoning of cattle or similar hemorrhagic syndromes to be trichothecene toxicoses. Experimental peroral administrations of T-2 toxin to calves (Pier et al., 1976; Matthews et al., 1977; Ribelin, 1977) have not caused hemorrhagic diseases, although repeated intramuscular injection of crude T-2 toxin has caused death with hemorrhage into the intestine after 65 days in one cow (Kosuri et al., 1970). Nevertheless, in spite of the lack of field and experimental evidence, the bovine hemorrhagic syndrome has been accepted as a T-2 toxicosis in recent textbooks (Ribelin, 1977).

#### 2.2.6 Miscellaneous diseases

Trichothecenes have been suggested as the cause of several other mouldy food toxicoses of man and animals in various parts of the world. In Japan, a syndrome of headache, nausea and diarrhea has frequently occurred in people after consumption of wheat, barley, oats, or rye

contaminated with Fusarium moulds (Tsunoda, 1970). This condition, known as "Akakabi-byo" (red mould disease) has been attributed to toxigenic F. nivale, strains of which were found to produce nivalenol and fusarenon-X in culture (Tatsuno, 1968; Ueno et al., 1969). Several isolates of F. roseum from toxic wheat have recently been shown to produce deoxynivalenol (Yoshizawa and Morooka, 1977). Thus, akakabi-byo of man appears similar to the emesis and food refusal syndrome of swine (Section 2.2.1). However, trichothecenes have not yet been demonstrated in mouldy cereals responsible for the condition in man.

A fatal neurological disease of horses fed mouldy bean-hulls has been recognized for many years in Japan. The condition is characterized by convulsions, involuntary movements and walking in circles (Ueno et al., 1972a). A toxigenic isolate of F. solani obtained from toxic bean hulls produced T-2 toxin and neosolaniol in culture (Ueno et al., 1972a), but there is presently no evidence that these trichothecenes are neurotoxic to horses.

"Fescue foot" is a disease of cattle caused by tall fescue grass and characterized by gangrene of the extremities (Garner and Cornell, 1977). The cause is not known. Many samples of toxic tall fescue were contaminated by F. tricinctum, so T-2 toxin and other trichothecenes were considered as a possible cause (Yates et al., 1969). However, the "fescue foot" condition has not been reproduced in cattle given T-2 toxin (Kosuri et al., 1970).

### 2.3 Toxic effects of trichothecenes

In this section, the published results of experimental toxicological studies on the effects of purified trichothecene mycotoxins will be reviewed. Studies on the effects of crude extracts of Fusarium or other fungi will not be considered unless the extracts have been shown to contain trichothecenes as a principal active component.

#### 2.3.1 Susceptible organisms

A large number of different biological species have been exposed to pure trichothecenes. Although degrees of susceptibility vary, plants, protozoa, fungi, insects, fish, avians, and mammals, including cell cultures, have exhibited lethal or growth-inhibitory effects in response to trichothecenes. Many examples of susceptibility have been presented previously in Table 7 (Section 2.1.4) which will not be further discussed here.

Only eukaryotic organisms are susceptible to trichothecenes; bacteria are resistant because trichothecenes have no effect on the function of prokaryotic ribosomes (Ueno et al., 1973b; Ueno, 1977b). Accordingly, although unsuitable as antibacterial antibiotics, trichothecenes have been considered as potential antifungal antibiotics (Godtfredsen and Vangedal, 1965) and as anticancer drugs (Goodwin et al., 1978).

#### 2.3.2 Mechanism of action

Nivalenol and fusarenon-X were found by Japanese investigators to be potent inhibitors of protein synthesis (<sup>14</sup>C-leucine incorporation) in rabbit reticulocytes, and they employed this activity as the basis of a bioassay for detection of trichothecenes (Ueno and Fukushima, 1968; Ueno and Shimada, 1974). Further studies showed that many different

trichothecenes inhibited protein synthesis in the rabbit reticulocyte system (Ueno et al., 1973b; Ueno, 1977b), and also in cultured intact mammalian cells (Ohtsubo et al., 1968) or protozoa (Ueno, 1977b).

The mechanism of inhibition of protein synthesis has been investigated in great detail since Stafford and McLaughlin (1973) recognized the ability of trichodermin to specifically inhibit the termination stage of polypeptide synthesis by eukaryotic ribosomes. Because of this novel activity, many other trichothecenes were studied in various ribosome function assays (Carrasco et al., 1973; Cundliffe et al., 1974; Schindler, 1974; Wei et al., 1974b).

As a group, the trichothecenes are the most potent known small molecules in their ability to inhibit eukaryotic ribosomal function (McLaughlin et al., 1977), but different mechanisms of action have been recognized for different trichothecenes. Schindler (1974) divided the trichothecenes into two groups; one group (I-type) which inhibited initiation of polypeptide synthesis, and another group (E-group) which inhibited the elongation and termination stages. Further studies by Cundliffe et al. (1974) and by McLaughlin et al. (1977) have supported this distinction, but the type of activity of any one trichothecene appears to depend on the concentration (Cundliffe and Davies, 1977). The designation of trichodermin as a specific inhibitor of the termination stage of polypeptide synthesis (Wei et al., 1974a) has been disputed by Carter et al. (1976) and by Cundliffe and Davies (1977) on the evidence that trichodermin inhibits both initiation and elongation depending on the concentration. It has since been found that trichothecenes are not true inhibitors of initiation; the initiation complex forms normally, but the first few

steps of polypeptide-bond formation are blocked (Cundliffe and Davies, 1977; Vazquez, 1979).

Trichothecenes have been shown to bind to the 60S subunit of eukaryotic ribosomes and in doing so, block the binding of other inhibitors of polypeptide-bond formation such as anisomycin and narciclasine (Vazquez, 1979). The I-type trichothecenes have a low binding affinity to polysomes, whereas the E-type trichothecenes will bind to polysomes and also to single ribosomes (Vazquez, 1979).

Although the mechanism whereby trichothecenes inhibit protein synthesis in cell-free or cell-culture systems is well understood, this activity has not been shown to be responsible for the toxic effects observed in intact animals or plants in vivo. Trichothecenes have been shown to inhibit synthesis of DNA in various cell-culture systems (Ueno and Fukushima, 1968; Ueno et al., 1971b), but this is possibly secondary to primary inhibition of protein synthesis (Ueno, 1977b).

In the protozoon Tetrahymena pyriformis, fusarenon-X inhibits the synthesis of neutral terpenoid lipids that are structurally related to trichothecenes, possibly by competitive inhibition as a structural analogue, and it also inhibits phosphate uptake (Chiba et al., 1972). These observations suggest that trichothecenes may interfere with cell-membrane function, but this possibility has not yet been investigated in mammalian cells. Russian investigators have reported inhibition of membrane-bound lysosomal  $\beta$ -glucosidase activity by poaefusarin, and release of lysosomal enzymes, suggesting that the active component of poaefusarin is capable of destroying lysosomal membranes (Pokrovskii et al., 1974, 1975).

Trichothecenes have an epoxide group which would be expected to bind readily to biological macromolecules, particularly to sulfhydryl groups or amino groups. However, trichothecenes lack the ability to attack DNA so they cause neither chromosomal breaks (Umeda et al., 1972) nor mutations (Ueno and Kubota, 1976; Ueno et al., 1978). T-2 toxin, fusarenon-X and neosolaniol inhibit some enzymes whose active sites contain sulfhydryl groups, including alcohol dehydrogenase, lactate dehydrogenase and creatine phosphokinase (Ueno, 1977a). However, trichothecenes appear to have no affinity for epoxidase or epoxide hydrolase enzymes in microsomes, nor do they inhibit glutathione transferase activity (Nakamura et al., 1977; Ueno, 1977a). These observations indicate that the epoxide group of trichothecenes, although necessary for acute toxicity in various systems (Bamburg, 1976), is not highly reactive with biological macromolecules that react readily with other epoxide and lactone moieties (Chu, 1977).

### 2.3.3 Biochemical effects

Few investigations have shown effects of trichothecenes on serum biochemical values. T-2 toxin fed at a level of 20 ppm to laying hens caused hypoproteinemia and hypolipemia after three weeks, but did not alter plasma glucose levels (Wyatt et al., 1975). In a similar feeding trial in laying hens conducted by Chi et al. (1977c), levels of T-2 toxin from 0.5 to 8.0 ppm did not influence the serum protein level, but higher levels (6-8 ppm) caused slight to moderate elevations in serum alkaline phosphatase (SAP) activity, lactate dehydrogenase (LDH) activity, and serum cholesterol levels. Serum activities of glutamate pyruvate transaminase (SGPT)\* and serum glutamate oxaloacetate transaminase (SGOT)\*\* were not affected. By comparison, broiler chickens fed diets containing between 0.2 and 4 ppm of T-2 toxin for three weeks exhibited dose-related decreases in SAP, LDH and cholesterol, but SGPT, SGOT, and total protein values were not affected (Chi et al., 1977b). Broiler chickens and laying hens had no significant alterations in SGOT, SGPT, LDH, SAP, total protein or cholesterol at both 10 and 30 days after single peroral doses of T-2 toxin greater than the LD<sub>50</sub> (Chi et al., 1977a). The observations both of Chi et al. and Wyatt et al. indicate that dietary T-2 toxin causes minimal injury to liver, kidney and muscle of chickens, irrespective of the age, route of administration and duration of exposure.

Serum values of LDH, SGOT, SGPT, SAP, cholesterol, and total protein were not altered in pigs given single intravenous doses of T-2 toxin (Weaver et al., 1978b) or diacetoxyscirpenol (Weaver et al., 1978a),

\* Alanine transaminase (ALT)

\*\* Aspartate transaminase (AST)

nor after 8 weeks on diets containing up to 8 ppm of T-2 toxin (Weaver et al., 1978c). Elevations in SGOT occurred after 15 days in calves given daily intraruminal doses of T-2 toxin (0.32 and 0.64 mg/kg), but bromsulfalein clearance half-time remained normal. After single intraperitoneal doses of T-2 toxin, rats exhibited moderate elevations in concentrations of serum lactate, cholesterol and plasma lipids (Kosuri et al., 1971).

Transient, marked elevations in activities of SGOT, SGPT, LDH occurred in six-week-old chickens between 0 and 48 hours after single intramuscular doses of T-2 toxin in ethanol (dose approximately 0.7 mg/kg) (Pearson, 1978). The elevations of SGOT and LDH, in conjunction with an observed increase in hepatic weight, were interpreted as being due to hepatotoxic effects of T-2 toxin. However, similar but less marked elevations in these enzyme activities occurred in control chickens treated with ethanol, so this interpretation is equivocal.

In general, biochemical studies so far conducted in various species indicate that trichothecene toxicosis is not accompanied by consistent or marked serum biochemical alterations.

#### 2.3.4 Physiological effects

##### 2.3.4.1 Food consumption

Few trichothecenes have been experimentally administered in the feed to laboratory or farm animals, but those that have were potent inhibitors of voluntary food consumption. This effect has been best demonstrated with deoxynivalenol in swine (Vesonder et al., 1976; Forsyth et al., 1977), but has also been shown for T-2 toxin in rats (Kotsonis et al., 1975b), swine (Weaver et al., 1978c), broiler chickens (Chi et al., 1977b), and laying hens (Wyatt et al., 1975; Chi et al., 1977c; Speers et al., 1977).

The mechanism by which trichothecenes decrease food consumption is unknown. High levels of T-2 toxin (greater than 4 ppm) cause reduced food consumption in chickens and also cause stomatitis and perioral dermatitis (Wyatt et al., 1972b; Chi et al., 1977b), so it is likely that reduced intake is partly due to anorexia secondary to stomatitis. However, high levels of deoxynivalenol (>40 ppm) and T-2 toxin (>24 ppm) are capable of almost completely deterring swine from ingesting contaminated food (Forsyth et al., 1977; Weaver et al., 1978c) which suggests that trichothecenes also reduce palatability or acceptability of feed.

##### 2.3.4.2 Growth, development and production

Diets containing high levels of trichothecenes caused reduced growth of various species as would be expected from the reductions in food intake outlined in the previous section (2.3.4.1). Reduced weight gain has also been reported in young rats fed T-2 toxin (15 ppm) or fusarenon-X (7 ppm) (Ohtsubo and Saito, 1977). In studies in chicks and turkey poults in which the inanition effect of dietary T-2 toxin (10 ppm)

was controlled by giving control birds the same amount of food as was consumed by the birds on the toxic diet, dietary T-2 toxin caused lower weight gains and lower feed efficiency than could be attributed to the reduced food consumption alone (Richard et al., 1978). In this experiment, T-2 toxin at a low level (2 ppm) reduced weight gains and feed efficiency in comparison with ad lib fed controls, without causing oral lesions (Richard et al., 1978). These observations indicate that T-2 toxin may cause reduced growth performance in the absence of other clinical effects. In another experiment by Chi et al. (1977b) using broiler chicks, dietary T-2 toxin at 2 ppm did not affect growth or efficiency of feed utilization, but frequently caused oral lesions, whereas the 4-ppm level caused reduced weight gain and more severe stomatitis without reducing the efficiency of feed utilization. This latter experiment suggests that reduction in growth by low levels of dietary T-2 toxin was due solely to reduced food consumption secondary to stomatitis. Weaver et al., (1977c) reported slight reductions in growth and food consumption of swine fed T-2 toxin (8 ppm), but feed efficiency was not affected and no oral lesions nor signs of systemic toxicity were observed. Thus, the effects of dietary T-2 toxin on the growth of swine are probably due solely to the food refusal effects.

Effects of dietary T-2 toxin on reproductive performance of poultry and swine have recently been examined. Wyatt et al. (1975) observed reduced egg production in approximately 20 per cent of hens fed dietary T-2 toxin (20 ppm) for three weeks, whereas Chi et al. (1977c) reported reduced egg production between 4 to 8 weeks on diets containing 2 to 8 ppm of T-2 toxin. In both studies, reduction in egg production was associated

with a decrease in shell thickness. Palyusik and Koplic-Kovács (1975) examined the effects in geese of corn infected with Fusarium sporotrichioides which was added to the ration so that the geese consumed between 1.5 and 3 ppm of T-2 toxin. Egg production ceased within 10 days, and hatchability of fertilized eggs was reduced, although only 13 eggs were available for incubation from the geese fed the toxic diet.

The effect of dietary T-2 toxin on porcine reproduction has received preliminary investigation by Weaver et al. (1978d). Dietary T-2 toxin (12 ppm) fed to two pregnant sows during the last third of pregnancy did not affect litter size, the health of the piglets, nor the subsequent estrus cycles, but neither sow became pregnant in spite of breeding for 3 and 5 cycles respectively. Another sow that was placed on the same toxic diet soon after weaning a litter became pregnant on the second cycle, but produced only four piglets, two of which died during the first day. Although these results were not obtained in a controlled experiment, they suggest that dietary T-2 toxin might adversely affect the reproductive efficiency of swine.

#### 2.3.4.3 Immunity and resistance to disease

Although trichothecenes have been shown to be acutely toxic to lymphoid and hematopoietic tissues (Saito et al., 1969; see Section 2.3.5.2) few studies have been conducted to assess the functional effects of trichothecenes on immunocytic or phagocytic defence mechanisms during dietary exposure to the toxins. Broiler chickens fed dietary T-2 toxin (16 ppm) became susceptible to lethal infections by Salmonella worthington, S. thompson, S. derby, and S. typhimurium, whereas neither the toxic diet nor the bacterial inocula produced mortality (Boonchuvit et al., 1975).

Increases in serum titres of anti-Salmonella agglutinins were not affected by dietary T-2 toxin. A recent study by Richard et al. (1978) found no effect of dietary T-2 toxin (10 ppm) on production of agglutinating antibody to inoculated Pasteurella multocida-bacterin by young chicks or turkey poults. Thymic atrophy occurred in the turkey poults fed the 10-ppm level of T-2 toxin, but not in poults fed T-2 toxin at a 2-ppm level.

Immunosuppressive effects of parenterally administered T-2 toxin and DAS have been examined in mice. Both toxins, when given daily for 7 days (i.p.), reduced response titres of hemagglutinins to sheep red blood cells. This reduction by T-2 toxin was dose-related; the lowest effective daily dose was 0.5 mg/kg/day, and the higher doses used (>1 mg/kg/day) completely suppressed the antibody response (Rosenstein et al., 1979). Similar but less marked dose-related decreases in titres were produced by DAS. The reduction in antibody titre in mice treated with T-2 toxin was associated with a dose-related reduction in thymic weight and also in the number of splenic cells secreting anti-sheep-red-cell antibody, as measured in a plaque-forming-cell (PFC) assay. The depressions of antibody response and thymic weight were transient because no effects occurred if more than 12 days had elapsed since the last of the seven daily treatments with T-2 toxin (Rosenstein et al., 1979).

Rosenstein et al. (1979) also found that T-2 toxin at a dose of 0.75 mg/kg/day given 3 or 4 times a week delayed the rejection of skin allografts in mice, and also reduced the numbers of macrophages and lymphocytes in histological sections of the grafts. Further evidence that trichothecenes are immunosuppressive was provided by Lafarge-Frayssinet et al. (1979) who found that, in vitro, T-2 toxin and DAS

strongly inhibited mitogenic responses of thymic and splenic lymphocytes to phytohemagglutinin (PHA, a T-cell mitogen) and to lipopolysaccharide (LPS, a B-cell mitogen). Low levels of T-2 toxin (less than 2 ng/ml) had an opposite effect; mitogenic responses of splenic and especially thymic cells to PHA were increased in the presence of low levels, which suggests that T-2 toxin may be active against suppressor T cells under these conditions. This interpretation is supported by the work of Masuko et al. (1977) who demonstrated enhanced delayed-hypersensitivity due to interference of suppressor-cell effects in mice given T-2 toxin.

Although these in vitro studies have demonstrated that T-2 toxin and other trichothecenes are highly toxic to lymphocytes, they do not establish that trichothecenes are immunotoxic when consumed in the diet. The absence of histological abnormalities in lymphoid tissues of poultry (Chi et al., 1977b; 1977c) and swine (Weaver et al., 1978c) fed diets containing trichothecenes suggests that under natural conditions of exposure, trichothecenes do not necessarily depopulate lymphoid tissues of these species. Further investigations should determine if lymphocytes of animals exposed to dietary trichothecenes are functionally impaired, or if lymphoid tissues are depleted by dietary trichothecenes under certain conditions. Such information is needed to determine if dietary trichothecenes are capable of predisposing animals to infectious diseases.

#### 2.3.4.4 Hematology

The implication that trichothecenes cause alimentary toxic aleukia, stachybotryotoxicosis, and the hemorrhagic syndromes of livestock, has led to examination of the hematological effects of acute and subacute exposure to pure trichothecenes. However, many of the recorded effects occurred in experiments in which the toxins were administered by repeated single doses; few effects have been observed in animals fed trichothecenes in the diet.

Wyatt et al. (1975) reported 30% reductions in total leukocyte counts in laying hens fed dietary T-2 toxin (20 ppm) for three weeks, but hematocrit, hemoglobin concentrations, and erythrocyte counts were not altered. Dietary T-2 toxin did not affect these red cell values in broiler chickens at levels up to 4 ppm for 9 weeks (Chi et al., 1977b), nor in laying hens fed levels up to 8 ppm for as long as 8 weeks (Chi et al., 1977c). However, in these two studies, neither total nor differential leukocyte counts were altered by T-2 toxin. Speers et al. (1977) reported normal erythrocyte and leukocyte values in laying hens after 4 weeks on diets containing corn infected with Fusarium tricinctum (giving up to 16 ppm T-2 toxin) or F. roseum "Gibbosum" (giving up to 50 ppm monacetoxyscirpenol).

Hematological abnormalities were not detected in routine complete blood analyses of swine fed dietary T-2 toxin (up to 8 ppm) for 8 weeks (Weaver et al., 1978c), nor in adult sows fed diets containing 12 ppm T-2 toxin for up to 220 days (Weaver et al., 1978d).

Rats fed dietary fusarenon-X (7 ppm) for up to 102 weeks did not become leukopenic (Ohtsubo and Saito, 1977). However, mice became

leukopenic when fed dietary T-2 toxin for 24 days at levels of 10 and 20 ppm, and when fed dietary fusarenon-X at levels above 10 ppm for 10, 24, and 61 days (Ohtsubo and Saito, 1977). These leukopenias were in the range of 40 to 70% of control values, but were not characterized by differential counts.

No abnormalities have been found in bone marrow sections or smears taken from swine (Weaver et al., 1978c; 1978d), nor chickens (Chi et al., 1977b; 1977c) fed dietary T-2 toxin in the studies mentioned above.

Several investigators have reported hematological alterations in experimental animals after single or multiple doses of trichothecenes, administered by various routes. Sato et al. (1975) administered several subcutaneous doses of T-2 toxin (0.05 mg/kg) to two cats over a 4-week period and observed leukopenia in the order of 7-16% of initial values, but differential cell counts were not made. Erythrocyte values also decreased to about 75% of initial values. Similar results were obtained by Lutsky et al. (1978) in a controlled experiment in which cats were given peroral doses of T-2 toxin (0.08 mg/kg or 0.1 mg/kg) every 48 hours. These cats survived an average of 15.0 or 13.5 days for the respective doses, and developed marked neutropenia, lymphopenia and thrombocytopenia, although only slight decreases in hemoglobin and hematocrit occurred.

Decreases in total leukocyte counts and in lymphocyte counts have also been reported in various mammalian species given multiple intravenous doses of trichothecenes, including diacetoxyscirpenol in rats and dogs (Stähelin et al., 1968) and verrucarín A in dogs, pigs, rats and monkeys (Rüsch and Stähelin, 1965). Undifferentiated leukopenias have been detected in mice given multiple daily oral doses of T-2 toxin (above

2 mg/kg/day) (Sato et al., 1978) and in rats given single intraperitoneal doses of T-2 toxin (Kosuri et al., 1970).

Guinea-pigs given oral T-2 toxin (0.9 mg/kg/day) for 27 days developed marked lymphopenia and a slight reduction in erythrocyte counts (DeNicola et al., 1978). Abnormalities in erythropoiesis including anisocytosis and basophilic stippling were observed which suggested that erythropoiesis was hyperplastic. Repeated exposure to T-2 toxin has caused minimal decrease in erythrocyte counts in cats (Lutsky et al., 1978) and mice (Sato et al., 1978), but the activity of erythropoiesis was not assessed. The failure of T-2 toxin to cause anemia during these short experimental studies (less than 4 weeks) is most likely due to the long half-life of erythrocytes. However, Lutsky et al. (1978) noted that those cats that survived the longest (up to 24 days) while being given 0.08 mg of T-2 toxin per kg every 48 hours exhibited signs of erythropoietic regeneration in the bone marrow, indicating that erythropoiesis was not suppressed at that stage.

The differences between the hematological effects of trichothecenes in different species, for different periods of exposure, for different toxins, and for different routes of administration make it impossible to predict if trichothecenes might suppress hematopoiesis when given by the dietary route. Effects observed after repeated single doses may not represent effects of trichothecenes consumed continuously in the diet. Peak blood levels of toxin could be much higher after single doses, thereby affecting greater proportions of germinal hematopoietic cells.

### 2.3.5 Morphological effects

#### 2.3.5.1 Cutaneous inflammation

The cutaneous-irritant activity of trichothecenes is important, both as a means of biologically detecting trichothecenes (see Section 2.1.4, Table 7), and also as the probable cause of stomatitis, perioral dermatitis and irritation of the upper alimentary tract during natural and experimental consumption of trichothecenes in the diet (Wyatt et al., 1972b). However, neither the mechanism by which trichothecenes evoke the inflammatory response, nor the pathogenesis of the cutaneous lesions, has been determined.

Trichothecenes are extremely irritant; amounts of T-2 toxin as low as 0.01  $\mu\text{g}$  produce visible hyperemic reactions on the skin of rabbits within 24 hours after application (Chung et al., 1974). Rats are slightly less sensitive, but will react to 0.05  $\mu\text{g}$  of T-2 toxin (Wei et al., 1972). Low doses produce hyperemia which subsides after 24 hours (Chung et al., 1974), whereas larger doses (up to 100  $\mu\text{g}$ ) produce swollen plaques with central pallor due to edema which increases until the second or third day, when a moist exudate comes to the surface and dries to form a scab (Bamburg, 1969). Extremely high doses on rats (greater than 0.25 mg) produce pale swollen skin lesions that do not change much in appearance beyond 24 hours after application; instead, rats become lethargic and may die after 3 to 5 days (Bamburg, 1969).

T-2 toxin (Bamburg, 1969), glutinosin (an unpurified mixture of verrucarins) (Brian et al., 1947), and roridin A (Mortimer et al., 1971) have been reported to be irritant to human skin. About 4 hours after accidental exposure of the hands to a crude extract containing T-2 toxin,

two people developed a transient burning sensation that lasted for one day. This was followed by a sense of numbness that did not completely resolve until 18 days. During this time, the skin of the fingers became "thick and white" (Bamburg, 1969).

There are only two reports of microscopic changes in the skin of laboratory animals after topical application of pure trichothecenes. Marasas et al. (1969) reported that topical doses of T-2 toxin between 0.12 and 1.3 mg produced severe cutaneous inflammation. When the sites of application were examined microscopically 4 days after treatment, both dermis and epidermis had undergone extensive coagulation necrosis. Ueno et al. (1970) microscopically examined the skin of guinea-pigs exposed 2 and 3 days previously with diacetoxyscirpenol (0.2-100 µg), fusarenon-X (0.2-100 µg), or nivalenol (1.0-100 µg), but provided only brief descriptions of the lesions. All levels of diacetoxyscirpenol caused epidermal necrosis and increased mitotic activity in the epidermis, whereas the other two toxins were less irritant and caused vacuolation of basal epidermal cells, dermal infiltration, and increased mitotic activity of the epidermis. Neither of these two microscopic studies included examinations made during the first 48 hours after application.

Schoental and Joffe (1974) described the microscopic appearance of severe necrotizing cutaneous lesions in rabbits after topical application of crude extracts of Fusarium sporotrichioides and F. poae, each of which has since been recognized as a producer of T-2 toxin (Yagen and Joffe, 1977). These extracts caused total necrosis of the epidermis and underlying dermis, and exudation of neutrophils into the epidermis and adjacent dermis.

#### 2.3.5.2 Acute toxicosis

Japanese workers demonstrated that nivalenol and fusarenon-X caused a radiomimetic pattern of tissue injury in mice and rats after oral or parenteral administration of lethal doses (Saito et al., 1969). Animals died between 12 and 72 hours after administration and exhibited karyorrhexis in the germinal cells of intestinal crypts, lymphoid follicles, thymus, and bone marrow. Cellular damage in these target tissues was evident by 6 hours, peaked between 6 and 24 hours, and had decreased by 48 hours, at which time regenerative activity was noticed in the bone marrow (Saito and Okubo, 1970).

Similar acute histopathological changes were recognized to be caused by other trichothecenes, including T-2 toxin and neosolaniol in mice (Ueno et al., 1972a); T-2 toxin in cats (Sato et al., 1975), pigs (Weaver et al., 1978c) and guinea-pigs (DeNicola et al., 1978); and diacetoxyscirpenol in pigs (Weaver et al., 1978b). In each of these studies, microscopic examination of lesions was conducted only on animals that died or were moribund after receiving a lethal dose. Neither the pathogenesis of the lesions nor the acute effects of sublethal doses were investigated.

Some reports indicate that high oral doses of trichothecenes cause necrosis and inflammation of the mucosa of the lower alimentary tract. Mortimer et al. (1971) observed hemorrhagic and necrotizing gastroenteritis in lambs that died within 48 hours after being given either roridin A or verrucarín A by the oral route, but the enteric lesions were not examined microscopically. Acute necrotizing enteritis was suspected to have occurred in trout fingerlings given a single feeding of diets

containing T-2 toxin at levels of between 2 and 8 ppm. Affected fish shed mucoid intestinal casts comprised of fragments of sloughed intestinal mucosa on the day after first receiving the toxic diet (Marasas et al., 1969). However no mortality occurred and the intestinal mucosa had regenerated after 10 weeks.

The effects of T-2 toxin, fusarenon-X, and nivalenol on the bursa of Fabricius of day-old chicks was examined histologically and ultra-structurally by Terao et al. (1978). These investigators observed necrosis of lymphocytes in follicles, and autophagocytosis in follicle-associated epithelium in chickens killed 60 minutes after inoculation of any one of these three trichothecenes into the yolk sac. The follicular lymphocytes and the epithelium overlying the follicles were destroyed within 6 hours, but neither the surrounding surface epithelium nor the lymphoepithelial cells of the follicles were affected. This study demonstrated the high susceptibility of lymphoid tissues to trichothecenes, and the rapidity with which the susceptible cells are destroyed.

Although these reported studies have identified the target tissues injured by various trichothecenes in various species of animals, they do not provide enough information from which to assess the role of the acute radiomimetic effects of trichothecenes in naturally occurring mycotoxicoses. Acute high-level exposure leading to death within several days is unlikely to occur under natural circumstances because of the reluctance of animals to consume food containing moderately high levels of trichothecenes (see Section 2.3.4.1).

### 2.3.5.3 Subacute toxicosis

In the studies of subacute toxicity of dietary T-2 toxin in poultry (Wyatt et al., 1972b, 1975; Chi et al., 1977b, 1977c; Richard et al., 1978) which have been previously outlined in Sections 2.3.4.1, 2.3.4.2, and 2.3.4.4, very few morphological lesions were observed. The most consistent observation was stomatitis, characterized by the appearance of caseous, yellow-white, proliferative plaques at the angles of the beak, on the tongue, and less frequently on the inside of the beak. The severity of the lesions and the frequency of affected birds were dose-related (Wyatt et al., 1972b) and the lowest effective concentration was approximately 2 ppm (Chi et al., 1977b). Similar lesions have been produced by diacetoxyscirpenol and crotoxin when fed to chickens (Chi and Mirocha, 1978). Richard et al. (1978) microscopically examined typical oral lesions and found them to be composed of a crust of alternating layers of necrotic epithelium and heterophils in association with a few small ulcerations of the epithelium.

Perioral dermatitis has also been recognized in rats fed diets containing 15 ppm of T-2 toxin (Marasas et al., 1969; Ohtsubo and Saito, 1977), but was not reported in pigs (Weaver et al., 1978c) nor in mice (Ohtsubo and Saito, 1977) fed diets containing up to 8 ppm or 15 ppm of T-2 toxin respectively.

Richard et al. (1978) reported inflammatory lesions in the crop of turkey poults fed dietary T-2 toxin. In all other studies in poultry given pure T-2 toxin in the diet, lesions have not been present in the esophagus or lower alimentary tract (Wyatt et al., 1975; Chi et al., 1977b, 1977c). Witlock et al. (1977), using scanning electron

microscopy, did not observe any change in villous structure of the duodenum nor ileum of chickens that were fed dietary T-2 toxin for 3 weeks. Neither oral nor gastrointestinal lesions were found in pigs fed 12 ppm of T-2 toxin for up to 8 weeks (Weaver et al., 1978c).

In some species, repeated ingestion of trichothecene mycotoxins, either in the food or by direct administration, has been associated with destructive and/or proliferative lesions in the upper alimentary tract. Mice and rats developed hyperkeratosis, acanthosis, and focal, papillary, epithelial proliferation of the squamous mucosa of the fore-stomach (pars esophagea) after having consumed T-2 toxin in the diet at levels of 10 and 15 ppm for several weeks (Ohtsubo and Saito, 1977). Similar lesions in the stomach had been previously reported in rats given crude extracts of Fusarium sporotrichioides and F. poae (Schoental and Joffe, 1974). Rats given repeated near-lethal doses of T-2 toxin (1 to 4 mg/kg) intragastrically at approximately monthly intervals also developed these gastric lesions (Schoental et al., 1979). Severe hemorrhagic abomasitis was observed in a sheep which died after 6 daily intraruminal doses of a mixture of roridin A and verrucarin A (1 mg/kg) (Mortimer et al., 1971). Two calves given daily oral doses of T-2 toxin (approximately 0.3 and 0.6 mg/kg/day) developed bloody feces, mild enteritis, abomasitis with ulceration, and ulcerative rumenitis after 20 to 30 days (Pier et al., 1976). Cats given oral doses of T-2 toxin (0.08 and 0.10 mg/kg/48 hours) developed hemorrhagic diarrhea, necrosis of the gastric mucosa, and dilation of crypts of Lieberkuhn in the small intestine (Lutsky et al., 1978). The pathogenesis of these various reported lesions in the lower alimentary tract has not been established;

they might be due to the irritancy of the toxins, or to selective destruction of the germinal cells of the mucosa.

Few other morphological effects of subacute toxicosis have been recognized. Thymic atrophy was produced in mice given seven daily intraperitoneal injections of T-2 toxin or diacetoxyscirpenol (Rosenstein et al., 1979) and also in turkey poults fed T-2 toxin in the diet for 4 weeks at 10 ppm (Richard et al., 1978). The latter authors found less severe thymic atrophy in control poults fed at the rate at which treated birds consumed the toxic diet and concluded that thymic atrophy was probably partly due to reduced food intake. The functional significance of this effect of T-2 toxin on the thymus is not known (see Section 2.3.4.3).

#### 2.3.5.4 Chronic toxicosis

Few long term experiments on toxicity of trichothecene mycotoxins have been reported. Rats were fed fusarenon-X at levels of 3.5 and 7 ppm for either 1 or 2 years, after which they were subjected to complete postmortem examination (Ohtsubo and Saito, 1977). Fusarenon-X reduced the body weights and survival times; most rats died of chronic bronchopneumonia, but the postmortem findings were not described. Some of the treated rats that died had atrophic lymphoid organs, which the investigators considered may have been either an effect of the toxin or secondary to pneumonia. Mice fed dietary T-2 toxin at levels of 10 and 15 ppm for periods of 3, 6, 9 and 12 months exhibited only gastric hyperkeratosis (Ohtsubo and Saito, 1977).

Schoental et al. (1979) reported an experiment in which rats were given repeated near-lethal, intragastric doses of T-2 toxin at approximately monthly intervals. About two-thirds of the rats died from the acute effects of one of the injections during the course of the study, but rats which survived for more than 12 months and died at times unrelated to the dose were necropsied. Most of these rats had developed hyperkeratosis of the pars esophagea of the stomach, interstitial nephritis with urolithiasis, arteriosclerosis, and focal myocardial degeneration and fibrosis. Various neoplasms were also observed which are considered in Section 2.3.5.6. Few control rats were used in this study and most were reported to have slight to moderate thickening of arterial walls, myocardial lesions and nephrotic kidney changes. Because of the unusual experimental design in which survivors were repeatedly selected, and because the treatment schedule does not resemble naturally-occurring patterns of exposure, the reported chronic effects should not be considered to typify the chronic effects of dietary T-2 toxin.

#### 2.3.5.5 Carcinogenesis and mutagenesis

Most of the information so far reported suggests that trichothecene mycotoxins are not carcinogenic. At least seven trichothecenes have been examined in vitro in Ames mutation assays, and they have been consistently non-mutagenic (Ueno et al., 1978 ; Kuczuk et al., 1978; Wehner et al., 1978), whereas the known carcinogenic mycotoxins such as aflatoxins, sterigmatocystin and patulin are consistently mutagenic (Ueno et al., 1978 ; Kuczuk et al., 1978). Furthermore, fusarenon-X did not induce breaks in DNA strands in the HeLa cells, whereas aflatoxin B<sub>1</sub>, penicillic

acid and patulin did (Umeda et al., 1972). Neither fusarenon-X nor T-2 toxin injured DNA in recombination-deficient mutants of Bacillus subtilis, in contrast to the positive effects of known carcinogens, aflatoxin B<sub>1</sub>, citrinin, patulin, sterigmatocystin, and penicillic acid (Ueno and Kubota, 1976).

The generally reliable assays for mutagenic effects in prokaryotes might be inappropriate for predicting the carcinogenic potential of trichothecenes which are selectively toxic to eukaryotes (see Section 2.3.2). In vitro mutagenicity assays employing an eukaryotic yeast, Saccharomyces cerevisiae, have failed to detect mutagenic activity of diacetoxyscirpenol and T-2 toxin (Kuczuk et al., 1978). However, Ueno et al. (1971b) reported that fusarenon-X induced low frequencies of mutations in respiratory-deficient mutants of S. cerevisiae.

Experiments in which trichothecenes have been administered to experimental animals for long periods have failed to clearly demonstrate carcinogenicity of trichothecenes. T-2 toxin neither induced nor promoted epithelial papillomas when applied to the skin of mice for up to 22 weeks (Marasas et al., 1969; Lindenfelser et al., 1974). No significant increase in incidence of neoplasms occurred in rats fed fusarenon-X (at levels of 3.5 or 7 ppm) for periods of 1 or 2 years, nor in mice fed T-2 toxin (10 or 15 ppm) for 12 months (Ohtsubo and Saito, 1977). Long-term exposure (up to 12 months) to T-2 toxin, at levels of up to 0.4 ppm in the diet, did not cause hepatic neoplasia in trout (Marasas et al., 1969). The papillomatous structures observed on the esophageal gastric mucosa in rats given intragastric doses of crude fusarial extracts were considered as a possible indication of tumorigenicity of

irritant fusarial toxins (Schoental and Joffe, 1974). However, similar lesions in mice and rats fed pure trichothecenes were not regarded as preneoplastic (Ohtsubo and Saito, 1977).

After repeated administration of high doses of trichothecenes, conflicting results and interpretations of carcinogenicity have been published. Saito and Ohtsubo (1974) observed very few neoplasms in rats and mice that were given 50 weekly oral doses or between 10 and 22 weekly subcutaneous doses of fusarenon-X (0.4 mg/kg), but because only 18 rats and 34 mice were examined, weak carcinogenic activity might not have been detected. In the chronic toxicity study by Schoental et al. (1979) that has been outlined in the previous Section (2.3.5.4), some rats developed benign and malignant tumours of the stomach, duodenum, pancreas and brain. The neoplasms reported occurred in rats that survived for up to 26 months after repeated, near lethal, intragastric doses of T-2 toxin. However, the role of T-2 toxin in causing the neoplasms observed is equivocal because few tumours were observed, few controls were maintained, and because the high levels of toxin administered caused necrosis of the gastrointestinal mucosa which may have facilitated absorption of other carcinogens. Furthermore, since rats were possibly immunosuppressed after high doses of T-2 toxin, the neoplasms observed in rats by Schoental et al. (1979) may have developed due to immunosuppression by high doses of T-2 toxin. Nevertheless, the observation of neoplasms in rats treated with T-2 toxin indicates that the question of carcinogenicity of trichothecenes still requires a definitive answer.

#### 2.3.5.6 Teratogenesis

Pregnant mice were given intraperitoneal doses of T-2 toxin (0.5, 1.0 or 1.5 mg/kg) by Stanford et al. (1975) on day 7, 8, 9, 10 or 11 of gestation. The highest two doses decreased prenatal fetal survival and caused some maternal mortality. Approximately 37% of fetuses from mice treated on day 10 with either of the highest two dose levels were born with grossly visible malformations, including short or missing tails; limb malformations, including syndactyly and oligodactyly; exencephaly; open eyes; and retarded development of jaws. A low percentage of fetuses from these mice had fused or malformed vertebrae and ribs. Similar teratogenic effects were reported by Hood et al. (1978), who treated mice on day 10 with T-2 toxin at 0.5 mg/kg. The incidence of malformations in these mice was potentiated by co-administration of ochratoxin A, a mycotoxin that caused a high frequency of malformations when given alone on day 8 (Hood et al., 1978).

Pregnant mice in each of these studies were given single, intraperitoneal, high doses of T-2 toxin. The teratogenicity of T-2 toxin under such circumstances is not surprising because many anti-neoplastic drugs that resemble trichothecenes in their acute cytotoxic effects are also teratogenic when administered as a single high dose during embryogenesis (Cahen, 1964). It remains to be seen whether trichothecenes are teratogenic when administered in the diet. Weaver et al. (1978d) fed T-2 toxin (12 ppm) to pregnant sows during late pregnancy but did not observe abnormalities in the piglets. One other sow fed throughout breeding and pregnancy had a small litter of only 4 piglets, two of which died soon after birth, but no physical or histological abnormalities were detected in these piglets.

These investigators also reported the occurrence of abortion in three pregnant sows soon after being given intravenous doses of T-2 toxin (0.41 mg/kg), but the fetuses were reported to be grossly and histologically normal.

#### 2.3.6 Neurotoxic and other effects

In some animals, clinical signs have been observed which suggest that trichothecenes cause neurological disturbances. The most frequently reported effect to consider as neurotoxic is the emetic response to oral or parenteral administration of various trichothecenes including T-2 toxin, diacetoxyscirpenol and fusarenon-X. This effect has previously been discussed in Section 2.2.1 in relation to the naturally occurring syndrome of emesis and refusal of F. roseum-contaminated feed. Experimentally, an emetic response has been observed in various species including ducklings and cats (Ueno et al., 1971b), pigeons (Ellison and Kotsonis, 1973), and pigs (Vesonder et al., 1973; Weaver et al., 1978b) given doses of trichothecenes. Emesis occurs after either alimentary or parenteral administration (Sato et al., 1975; Weaver et al., 1978b), suggesting that trichothecenes induce vomiting by an effect on the central nervous system, rather than locally on the stomach; however, the mechanism has not been determined.

Neural disturbances have been observed in young broiler chickens fed dietary T-2 toxin at levels of 4 to 16 ppm for between 13 and 21 days. Affected birds developed an abnormal posture, abnormal righting reflexes, and seizures in response to loud noises (Wyatt et al., 1973). Neurological signs, which were clearly associated with consumption of T-2 toxin in this study by Wyatt et al., have not been reported in other studies in swine or poultry.

## 2.4 Toxicology of trichothecenes

### 2.4.1 Toxicity

As a group, the trichothecenes are potent chemical toxins; the most toxic members such as verrucarin A, roridin A, fusarenon-X, T-2 toxin, and HT-2 toxin have acute LD<sub>50</sub> values in the range of 1 to 10 mg/kg in most small experimental animals (Bamburg and Strong, 1971). The least toxic members of the group such as trichodermin, trichothecin and trichodermol have acute LD<sub>50</sub> values in the range of 250 to 1000 mg/kg (Bamburg and Strong, 1971).

Partial lists of the relative toxicities of the different trichothecenes in various biological systems have been compiled by various authors including Bamburg and Strong (1971), Bamburg (1976), and Sato and Ueno (1977). Median effective doses or concentrations of different toxins in biological systems used as bioassays are generally available in the original reports of the various techniques; these bioassays and references have been tabulated in Section 2.1.4 (Table 7).

### 2.4.2 Biotransformation and toxicokinetics

Until very recently, little was known of the metabolic alterations and movements of trichothecenes in vivo because of the limitations in quantitative analytical methods suitable for measuring small concentrations of trichothecenes in biological fluids. However, since isotope-labelled trichothecenes have been synthesized (Ueno et al., 1971b; Wallace et al., 1977; Matsumoto et al., 1978), several reports of the in vivo movement of T-2 toxin have been published.

Homogenized bovine or human liver was reported by Ellison and Kotsonis (1974) to metabolize T-2 toxin to HT-2 toxin, by removing the

acetyl group from the C-4 position. Ueno et al. (1971b) reported that mice injected subcutaneously with  $^3\text{H}$ -fusarenon-X excreted most of the label in the urine within 12 hours, after temporary concentration in the liver at 0.5 hours. The excreted radioactivity was not present as the parent compound, but the identity of the metabolite was not determined. Chi et al. (1978a) measured the distribution of radioactivity over 48 hours in chickens given single oral doses of  $^3\text{H}$ -T-2 toxin, and reported that over 80% of the radioactivity was excreted within 48 hours, most of it via the bile and the intestine. The chickens used had been fed diets containing non-radioactive T-2 toxin at levels of 2 to 8 ppm for 5 weeks before the distribution study, so this reported pattern of excretion may not be typical for previously unexposed chickens. Mice have also been found to excrete the radiolabel from  $^3\text{H}$ -T-2 toxin into the intestine by Matsumoto et al. (1978), but of 68% of the initial dose of radioactivity recovered over 72 hours, 51% was present in the feces and 17% in the urine. Small fractions of the fecal radioactivity were found as T-2 toxin and HT-2 toxin, but the majority was present as unknown compounds that were neither T-2 toxin nor HT-2 toxin. These investigators thus demonstrated a pattern of distribution in mice that differed from that of the chicken. It remains to be seen if mice excrete T-2 toxin more quickly if they have been previously exposed to T-2 toxin.

Because of the rapid excretion of the radiolabel from the body, small residues of label were found in the edible portions of chickens by 24 hours after treatment (Chi et al., 1978a). These investigators predicted that chickens on a diet containing 2 ppm of T-2 toxin would

accumulate residues of approximately 0.019 ppm in the muscle, based on their measurements of residual radioactivity in muscle after treatment. Similar levels of radioactivity (approximately 0.018 ppm) were found in eggs of chickens given daily oral doses of  $^3\text{H}$ -T-2 toxin equivalent to 1.6 ppm of dietary T-2 toxin (Chi et al. 1978b). A Holstein cow given daily oral doses of 182 mg of T-2 toxin, equivalent to a feed concentration of 50 ppm, excreted up to 0.016 ppm of unchanged T-2 toxin in the milk, as measured by GC-MS using SIM (see Table 6). However, most samples contained between 0.001 and 0.008 ppm (Robison et al., 1979). The toxicological significance of such low residues of trichothecenes in meat and milk products consumed by humans is presently unknown.

#### 2.4.3 Factors influencing toxicity

The small number of toxicological studies so far conducted for members of the trichothecene group have not been designed to consider the possible influence of nutrients or other dietary components on the toxicity of trichothecenes. However, several reported observations indicate that such influences might be important during natural exposure to trichothecene mycotoxins.

Grain experimentally infected with a toxigenic fungus, or crude extracts of toxin, produce more severe toxic effects in experimental animals than diets containing the same level of pure trichothecene. For example, Palyusik and Koplík-Kovács (1975) found that geese were severely affected by extracts of F. sporotrichioides added to the feed to give a level of only 3 ppm of T-2 toxin. Reduction of the level to 1.5 ppm still had marked toxic effects and most geese died after several weeks. Such low dietary levels of pure T-2 toxin in feed caused few toxic effects

in chickens and turkeys (Chi et al., 1977b, 1977c; Richard et al., 1978). Speers et al. (1977) compared the effects of diets containing Fusarium tricinatum-contaminated corn (giving levels 9 and 16 ppm of T-2 toxin), with the effects of diets containing similar levels of pure T-2 toxin. The latter diets caused less effect on body weight, feed consumption and egg production than did the diets containing mouldy corn. These observations suggest that other fungal metabolites, such as other mycotoxins, or even non-toxic substances, might potentiate the toxicity of T-2 toxin.

Suboptimal levels of various nutrients, especially protein, are known to potentiate the toxicity of many toxicants (McLean and McLean, 1969; Campbell and Hayes, 1976). Several authors have noted that outbreaks of naturally occurring mycotoxic diseases such as ATA (Mayer, 1953a, 1953b) and mouldy-corn poisoning of cattle (Bamburg, 1969) are more severe in individuals that were malnourished. Accordingly, the possibility that trichothecenes might require other contributing factors such as a nutritional deficiency or other toxins before they can cause potentially lethal effects on hematopoietic or immunocytic systems should be investigated.

**EXPERIMENTAL STUDIES**

### 3.0 MORPHOLOGY OF DERMATITIS INDUCED BY TOPICAL T-2 TOXIN

#### 3.1 Abstract

Cutaneous inflammatory reactions in rats and rabbits topically exposed to T-2 toxin were grossly and histologically examined. Rats were exposed to 0.24 µg of T-2 toxin in ethyl acetate (80 µg/ml) and were examined at 3, 6, 9, 12, 15, 18, 21, 24, 30, 36, 48 and 72 hours, and at 6, 9, 12 and 14 days after application. Rabbits were exposed to 0.16 µg of T-2 toxin and examined at 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 36, 42 and 48 hours. The sequential development of lesions induced by T-2 toxin in corn oil/ethyl acetate (9:1; v/v) was compared with reactions to croton oil at 2, 4, 6, 8, 10, 12, 15, 18, 21, 24, 27, 30, 33, 36, 42, 48, 60, 72 and 96 hours after application.

Cutaneous reactions to T-2 toxin were macroscopically visible as hyperemic plaques which became more intensely red and edematous until 48 hours. They subsequently became covered with scaly dry exudate which broke away after 6 days, leaving a pale pink hairless scar. Microscopically, a sequence of acute inflammatory changes in the dermis was apparent, beginning as soon as 3 hours and gradually increasing in intensity until 48 hours. Many neutrophils infiltrated the dermis and subsequently migrated through the epidermis by 24 hours. In most reactions, degenerative changes were not evident in the epidermis, although in some, focal epidermal necrosis occurred in association with a more severe neutrophilic infiltration.

Similar reactions were observed in rabbits in response to T-2 toxin. The sequence of development of cutaneous lesions in response to croton oil was similar to that caused by T-2 toxin. Reactions to T-2 toxin in the corn oil vehicle were milder than were observed when ethyl acetate was used. The lesions caused by T-2 toxin were indistinguishable from those produced by other chemical irritants and no specific changes peculiar to T-2 toxin were detected.

### 3.2 Introduction

Trichothecene mycotoxins are highly irritant to the skin and to mucous membranes. This activity may be responsible for the cutaneous and oral inflammatory lesions observed in fusariotoxicosis (Wyatt et al., 1972a), stachybotryotoxicosis (Forgacs and Carll, 1962; Rodricks and Eppley, 1974), and alimentary toxic aleukia (Joffe, 1971). Trichothecenes are commonly detected in feed extracts using bioassays based on cutaneous inflammation (Bamburg and Strong, 1971). Long before trichothecenes were incriminated in the diseases above, stachybotryotoxin (Forgacs and Carll, 1962; Palyusik, 1970) and poaeufusarin (Joffe, 1971) were recognized as irritant extracts of toxic feed. More recently, trichothecenes have been detected in Fusarium-contaminated cereals using cutaneous irritation as a screening test (Eppley, 1974; Pathre and Mirocha, 1977). However, even though the irritant activity of trichothecenes is important both in the pathogenesis and the diagnosis of trichothecene mycotoxicoses, this activity has not been fully investigated.

Descriptions of cutaneous changes after topical application of pure trichothecenes (Marasas et al., 1969; Ueno et al., 1970) or crude extracts (Joffe, 1971; Schoental and Joffe, 1974) are limited to observations made

two days after application. Furthermore, the reactions examined were severe responses to high doses of toxins, whereas mild reactions are most commonly encountered in testing extracts from cereals (Chung et al., 1974).

The cutaneous injury produced by a low dose of pure T-2 toxin (0.24 µg) was examined to determine: (1) the most likely mechanism of cutaneous injury; (2) the morphological changes most suitable as quantifiable endpoints in a cutaneous irritation bioassay; and (3) the histological changes that might be specific for trichothecenes, thereby enabling a specific bioassay to be designed.

### 3.3 Materials and Methods

#### 3.3.1 Animals and management

Female Wistar rats (approximately 150 g) and young female white New Zealand rabbits (approximately 1.5 kg) from Canadian Breeding Farms and Laboratories, St. Constant-Laprairie, Québec, were used in these studies. Fur growth on the back was usually inactive in animals at these weights. Animals were housed in conventional screen-bottomed steel cages with pelleted laboratory animal diets and water supplied ad lib. All treatments and management procedures conformed to the guidelines of the Canadian Council on Animal Care.

Prior to application of the toxins, the fur over the back was clipped with a No. 40 surgical clipper blade (Oster Corporation, Milwaukee, Wisconsin, U.S.A.) within 24 hours before application of solutions of toxins. To identify application sites, grid lines were marked on the skin with a felt-tip pen containing insoluble ink that did not produce a cutaneous reaction.

### 3.3.2 Toxins

Crystalline T-2 toxin and diacetoxyscirpenol (DAS) were used. T-2 toxin was supplied initially by Dr. H.R. Burmeister (Northern Regional Research Laboratory, Peoria, Illinois, U.S.A.) DAS and additional supplies of T-2 toxin were purchased (Makor Chemicals, Jerusalem, Israel). Toxins were dissolved in either reagent-grade ethyl acetate (EAC) (J.T. Baker Co., Phillipsburg, New Jersey, U.S.A.), or corn oil and applied directly to the skin using a variable-dose microsyringe (CR700-20, Hamilton Co., Reno, Nevada, U.S.A.). Preliminary tests with ethyl acetate alone ensured that this vehicle did not cause macroscopic or microscopic lesions in the skin.

### 3.3.3 Experimental designs

Reactions on the skin both of rats and rabbits were examined sequentially after application of T-2 toxin. Subsequently, reactions of rats to croton oil were compared. All applications were applied randomly and were appraised under incandescent light without knowledge of the treatment given.

#### 3.3.3.1 Effects of topical T-2 toxin on rats

Nine rats were divided into 3 groups. T-2 toxin (80 µg/ml) was applied in 3 µl volumes of EAC at intervals of 3, 6, 9, 12, 24 and 36 hours before necropsy of group A; 15, 18, 21, 30 and 48 hours before necropsy of group B, and 3, 6, 9, 12 and 14 days before necropsy of group C. Eight treatment sites on each rat were used, allowing three separate applications for each time, with one site left untreated.

### 3.3.3.2 Effects of topical T-2 toxin on rabbits

A similar experiment was conducted on 6 rabbits using 2 µl applications of T-2 toxin (80 µg/ml) in EAC at intervals of 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 36, 42 and 48 hours before necropsy. Three replicate applications of each dose were used.

### 3.3.3.3 Comparison of effects of topical T-2 toxin and croton oil on rats

To compare the nature of the irritation effects of T-2 toxin with croton oil (Sigma Chemical Co., St. Louis, Missouri, U.S.A.), T-2 toxin was dissolved in ethyl acetate/ corn oil (1:9, v/v) to a concentration of 50 µg/ml. This was applied to the skin of rats in volumes of 2 µl, whereas croton oil was applied in volumes of 4 µl, each at intervals of 2, 4, 6, 8, 10, 12, 15, 18, 21, 24, 27, 30, 33, 36, 42, 48, 60, 72 and 84 hours prior to necropsy examination, according to the plan in Table 9.

### 3.3.4 Necropsy procedures

In each trial the macroscopic appearance of each test site was evaluated before the animals were killed either by intravenous sodium pentobarbitone or by ether inhalation. The treated areas of skin were removed, stapled to wooden tongue depressors and fixed by immersion in 10% neutral buffered formalin. Blocks of skin through the diameter of the application site were embedded in paraffin, sectioned at 7-8µm and stained with haematoxylin and eosin for microscopic examination.

## 3.4 Results

### 3.4.1 Effects of topical T-2 toxin on rats

The macroscopic and microscopic observations are summarized on Table 10.

Table 9 : Plan for test applications of T-2 toxin and croton oil on rats prior to histopathological comparison of reactions. (Section 3.3.3.3).

Rat No.	Site No.									
	1	2	3	4	5	6	7	8	9	10
1	C2 <sup>a</sup>	T8	C4	T4	C6	T10	C8	T6	C10	T2
2	T8	C6	C8	T4	T2	C4	T10	C10	T6	C2
3	C6	T8	C2	C10	T2	T6	C4	C8	T10	T4
4	C12	T18	C15	T21	C18	C21	T24	T15	C24	T12
5	C24	T15	C15	T12	C12	T21	C18	T24	C21	T18
6	T12	C18	T15	C24	T24	T21	C21	C12	T18	C15
7	T27	M12	T33	C30	T30	C33	T36	C27	M24	C36
8	C36	T36	T30	M6	M0	C30	T33	T27	C33	C27
9	C84	T42	C42	C60	T84	C72	T72	T48	T60	C48
10	C42	T60	T72	T84	C48	C84	T42	C60	C72	T48

<sup>a</sup> C = croton oil; T = T-2 toxin in ethyl acetate - corn oil;  
M = ethyl acetate - corn oil vehicle; Numeral = hours between application and necropsy.

Table 10 : Sequential changes in the skin of rats after topical application of 240 µg of T-2 toxin in 3 µl of ethyl acetate.

Time after application	Macroscopic changes	Microscopic changes	
		D = dermis	E = epidermis
3 hrs.	None	D. Neutrophils in veins and subepidermal capillaries. Pyknosis of individual cells in dermis in some.	E. None.
6 hrs.	None	D. Pyknosis and karyorrhexis of fibroblasts. Neutrophils in prominent subepidermal capillaries.	E. None.
9 hrs.	None	D. As for 6 hrs.	E. None
12 hrs.	Slight hyperemia	D. Emigration of neutrophils into dermis. Focal necrosis of dermal cells. Subepidermal edema. Prominent capillaries.	E. Spongiosis in association with sub-epidermal edema. Pyknosis of isolated basal cells.
15 hrs.	Hyperemia Slight edema	D. Prominent capillaries. Dense neutrophil margination and infiltration. Focal necrosis of dermal cells. Subepidermal edema.	E. Spongiosis or focal necrosis.
18 hrs.	Hyperemia Edema	D. Dense neutrophil infiltration throughout. Focal necrosis of dermal cells. Prominent capillaries.	
21 hrs	Hyperemia Edema Central pallor	D. Dense neutrophil infiltration below epidermis and into epidermis. Prominent capillaries.	E. Focal necrosis, with neutrophils.
24 hrs	Hyperemia Edema Central pallor Moist exudate in some	D. As for 21 hrs.	E. Focal necrosis with neutrophils. Increased mitotic activity.
30 hrs.	Marked hyperemia Moist exudate	D. Most neutrophils in and below epidermis.	E. Prominent proliferation. Accumulation of neutrophils in epidermis.

Continued -

Table 10 - continued. Sequential changes in the skin of rats after topical application of 240  $\mu$ g of T-2 toxin in 3  $\mu$ l of ethyl acetate.

Time after application	Macroscopic changes	Microscopic changes	
		D = dermis	E = epidermis
36 hrs	Intensely hyperemic Edematous Moist exudate	D.	As for 30 hrs. Active cells in connective tissue.
		E.	Hyperplastic. Accumulation of neutrophils in epidermis.
48 hrs.	Intense hyperemia Edema Moist exudate	D.	As for 36 hrs.
		E.	Marked hyperplasia. Accumulation of neutrophils in epidermis.
72 hrs.	Less hyperemia Minimal edema Dry flaking exudate	D.	Active nuclei in dermal fibroblasts. Absence of neutrophils.
		E.	Hyperkeratosis, parakeratosis. Aggregates of neutrophils in flakes on surface.
6 days	Removable flakes Pink hairless scar	D.	Subepidermal fibroplasia.
		E.	Hyperkeratosis and parakeratosis.
9 days	Small smooth pink scar	D.	Fibroplasia and contraction.
		E.	Thickened but proliferation absent.
12 days	Small pink scar	D.	As for 9 days.
		E.	Regeneration of hair follicles.
14 days	Smooth scar	D.	Pronounced subepidermal fibroplasia.
		E.	As for 12 days.

Reactions to topically applied T-2 toxin became visible by 12 hours as flat hyperemic plaques that subsequently increased in redness to a maximum at 48 hours. Plaques became swollen and centrally pale by 18 hours and covered with moist exudation by 24-30 hours. After 48 hours, redness subsided and the plaques became covered with a dry friable exudate. Scaley superficial flakes were frequently present over the reaction sites at 72 hours, but these broke away by 6 days leaving a small, smooth, pink, hairless area which gradually diminished over the next week to be almost inapparent at 14 days.

Microscopically, neutrophils were sequestered in dermal capillaries and at the luminal margins of venules by 3 hours (Fig. 2A, 2B). Between 6 and 12 hours, nuclei of fibroblasts in the interfollicular dermis were pyknotic and karyorrhectic (Fig. 2C). Neutrophils had infiltrated throughout the inner dermis by 15 hours (Fig. 2D), into the outer regions of the dermis by 24 hours (Fig. 3A, 3B), and were concentrated in and below the epidermis at 36 hours (Fig. 3C) and 48 hours (Fig. 3D). Few were observed at 72 hours or later.

Changes in the epidermis and hair follicles were variable during the first 24 hours. Isolated pyknotic nuclei were infrequently found in the basal layer of the epidermis. Epidermal spongiosis and subepidermal edema were evident in some sites (Fig. 2C). By 24 hours, focal areas of coagulation necrosis of epidermis were found in the more severe reactions (Fig. 3A), but in milder reactions, the epidermis overlying the dermal inflammatory response was intact (Fig. 3B). Hyperplasia of the epidermis, which began after 24 hours, became prominent by 48 hours (Fig. 3D), and persisted throughout the 14 day observation period. No inflammatory changes were observed in control sites (Fig. 4A). Subepidermal fibroplasia

Figure 2

Early microscopic changes in the skin of rats after topical application of T-2 toxin (240 µg) in ethyl acetate (3 µl).

- A. 3 hours.  
The epidermis and dermis appear normal except for a few dilated venules. A few leukocytes, especially neutrophils (arrow) and occasionally eosinophils, are sequestered in dilated vessels.
- B. 6 hours.  
Few changes are evident other than dilated venules (long arrow) or capillaries (short arrow) containing leukocytes.
- C. 12 hours.  
Microscopic appearance of more severely affected lesion. Note the pyknosis and karyorrhexis of dermal fibroblasts (arrows), and subepidermal edema (E). There is less separation of collagen bundles, probably as a result of edema.
- D. 15 hours.  
The dermal inflammatory response is more pronounced at this stage.  
Note the increased cellularity of the dermis due to infiltration of neutrophils and enlargement of fibroblasts and endothelial cells. Capillaries are dilated (arrow).

Hematoxylin and eosin; x 300.

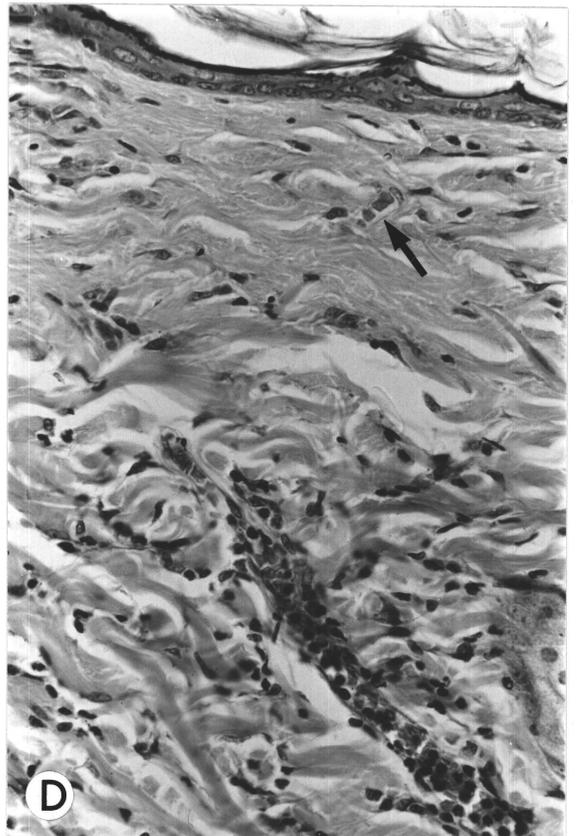
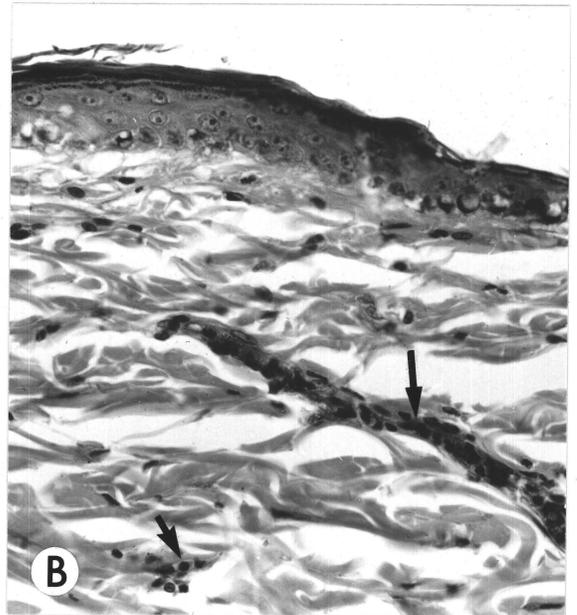


Fig. 2

Figure 3

Microscopic changes in the skin of rats 24 to 48 hours after topical application of T-2 toxin (240  $\mu$ g) in ethyl acetate (3  $\mu$ l).

- A. 24 hours.  
There is a dense infiltration of neutrophils throughout the dermis, and also into the epidermis, particularly in areas of focal epidermal necrosis (N).  
x 120.
- B. 30 hours.  
In some reactions, the dermis was densely infiltrated by neutrophils, but the overlying epidermis was intact.  
x 120.
- C. 36 hours.  
Higher magnification of an area of focal epidermal necrosis (N) that is densely infiltrated by neutrophils. Basal epithelial cells (arrow) at the top of the hair follicle have enlarged and have begun to proliferate.  
x 300.
- D. 48 hours.  
The cellular inflammatory response is mild in this reaction, but some neutrophils are concentrated beneath and in the epidermis, which is now hyperplastic (arrow).  
x 120.

Hematoxylin and eosin; magnification factors are given in each legend.

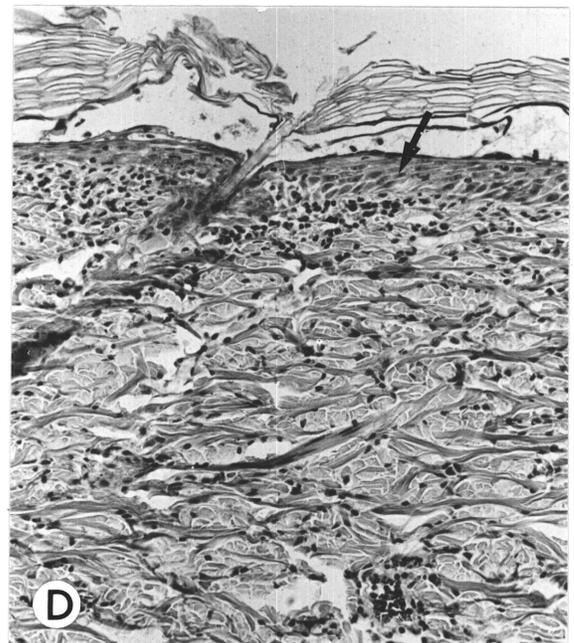
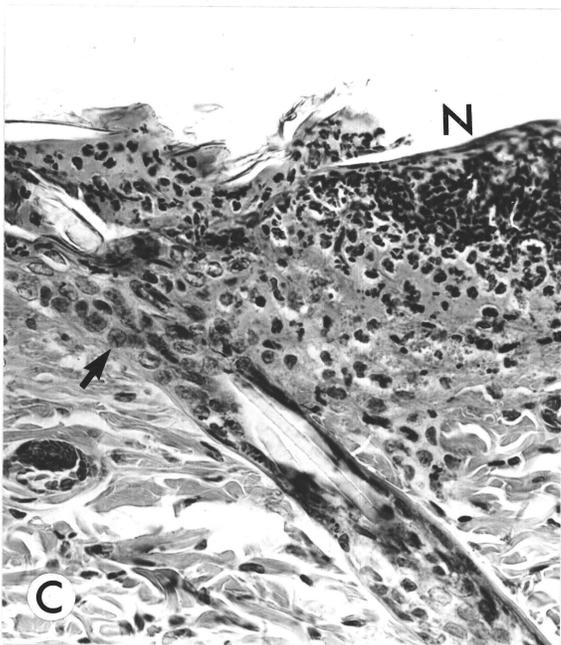
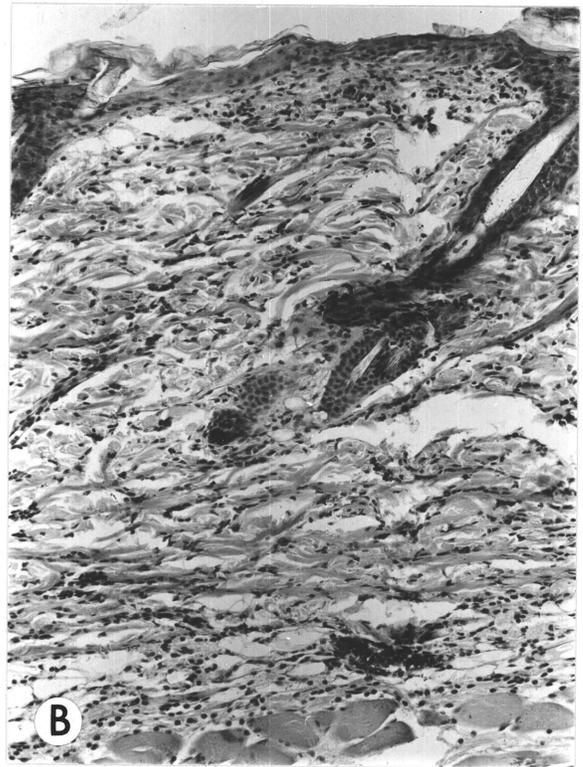


Fig. 3

Figure 4

Microscopic changes in the skin of rats at 6 and 9 days after topical application of T-2 toxin (240  $\mu$ g) in ethyl acetate (3  $\mu$ l).

A. Control.

B. 6 days after treatment.

Note the loosely attached plaque of neutrophilic exudate (E), and a slight increase in the number of subepidermal fibroblasts (arrows). The cellular inflammatory response has completely subsided.

C. 9 days after treatment.

Note the subepidermal fibrosis (arrow) which is in the process of contracting.

Hematoxylin and eosin; x 48.

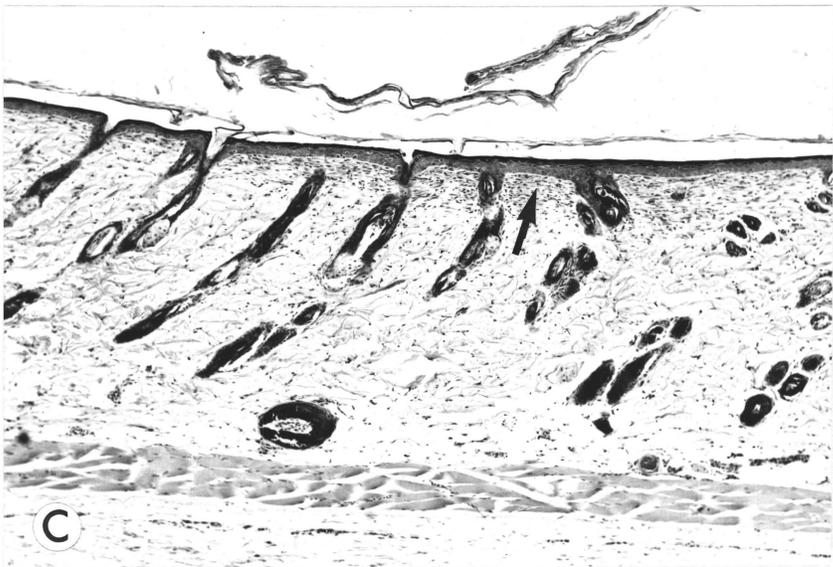
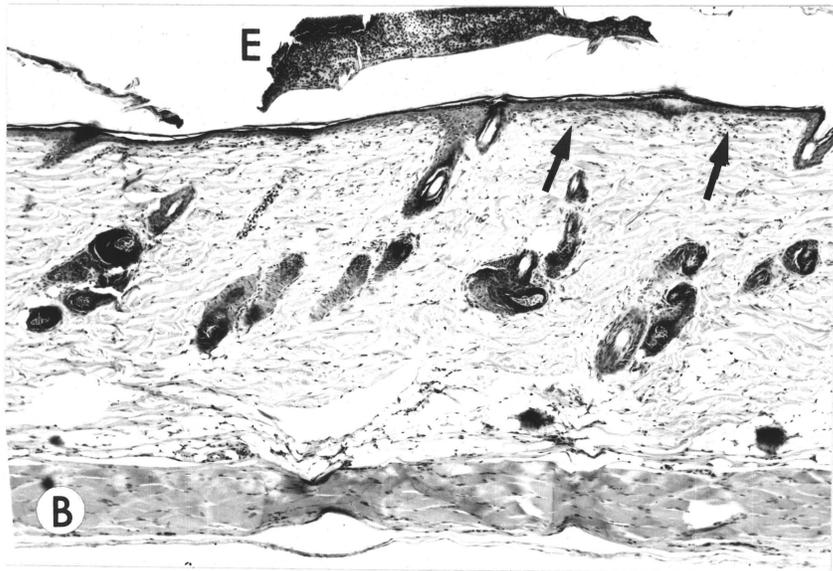
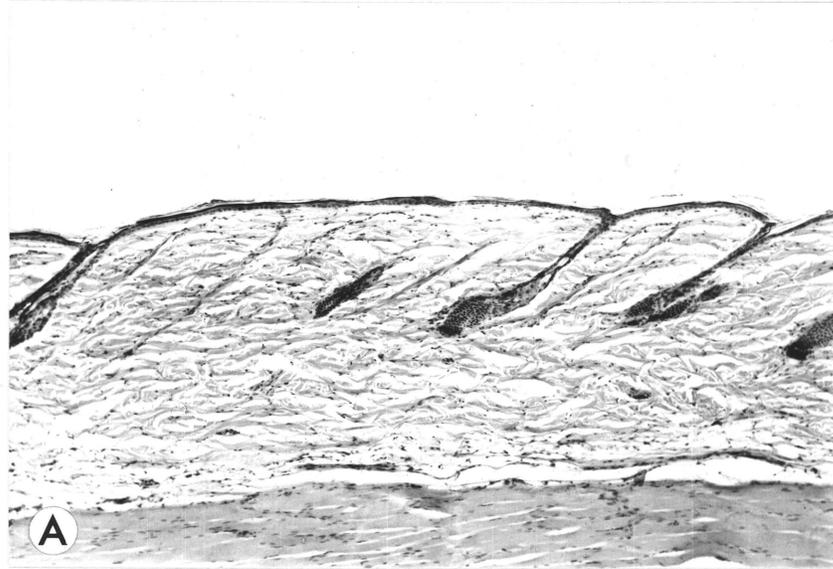


Fig. 4

appeared by 6 days (Fig. 4B) and subsequently increased (Fig. 4C).

The intensity of reactions was variable among the different rats. Some reactions were mild with minimal epidermal necrosis, whereas others which were most severe grossly had foci of dermal and epidermal necrosis and a more intense cellular reaction.

#### 3.4.2 Effects of topical T-2 toxin on rabbits

The macroscopic and microscopic changes observed in rabbits are summarized in Table 11 (pages 91-92). Essentially similar changes occurred as were described for rats in Section 3.4.1. In rabbits, dilated capillaries and venules were more conspicuous and infiltration of neutrophils (heterophils) occurred more rapidly, resulting in emigration through the epidermis by 18-21 hours. Focal areas of dermal and epidermal necrosis were observed at stages similar to those in rats.

#### 3.4.3 Comparison of effects of topical T-2 toxin and croton oil on rats

Reactions were not observed in response to corn oil applied to control sites. The macroscopic and microscopic findings in response to T-2 toxin and croton oil are summarized in Table 12 (pages 93-95).

Reactions produced by T-2 toxin in the corn-oil vehicle were similar, but less intense than those in response to T-2 toxin in ethyl acetate described in Sections 3.4.1 and 3.4.2. These weaker reactions faded after 24 hours, and neutrophil exudation through the epidermis was minimal, in contrast to the previously observed stronger reactions which intensified between 24 and 48 hours. The macroscopic and microscopic findings during the sequential development of reactions to croton oil were essentially similar to those observed for T-2 toxin. However, croton oil produced more severe reactions and a more pronounced

hyperplastic response in the epidermis. Focal coagulation necrosis of the epidermis, which was frequently observed in sites treated with croton oil, was rarely observed in sites treated with T-2 toxin. Inflammatory infiltrates were concentrated around hair follicles in reactions to T-2 toxin in corn oil, or to croton oil, whereas reactions were more evenly spread through the dermis in the previous trials (Sections 3.4.1 and 3.4.2) in which ethyl acetate was used as the vehicle.

Table 11: Sequential changes in the skin of rabbits after topical application of 160 µg of T-2 toxin in 2 µl of ethyl acetate.

Time after application	Macroscopic changes	Microscopic changes	
		D = dermis	E = epidermis
3 hrs.	None	D. Focal accumulation of neutrophils in capillaries.	E. None.
6 hrs.	None	D. Pyknotic and karyorrhectic fibroblasts. Prominent capillaries. Few neutrophils in capillaries.	E. Isolated pyknotic basal cells.
9 hrs.	None	D. Dilated capillaries. Margination of neutrophils. Pyknotic and karyorrhectic fibroblasts.	E. Hydropic degeneration of keratinocytes. Focal areas of coagulation necrosis.
12 hrs.	Hyperemia	D. Many neutrophils throughout dermis. Dilated capillaries and venules. Necrosis of fibroblasts.	E. Neutrophils in dermo-epidermal zone. Swollen keratinocytes.
15 hrs.	Hyperemia Edema	D. As for 12 hrs. Many more neutrophils.	E. Neutrophils infiltrating epidermis.
18 hrs.	Hyperemia Edema Central pallor	D. As for 15 hrs. Dermis is edematous.	E. Many neutrophils in epidermis.
21 hrs.	Hyperemia Edema Central pallor	D. Dilated capillaries. Many neutrophils throughout. Focal subepidermal necrosis. Edema.	E. Focal necrosis. Dense neutrophilic exudation.
24 hrs.	As for 21 hrs.	D. As for 21 hrs.	E. As for 21 hrs. Epidermal proliferation.

Continued -

Table 11- continued. Sequential changes in the skin of rabbits after topical application of 160 µg of T-2 toxin in 2 µl of ethyl acetate.

Time after application	Macroscopic changes	Microscopic changes	
		D = dermis	E = epidermis
27 hrs	As for 24 hrs., but more exudate.	D. Neutrophils mostly in subepidermal zone. Focal epidermal and dermal necrosis. Dermal edema.	E. Aggregates of neutrophils in epidermis. Hyperplasia of basal cells.
30 hrs.	Hyperemia Moist exudate Edema	D. Neutrophils in subepidermal zone. Less cellular infiltration. Slight edema.	E. Hyperplastic response. Aggregates of neutrophils in epidermis.
36 hrs.	Hyperemia Edema Moist exudate	D. Fewer neutrophils - mostly subepidermal. Dermal edema still evident.	E. Hyperplastic response. Aggregates of neutrophils below stratum corneum.
42 hrs.	As for 36 hrs.	D. As for 36 hrs., but less intense.	E. Exudate concentrated in epidermis. Hyperplastic basal cells.
48 hrs.	Hyperemia Edema Moist exudate	D. As for 42 hrs., but less intense.	E. Hyperplasia. Neutrophilic exudate in epidermis.

Table 12 : Comparison of microscopic changes in the skin of rats after application of T-2 toxin in corn oil, and of croton oil.

Time after application	T-2 toxin	Croton oil
2 hrs.	Dilated capillaries. Marginated neutrophils. Degranulation of mast cells.	Dilated capillaries. Marginated neutrophils.
4 hrs.	As for 2 hrs.	More neutrophils in dermis. Epidermal spongiosis. Prominent capillaries.
6 hrs.	As for 4 hrs. Neutrophilic response still weak. Some karyorrhexis in dermis.	As for 4 hrs. Karyorrhexis in dermis.
8 hrs.	Moderate dermal neutrophilic infiltration. Capillaries prominent in some. Epidermal spongiosis in some.	Epidermal spongiosis. Stronger neutrophilic infiltration. Prominent capillaries.
10 hrs.	Pyknotic basal keratinocytes. Epidermal spongiosis Similar neutrophilic infiltrate.	Focal epidermal necrosis. Subepidermal edema. Epidermal spongiosis. Dermal necrosis in some.
12 hrs.	Pyknotic basal keratinocytes. Increased mitotic activity. Focal epidermal necrosis in some. Similar mild infiltration.	Epidermal necrosis in some. Increased mitotic activity. Neutrophils beneath epidermis. Dermal necrosis.
15 hrs.	Inflammatory response is mild. Karyorrhexis in dermis. Prominent capillaries. Epidermal spongiosis in some. Pyknotic basal keratinocytes. Focal necrosis in some.	Epidermal necrosis. Increased mitotic activity. Epidermal spongiosis. Dermal necrosis. Dense neutrophil response.
18 hrs.	Moderate neutrophilic infiltrate. Increased mitotic activity. Pyknotic basal keratinocytes.	Neutrophils in subepidermal zone. Mitotic activity. Epidermal spongiosis. Some dermal necrosis.

Continued -

Table 12 - continued. Comparison of microscopic changes in the skin of rats after application of T-2 toxin in corn oil, and of croton oil.

Time after application	T-2 toxin	Croton oil
21 hrs.	Epidermal proliferation. Epidermal spongiosis. Pyknosis in epidermis in some. Neutrophils below epidermis. Prominent capillaries.	Marked epidermal proliferation. Epidermal necrosis. Epidermal spongiosis. Neutrophils below epidermis. Prominent capillaries.
24 hrs.	As for 21 hrs. Neutrophils around hair follicles.	As for 21 hrs. No epidermal necrosis, but marked neutrophilic infiltration.
27 hrs.	Neutrophils in epidermis. Epidermal necrosis in some. Marked hyperplasia of epidermis.	Neutrophils in epidermis. Marked proliferation of epidermis. Minimal epidermal necrosis.
30 hrs.	Epidermal hyperplasia. Neutrophils in epidermis. Focal epidermal necrosis in some.	Neutrophil response is decreased. Pronounced epidermal hyperplasia. Some proliferation of fibroblasts.
33 hrs.	Few neutrophils in dermis. Mild proliferative response.	Few neutrophils in dermis. Marked proliferative epidermal response.
36 hrs.	As for 33 hrs.	As for 33 hrs.
42 hrs.	Minimal inflammation. Marked epidermal proliferation.	Epidermal necrosis. Hyperplastic epidermis. Parakeratosis Active dermal fibroblasts. Few neutrophils.
48 hrs.	Minimal inflammation. Hyperplasia of epidermis.	Hyperplasia. Hyperkeratosis and parakeratosis. Minimal inflammation. Active dermal fibroblasts.

Continued -

Table 12 - continued. Comparison of microscopic changes in the skin of rats after application of T-2 toxin in corn oil, and of croton oil.

Time after application	T-2 toxin	Croton oil
60 hrs.	Hyperkeratosis. Parakeratosis.	Hyperkeratosis. Dermal fibroplasia. Minimal inflammation.
72 hrs.	Normal or slight hyperplasia.	Hyperkeratosis. Parakeratosis. Dermal fibroplasia.
84 hrs.	Normal.	Hyperplasia of epidermis. Dermal fibroplasia.

### 3.5 Discussion

The macroscopic and microscopic changes observed in the skin after topical application of T-2 toxin were consistent with those described in the cutaneous reactions to a variety of physical (Logan and Wilhelm, 1966; Sholley et al., 1977) and chemical injurious agents (Rostenberg, 1957; Steele and Wilhelm, 1966; 1970). Accordingly, the effects of T-2 toxin on the skin can be considered to result from primary contact chemical irritation. This interpretation is further supported by the observed similarity between cutaneous reactions to T-2 toxin and croton oil, the latter being a well-recognized primary cutaneous irritant (Houck, 1963).

The mechanism of cutaneous injury by T-2 toxin is unknown. In mild reactions, T-2 toxin caused minimal damage in the epidermis, but evoked an intense dermal inflammatory reaction, with hyperemia, edema and neutrophilic infiltration. This suggests that T-2 toxin is capable of causing dermal inflammation without necessarily causing epidermal necrosis, although in stronger reactions, focal coagulation necrosis of epidermis was observed.

In both mild and severe reactions, focal dermal necrosis, with karyorrhexis of spindle-shaped cells identified as fibroblasts, was frequently recognized within 4-6 hours after application of T-2 toxin or of croton oil. Similar changes have been observed in skin exposed to irritant organic solvents (Steele and Wilhelm, 1966; 1970), ultraviolet radiation (Logan and Wilhelm, 1966), and thermal injury (Sholley et al., 1977). In some sites, such dermal necrosis was observed beneath an edematous but otherwise intact epidermis (see Fig. 2C).

The mechanisms by which chemicals evoke dermal inflammation have been only partly determined. Inflammation may be initiated by direct injury to dermal capillaries with alteration of permeability of venules, in combination with activation of the kinin and complement systems, and by release or formation of neutrophil-chemotactic factors by necrotic cells (Ryan and Majno, 1977). Further cutaneous injury may then occur due to intravascular thrombosis (Steele and Wilhelm, 1970), subepidermal edema (Rostenberg, 1957), or by degradative enzymes in infiltrating neutrophils or other leukocytes (Ryan and Majno, 1977). However, many aspects of the cutaneous inflammatory response, including identification of the target of chemical injury, have not yet been determined. Present evidence is insufficient to indicate that trichothecenes evoke dermal inflammation by cytotoxic injury to specific populations of either dermal or epidermal cells. The mechanism of cutaneous injury by trichothecenes might be different from that involved in cytotoxic injury to germinal populations of cells in the lymphoid, hematopoietic, or epithelial tissues.

Other investigators have emphasized the necrotizing effects of high doses of trichothecenes. T-2 toxin in topical doses of 0.12 to 1.3 mg on rats caused severe necrosis of the skin, with sloughing of the epidermis and dermis (i.e., eschar formation) after several days (Marasas et al., 1969). Necrotizing lesions have also been described in the skin of guinea-pigs 2 and 3 days after application of moderately high doses of other trichothecene mycotoxins, namely diacetoxyscirpenol (DAS), fusarenon-X and nivalenol (Ueno et al., 1970). Necrosis also occurs in the skin of rabbits and mice after applications of crude

extracts of Fusarium poae and F. sporotrichioides (Joffe, 1971; Schoental and Joffe, 1974) which are now known to contain high levels of T-2 toxin (Yagen and Joffe, 1976). Ueno et al. (1970) found that DAS, the most irritant of the three trichothecenes examined, caused leukocytic infiltration into the epidermis after 2 days, with deposition of necrotic debris on the surface after 3 days. These investigators also observed that low doses of DAS, or of the other two less-irritant trichothecenes, resulted in similar changes, but with less destruction in the epidermis. These reported lesions are generally consistent with those observed in the present experiments. Reactions to T-2 toxin might be identical to those produced by other trichothecenes, but this is not yet known because the cutaneous effects of T-2 toxin and other trichothecenes have not been examined under similar experimental conditions.

Severe necrotizing lesions caused by high doses of trichothecenes result in cutaneous ulcers which are still inflamed 8 days after application (Joffe, 1971). The lower doses used in the present study caused reactions that diminished after 48 hours. High doses clearly illustrate the toxicity of trichothecenes to the skin, but the resulting lesions bear little resemblance to the mild reactions frequently encountered in routine use of dermal irritation as a bioassay to detect feed extracts containing trichothecenes (Eppley et al., 1974; Chung et al., 1974). Low doses might evoke inflammation without causing dermal or epidermal necrosis.

The non-specific nature of the inflammatory reaction induced in skin by T-2 toxin suggests that neither the specificity nor the sensitivity of skin irritation bioassays would be greatly improved by histological evaluation of the application sites. The dose-response relationships of the various histopathological changes in skin exposed to trichothecenes have not been examined. However, in the present studies, both the neutrophilic response and the degree of dermal and epidermal necrosis appeared to be correlated with the grossly visible intensity of skin reactions. Because each of these components of the skin response subsided after 48 hours, reactions in dose-response studies might be more reliably evaluated at some stage during the first 48 hours after application of toxins.

#### 4.0 TOXICOLOGICAL ASPECTS OF CUTANEOUS IRRITATION BY TRICHOHECENE MYCOTOXINS

##### 4.1 Abstract

Various aspects of the dose-response relationship of cutaneous irritation by T-2 toxin and diacetoxyscirpenol (DAS) were examined to determine ways of improving the irritation bioassay for the trichothecene group of toxins. Reactions, when appraised quantitatively by the Draize scoring technique, were found to be dose-dependent after 48 hours in both rats and rabbits. Rabbits were more sensitive than rats, but exhibited wider variation in intensity of reactions to a given dose among different individuals. At 24 hours, no dose-response relationship was evident in rabbits. Rats were considered to be suitable animals for bioassays because they were more conveniently managed and because the intensity of cutaneous reactions correlated well with topical dose.

Dose-response relationships for both DAS and T-2 toxin were assessed at 24, 48 and 72 hours in rats by determining frequency of response to a graded series of doses. Determinations of median effective (irritant) dose ( $ID_{50}$ ) were similar for both toxins, but were imprecise. Wide variation in individual sensitivity was evident in rats, with some responding to concentrations of T-2 toxin as low as 5  $\mu\text{g}/\text{ml}$ , whereas others did not respond to concentrations as high as 25  $\mu\text{g}/\text{ml}$ . These observations indicated that variation in individual sensitivity should be avoided or controlled if cutaneous irritation were to be employed as a quantifiable end point to accurately assay concentrations of trichothecenes.

The influence of several factors on the dose-response relationship was examined. Reactions to a given dose were highly consistent on the same rat, as were graded responses to different dose levels. T-2 toxin in either ethyl acetate or methanol was more irritant than when dissolved in corn oil or dimethylsulfoxide (DMSO). Intensity of reaction correlated more closely with the quantity of toxin applied than with the concentration.

#### 4.2 Introduction

Several versions of a bioassay based on cutaneous irritation have been described for the detection of trichothecene mycotoxins (Gilgan et al., 1966; Bamburg, 1969; Marasas et al., 1969; Ueno et al., 1970; Wei et al., 1972; Chung et al., 1974). Similar tests have been employed for detection of toxicity of crude extracts of Fusarium poae, F. sporotrichioides (Joffe, 1971), and Stachybotrys alternans (Forgacs and Carll, 1962; Palyusik, 1970; Korpinen, 1974). These tests have been designed as sensitive detection assays, with as little as 10 ng of T-2 toxin being detectable by Chung et al. (1974).

The published methods are generally simple to perform and highly sensitive, but are semiquantative at best (Eppley, 1975). Estimates of quantity of toxin in test extracts are based on the relationship between visible or otherwise measurable intensity of reaction, and the dose of toxin applied. Various quantities of extract have been used under various conditions of application and appraisal, but optimal conditions for performance of the irritation bioassay have not been defined. Variation in sensitivity among different test animals could be expected to contribute to the error of these estimates. In addition, effects of self-mutilation

and secondary bacterial dermatitis would exaggerate measurements obtained.

To determine optimal conditions under which a quantitative dermal irritation bioassay should be performed, the dose-response relationships of cutaneous irritation by T-2 toxin and DAS were examined in rabbits and rats. Responses to graded doses were measured both quantitatively, by subjective scoring of visible intensity of reactions, and quantally, by determining the frequency of response. Influences of species and of individual sensitivity, dose and concentration of toxin applied, vehicle, and age of lesion on these relationships were also examined.

#### 4.3 Materials and methods

Two separate methods were employed to measure irritation in response to topically applied toxins. Rabbits and rats were used as described in Sections 3.3.1. T-2 toxin and diacetoxyscirpenol (DAS) (Makor Chemicals, Jerusalem, Israel) were used in ethyl acetate (EAC) as described in Section 3.3.2, except where indicated otherwise. All applications were randomized and were appraised under incandescent lighting, without knowledge of the treatment plan.

#### 4.3.1 Experimental designs

##### 4.3.1.1 Quantitative measurement of dose-response

In this experiment, the relationship between intensity of reaction and concentration of toxin was examined. Six solutions of T-2 toxin at concentrations of 0, 20, 40, 80, 160 and 320 µg/ml were applied randomly in 2 µl volumes to each of 10 rats and 6 rabbits. The reactions were scored at 24, 48 and 72 hours by the method of Draize, Woodard and Calvery (1944) in which both hyperemia and edema were subjectively scored on a 0-4 scale of increasing intensity (Table 13). The two scores were added to give the Draize score (maximum score = 8). Mean scores for reactions to each dose were determined and analysed by regression against the logarithm of the concentration of toxin applied. Dose-response relationships at the three times of evaluation were compared, as were the responses between the two species.

TABLE 13: Method for scoring the intensity of cutaneous inflammatory responses to primary irritants, according to Draize et al., 1944.

---

A. Erythema and Eschar Formation	
Very slight erythema (barely perceptible)	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
Total possible erythema score	4
B. Edema Formation	
Very slight edema (barely perceptible)	1
Slight edema (edges or area well defined by definite raising)	2
Moderate edema (area raised approximately 1 mm)	3
Severe edema (raised more than 1 mm and extending beyond area of exposure)	4
Total possible edema score	4
Total possible score for primary irritation	8

---

#### 4.3.1.2 Quantal measurement of dose-response

The relationship between concentration of toxin and the frequency response was measured using the method described by Kligman and Wooding (1967). This is a procedure for mathematically evaluating the frequency response of irritation of a number of individual subjects each of which is exposed to all dose levels applied randomly to different areas of skin. T-2 toxin at 2  $\mu$ l volumes at concentrations of 2.5, 5, 10, 15, 20, 40 and 80  $\mu$ g/ml was applied to each of 10 rats. The response was appraised quantally (i.e., present or absent) after 24 and 48 hours, and the frequency response to each dose level was determined. A similar trial was conducted using levels of 5.3, 8, 12, 18, 26.7, 40, 60 and 90  $\mu$ g/ml of both T-2 and DAS, each on separate groups of 10 rats and read at 24, 48 and 72 hours. Using normograms adapted from Litchfield and Wilcoxon (1949) to measure median effective dose as described by Kligman and Wooding (1967), the median irritant dose ( $ID_{50}$ ) and slope function (S) of the probit-plotted frequency of response against the logarithm of concentration were determined for each toxin. Irritancy of T-2 toxin and DAS were compared, and the degree of variation in response between individuals, as indicated by the slope of the response curves, was assessed for both 24 and 48 hour readings.

#### 4.3.1.3 Factors affecting dose-response

The consistency of intensity of reactions by individual rats, and of gradation in response to a series of different doses applied to an individual rat, were examined. Three dose levels of DAS (10, 15 and 30  $\mu$ g/ml) in 2  $\mu$ l volumes were each applied seven times in parallel along the rows of a 7 x 3 grid marked on each of ten rats. Reactions

were assessed at 24 and 48 hours and scored on a 0-4 scale for hyperemia according to the method of Draize et al., (1944). Within each row of seven identical applications, those reactions scored above or below the most common score were counted and the overall frequency of such atypical reactions was determined to measure inconsistency of response by a rat to a given dose. In addition, each of the seven panels of three side-by-side sites was examined for the presence of a response graded with dose. The frequency of correct differentiation of the doses was determined to measure the consistency of dose-response relationship.

To examine the effect of the vehicle on the cutaneous irritation response, 2  $\mu$ l volumes of solutions of T-2 or DAS in ethyl acetate, methanol, dimethyl sulfoxide (DMSO), or corn oil, at a constant concentration (100  $\mu$ g/ml) were each applied once to each of six rats. The intensities of the reactions at 24 and 48 hours were determined by Draize scoring and compared for each vehicle.

To determine whether the concentration of toxin or the absolute amount applied was responsible for the production of a reaction, T-2 toxin was applied twice to each of nine rats in three ways: (1) 0.03  $\mu$ g in 2  $\mu$ l; (2) 0.03  $\mu$ g in 4  $\mu$ l, and (3) 0.06  $\mu$ g in 4  $\mu$ l. Thus (1) and (3) were the same in concentration, whereas (1) and (2) were the same in quantity applied. The frequency of reactions determined for each at 24 hours and 48 hours were compared.

The effect of age of reaction on the relationship of both frequency response and intensity of response to dose was examined as described in Sections 4.3.1.1 and 4.3.1.2.

#### 4.4 Results

##### 4.4.1 Quantitative measurement of dose-response

Mean Draize scores of both rabbits (Table 60 , Appendix A ) and rats (Table 61, Appendix A ) increased with increasing concentration of T-2 toxin, except at 24 hours, when no relationship with dose was evident in rabbits (Fig. 5 ). At 48 hours, significant dose-response regressions were evident in rabbits ( $p \leq 0.05$ ) and rats ( $p \leq 0.01$ ) but, for a given dose, scores were greater in rabbits, indicating that they were more sensitive to the toxins. Scores were higher at 48 hours than at 24 and 72 hours for both species. Variation in scores among rabbits, indicated by the standard deviations, was greater than among rats (Fig. 5). Ethyl acetate alone did not cause visible reactions in any rats or rabbits (Tables 60, 61, Appendix A).

##### 4.4.2 Quantal measurement of dose-response

Frequencies of response observed at 24, 48 and 72 hours after application of T-2 toxin and DAS are presented in Table 62, Appendix A. The median effective concentration ( $ID_{50}$ ) values (Table 14) measured quantally by the method of Kligman and Wooding (1967) demonstrated that the skin of rats was extremely sensitive to both T-2 toxin and DAS. Marked variation in sensitivity among individual rats was evident in the relatively flat dose-response gradients (Fig. 6 ) and high slope-function values (Table 14). Most rats responded to concentrations above 15  $\mu\text{g/ml}$ . It was not possible to distinguish between the irritancy of T-2 toxin and DAS by this method because confidence intervals of the  $ID_{50}$  and slope function were wide (Table 14).

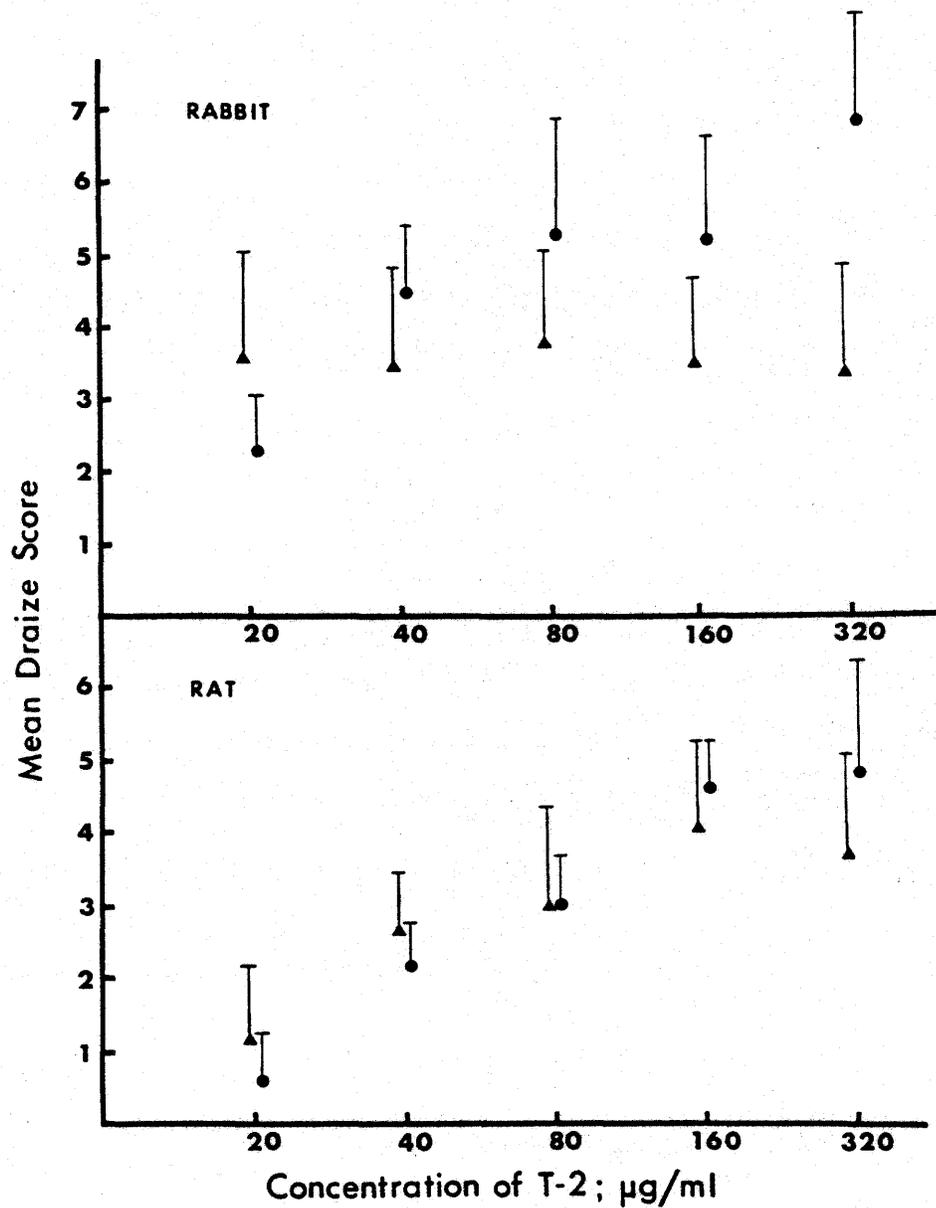


Fig. 5: Comparison of mean Draize scores of rabbits and rats at 24 hours (▲) and 48 hours (●) after topical application of graded doses of T-2 toxin in ethyl acetate. Note the higher scores of rabbits, indicating greater sensitivity and the absence of a dose-response relationship at 24 hours in rabbits. The wide SD bars illustrate the wide variation in intensities of reactions by different animals.

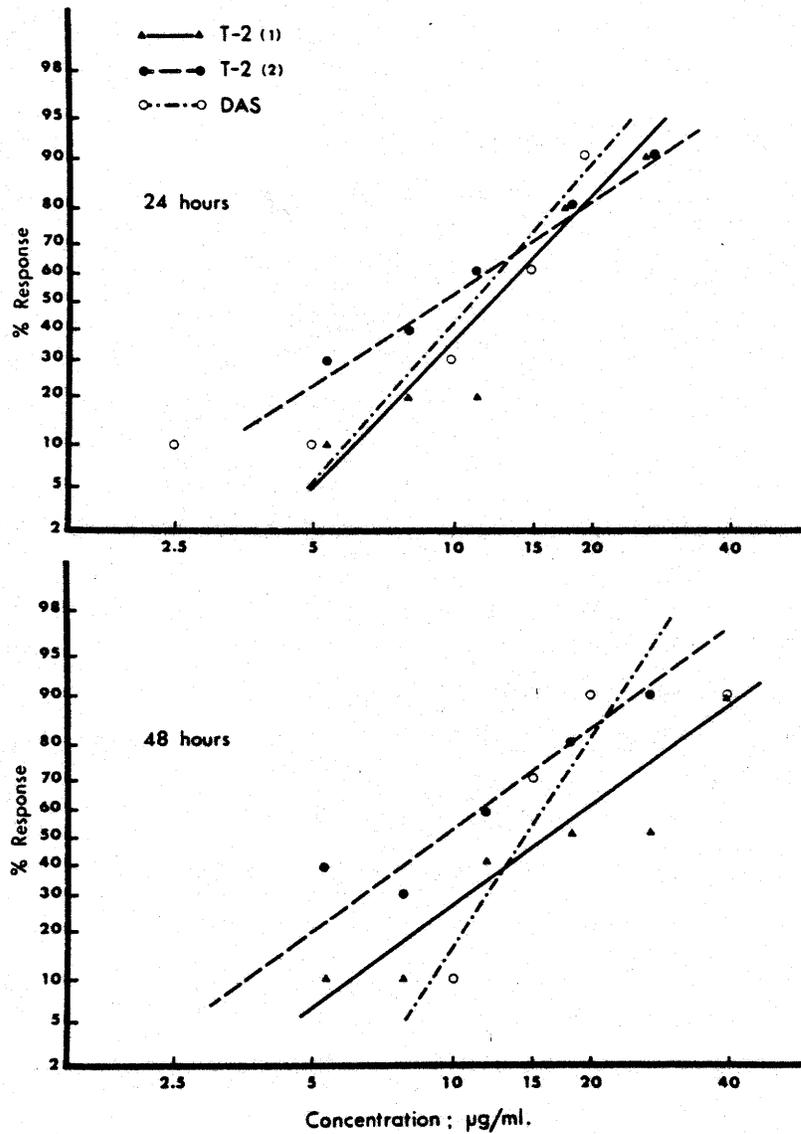


Fig. 6: Frequency response relationships of cutaneous reactions by rats topically exposed to graded doses of T-2 toxin (●---●) and (▲---▲), and DAS (○---○), as determined at 24 hours (above) and 48 hours (below).

TABLE 14: Values<sup>a</sup> of median irritant concentrations (ID<sub>50</sub>) and slope functions (S) for topically applied T-2 toxin and diacetoxyscirpenol (DAS) measured by quantal assay in rats.

Toxin	Time examined	ID <sub>50</sub> (µg/ml)	95% confidence limits	S	95% confidence limits
T-2 <sup>b</sup>	24 hours	11.0	8.1 - 15.0	1.64	1.30 - 2.07
T-2 <sup>c</sup>	24 hours	7.4	4.3 - 12.7	2.31	1.46 - 3.68
DAS <sup>d</sup>	24 hours	11.0	7.9 - 15.4	1.74	1.34 - 2.26
T-2 <sup>b</sup>	48 hours	13.5	10.6 - 17.3	1.49	1.25 - 1.77
T-2 <sup>c</sup>	48 hours	8.5	5.6 - 13.0	1.84	1.27 - 2.67
DAS <sup>d</sup>	48 hours	14.5	9.3 - 22.6	2.04	1.44 - 2.90

<sup>a</sup> Determined according to the method of Kligman and Wooding (1967), for frequency response of skin irritation to multiple levels of test irritants exposed to each test individual.

<sup>b,c,d</sup> Each conducted in separate groups of 10 rats.

#### 4.4.3 Factors affecting dose-response

Frequencies of responses of rats to each of the three concentrations of DAS are presented in Table 63 , Appendix A . Inconsistencies in intensity of response, and in gradation of response with dose are summarized in Table 15.

Reactions to each dose of DAS were highly consistent for individual rats, in contrast to the previously observed variation in reactions by different rats. Atypical responses were uncommon at both 24 and 48 hours (Table 15. For each time of examination, approximately 20% of reactions were scored either above or below the most common score (Table 15).

Correct gradation of reaction with dose occurred at 52 of the 70 test panels at 24 hours and in 32 of the panels at 48 hours. The lower frequency at the later time was largely due to a higher frequency of negative responses (Table 15).

Reactions to both T-2 toxin and DAS (Table 16) at either 24 or 48 hours were similarly frequent and intense for ethyl acetate and methanol solvents. The reaction intensities and frequencies were much lower when corn oil was used as the vehicle. In DMSO, DAS produced no reactions and T-2 toxin produced very mild reactions.

Reactions to T-2 toxin at 24 hours were similarly frequent for each concentration, but the larger quantity of toxin (0.06  $\mu\text{g}$  in 4  $\mu\text{l}$ ) evoked more reactions (Table 17). At 48 hours, a similar relationship was observed, although the overall frequencies of reactions observed had decreased (Table 17).

TABLE 15: Frequencies of inconsistent responses of rats to multiple applications of diacetoxyscirpenol at constant doses.

	24 hours	48 hours
Frequency of reactions	104/210	74/210
Number of atypical reactions	7	11
Number of atypically intense reactions	10	9
Number of atypically weak reactions	8	5
Frequency of correct gradation of responses	52/70	32/70
a. Partial differentiation - two lower doses gave zero scores	27	29
b. Complete differentiation of all three dose levels	25	3
Frequency of poor gradation of responses	18/70	38/70
a. No differentiation - all negative	8	20
b. No differentiation - similar scores for erythema for different doses	10	15
False differentiation - scores ranked contrary to dose	0	3

TABLE 16: Draize scores of reactions to topical T-2 toxin and DAS applied to rats at constant concentrations (100 µg/ml) in four different solvents.

Toxin	Solvents <sup>a</sup>	Rat Number						Total Scores
		1	2	3	4	5	6	
T-2 toxin	Ethyl acetate	3,NA <sup>b</sup>	3,6	4,6	4,4	4,7	1,6	19,29
	Methanol	1,7	3,3	4,6	3,2	2,0	4,5	17,25
	DMSO	0,0	2,1	1,0	1,0	1,0	0,0	5, 1
	Corn oil	0,0	2,2	2,2	1,0	1,1	1,0	8, 5
DAS	Ethylacetate	0,0	4,4	4,3	2,2	2,5	4,3	16,17
	Methanol	2,5	4,4	3,3	0,0	3,6	5,5	17,23
	DMSO	0,0	0,0	0,0	0,0	0,0	0,0	0, 0
	Corn oil	0,0	2,0	1,1	0,0	0,0	0,0	3, 1

<sup>a</sup> Applications were made in 2 µl of solvent.

<sup>b</sup> Values are total Draize scores at 24 and 48 hours respectively.  
NA = not available.

TABLE 17: Frequencies of cutaneous reactions on rats exposed to different concentrations and quantities of T-2 toxin.

	Solvent (2 µl)	0.03 µg in 2 µl	0.03 µg in 4 µl	0.06 µg in 4 µl
At 24 hours	0/9	14/18	14/18	17/18
At 48 hours	0/9	10/18	10/18	14/18

#### 4.5 Discussion

Significant dose-response relationships were observed when effects were measured by intensity of reaction or by frequency response. However, several observations indicated conditions under which cutaneous irritation might best be used to quantitate the level of toxin in an unknown sample.

##### 4.5.1 Quantitative measurement of dose-response

Scoring of reactions by the method of Draize et al. (1944) was readily performed and the response increased over a wide range of effective concentrations. However, variability in intensity of responses both of rabbits and rats was so wide that 4-fold increases in concentration often produced reactions that could not be distinguished from those produced by the lower dose (Fig. 5).

Mean Draize scores for a given dose of T-2 toxin were consistently higher in rabbits than in rats (Fig. 5), indicating that rats were less sensitive to T-2 toxin. Rabbits are generally recognized as being more sensitive than other laboratory animals to most strong topical irritants (Draize et al., 1944; Marzulli and Maibach, 1975). Chung et al. (1974), who compared the effects of topical T-2 toxin on Fisher rats and rabbits, reported minimum sensitivities of 20 µg/ml and 5 µg/ml for each species respectively. Therefore, they concluded that rabbits were superior to rats in bioassays for trichothecenes because of their greater sensitivity. However, in the present study, rabbits varied more in sensitivity than did rats, and rabbits, after 24 hours, exhibited poor correlation between response and dose (Fig. 5). This species difference may have been due to differences between the dose-response relationships

for strong and weak reactions. Rats exhibited a more linear relationship and less variation, but mean scores were mostly less than 4. However, the range of doses used on rabbits produced reactions with mean scores mostly greater than 5. Nevertheless, the variation in intensities of reactions to a given dose by different individuals of either species suggests that the absolute score of a reaction to a test application may be an unreliable indication of the dose. Rats can be more easily managed and may be suitable animals for bioassays if their low sensitivity is not limiting, and if errors due to variation among rats can be minimized.

#### 4.5.2 Quantal measurement of dose-response

The quantal dose-response relationships of reactions by rats to T-2 toxin and DAS demonstrated wide variation in sensitivity among different rats (Fig. 6). Although some rats had reacted at 24 hours to as little as 5 µg/ml, the majority did not respond to concentrations less than 15 µg/ml, in agreement with observations of Wei et al. (1972), who found that most rats would exhibit reactions at 24 hours to quantities of T-2 toxin above 25 µg/ml. This variation in sensitivity meant that slopes of response lines were not sufficiently steep to enable precise measurement of irritancy based on frequency response. In one trial, T-2 toxin was twice as irritant as DAS, whereas in another, they were indistinguishable (Table 14). Because many rats were needed to obtain an ID<sub>50</sub> measurement of irritancy, and because the ID<sub>50</sub> was imprecise, the frequency response appears to be an unsuitable basis of a quantitative bioassay for trichothecene toxins. The variation in sensitivity among rats in the present experiment further indicates that a quantitative bioassay should exclude errors due to differences among test animals.

#### 4.5.3 Factors affecting dose-response

Reactions to multiple applications of the same dose were found to be highly consistent on any one rat, although some atypical reactions occurred (Table 15). The dose-response relationship was also consistent. Reactions were dependent on the type of vehicle used and on the dose rather than the concentration applied. An optimal quantitative bioassay should therefore use constant volumes both of standards and of test solutions in the same solvent. By using small volumes (2  $\mu$ l), up to 18 applications could be made onto the back of a rat. Because of the consistency of reactions within any one individual rat, it would be possible to compare reactions to unknowns directly with reactions to standards applied to the same rat. However, variation in sensitivity among rats would mean that the overall degree of inflammation would vary from rat to rat. Accordingly, reactions would be additive from rat to rat only if they were appraised in units directly related to dose, rather than related to the degree of inflammation.

Differences in dose-response relationships for intensity of reactions at 24 and 48 hours indicated that the optimal time to appraise reactions depended on the range of doses applied. Reactions to low doses of T-2 toxin or DAS (10 to 30  $\mu$ g/ml) were often greater at 24 hours than at 48 hours, when some reactions had disappeared. For slightly higher doses between 30 and 100  $\mu$ g/ml, reactions generally increased to a peak intensity at 48 hours and were more closely graded with dose. Similar differences in dose-response were observed for test extracts applied to rabbits by Chung et al. (1974), who suggested that fading

of mild reactions after 24 hours indicated a non-specific irritant, whereas increasing inflammation until 48 hours more likely indicated the presence of trichothecene toxins in feed extracts. However, because both types of reactions were observed in response to T-2 toxin in the present study, such a difference in reactions can apparently be due solely to differences in concentration of the irritant applied. Intensification of reactions between 24 and 48 hours in the previous chapter (Section 3.4.1) was associated with epidermal and dermal necrosis, and with an intense neutrophilic inflammatory response. Because both the inflammatory response and the visible intensity of reactions subsided after 48 hours, reactions in a dermal-irritation bioassay probably should be evaluated within 2 days after application of toxins.

## 5.0 EVALUATION OF A QUANTITATIVE ASSAY FOR IRRITANCY OF TRICHOHECENES

### 5.1 Abstract

An assay method based on cutaneous irritation by T-2 toxin in rats was designed to enable accurate and sensitive measurement of concentration of solutions containing irritant trichothecene mycotoxins. The influence of variation in sensitivity among individual rats was minimized by applying a graded series of six standard solutions of T-2 toxin in ethyl acetate to each test rat. Twelve test applications were applied blindly to the remaining 12 areas on a 6 x 3 grid of squares on each rat used. Test sites were examined "blindly" at 24 and 48 hours after application. The intensity of each reaction was estimated in equivalent concentration of T-2 toxin by comparison with the graded series of reactions to standards. In this way, estimates from multiple replicate tests applied to different animals could be pooled, with the average being the measured concentration. Several series of masked solutions of T-2 toxin were assayed, and determined concentrations were compared with the actual concentrations. Regression coefficients were significant and close to unity for both 24- and 48-hour measurements, but the linear relationship was much closer for the latter time. For 48-hour determinations, errors in measurement were consistently small, being less than 10  $\mu\text{g}/\text{ml}$  in the effective range of the test (5 to 55  $\mu\text{g}/\text{ml}$ ).

### 5.2 Introduction

A precise, sensitive, and accurate bioassay for evaluating cutaneous irritation would be useful in the experimental investigation of the irritancy of trichothecene mycotoxins. Existing methods (Gilgan et al.,

1966; Bamburg et al., 1969; Ueno et al., 1970; Wei et al., 1972; Chung et al., 1974) are semi-quantitative and are based on estimations of the intensity of cutaneous inflammation. Studies on the pathogenesis and dose-response relationships of cutaneous irritation by trichothecenes in Sections 3 and 4 indicated some conditions under which skin reactions could be used to accurately and precisely measure a topical dose of T-2 toxin. In this chapter, the performance of a method based on the previous findings was evaluated.

### 5.3 Materials and methods

#### 5.3.1 Design of a quantitative bioassay

Young female Wistar rats (approximately 150 g) from Canadian Breeding Farms and Laboratories, St. Constant-Laprairie, Québec, were used as described in Section 3.3.1. Rats were selected because they were more easily managed and because variation in sensitivity was found to be lower than for rabbits in the previous experiments.

A 6 x 3 grid of square areas, each approximately 2 cm x 2 cm, was marked with permanent ink on the shaved skin of the back of each rat. Along one randomly selected row, in either direction, a series of six standard concentrations of T-2 toxin (10, 20, 30, 40, 50 and 60 µg/ml) in 2 µl were applied in sequence, as illustrated in Fig. 7. The 12 remaining sites on each rat were used for test applications. Each test solution was randomly applied at least 10 times, with no more than four applications of the same test solution being applied to any one rat.

Fig. 7: Schematic representation of pattern of application of standard and test solutions of T-2 toxin on the skin of a rat.

10µg/ml	20µg/ml	30µg/ml	40µg/ml	50µg/ml	60µg/ml	Standard solutions
A	D	C	B	F	C	Test solutions
E	F	A	E	B	D	

A, B, C, D, E and F represent test samples applied at random. Each should be applied twice to at least five rats.

At 24 and 48 hours after application, the inflammatory reactions on test sites were compared with those in response to the known standard series of reactions. The concentration applied to each test site was estimated directly by comparison with the intensities of reactions in the standard scale. The average of all estimates of the concentration of the unknown was the assayed concentration, measured as equivalent concentration of T-2 toxin.

### 5.3.2 Performance of bioassay

Solutions of T-2 toxin in ethyl acetate at various concentrations close to the minimum effective concentration were assayed. Solutions were masked and assayed as unknowns, and the assayed concentrations were compared with the actual concentrations. Five series of assays (A-E), as described below, were conducted to determine accuracy and precision under different conditions. In series A, four solutions with concentrations of 20, 30, 45 and 60 µg/ml were selected at random from a total of 12 masked solutions ranging in concentration from 10 to 70 µg/ml, and each was applied three times to each of 10 rats. In series B, 10 solutions with concentrations of 8, 14, 20, 26, 30, 36, 42, 50, 54 and 70 µg/ml were

assayed at both 24 and 48 hours, with each test solution being applied three times to each of four rats. In series C, 12 solutions with concentrations of 4, 7, 8, 11, 14, 16, 22, 28, 32, 44, 56 and 64  $\mu\text{g/ml}$  were each applied twice to each of five rats and assayed at 24 and 48 hours. In series D, the same set of unknowns used in series C was reassayed, with readings made only at 48 hours. In series E, five solutions with concentrations of 6, 12, 24, 36 and 48  $\mu\text{g/ml}$  were each divided into 10 replicate samples, all of which were coded and assayed at 48 hours. In series E, all reactions were read by two different people, neither of whom knew the range of the concentrations of the samples. Precision was assessed from the distribution of 10 assayed values in series E, and accuracy was evaluated in each series by comparing measured concentrations with actual concentrations.

#### 5.4 Results

In each series of bioassays, the reactions to standard solutions of T-2 toxin were graded with dose, except that many rats exhibited one or two reactions which appeared to be out of sequence in the standard range. Where such atypical reactions were encountered, they were ignored, and estimations of unknowns were made by comparison with the rest of the reactions in the standard range. A few rats that were not sensitive to concentrations below 30  $\mu\text{g/ml}$  were rejected. Gradation of reactions to standards was less evident at 24 hours than at 48 hours because there were more atypically intense reactions to low concentrations visible at 24 hours.

Intensities of reactions to all test solutions used in series A, B, C and D, as measured in equivalent concentrations of T-2 toxin, increased in significant linear fashion with increasing dose applied ( $p \leq 0.001$  by  $t$  tests on regression coefficients). However, at 48 hours, measured concentrations (M) were more closely related to actual concentrations (A) than at 24 hours. The regression equations, derived from all measurements in series A, B, C and D were:

$$\begin{aligned} \text{At 48 hours: } M &= 7.2 + 0.74 A \\ &\text{coefficient of determination} = 0.86 \end{aligned}$$

$$\begin{aligned} \text{At 24 hours: } M &= 10.4 + 0.65 A \\ &\text{coefficient of determination} = 0.73 \end{aligned}$$

The errors in measurement of the 4 unknown solutions each applied 30 times in series A were generally small, being less than 30% of the actual concentrations, and in absolute terms, less than 10  $\mu\text{g/ml}$ . Similar performance was achieved when measurements were taken from 5 rats randomly chosen from the 10 used, so reducing the number of applications of toxin from 30 to 15 (Table 18).

Table 18: Comparison of actual concentrations of test solutions with values determined by bioassay in series A, Section 5.4

Sample	Actual concentration	Measured concentration			
		30 replicates		15 replicates	
1	20 $\mu\text{g/ml}$	26 <sup>a</sup>	24 <sup>b</sup>	26	24
2	45 $\mu\text{g/ml}$	39	36	36	33
3	60 $\mu\text{g/ml}$	51	50	53	49
4	30 $\mu\text{g/ml}$	38	35	37	31

<sup>a</sup> Measured at 24 hours.

<sup>b</sup> Measured at 48 hours.

In series B, only 9 applications for each of the test solutions were measured because three of the 10 rats were rejected due to low sensitivity to the standard range of solutions. Measurement errors in series A, B and C were consistently small, with the exception that concentrations of all solutions greater than 55  $\mu\text{g/ml}$  were underestimated by more than 10  $\mu\text{g/ml}$  (Fig. 8). This plateau effect was brought about by difficulties in assessing dose-equivalent measurements for reactions that exhibited similar or greater intensity than was produced by the strongest standard concentration (60  $\mu\text{g/ml}$ ). Accuracy of assayed concentrations, as indicated by standard deviations from the expected regression lines, and by 95% confidence limits was greater when samples at the upper end of the range (greater than 55  $\mu\text{g/ml}$ ) were excluded (Table 19).

The replicate samples in series E were assayed precisely and accurately; the largest error among the 50 assays was 11  $\mu\text{g/ml}$  (Table 20). Correlation between results obtained by different observers was high (Fig. 9). Standard deviations of assayed concentrations were less than 5.0  $\mu\text{g/ml}$ . Precision was sufficient to distinguish between solutions differing by 12  $\mu\text{g/ml}$ , but there was considerable overlap in the ranges of concentrations assayed for the two solutions differing by only 6  $\mu\text{g/ml}$ . Means of assayed concentrations of each set of 10 replicate samples were within 4.6  $\mu\text{g}$  of the actual concentrations (Table 20).

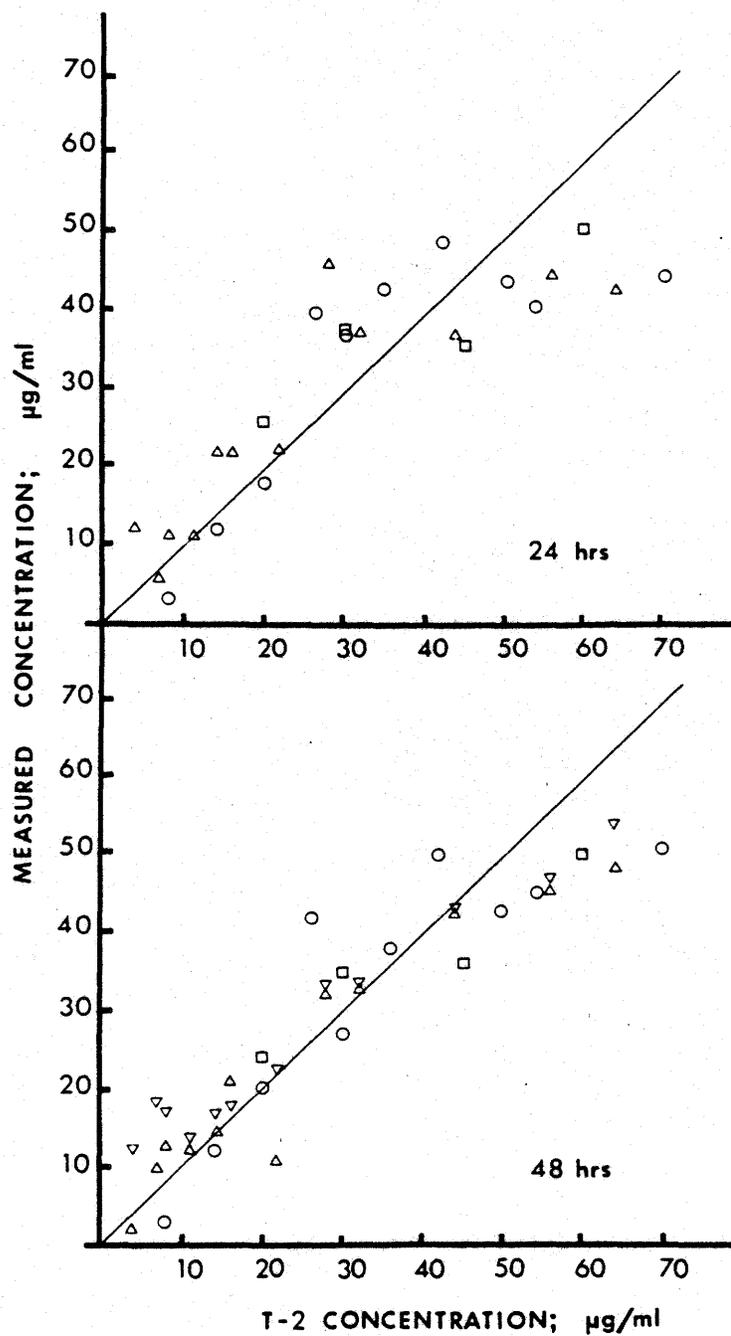


Fig. 8: Plots of measured values obtained from solutions of T-2 toxin assayed in series A ( $\square$ ), B ( $\circ$ ), C ( $\triangle$ ), and D ( $\nabla$ ). The bioassay was more accurate when reactions were read at 48 hours. The lowest concentration assayed was 4  $\mu\text{g/ml}$ .

Distribution of values ( $\bar{x} + SD$ ) of 10 replicate assays  
from 5 concentrations of T-2 toxin

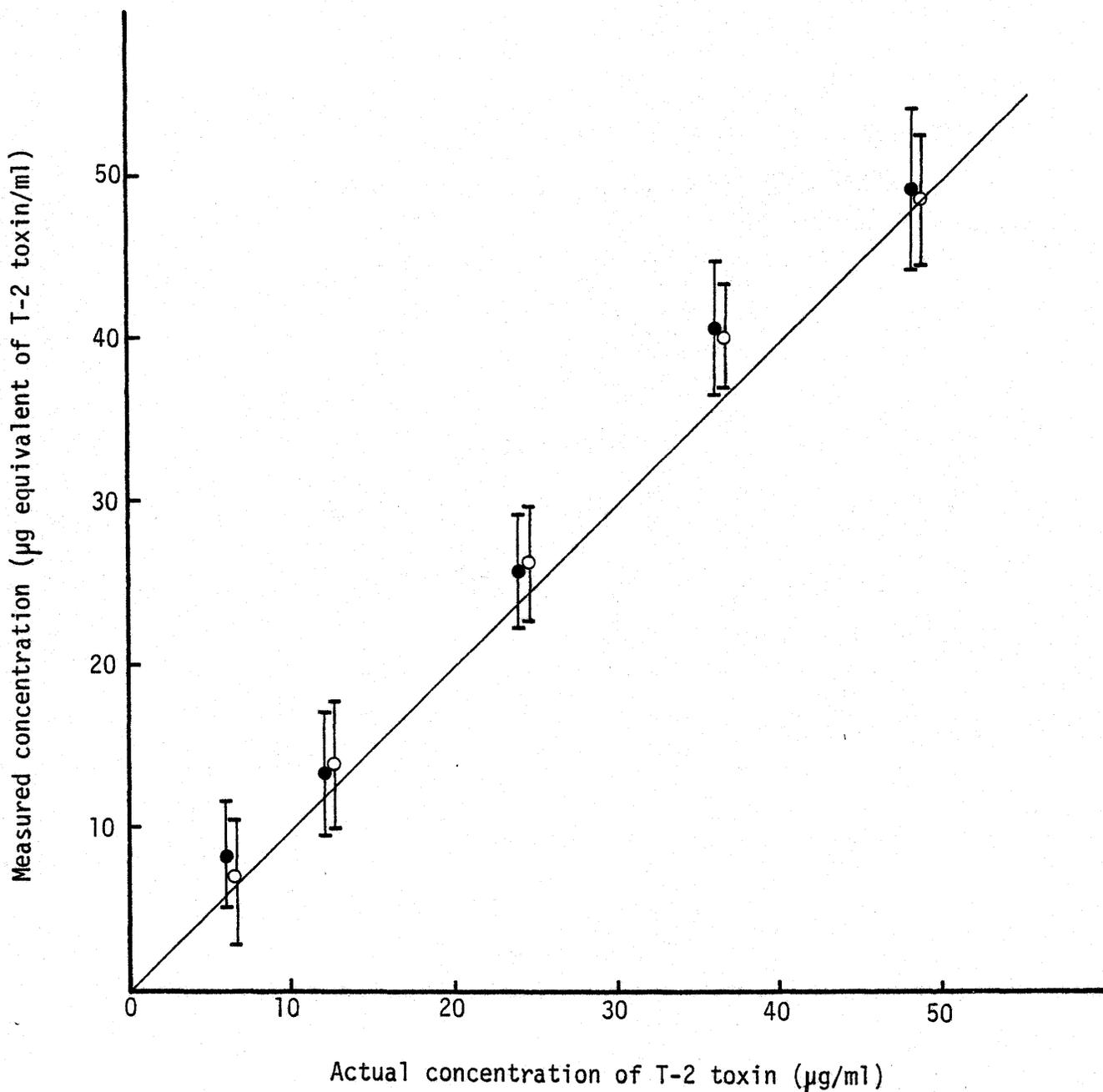


Figure 9. Comparison of concentrations of T-2 toxin determined by two people who each read the same series of assays. Each point is the mean ( $\pm$  SD) of 10 replicate assays. The line shows the expected measurements.

Table 19: Accuracy of measurement of concentrations of T-2 toxin by the quantitative dermal-irritation bioassay in Section 5.4.

Concentrations of samples assayed ( $\mu\text{g/ml}$ )	Number of replicate applications	24 hour readings		48 hour readings	
		SD <sup>a</sup> ( $\mu\text{g/ml}$ )	95% C.L. <sup>b</sup> ( $\mu\text{g/ml}$ )	SD ( $\mu\text{g/ml}$ )	95% C.L. ( $\mu\text{g/ml}$ )
<u>Series B</u>					
8;14;20;26;30;	9	12.0	<u>+23.5</u>	9.5	<u>+18.6</u>
36;42;50;54;70	9 <sup>c</sup>	8.5	<u>+16.7</u>	9.1	<u>+17.8</u>
<u>Series C</u>					
4;7;8;11;14;16;22;	10	9.3	<u>+18.1</u>	7.5	<u>+14.7</u>
28;32;44;56;64	10 <sup>c</sup>	6.3	<u>+12.3</u>	5.1	<u>+10.1</u>
<u>Series D</u>					
4;7;8;11;14;16;22;	10	-	-	6.8	<u>+13.3</u>
28;44;56;64	10 <sup>c</sup>	-	-	6.0	<u>+11.8</u>
Combined results		9.8	<u>+19.2</u>	8.1	<u>+15.9</u>
from all series		7.1	<u>+14.0</u>	6.4	<u>+12.5</u>

<sup>a</sup> Standard deviation of measured values from actual concentrations.

<sup>b</sup> 95% confidence limits (=  $\pm 1.96$  SD).

<sup>c</sup> Measurements of samples above 55  $\mu\text{g/ml}$  excluded from calculations.

Table 20: Performance of bioassay in determining concentrations of replicate solutions of T-2 toxin in ethyl acetate.

Actual concentration (µg/ml)	Mean assayed concentration (µg/ml) <sup>a</sup>	Range (µg/ml)	Standard deviation (µg/ml)	Standard error (µg/ml)	Coefficient of variation (%)
48	49.1 <sup>b</sup>	43-59	5.0	1.6	10.2
	48.3 <sup>c</sup>	42-55	4.0	1.3	8.3
36	40.6	35-46	4.2	1.3	10.3
	39.1	32-43	3.3	1.0	8.4
24	25.9	20-30	3.8	1.2	14.7
	26.2	19-30	3.9	1.2	14.9
12	13.1	9-15	3.5	1.1	26.7
	13.9	9-19	4.7	1.5	33.8
6	8.3	4-14	3.1	1.0	37.3
	6.9	3-16	3.8	1.2	55.1

<sup>a</sup> Of 10 replicate samples.

<sup>b,c</sup> Readings obtained by different people.

## 5.5 Discussion

The bioassay in rats proved to be a sensitive and accurate method of measuring the irritant activity of T-2 toxin. Concentrations as low as 4 µg/ml were accurately measured. This degree of sensitivity compares favourably with the detection limit of approximately 5 µg/ml using rabbits (Chung, et al., 1974). Measurements of reactions were independent of variations in the intensity of inflammatory reactions among rats. By using a total of 18 application sites on each rat, thus allowing the series of standard solutions to be applied to each rat, test reactions were appraised in units of concentration of toxin, rather than by the subjective measurement of intensity of inflammation used on other systems (Ueno et al., 1970; Joffe, 1971; Wei et al., 1972; Chung et al., 1974). Because measurements were independent of the absolute intensity of inflammation, readings from different rats were additive. Accordingly, this assay should produce consistent results even with different strains of rats, provided that the range of concentrations of the standards corresponds to the lowest effective concentrations for the rats used.

The accuracy of measurement of concentration of T-2 toxin (as an average of the readings from 9 or 10 replicate applications) was consistently high across most of the test range used, and was greater when readings were made at 48 hours than at 24 hours (Table 19). The tendency to underestimate the equivalent concentration for test reactions stronger than those observed in the standard range resulted in errors greater than 10 µg/ml. However, when measurements from test solutions over 55 µg/ml were excluded, accuracy improved (Table 19). Precision

was high for a bioassay, and was sufficient to allow differences in concentration in order of 10  $\mu\text{g}/\text{ml}$  to be detected. The mean concentrations of 10 assayed replicates were within 13% of the actual concentrations, indicating that both accuracy and precision are likely to improve when more than 10 applications per assay are used.

The assay was not difficult to perform and many samples could be applied in a short time. Because better results were consistently achieved when reactions were read at 48 hours, only one reading at this time was necessary. The measurements obtained by different people were in close agreement. A further advantage of the assay was that only mild reactions were produced by the standards. Severe reactions are undesirable, both for humane reasons and because they could lead to self-mutilation which would interfere with the relationship between the intensity of reactions and the dose. Furthermore, mild reactions quickly subside so rats may be re-used after 3 or 4 weeks.

## 6.0 EFFECTS OF ACUTE ORAL T-2 TOXICOSIS IN MICE

### 6.1 Abstract

T-2 toxin (2.5 mg/kg) was given intragastrically to young male white Swiss mice weighing  $17.9 \pm 2.4$  g. Groups of five treated mice were examined at intervals of 1, 2, 3, 4, 5, 6, 9, 12, 18, 24, 48 and 96 hours after treatment with T-2 toxin. Control groups of six mice given the propylene glycol vehicle were examined at intervals of 3, 6, 9 and 12 hours after administration. T-2 toxin caused necrosis in all lymphoid tissues, hematopoietic tissues, and in germinal regions of intestinal crypts. Mitotic activity in intestinal crypts ceased within 2 hours, after which numerous round bodies in the cytoplasm of intestinal crypt epithelial cells appeared by 3 to 4 hours. Many such bodies were evident by 12 hours and often they formed into plugs of necrotic debris at the base of crypts. Regenerative activity was evident by 9 hours and increased until crypt epithelium became hyperplastic between 24 and 48 hours. Destruction of lymphoid cells in the lamina propria of the intestine occurred within 1 hour. Karyorrhexis in lymphoblasts of follicles in the spleen and lymph nodes occurred within 1 to 2 hours. Karyorrhexis of nuclei in the thymic cortex was minimal until 9 hours, after which complete necrosis was observed. This was followed by thymic atrophy, but by 96 hours regenerative activity was observed in the atrophic regions of the thymus. Mice given T-2 toxin developed a transient but marked lymphocytosis within 3 hours. Necrosis in hematopoietic tissue of the spleen and bone marrow was visible by 6 hours. Regeneration of megakaryocytes and other undifferentiated hematopoietic cells occurred in spleen and bone marrow by 48 hours. The severity of

lesions, and the rate of recovery was variable among different mice.

Groups of 10 mice were given intragastric doses of T-2 toxin at rates of 0.125, 0.25, 0.5, 1.0, 2.0 or 4.0 mg/kg and tissues were examined microscopically 7 hours later to determine the dose-response relationship of the injury to the various susceptible tissues. The degree of karyorrhexis in intestinal crypts, lymphoid follicles, splenic red pulp, bone marrow, and of mononuclear cells in the lamina propria of the small intestine increased in proportion with doses above 0.5 mg/kg.

## 6.2 Introduction

High doses of trichothecene mycotoxins are acutely cytotoxic to the germinal cells of lymphoid, hematopoietic and alimentary tissues. Saito et al. (1969) described necrosis and karyorrhexis in such tissues in mice 6 hours after being given nivalenol or fusarenon-X. Ueno et al. (1971b) found that fusarenon-X caused a similar pattern of lesions in rats, guinea-pigs, cats and ducklings. These workers subsequently observed that T-2 toxin, or crude fungal extracts containing T-2 toxin, caused similar lesions in mice (Ueno et al., 1972a, 1972c), and cats (Sato et al., 1975). T-2 toxin also caused such radiomimetic patterns of lesions in guinea-pigs (DeNicola et al., 1978), and swine (Weaver et al., 1978c).

The mechanism by which T-2 toxin or other trichothecenes cause acute cellular injury is unknown. Acutely toxic doses either of ionizing radiation or of any one of a wide range of different antitumor agents affect the same tissues that are susceptible to trichothecenes (Fuska and Proska, 1976). However, many different biochemical processes involved in replication and transcription of DNA, or in ribosomal function,

may be the target for these injurious agents (Farber, 1971; Bhuyan et al., 1972). Differences in the relative susceptibilities of different proliferative cells, and in the sequential changes in tissues following a single exposure have been recognized depending on the mechanism of injury caused by the various agents. Agents that permanently injure DNA (e.g., ionizing radiation and alkylating agents; Lieberman et al., 1970) cause continued destruction over a period of 24 hours. Other agents such as the antimetabolites that specifically inhibit enzymic steps in the synthesis of DNA (e.g., cytosine arabinoside; Verbin et al., 1973), or protein (e.g., cycloheximide; Verbin et al., 1971b), cause sudden but transient injury with recovery of damaged tissues occurring within several hours. Some antimetabolites that affect many metabolic processes in a cell, such as the antifolate antineoplastic agents, cause a more prolonged period of cellular destruction (Philips et al., 1975).

In the following experiment, the changes in susceptible tissues of mice given a single, sublethal intragastric dose of T-2 toxin were examined sequentially in an attempt to determine the pathogenesis of damage, and the rate of resolution in the susceptible germinal regions of the thymus, spleen, bone marrow, lymphoid tissues and intestine. The relationship between severity of these effects and the dose was also examined to determine if there were variations in the susceptibility of these tissues to T-2 toxin.

### 6.3 Materials and methods

#### 6.3.1 Sequential changes

Ninety-six young male outbred white Swiss mice weighing  $17.9 \pm 2.4$  g (Animal Resources Centre, University of Saskatchewan, Saskatoon) were assigned randomly to treatment groups of five or six mice. Mice were housed on softwood shavings in screen-top shoe-box cages and were supplied ad libitum with water and pelleted, natural-ingredient diet during a settling-in period of at least 24 hours. Food was then withdrawn 10 hours before treatment. Principal groups of five mice were lightly anesthetized with ether and given, by gastric intubation, T-2 toxin (crystalline, Makor Chemicals, Jerusalem, Israel) dissolved in propylene glycol (0.5 mg/ml) at a rate of 2.5 mg/kg. Control groups of six mice were given propylene glycol (50 ml/kg). Food was resupplied 3 hours after administration of solutions.

Principal groups were examined at intervals of 1, 2, 3, 4, 5, 6, 9, 12, 18, 24, 48 and 96 hours after treatment. Control groups were examined at 3, 6, 9 and 12 hours after treatment. Two further groups of mice were examined after 12 hours; one received only the ether sedation, whereas the other was subjected to food withdrawal alone.

Mice were killed by decapitation and exsanguination. Samples of blood were collected in anticoagulant (EDTA) and automatically analysed (Coulter-S, Coulter Electronics Inc., Hialeah, Florida, U.S.A.) to determine erythrocyte count (RBC), hemoglobin concentration (HGB), mean corpuscular volume (MCV), and total leukocyte count (WBC). Whole blood was stained in methylene blue and smeared for determination of percentages of reticulocytes. Direct blood smears were stained with Wright's-Giemsa

for morphological examination and for differential leukocyte counts.

At necropsy, tissues were examined grossly and samples of thymus, spleen, mesenteric lymph node, stomach, duodenum, jejunum, ileum, cecum, colon, testis, kidney, bladder, heart, lung, muzzle skin, bone marrow and muscle were collected in Heidenhain's Susa fixative. After 24 hours, tissues were stored at 70% ethanol before being embedded in paraffin, sectioned at 6  $\mu$ m and stained with hematoxylin and eosin. Some sections were also stained by the PAS and Feulgen methods.

All tissues were microscopically examined qualitatively, but some changes were quantified. For the thymic cortex, mitoses in a randomly selected high-magnification microscopic field (400x) were counted. For the squamous mucosa of the stomach, and the mucosa of the duodenum and the ileum, mitoses were counted in five high-magnification fields, each of which was selected so that the muscularis mucosae ran diametrically through the field. The number of mitoses in five randomly selected germinal centres of splenic follicles was determined. The degree of destructive injury in thymic cortex, splenic follicles, duodenal and intestinal crypts, splenic red pulp, and bone marrow was graded for severity according to the following scale:

- 0 = no change;
- 1 = minor injury, fewer than 10% of cells destroyed;
- 2 = moderate injury, but structural integrity maintained;
- 3 = severe injury; few normal cells remaining;
- 4 = total destruction of all susceptible cells.

All semiquantitative and quantitative assessments on histological sections were performed without knowledge of the treatment.

### 6.3.2 Dose-related changes

To examine the influence of dose of T-2 toxin on the occurrence and severity of lesions, six groups of 10 male Swiss mice (approximately 20 g) were each given 0.1 ml/20 g of T-2 toxin in propylene glycol-water-ethanol (50:47.5:2.5) at various concentrations so that doses of 0.125, 0.25, 0.5, 1.0, 2.0 and 4.0 mg/kg were administered in similar volumes. Mice were treated after withdrawal of food as described in Section 6.3.1 and were killed after 7 hours. Samples of spleen, duodenum, jejunum, mesenteric lymph node and bone marrow were processed for histological examination as described in Section 6.3.1. The severity of tissue injury in splenic red pulp, lymph follicles and bone marrow was graded on a 0-4 scale of increasing severity as described in Section 6.3.1. For the duodenal mucosa, the numbers of round phagosomes in 20 crypts, selected at random from at least two blocks of tissue, were counted. Mean numbers of phagosomes per crypt were calculated for each dose.

## 6.4 Results

### 6.4.1 Sequential changes

#### 6.4.1.1 General physical changes

Clinical or morphological abnormalities were not observed in any mice in the control groups. Several treated mice became depressed between 3 and 12 hours after administration of T-2 toxin. Most subsequently improved, but three developed hemorrhagic diarrhea; one of these was killed at 12 hours, one died at 15 hours, and the third died at 23 hours.

#### 6.4.1.2 Hematological findings

All hematological values in which changes were observed are listed in Table 21. After treatment with T-2 toxin, hemoglobin concentrations and erythrocyte counts were slightly elevated between 5 and 12 hours inclusive. By 48 hours, they were both slightly decreased. Reticulocyte counts were high at 9 and 12 hours, but were also elevated in vehicle-treated controls at 12 hours. Otherwise, no consistent changes occurred in numbers of reticulocytes in circulation. Total leukocyte counts were elevated within 1 hour, peaked at 4 hours after administration of toxin, and rapidly declined until they reached subnormal levels after 18 hours. Elevations were due to an initial rise in numbers of circulating lymphocytes by 1 hour, and a subsequent elevation in neutrophil counts by 4 hours. Lymphocyte counts remained elevated until 6 hours, after which they declined and reached subnormal levels by 18 hours. Neutrophil counts remained elevated from 3 to 12 hours, and then declined to subnormal levels by 24 hours after treatment with T-2 toxin.

#### 6.4.1.3 Macroscopic findings

At 9 hours, the small intestine was dilated and fluid-filled in three of five mice. Similar changes were found in several mice killed at 12 and 18 hours, by which times colonic contents were also fluid. Intestinal contents were blood-tinged throughout the lower intestinal tract in those mice that died. At 48 hours, the spleen appeared small, but by 96 hours, splenomegaly was evident in two mice. The thymus was consistently small in all treated mice at both 48 and 96 hours.

TABLE 21: Hematological values of young Swiss mice at different intervals after intragastric treatment with T-2 toxin (2.5 mg/kg).

Hours post-treatment	Hemoglobin g/dl	Erythrocytes $\times 10^6/\mu\text{l}$	Reticulocytes %	Leukocytes $\times 10^3/\mu\text{l}$	Neutrophils $\times 10^3/\mu\text{l}$	Lymphocytes $\times 10^3/\mu\text{l}$
1	14.6 $\pm$ 0.5 <sup>a</sup>	7.8 $\pm$ 0.3	4.6 $\pm$ 3.0	9.5 $\pm$ 0.5	1.1 $\pm$ 0.3	8.1 $\pm$ 0.7
2	13.6 $\pm$ 0.3	7.7 $\pm$ 0.2	8.8 $\pm$ 2.9	9.2 $\pm$ 1.3	1.8 $\pm$ 0.4	7.4 $\pm$ 1.0
3	13.4 $\pm$ 0.4	7.7 $\pm$ 0.2	7.1 $\pm$ 1.9	10.9 $\pm$ 2.6	2.6 $\pm$ 1.0	8.3 $\pm$ 1.7
3(control)	13.7 $\pm$ 0.3	7.5 $\pm$ 0.2	10.9 $\pm$ 2.2	3.8 $\pm$ 1.3	0.2 $\pm$ 0.1	3.3 $\pm$ 1.3
4	14.5 $\pm$ 0.5	8.2 $\pm$ 0.3	15.5 $\pm$ 2.3	17.2 $\pm$ 4.2	5.1 $\pm$ 1.9	12.0 $\pm$ 3.0
5	15.3 $\pm$ 0.2	8.2 $\pm$ 0.2	7.3 $\pm$ 0.8	13.2 $\pm$ 2.1	2.9 $\pm$ 0.6	10.1 $\pm$ 1.7
6	15.3 $\pm$ 0.4	8.3 $\pm$ 0.3	6.0 $\pm$ 1.8	6.8 $\pm$ 1.6	1.7 $\pm$ 0.6	5.0 $\pm$ 1.3
6(control)	14.7 $\pm$ 0.2	7.8 $\pm$ 0.1	12.6 $\pm$ 1.4	4.5 $\pm$ 0.6	0.6 $\pm$ 0.1	3.9 $\pm$ 0.5
9	15.4 $\pm$ 0.7	7.9 $\pm$ 0.4	19.9 $\pm$ 4.3	5.9 $\pm$ 1.1	3.3 $\pm$ 0.4	2.6 $\pm$ 1.6
9(control)	12.4 $\pm$ 0.5	7.3 $\pm$ 0.2	15.8 $\pm$ 1.2	5.1 $\pm$ 0.8	0.8 $\pm$ 0.2	4.2 $\pm$ 0.8
12	16.2 $\pm$ 0.5	9.0 $\pm$ 0.4	25.5 $\pm$ 7.0	7.2 $\pm$ 1.3	3.7 $\pm$ 0.7	2.8 $\pm$ 0.5
12(control- vehicle treated)	13.3 $\pm$ 0.4	6.9 $\pm$ 0.2	19.4 $\pm$ 4.6	6.0 $\pm$ 0.9	1.2 $\pm$ 0.2	4.9 $\pm$ 0.6
12 (non- restricted food)	13.9 $\pm$ 0.4	7.0 $\pm$ 0.2	9.9 $\pm$ 2.2	4.9 $\pm$ 0.6	0.8 $\pm$ 0.2	4.1 $\pm$ 0.6
12 (non anesthe- tized)	12.8 $\pm$ 0.6	6.7 $\pm$ 0.1	12.7 $\pm$ 4.4	4.6 $\pm$ 0.5	0.9 $\pm$ 0.1	3.7 $\pm$ 0.5
18	13.5 $\pm$ 0.3	7.6 $\pm$ 0.2	9.3 $\pm$ 1.7	3.1 $\pm$ 0.3	1.7 $\pm$ 0.2	1.6 $\pm$ 0.1
24	13.4 $\pm$ 0.9	7.9 $\pm$ 0.6	9.3 $\pm$ 0.9	2.7 $\pm$ 0.2	0.9 $\pm$ 0.2	1.8 $\pm$ 0.3
48	12.6 $\pm$ 0.4	6.8 $\pm$ 0.2	3.1 $\pm$ 1.3	2.2 $\pm$ 0.3	0.4 $\pm$ 0.1	1.7 $\pm$ 0.3
96	12.4 $\pm$ 0.7	6.7 $\pm$ 0.3	7.2 $\pm$ 2.1	2.8 $\pm$ 0.3	3.7 $\pm$ 0.7	2.5 $\pm$ 0.3

<sup>a</sup> Values are means ( $\pm$ SEM) for separate groups of 5 or 6 mice.

#### 6.4.1.4 Microscopic findings

Mice treated with T-2 toxin developed mitotic arrest, quickly followed by karyorrhexis in germinal regions of the intestinal mucosa (Fig. 10), lymph nodes, thymic cortex (Fig. 11), splenic white and red pulp, and bone marrow. Karyorrhexis was also observed in the intra-epithelial and subepithelial populations of lymphocytes of the small intestinal villi and particularly in the Peyer's patches. Soon afterwards, mitotic activity resumed in the damaged tissues and cell debris was rapidly cleared. Compensatory hyperplasia was often observed in previously damaged tissues during the latter half of the 4-day observation period. Rates of onset of karyorrhexis and recovery were variable among the different tissues and the severity of injury was variable among different mice (Tables 22 and 23). In any single mouse, the degree of injury to the intestinal mucosa varied from level to level and, in some individuals, from crypt to crypt.

Intestinal epithelium: Mitotic activity in the duodenum (Fig. 12A), jejunum and ileum decreased completely during the first 2 hours after treatment (Fig. 12B). By 2 hours, epithelial cells in the crypts lacked clear cellular detail and some contained small (approximately 1-2  $\mu\text{m}$ ), eosinophilic, intracytoplasmic particles (Fig. 12C). The numbers of these particles increased markedly until 12 hours, but subsequently decreased. Intracellular bodies were generally round, multiple within a cell, and located in the lower half of the crypts. Some bodies contained basophilic debris (Fig. 12C) which was Feulgen positive. In severely affected crypts, large numbers of round bodies were present in an aggregate that became extruded into the lumen of the crypt (Fig. 12D).

Fig. 10: Sequential changes in mitotic rate ( $\blacktriangle$ ) and degree of tissue damage ( $\square$ ) in duodenal and ileal crypts of mice after a single intragastric dose of T-2 toxin (2.5 mg/kg).

The degree of tissue damage is a mean of scores from five mice examined at each of the times. Scores were assigned on a 0-4 scale of increasing severity, as described on page 137.

Fig. 11: Sequential changes in mitotic activity ( $\blacktriangle$ ) and degree of karyorrhexis ( $\square$ ) in thymic cortex and germinal centres of splenic follicles of mice after a single intragastric dose of T-2 toxin (2.5 mg/kg).

The degree of karyorrhexis is a mean of scores from five mice examined at each of the times. Scores were assigned on a 0-4 scale of increasing severity, as described on page 137.

Fig. 10

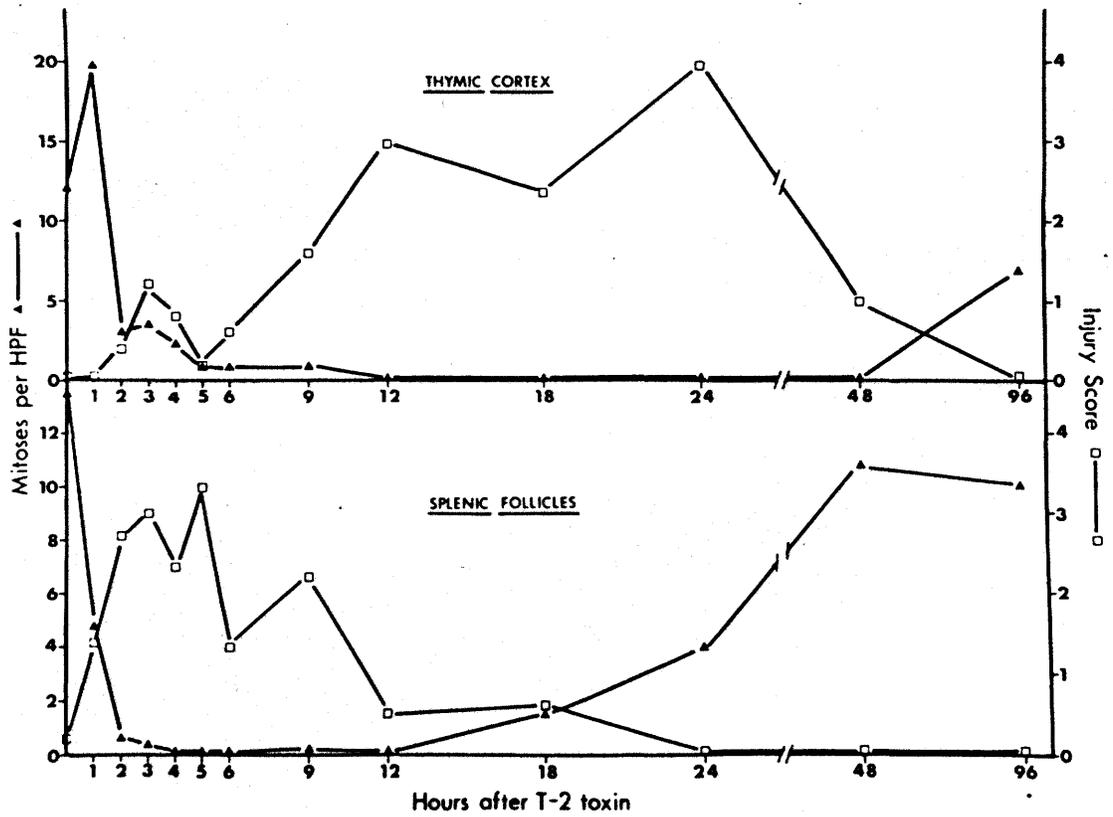
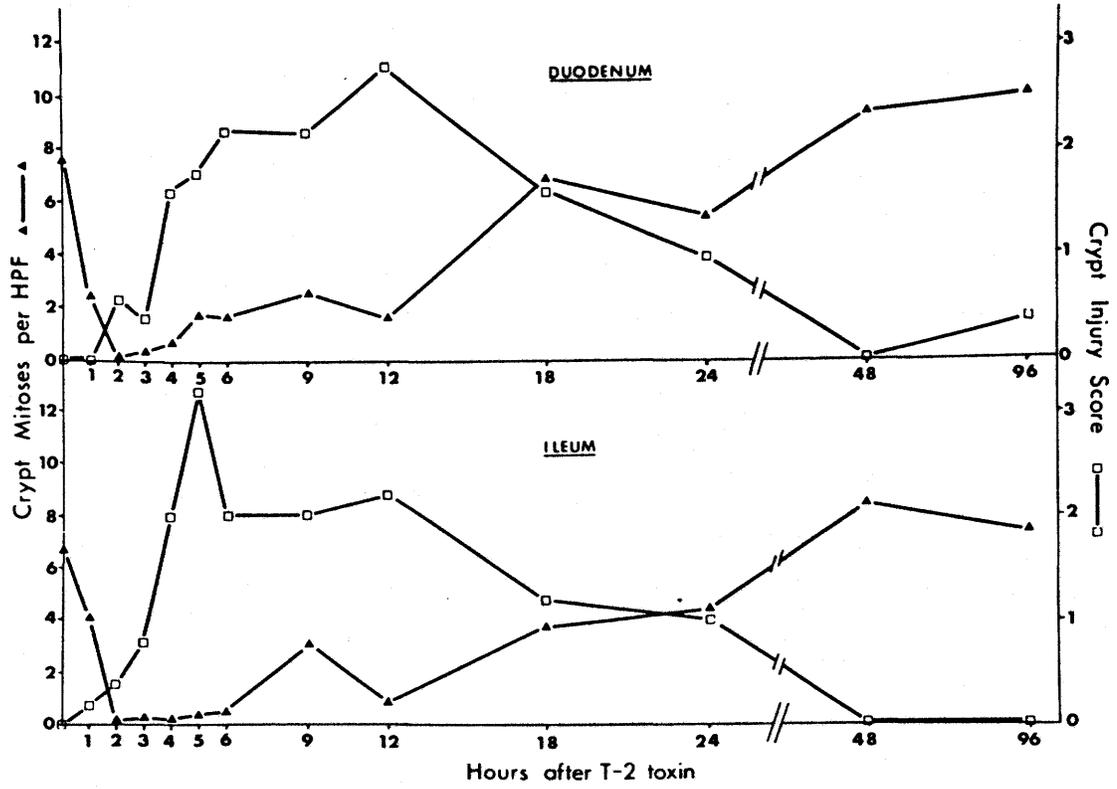


Fig. 11

TABLE 22: Mitotic rates and injury scores in the duodenum and ileum of mice after a single intragastric dose of T-2 toxin (2.5 mg/kg).

	Hours after treatment												
	0	1	2	3	4	5	6	9	12	18	24	48	96
DUODENUM	21	19	1	1	7	3	22	24	0	45	37	81	69
mitotic rate /5 HPF	36	5	0	3	0	11	17	35	3	38	X*	36	23
	51	3	0	6	8	5	0	0	17	89	24	38	44
	53	28	0	0	2	3	1	5	15	6	8	39	60
	32	9	0	0	3	25	5	5	X	0*	43	43	57
	38												
injury score	0	0	1	1	2	2	1	1	4	1	1	0	0
	0	0	0	0	1	1	1	1	2	1	X*	0	0
	0	0	1	0	1	2	4	4	3	1	1	0	1
	0	0	0	0	2	3	3	2	2	2	1	0	0
	0	0	0	1	2	1	2	3	X	3*	1	0	1
0													
ILEUM	14	13	0	3	1	1	4	39	0	47	22	69	48
mitotic rate / 5 HPF	33	15	2	0	0	0	1	15	3	44	X*	54	41
	49	11	2	2	4	3	3	0	13	2	25	39	32
	22	10	0	1	0	0	3	6	0	2	27	20	25
	40	54	0	0	0	7	2	16	X	0*	15	29	41
	42												
injury score	0	1	0	1	2	2	2	1	4	0	2	0	0
	0	0	0	1	1	3	1	1	2	0	X*	0	0
	0	0	1	0	2	4	4	4	2	1	1	0	0
	0	0	0	1	3	4	2	2	3	2	0	0	0
	0	0	1	1	2	1	1	2	X	3*	1	0	0
0													

\* Died

X = tissue not examined.

TABLE 23: Mitotic rates and injury scores of thymus, spleen and bone marrow in mice after a single intragastric dose of T-2 toxin. (2.5 mg/kg).

	Hours after treatment												
	0	1	2	3	4	5	6	9	12	18	24	48	96
THYMUS	13	17	4	8	1	2	0	1	0	0	0	0	16
mitotic rate/HPF	14	23	2	1	6	0	1	1	0	0	0*	0	11
	5	15	6	5	2	0	0	0	0	0	0	0	0
	9	24	1	0	0	0	0	0	0	0	0	0	0
	6	X	3	X	3	2	3	0	0	0*	X	0	X
	24												
injury score	0	0	1	1	1	0	1	1	4	2	4	1	0
	0	0	0	2	1	0	0	1	3	2	4*	0	0
	0	0	1	1	1	0	1	2	2	2	4	0	0
	0	0	0	2	1	1	0	2	3	2	4	0	0
	0	X	0	X	0	0	1	2	X	4*	X	4	X
	0												
SPLENIC FOLLICLES	9	3	X	0	0	X	0	0	0	3	4	X	9
mitotic rate, /5 follicles	12	2	2	X	2	0	X	1	0	2	X*	27	11
	27	2	0	1	0	X	X	0	0	1	6	4	10
	8	5	0	0	X	0	0	0	0	2	X	4	X
	12	12	X	0	0	0	0	0	X	0*	2	8	X
injury score	0	1	X	2	2	2	3	2	0	1	0	X	0
	1	2	2	X	2	4	X	2	0	0	X*	0	0
	0	2	3	3	2	X	X	4	2	0	0	0	0
	0	1	3	3	X	3	1	2	0	1	X	0	0
	0	1	X	4	3	4	0	1	X	1*	0	0	X
BONE MARROW	0	0	1	0	1	2	2	2	3	3	2	4	0
injury score	0	0	0	1	0	3	2	0	3	2	4*	1	X
	0	0	0	1	0	3	2	2	2	X	4	1	0
	0	0	0	0	2	0	2	2	2	1	3	2	0
	0	0	0	1	0	1	0	1	X	4*	4	4	0
	0												

\* Died

X = tissue not examined.

Figure 12

Sequential changes in duodenal crypts after a single intragastric dose of T-2 toxin (2.5 mg/kg).

- A. Control.
- B. 1 hour.  
Mitotic activity is greatly reduced. Some crypts have a swollen, bulb-like conformation (arrow).
- C. 3 hours.  
Numerous, small, intracellular bodies are visible in the lower regions of the crypts. The smallest, barely-visible particles are eosinophilic, whereas the larger bodies are dense and basophilic (arrow).
- D. 9 hours.  
Density of intracellular bodies has increased. Most are basophilic. Some crypts are overloaded so that bodies have been extruded into the lumen of the crypt (arrow).
- E. 24 hours.  
Most of the bodies have disappeared. The depth of crypts has decreased, and some crypts contain residual casts of debris (arrow). Mitotic activity has resumed in some crypts.
- F. 96 hours.  
Some crypts have regenerated in a disorganized fashion and are lined by hyperplastic, undifferentiated epithelium. The lamina propria has increased cellularity due to disappearance of crypts and an inflammatory response.

Hematoxylin and eosin; x 300.

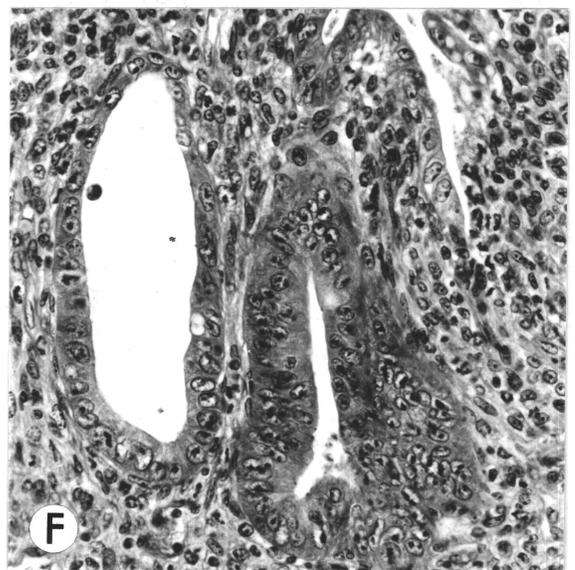
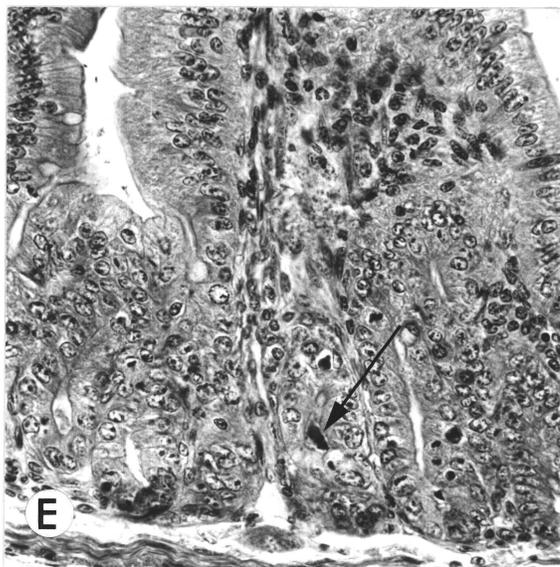
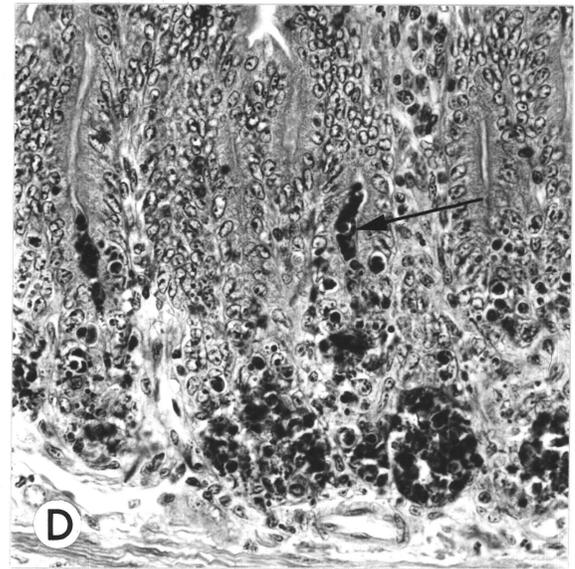
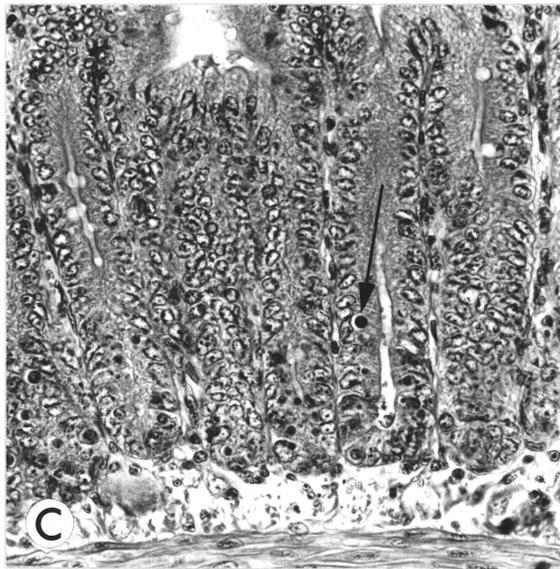
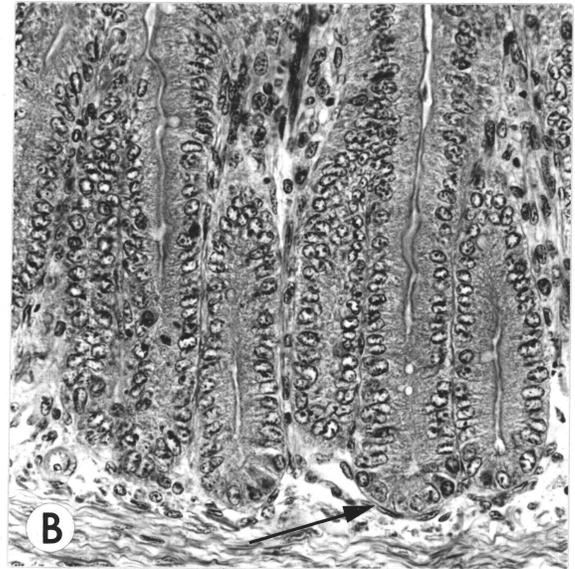
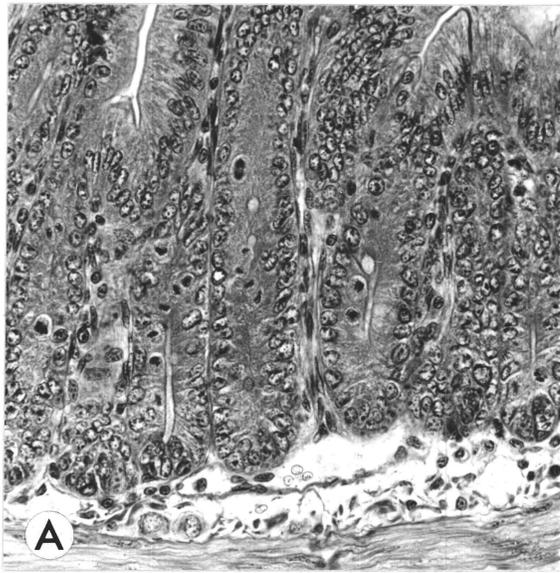


Fig. 12

Figure 13

Effect of a single intragastric dose of T-2 toxin  
(2.5 mg/kg) on intestinal lymphoid tissues of mice.

- A. Duodenal villi from a control mouse treated 3 hours previously with propylene glycol. Intraepithelial theliolymphocytes (arrow) and mononuclear cells of the lamina propria are intact.
  
- B. Duodenal villi from a mouse treated 1 hour previously with T-2 toxin. Note the karyorrhexis of cells in the lamina propria. Some theliolymphocytes are pyknotic (arrows).
  
- C. Duodenal mucosa of a mouse treated 2 hours previously with T-2 toxin. Karyorrhexis is evident in the lamina propria of a villus (asterisk), but intestinal crypts are still intact.
  
- D. Aggregate of gut-associated lymphoid tissue in the duodenum of a mouse treated 1 hour previously with T-2 toxin. Many lymphocytes in the subepithelial zone have undergone karyorrhexis, but the majority are unaffected.

Hematoxylin and eosin, x 300.

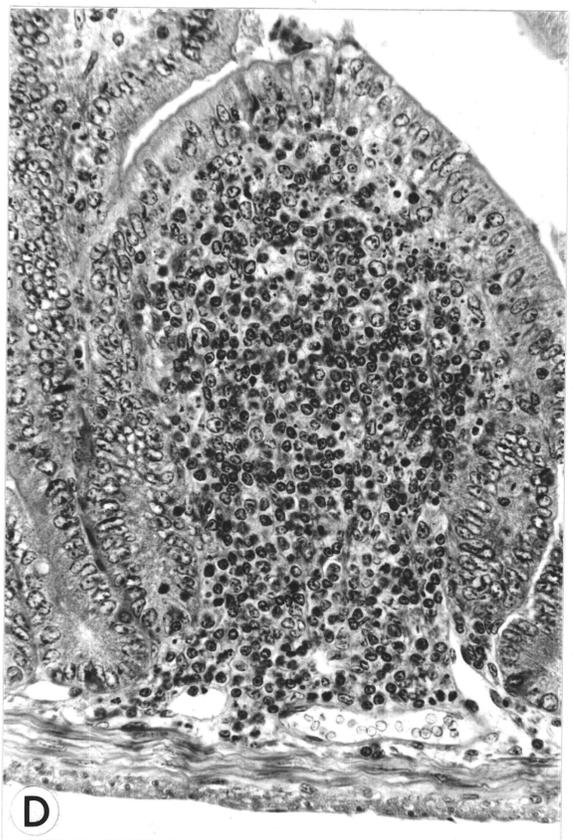
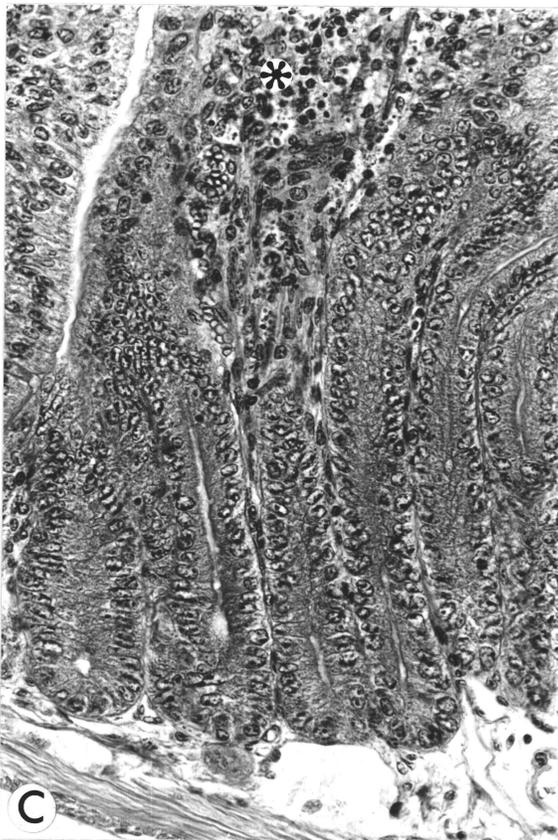
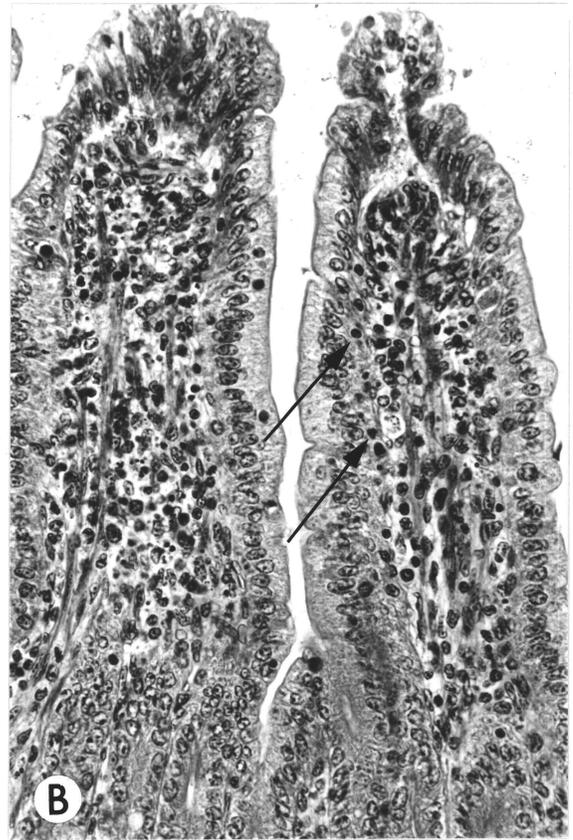
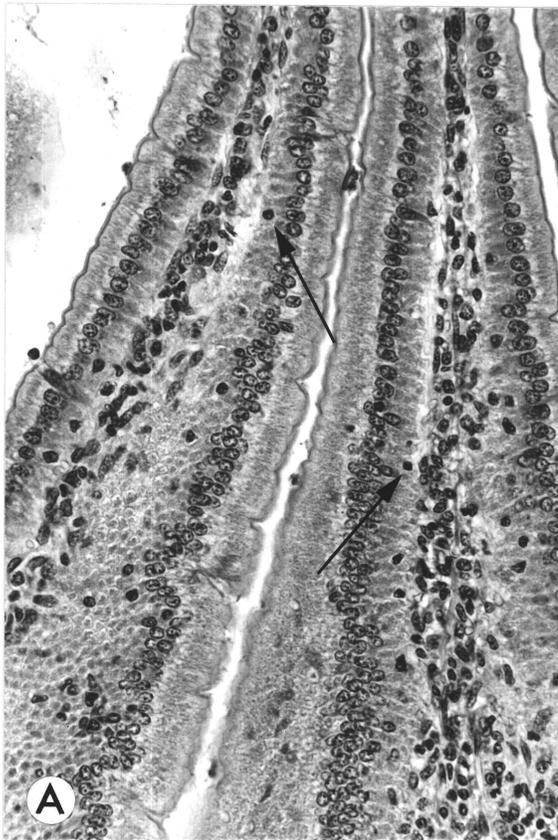


Fig. 13

Neutrophils had infiltrated the lamina propria by 3 hours and some appeared to have emigrated into the lumina of damaged crypts by 6 hours. Mitotic activity resumed in some mice within 5 or 6 hours at the duodenal level and within 9 hours at the ileal level (Fig. 10). There appeared to be a phasic pattern in the mitotic rate during regeneration. By 24 hours, minimal amounts of debris remained and some crypts were atrophic (Fig. 12E). Mitotic rates at 48 and 96 hours were higher than at earlier times or in control mice. At these stages, crypts were hyperplastic and distorted in shape and villi were shortened (Fig. 12F).

Few changes were observed in the epithelium of other regions of the alimentary tract. A few karyorrhectic cells were observed in gastric glands and colonic crypts of some mice at 6 and 9 hours and mitoses were rarely seen in the squamous gastric mucosa during the first 12 hours after treatment.

Intestinal lamina propria: At 1 hour, numerous pyknotic and karyorrhectic nuclei were found in the lamina propria of the villi and, to a lesser extent, between the crypts. Pyknotic cells which appeared to be intraepithelial lymphocytes were found in the villus epithelium (Fig. 13B). Fragments of karyorrhectic nuclei were present in clusters by 2 hours (Fig. 13C) and remained visible until 12 hours. In mice with severe crypt lesions, the lamina propria was edematous and increased numbers of neutrophils were observed in capillaries near damaged crypts. Pyknotic and karyorrhectic lymphoid nuclei were numerous in and around foci of intestinal lymphoid tissue and Peyer's patches (Fig. 13D). Karyorrhexis, and subsequent depletion, was also observed in germinal centres of Peyer's patches between 1 and 12 hours after treatment.

Thymus: Mitotic activity was absent in both the cortex and medulla within 2 hours (Fig. 11, 14B). Scattered karyorrhectic nuclei were present in the cortex during the first 6 hours (Fig. 14B), but by 12 to 24 hours the cortex was totally destroyed (Fig. 14C). Cellular fragments were picked up by macrophages and debris disappeared after 24 hours, so that by 48 and 96 hours, the cortex was reduced to a residuum of epithelial and reticular cells (Fig. 14D). Mitotic activity in the thymic medulla and cortex had resumed in some mice by 96 hours.

Splenic white pulp: Mitotic lymphoblasts were consistently seen in controls (Fig. 15A) but, within 1 hour of treatment, karyorrhexis was evident in follicles (Fig. 15B). By 12 hours, mitotic activity resumed (Fig. 11) and the cell debris was removed by macrophages.

Lymph nodes: Mitotic activity in germinal centres (Fig. 15C) was absent between 1 and 12 hours inclusive, but then resumed. Varying degrees of karyorrhexis were found in germinal centres of active follicles at 1 hour after treatment. Between 2 and 12 hours, pyknotic and karyorrhectic nuclei were infrequently observed in the paracortical and medullary regions of the nodes. Lymphoid cellular debris was aggregated in macrophages between 2 and 4 hours (Fig. 15D).

Splenic red pulp: Small fragments of karyorrhectic nuclei (Fig. 16B) were observed in hematopoietic populations within 3 hours. Numbers of immature hematopoietic precursors declined during this period. By 48 hours, regenerative activity was evident, firstly in megakaryocytes, followed then in myeloid cells (Fig. 16D, E). No increase in numbers of maturing erythroid cells was evident by 96 hours (Fig. 16F).

Figure 14

Sequential changes in the thymic cortex of mice after a single intragastric dose of T-2 toxin (2.5 mg/kg).

- A. Control mouse.  
The thymic cortex is densely cellular, and mitotic figures (arrows) are numerous.
  
- B. Thymic cortex 2 hours after treatment with T-2 toxin.  
No mitotic figures are present and a few cells have undergone karyorrhexis (arrows).
  
- C. Severe cortical necrosis at 24 hours after treatment with T-2 toxin.  
Almost all cortical lymphocytes are karyorrhectic or pyknotic, and many fragments have been phagocytosed by macrophages (arrows).
  
- D. Depleted thymic cortex, populated by epithelial cells and vascular stroma at 96 hours after treatment.  
The medulla is not depleted.

Hematoxylin and eosin, x 300.

Micrographs are orientated with the cortex above and the medulla below.

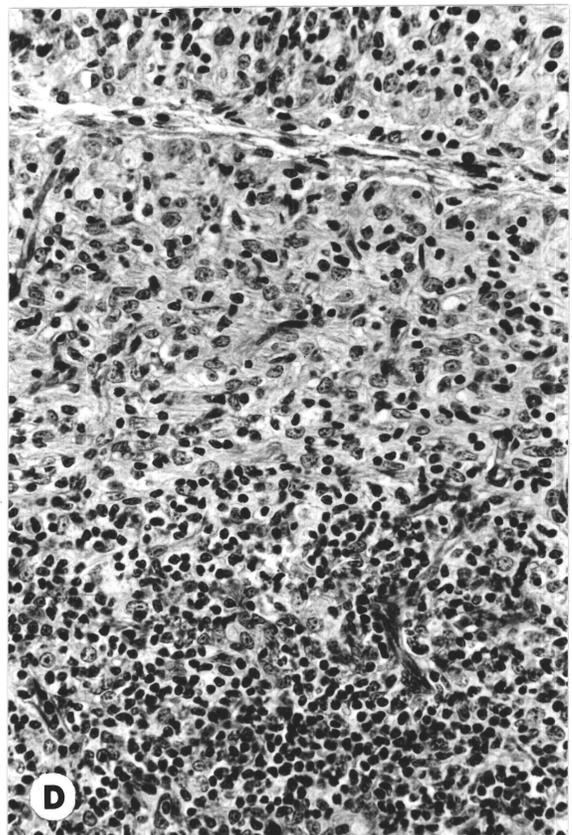
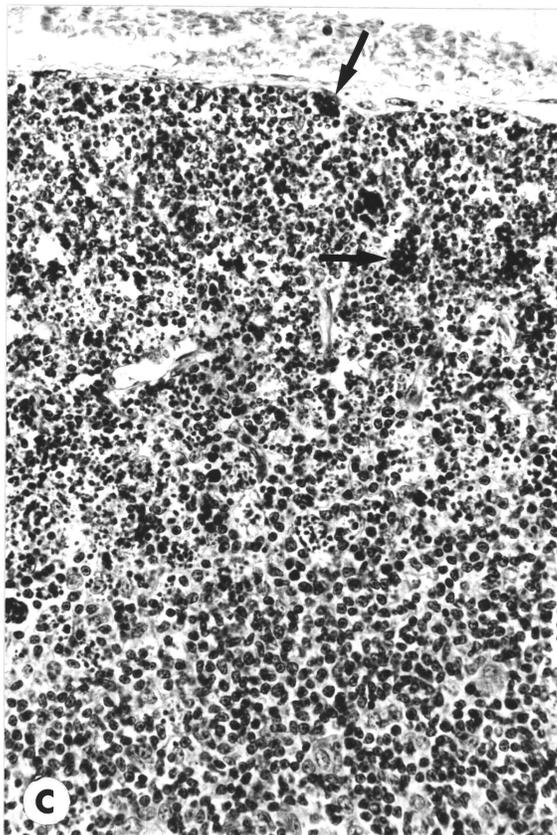
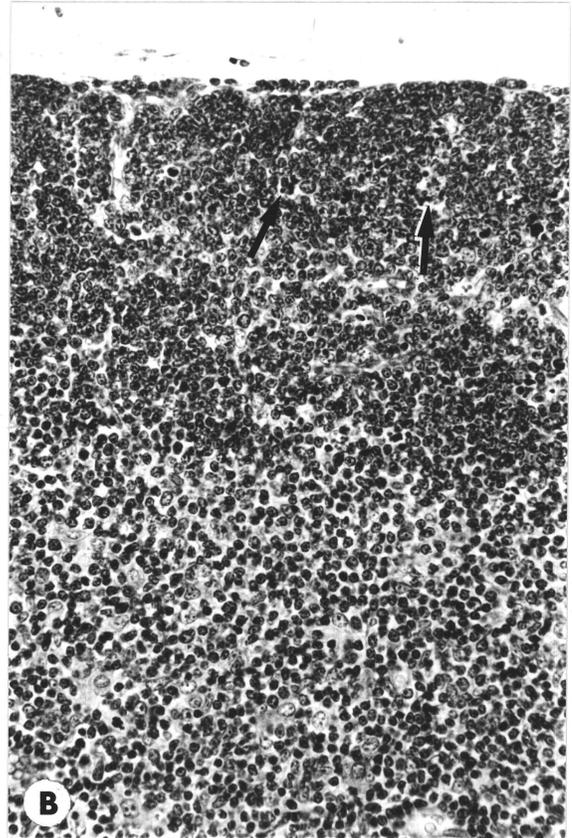
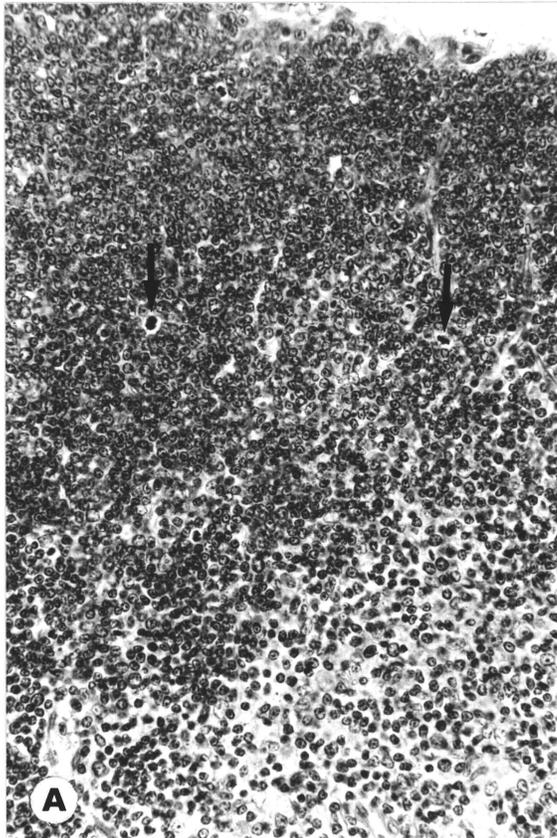


Fig. 14

Figure 15

Effect of a single intragastric dose of T-2 toxin  
on the germinal centres of lymphoid follicles of mice.

- A. Normal lymphoid follicle with germinal centre in the spleen of a control mouse.
  
- B. Depleted splenic germinal centre with karyorrhexis of lymphoblasts in a mouse treated 1 hour previously with T-2 toxin.
  
- C. Normal cortical zone of the mesenteric lymph node from a control mouse.
  
- D. Secondary follicle in the mesenteric lymph node of a mouse treated 4 hours previously with T-2 toxin.  
Note the karyorrhexis of nuclei of lymphoblasts and the aggregation of debris in reticular macrophages (arrow)

Hematoxylin and eosin, x 300.

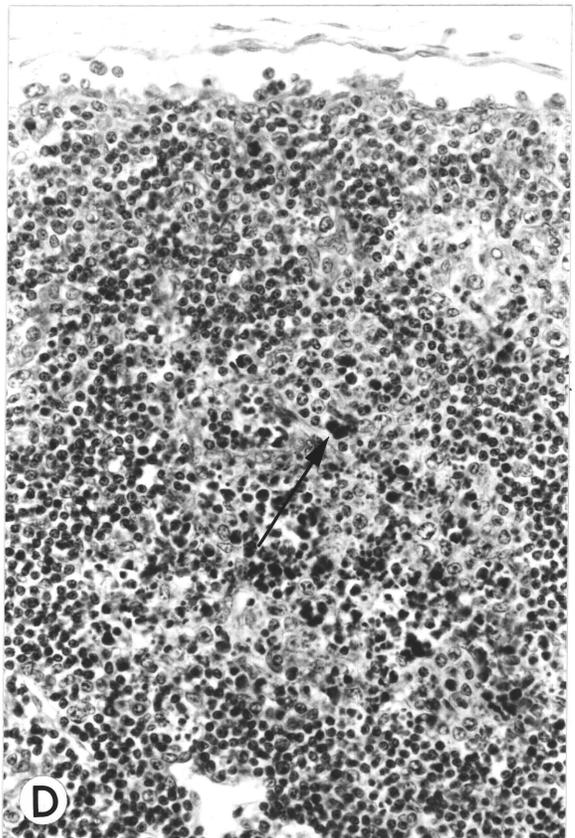
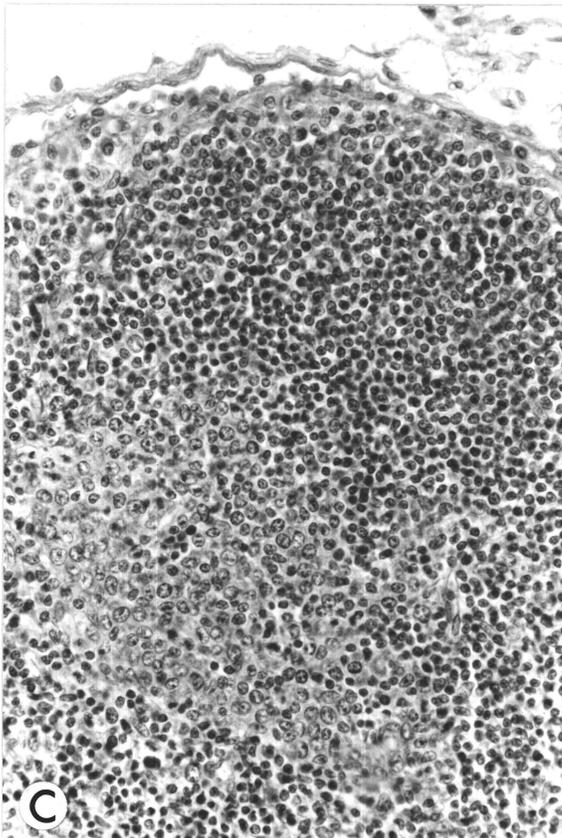
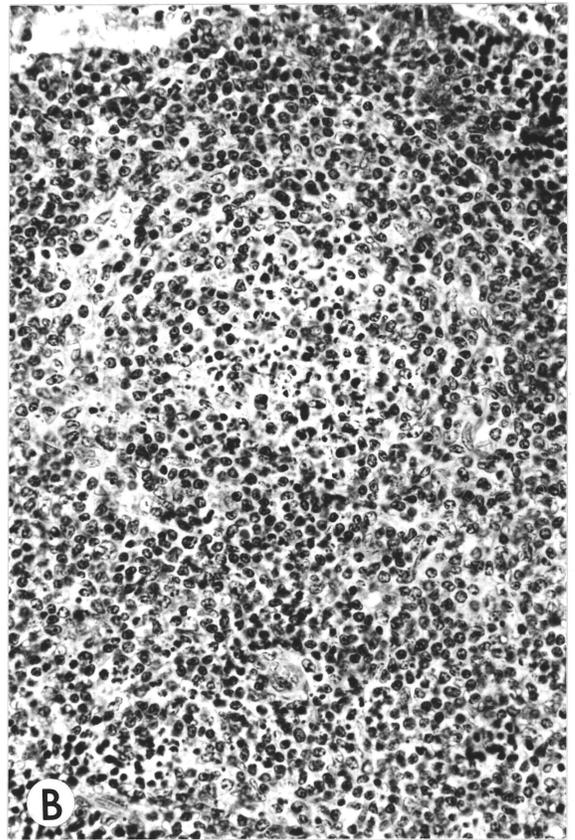
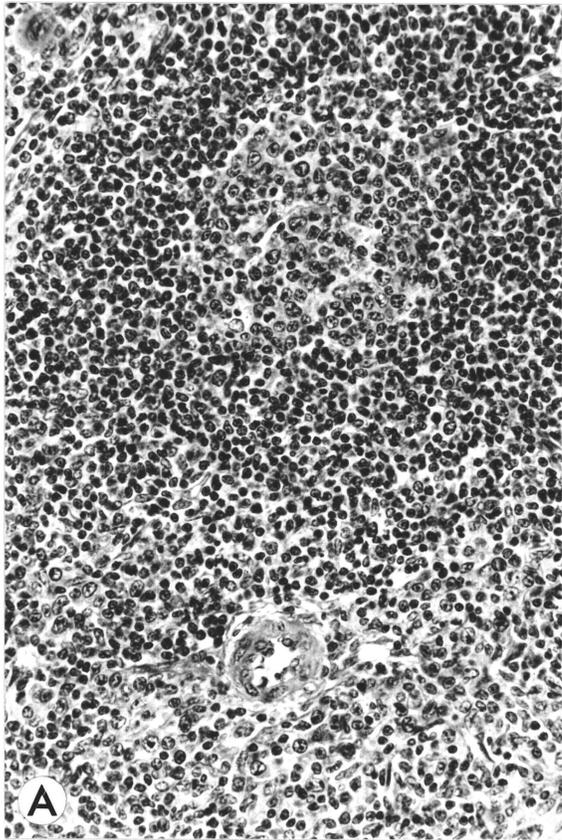


Fig. 15

Figure 16

Sequential changes in splenic red pulp of mice after receiving a single intragastric dose of T-2 toxin (2.5 mg/kg).

- A. Normal red pulp of a control mouse. Maturation of megakaryocytes (1), granulocytes (2), and erythrocytes (3) is normal.
  
- B. Appearance of red pulp 6 hours after T-2 toxin. Small fragments of karyorrhectic nuclei are present (arrows), and mitotic figures are absent.
  
- C. Atrophic splenic red pulp 24 hours after T-2 toxin. Few mature erythroid or myeloid stages are present, but mitotic activity (arrow) and large undifferentiated blast cells (arrowhead) are evident.
  
- D. Mitotic activity of megakaryocytes (1) in the atrophic red pulp 48 hours after treatment with T-2 toxin.
  
- E. Granulopoiesis (arrows) in red pulp 48 hours after treatment. Note the predominance of immature stages (arrowhead).
  
- F. Regeneration of hematopoietic tissue in the splenic red pulp 96 hours after treatment of T-2 toxin. Thrombopoiesis (1), myelopoiesis (2), and erythropoiesis (3) are evident, but immature blast cells are still present in large numbers (arrow).

Hematoxylin and eosin, x 300.

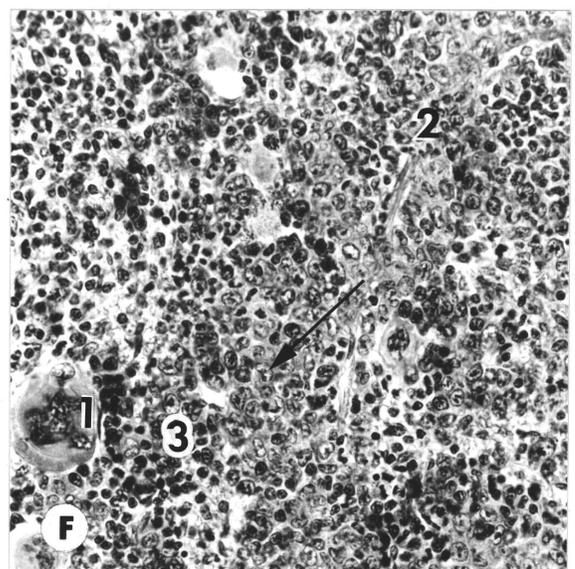
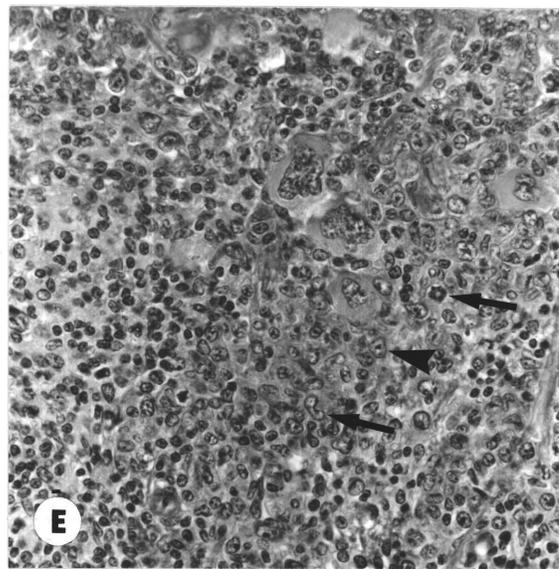
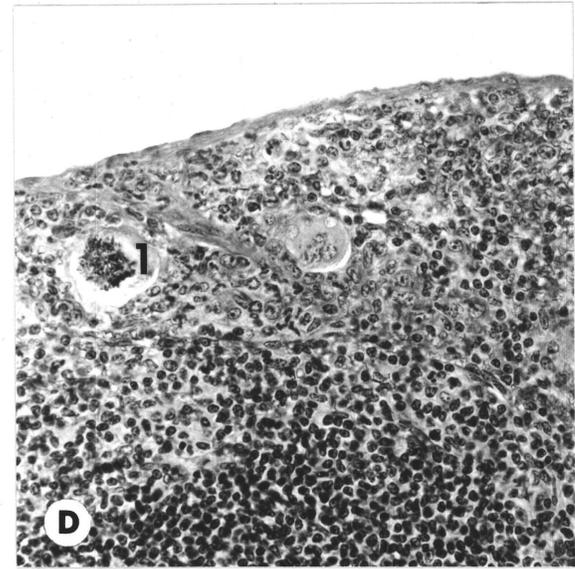
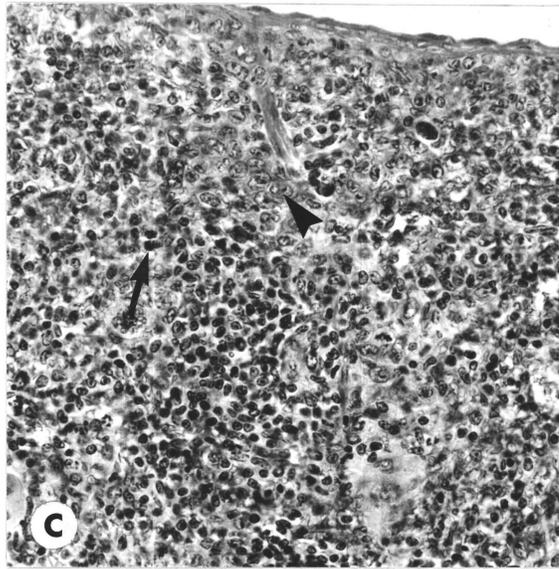
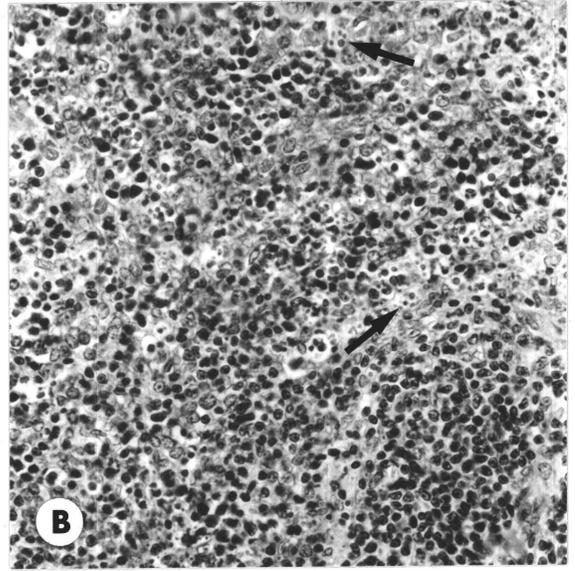
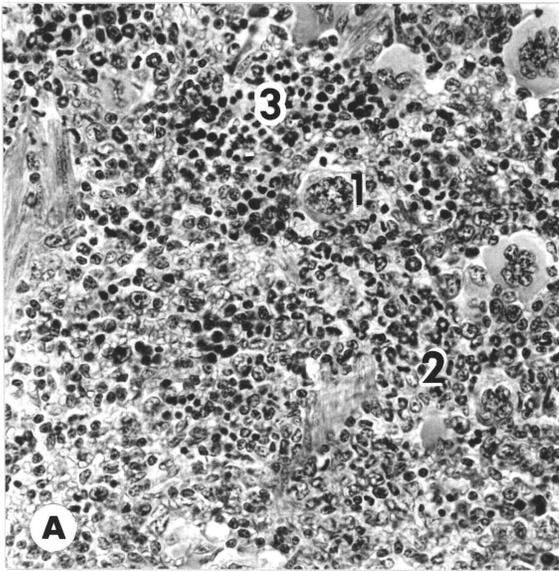


Fig. 16

Bone marrow: Fragmentation of nuclei was not visible in untreated mice (Fig. 17A), but steadily increased in severity in treated mice until 24 hours, at which stage the bone marrow was usually totally destroyed (Fig. 17B). Disruption was severe enough to cause hemorrhage into the medullary cavity in some regions. The sequential change in the severity of marrow damage is illustrated in Fig. 18. By 96 hours, when necrotic cells were infrequent, regenerative activity was evident.

Other tissues: No lesions were observed in the testis, kidney, adrenal gland, bladder, heart, lung or skeletal muscle. Pyknotic nuclei were occasionally found in epithelial cells of pancreatic acini and in the germinal regions of large hair follicles of the muzzle. The liver appeared normal during the early stages after treatment, but later, small hematopoietic colonies appeared in the sinusoids, especially in mice examined at 96 hours.

#### 6.4.2 Dose-related changes

The degree of destructive injury in intestinal crypts increased significantly with dose (Fig. 19). There was wide variation in numbers of phagosomes per crypt in any one mouse and also among the average counts from different mice (Table 24). Scores for severity of karyorrhexis in splenic follicles, splenic red pulp (Table 25), follicles in lymph nodes, and bone marrow were similarly dose-related. For each tissue, the lowest dose that caused recognizable injury in the majority of mice was 0.5 mg/kg.

Figure 17

Effect of a single intragastric dose of T-2 toxin  
(2.5 mg/kg) on bone marrow of mice.

A. Control.

B. Mouse treated 24 hours previously.  
There is widespread pyknosis and karyorrhexis of nuclei of  
hematopoietic cells. Sinusoids are dilated (arrow), but  
endothelial cells are necrotic and there is extensive  
hemorrhage into the bone marrow.

Hematoxylin and eosin, x 300.

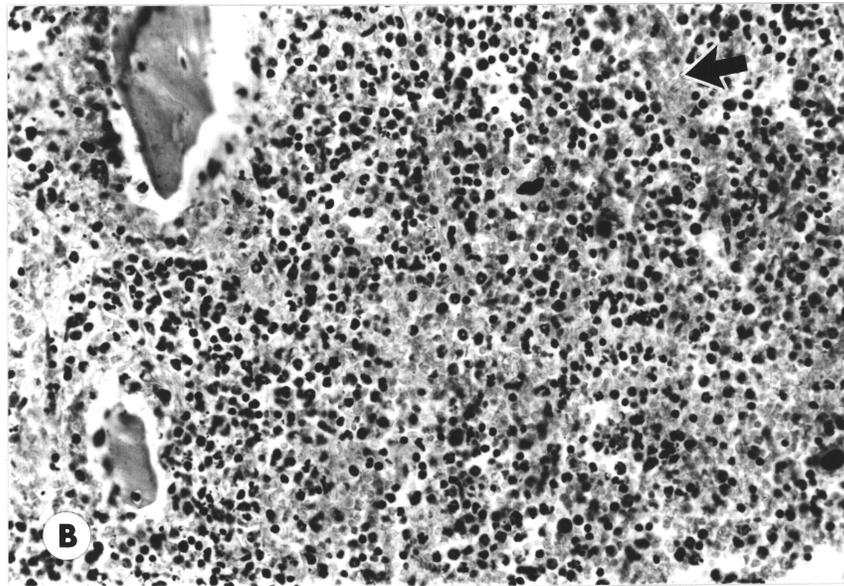
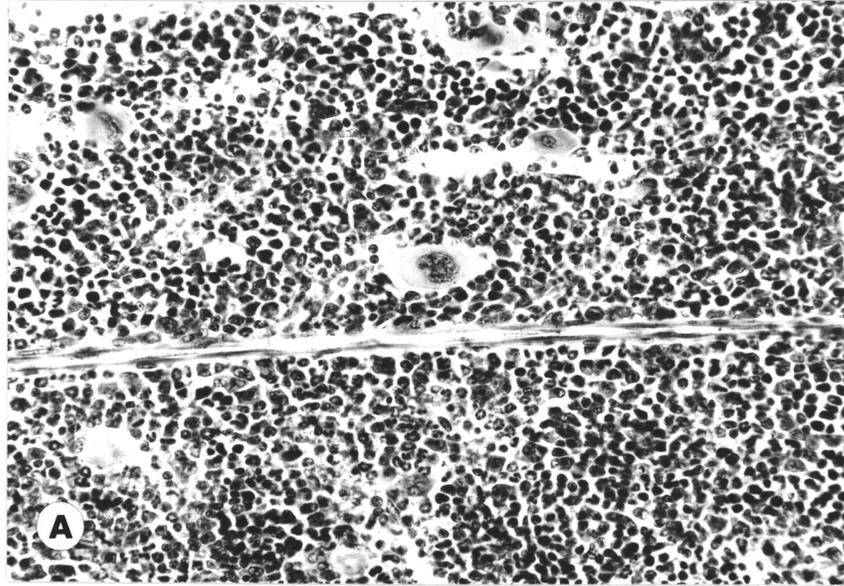


Fig. 17

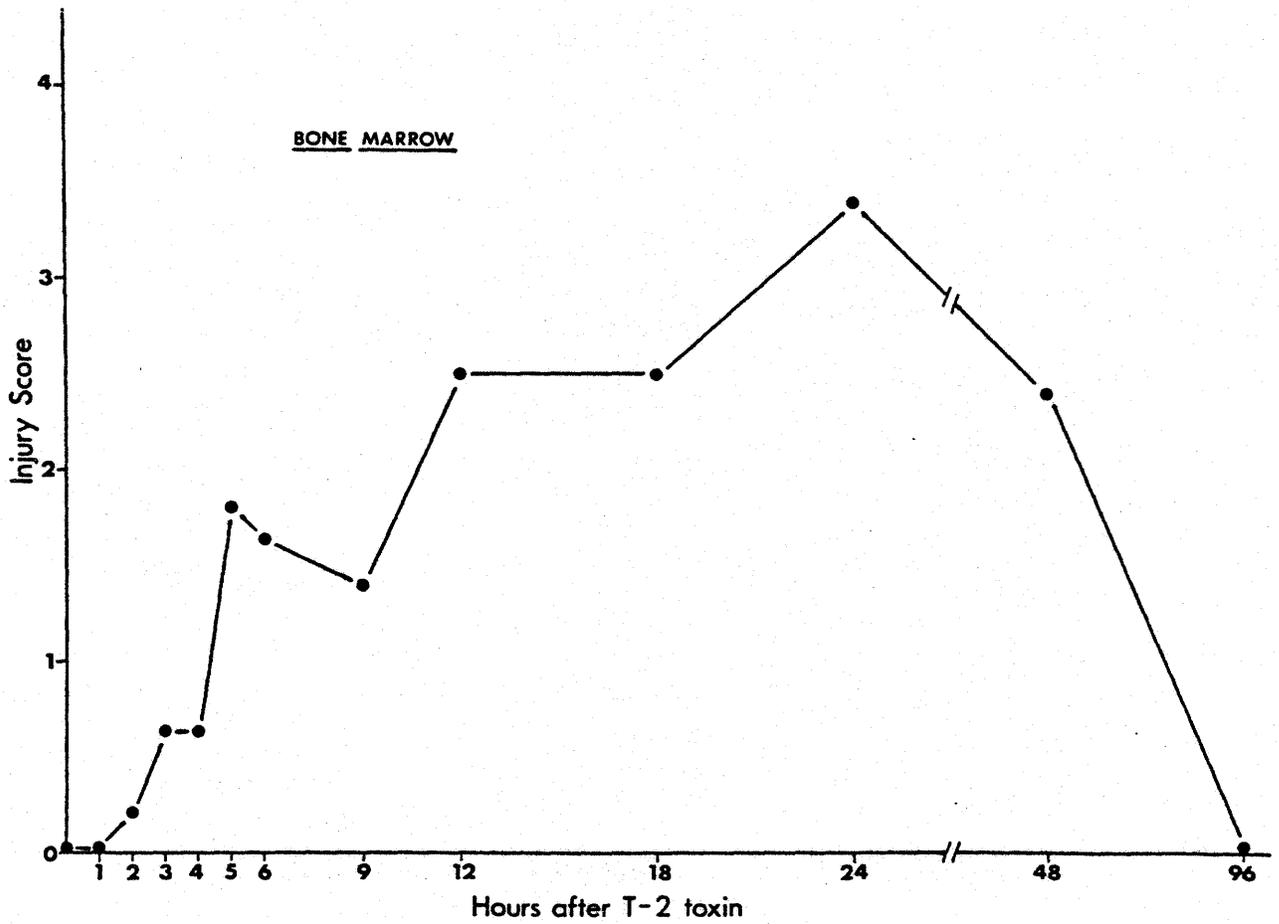


Fig . 18: Sequential change in the degree of necrosis visible in the bone marrow after treatment with T-2 toxin (2.5 mg/kg). Each point is the mean score of 5 mice examined at each time. Maximum possible score is 4.

Injury scores are ratings of injury on a scale of 0-4 of increasing severity, as described on page 137.

TABLE 24: Numbers of phagosomes in duodenal crypts of young Swiss mice 7 hours after receiving different doses of T-2 toxin.

Mouse	Dose (mg/kg)					
	0.125	0.25	0.5	1.0	2.0	4.0
1	0.3 $\pm$ 0.7 <sup>a</sup>	0.2 $\pm$ 0.5	7.6 $\pm$ 7.4	3.9 $\pm$ 4.6	19.6 $\pm$ 9.1	22.5 $\pm$ 10.6
2	0.0 $\pm$ 0.0	0.1 $\pm$ 0.2	1.6 $\pm$ 2.3	5.6 $\pm$ 5.8	25.7 $\pm$ 12.4	23.3 $\pm$ 8.0
3	0.0 $\pm$ 0.0	1.0 $\pm$ 2.3	5.3 $\pm$ 5.6	7.6 $\pm$ 8.4	21.6 $\pm$ 8.4	33.3 $\pm$ 8.9
4	0.1 $\pm$ 0.0	1.2 $\pm$ 1.8	2.4 $\pm$ 2.5	3.7 $\pm$ 3.6	21.3 $\pm$ 13.4	36.9 $\pm$ 6.4
5	0.0 $\pm$ 0.0	1.1 $\pm$ 2.8	3.5 $\pm$ 4.7	4.0 $\pm$ 3.8	26.1 $\pm$ 9.9	24.7 $\pm$ 5.6
6	0.9 $\pm$ 1.8	0.0 $\pm$ 0.0	1.0 $\pm$ 1.4	14.7 $\pm$ 9.4	17.6 $\pm$ 10.6	20.0 $\pm$ 10.1
7	0.1 $\pm$ 0.0	1.1 $\pm$ 2.5	6.0 $\pm$ 6.0	19.2 $\pm$ 6.9	35.3 $\pm$ 6.8	19.3 $\pm$ 8.9
8	0.6 $\pm$ 1.2	0.3 $\pm$ 0.6	2.2 $\pm$ 3.6	16.1 $\pm$ 12.3	16.8 $\pm$ 6.4	38.5 $\pm$ 3.7
9	0.0 $\pm$ 0.0	1.7 $\pm$ 2.9	0.3 $\pm$ 0.6	3.5 $\pm$ 3.0	26.8 $\pm$ 9.2	40.0 $\pm$ 0.0
10	0.0 $\pm$ 0.0	0.1 $\pm$ 0.4	0.2 $\pm$ 0.6	12.6 $\pm$ 6.9	27.3 $\pm$ 10.2	16.5 $\pm$ 5.8
Mean no. per crypt $\pm$ SD	0.2 $\pm$ 0.3 <sup>b</sup>	0.7 $\pm$ 0.6	3.0 $\pm$ 2.5	9.1 $\pm$ 6.0	23.8 $\pm$ 5.6	27.5 $\pm$ 8.8

<sup>a</sup> Values are mean numbers of phagosomes per crypt ( $\pm$ SD) where N = 20 crypts sampled over 3 blocks. Counts greater than 40 were listed as 40.

<sup>b</sup> Mean of the 10 means in the column above.

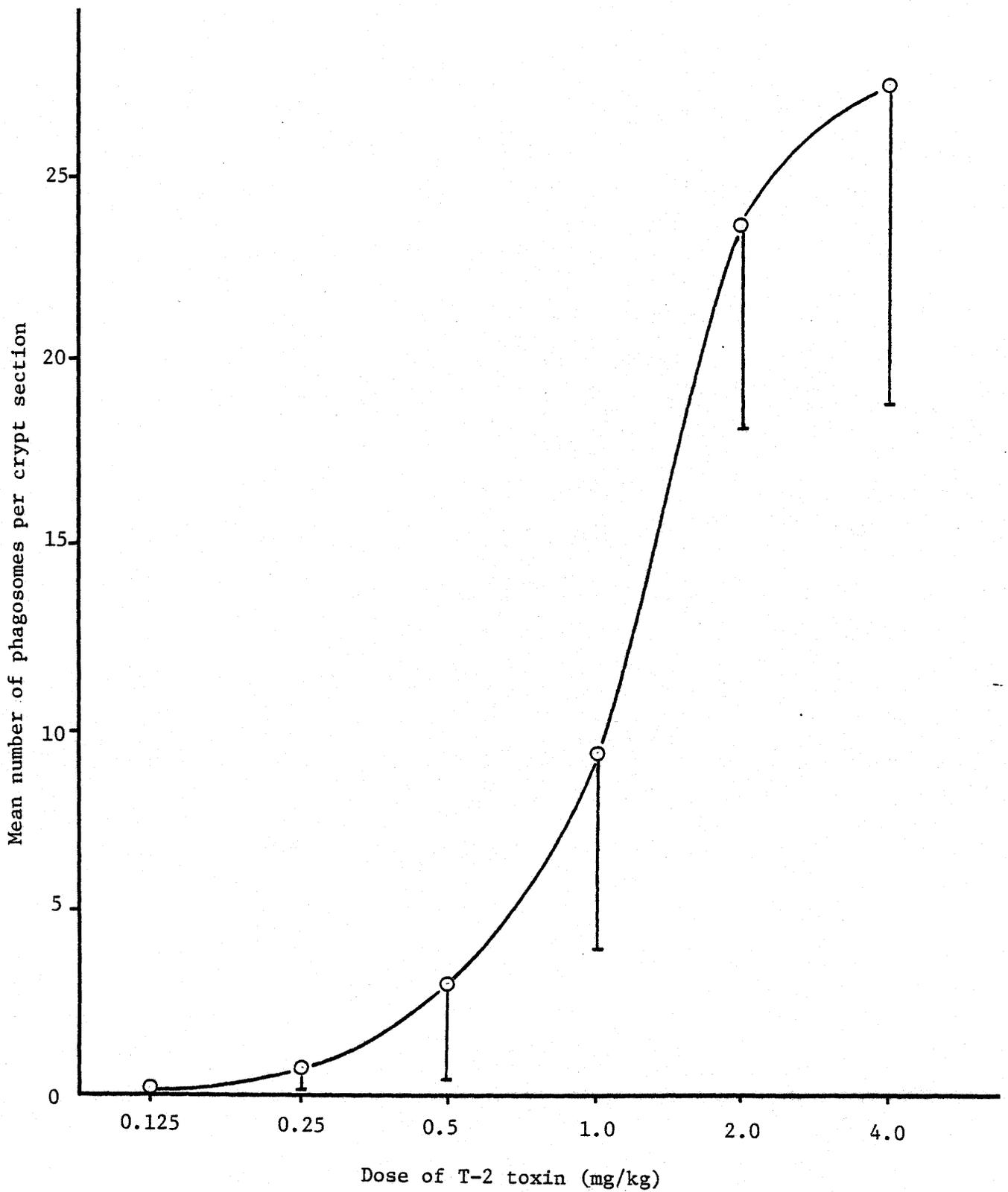


Fig. 19. Relationship between dose of T-2 toxin and acute necrosis in duodenal crypts of mice. Each point is the mean ( $\pm$ SD) of counts from ten mice. Counts were made in mice killed 7 hours after intragastric administration of T-2 toxin.

TABLE 25: Mean scores of karyorrhexis in the splenic red pulp and germinal centres of follicles of young Swiss mice 7 hours after being given graded doses of T-2 toxin.

	Dose (mg/kg)					
	0.125	0.25	0.5	1.0	2.0	4.0
Splenic follicles - karyorrhexis	0.1 <sup>a</sup>	0.3	0.4	1.5	2.1	3.4
Red pulp - karyorrhexis	0.1	0.4	0.5	1.7	2.3	3.5

<sup>a</sup> Values are means of scores (0-4) from groups of 10 mice.

## 6.5 Discussion

The distribution of lesions observed in tissues of mice exposed to a single sublethal oral dose of T-2 toxin resembled that occurring after exposure to ionizing radiation (Brecher et al., 1948; Montagna and Wilson, 1955) or to high doses of various anti-neoplastic chemicals, including alkylating agents (Graef et al., 1948), methotrexate (Trier, 1962), hydroxyurea (Philips et al., 1967), cytosine arabinoside (Lieberman et al., 1970; Verbin et al., 1973), or colchicine (Dinsdale, 1975). Thus, the acute lesions caused by T-2 toxin are non-specific. However, because most other common mycotoxins do not cause such radiomimetic lesions, this pattern of injury could be used as a means of tentatively distinguishing trichothecenes from other mycotoxins.

Similar distributions of lesions have been described in various experimental animals given single doses of various trichothecene mycotoxins, namely nivalenol in mice (Saito et al., 1969); fusarenon-X in mice (Saito and Okubo, 1970); neosolaniol in mice (Ishii et al., 1971; Ueno et al., 1972a); fusarenon-X in rats, guinea-pigs, cats and ducklings (Ueno et al., 1971b); and T-2 toxin in mice (Ueno et al., 1972a), cats (Ueno et al., 1973a; Sato et al., 1975), guinea-pigs (DeNicola et al., 1978), and swine (Weaver et al., 1978c). In most of these reports, the pathogenesis of the lesions was not determined; microscopic changes were usually described in moribund or dying animals that had been given lethal doses of mycotoxin. Saito and Okubo (1970) reported sequential changes in mice at 6, 12 and 24 hours after doses of fusarenon, fusarenon-X, nivalenol and crude Fusarium extracts, but these investigators did not describe the lesions in

detail. Thus, the observations in the present experiment provide a description of the early histological changes in acute sublethal T-2 toxicosis which is not available elsewhere.

Several morphological observations in this experiment provided important information on the acute effects of T-2 toxin in vivo. Of particular interest were the severe effects on the lymphoid system. The rapid onset within one hour of lympholysis in the intestinal epithelium, intestinal lamina propria, the Peyer's patches, and the germinal-centres of follicles illustrated that several populations of lymphocytes are exquisitely sensitive to this toxin at dose levels well below the median lethal dose. Thymic cortical lymphocytes were also extremely sensitive, but this population was not destroyed until 9 hours after exposure. Because regeneration of all damaged lymphoid tissues quickly followed the appearance of lesions, it is unlikely that single-dose exposure to T-2 toxin would cause a lasting impairment of immune function. This interpretation is consistent with the recent observations of Rosenstein et al. (1979) and of Lafarge-Frayssinet et al. (1979), who described transient depressions of both T- and B-cell functions after single-dose exposure to sublethal doses of T-2 toxin and diacetoxyscirpenol. Rosenstein et al. (1979) also observed dose-dependent thymic atrophy in mice treated with single doses of T-2 toxin, diacetoxyscirpenol, or crude extracts of Fusarium poae. The observation of regeneration in the thymic cortex 4 days after treatment of mice with T-2 toxin in the present experiment correlates well with the reported recovery of T-cell immune function after exposure to T-2 toxin (Rosenstein et al., 1979). These observations indicate that T-2 toxin and other

trichothecenes are potently toxic to the immune system, but that problems in immune function are more likely to be the result of continuous exposure to trichothecene mycotoxins, rather than as a result of single dose or short-term exposure.

The functional significance of the effects of T-2 toxin on the mature lymphoid cells in the intestinal epithelium (theliolymphocytes), in the intestinal lamina propria, and in the dome regions of Peyer's patches, is unknown. These various populations of lymphocytes are generally considered to be non-germinal, differentiated lymphocytes (Ferguson, 1977; Abe and Ito, 1978), although they are capable of division. The reason for their susceptibility to T-2 toxin is difficult to explain; most other susceptible cells are in S phase of the cell cycle, whereas many of these intestinal lymphocytes are not. It is possible that this effect could result in impaired local intestinal immune function in animals exposed continuously to trichothecenes. Intestinal lympholysis has not previously been recognized as an effect of T-2 toxin, probably because most of the cellular fragments disappear within hours of formation, and are difficult to visualize when fixation is less than ideal. Intestinal effects during the early stages of sublethal trichothecene toxicosis have not received detailed examination (Saito and Okubo, 1970), so this effect may have been overlooked. The effects of T-2 toxin on intestinal epithelial and lymphoid cells is the subject of ultrastructural examination and further discussion in the following chapter (Section 7).

The effects of T-2 toxin on the intestinal epithelium were also recognizable within a few hours of treatment. The earliest change was

the disappearance of mitotic figures, indicating that T-2 toxin prevents germinal cells from entering mitosis by arresting the cell cycle. Karyorrhexis, with phagocytosis of debris by neighboring healthy cells, occurred in germinal zones of intestinal crypts within 3 hours. The severity of this early injury to intestinal crypts by T-2 toxin as determined by the number of phagosomes was dose-related, but varied among different locations in the intestine, and among different mice. The pathogenesis of changes in injured crypts was also variable. In mildly affected mice, mitotic activity resumed within 12 hours, phagosomes rapidly disappeared within 18 hours, and the villous and crypt architecture was not altered. In others in which crypt damage was more severe, a central cast of cell debris formed in the lumen of the crypt, and this persisted beyond 24 hours. In these mice, resumption of mitotic activity was delayed, but when crypts did regenerate, they did so in an exaggerated and disorganized manner such that villi were atrophic at 48 and 96 hours after treatment.

The rate at which intestinal crypts regenerate after damage by a single, sublethal dose of an antineoplastic agent might reflect the biochemical mechanism by which the agent injures the susceptible cells. Antineoplastic drugs such as cytosine arabinoside (Verbin et al., 1973) and hydroxyurea (Philips et al., 1967) cause mild, transient intestinal injury after which mitotic activity returns to normal within 12 hours. This pattern of injury has been explained by the temporary, readily reversible inhibition of DNA synthesis by these two agents in the susceptible cells (Verbin et al., 1973). By comparison, other drugs such as actinomycin D and methotrexate interfere with several biosynthetic

processes, and cause a more prolonged impairment in DNA synthesis. This prolonged effect is considered responsible for the longer-lasting cytotoxic effects and mucosal atrophy observed in animals treated with these compounds (Philips et al., 1975). Ionizing radiation and alkylating agents may cause permanent damage to some germinal cells (Trainor and Morley, 1976) resulting in atypical growth and division, and a prolonged period of regeneration. However, in the present study, injured crypts regenerated rapidly in some mice, whereas in others, regeneration was prolonged. Thus, the same agent caused both transient and delayed patterns of injury, with the latter apparently a consequence of the most severe injury. If the rate of regeneration does reflect the biochemical lesion, then T-2 toxin might have more than one mechanism of action on crypt cells. Alternatively, the rate of regeneration may depend on the degree of damage, and the different responses to T-2 toxin in the present study may have occurred because of wide variation in susceptibility among the mice used.

The transient leukocytosis observed within several hours of treatment with T-2 toxin was similar to the observation of Sato et al. (1978), who found elevations as soon as 1 hour and peaks at 6 hours after administration of fusarenon-X, neosolaniol, or T-2 toxin to mice. These authors speculated that this elevation was due to inflammatory reactions induced by the irritant trichothecenes, but did not perform differential counts of circulating leukocytes. In the present study, differential counts of leukocytes 4 hours after treatment revealed neutrophilia, correlating with appearance of neutrophils in the lamina propria of the damaged intestine. Thus, the neutrophilia could be part of an inflammatory

response. However, lymphocyte counts were greatly elevated within 1 hour of treatment, and remained elevated for 6 hours, with a peak at 4 hours. Lymphocytosis at this stage could not be a result of the stress of treatment, as suggested by Sato et al. (1978), because cortisone causes lymphopenia within 4 hours (Schalm et al., 1975). The observed lymphocytosis may have been due to the cytotoxic effects of T-2 toxin on lymphoid tissues. However, other agents such as the alkylating agents, which are similarly toxic to lymphoid tissues, cause lymphopenia within a few hours of treatment. At present, the mechanism and functional significance of the lymphocytosis induced by T-2 toxin is unknown.

Hematopoietic populations in the spleen and bone marrow also exhibited necrosis within several hours of treatment with T-2 toxin. Numbers of circulating reticulocytes and granulocytes at 48 hours were below control values. At this stage, cells in depleted bone marrow and splenic red pulp had begun to proliferate, and colonies of immature precursors of myeloid, megakaryocytic, and erythroid cells were visible. Differentiation of megakaryocytes and segmented granulocytes was evident by 96 hours, but, at this stage, regenerating erythroid cells were still immature. These regenerating hematopoietic foci in the spleen resembled those described in mice after single exposure to radiation or to cytotoxic drugs (Curry and Trentin, 1967; Santos and Haghshenass, 1968). At 96 hours, extra-medullary hematopoietic foci were evident in the hepatic sinusoids, further demonstrating the activity of the recovery phase. These observations indicate that single dose exposure to T-2 toxin appears unlikely to cause a lasting depression of hematopoiesis.

## 7.0 ULTRASTRUCTURAL CHANGES INDUCED BY T-2 TOXIN IN THE SMALL INTESTINE AND SPLEEN OF MICE

### 7.1 Abstract

Ten young male Swiss mice (weighing approximately 16-18 g) were given T-2 toxin orally in a propylene glycol/water vehicle at a rate of 2 mg/kg. Six control mice received the vehicle alone. Samples of duodenum, jejunum and spleen were obtained at either 90 or 180 minutes after treatment. In mice treated with T-2 toxin, mitotic activity in intestinal crypts and lymphoid follicles had ceased within 90 minutes. Epithelial cells at the base of intestinal crypts, plasma cells and lymphocytes of the lamina propria, lymphoblasts of the splenic follicles and rubriblasts in the red pulp, all exhibited similar changes. By 90 minutes nuclear pyknosis and karyorrhexis occurred in these populations. Fragments of cytoplasm or nucleus were present in phagosomes in surviving epithelial cells and in macrophages. By 180 minutes, phagosomes had increased in size and number and many displayed various degrees of lysosomal degradation.

The ultrastructural changes in susceptible cells of the small intestine and the spleen were similar to those reported to occur in animals treated with antineoplastic chemotherapeutic agents and ionizing radiation. T-2 toxin probably does not induce fragmentation of cells solely by inhibition of polypeptide synthesis, because other inhibitors of polypeptide synthesis prevent this type of injury.

## 7.2 Introduction

Various trichothecene mycotoxins, including T-2 toxin, are acutely toxic to germinal cells of the intestinal mucosa, lymphoid follicles, and bone marrow (Saito et al., 1969; Saito and Okubo, 1970; Bamburg and Strong, 1971; Ueno et al., 1971a; 1972b; Sato et al., 1975; Lutsky et al., 1978; DeNicola et al., 1978). The resulting pattern of injury is described as "radiomimetic" (Saito et al., 1969; Ueno et al., 1972a) because lesions are similar to those caused by ionizing radiation.

The pathogenesis of lesions in acute trichothecene toxicosis is unknown because the microscopic changes during the first 6 hours after treatment have not been defined, except for the studies on acute T-2 toxicosis of mice in the previous experiment (Section 6). Because cell damage was evident in lymphoid tissues and in the intestinal mucosa as early as 1 hour after intragastric administration of T-2 toxin, the cytopathological events in these tissues during the first 3 hours after treatment were examined by electron microscopy.

## 7.3 Materials and methods

Sixteen young male albino Swiss mice weighing approximately 20 g (Animal Resources Centre, University of Saskatchewan) were housed in stainless-steel cages with tap water ad lib. Food was withdrawn 6 to 8 hours before treatment. Mice were lightly anesthetized with ether and given 0.1 ml/20 g of solution of T-2 toxin (0.4 mg/ml) in propylene glycol/water (1:1; v/v). The dose rate of T-2 toxin was 2 mg/kg. Control mice received similar quantities of the propylene glycol/water vehicle without T-2 toxin. Solutions were administered by gastric intubation.

Mice were killed by cervical dislocation and exsanguination either at 90 minutes or at 180 minutes after dosage, according to the schedule in Table 26. Samples (approximately 1 mm<sup>3</sup>) of duodenum, jejunum and spleen from 6 mice were fixed by immersion for 60 minutes in 2% osmium tetroxide buffered in 1.25% sodium bicarbonate adjusted to pH 7.4 with 0.1 M hydrochloric acid. Samples of duodenum and jejunum from the other 10 mice were opened, attached to wooden tongue depressors with the mucosal surface exposed, and immersed in 5% glutaraldehyde in 0.2 M s-collidine buffer (730 mOsm). After 30 minutes, tissues were trimmed into square pieces (approximately 1 mm<sup>2</sup>), returned to glutaraldehyde for a further 3½ hours, and stored overnight in s-collidine buffer at 4°C. These samples were secondarily fixed in 2% osmium tetroxide as described above.

Fixed tissues were dehydrated in graded ethanol solutions, cleared in propylene oxide, and embedded in Epon. Sections for light microscopy (1 µm) were stained with 1% toluidine blue for 3-5 minutes at 60°C. Ultrathin sections (silver-gold) were cut on glass or diamond knives on a Porter-Blum ultramicrotome (MT-2, I. Sorvall Inc., Newtown, CT.), collected on 300 mesh uncoated copper grids, stained in uranyl acetate and lead citrate, and examined on an Hitachi HS8 electron microscope at 50 KV.

#### 7.4 Results

In control mice, the fine structures of epithelial cells of crypts, interstitial cells of the lamina propria, and lymphoid and hematopoietic cells of the spleen, were similar to published descriptions (Jordan, 1967; Toner, 1968; Cheng and Leblond, 1974).

TABLE 26: Design of Experiment, Section 7.

Minutes after treatment	90		180	
Method of fixation	Osmium <sup>a</sup>	Glutaraldehyde <sup>b</sup>	Osmium <sup>a</sup>	Glutaraldehyde <sup>b</sup>
Number of mice given T-2 toxin (2mg/kg)	2	3	2	3
Number of mice given vehicle	1	2	1	2

<sup>a</sup> OsO<sub>4</sub> 2% in 1.25% sodium bicarbonate (pH = 7.4) for 60 minutes.

<sup>b</sup> Glutaraldehyde 5% in 0.2 M s-collidine buffer (730 mOsm) for 4 hours, then into osmium as above.

#### 7.4.1 Light microscopy

Observations at 90 minutes: Numerous pyknotic and karyorrhectic nuclei were visible in the lamina propria of the villi between the crypts of both the duodenum and the jejunum of treated mice (Fig. 20C), but not in controls (Fig. 20A; B). Some crypt epithelial cells were swollen, giving crypts a bulb-like conformation. Mitotic figures which were common in controls (Fig. 21A), were infrequently found in treated mice (Fig. 21B). In the spleen, pyknosis and karyorrhexis of nuclei of germinal cells of lymphoid follicles were more pronounced in treated mice; however, some tingible bodies were occasionally found in macrophages in follicles in control mice.

Observations at 180 minutes: In treated mice, pyknotic nuclei were observed in the intestinal lamina propria and splenic follicles. In the intestinal crypts, mitoses were infrequent in comparison with crypts of control mice. Numerous round bodies of variable size and intensity of staining were present within the cytoplasm of epithelial cells in some crypts. Most bodies were located towards the apical pole and were often multiple within any one cell (Fig. 21C). Smaller bodies were approximately 1  $\mu$ m in diameter and homogeneously stained dark blue, whereas larger bodies were up to 6  $\mu$ m in diameter and some of these contained irregular densely stained clumps (Fig. 21C). In the spleen, pyknosis and karyorrhexis were frequently observed in hematopoietic populations of the red pulp at 180 minutes.

#### 7.4.2 Electron microscopy

Observations at 90 minutes: In the lamina propria, nuclei of plasma cells and lymphocytes were pyknotic with crescent-shaped clumping of chromatin against the nuclear envelope (Fig. 22). Disruption of the nuclear envelope, and fragmentation of nuclei (karyorrhexis)

Figure 20

Light-microscopic changes in duodenal villi 90 minutes after intragastric administration of T-2 toxin (2 mg/kg).

- A. Control, x 300.
- B. Control, x 750.
- C. T-2 toxin-treated, x 300.
- D. T-2 toxin-treated, x 1200.

Note the pyknosis of mononuclear cells (long arrows) within the lamina propria of C and D.

Some karyorrhectic fragments have been phagocytosed by macrophages (asterisk).

Note the pyknosis of some theliolymphocytes (short arrows).

Code for identification of cells:

- 1. Lymphocytes
- 2. Theliolymphocytes (some are pyknotic in C)
- 3. Plasma cells
- 4. Granulocytes
- 5. Globule leukocyte
- 6. Macrophages
- 7. Epithelial cells

Toluidine blue; 1 micron, Epon-embedded sections.

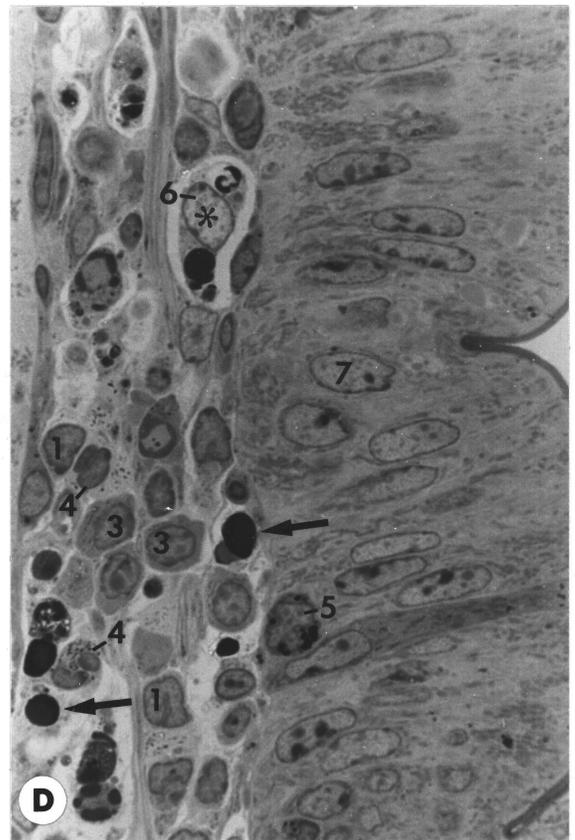
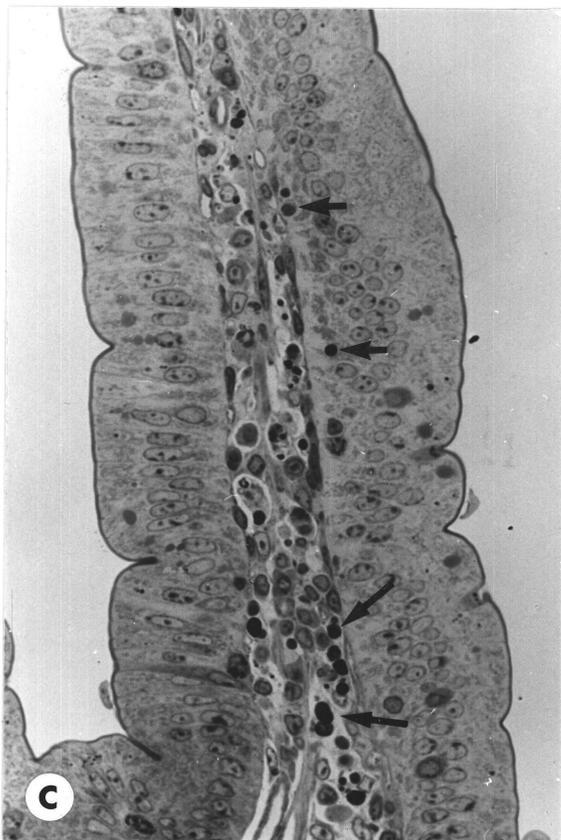
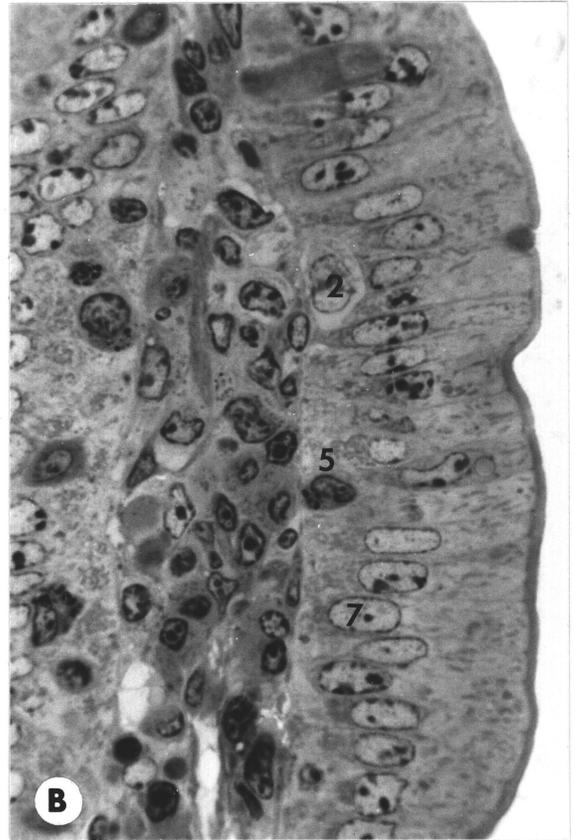
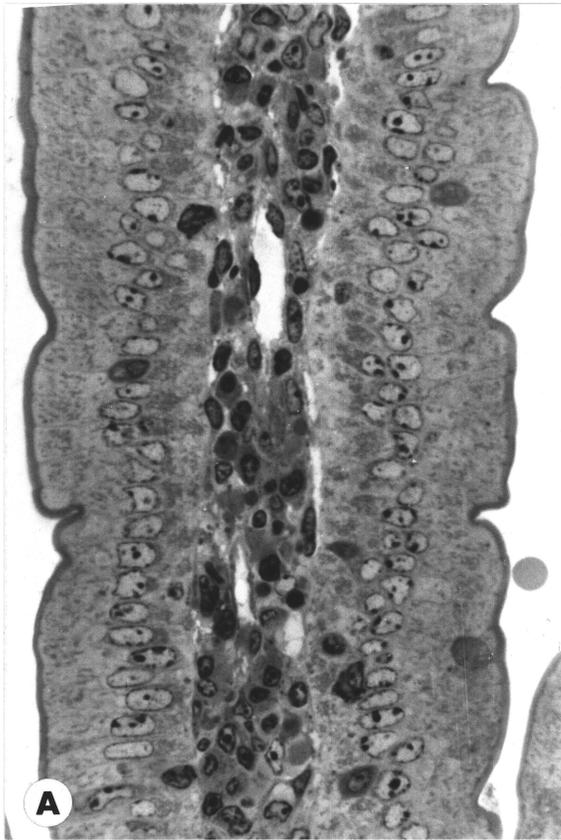


Fig. 20

Figure 21

Light-microscopic changes in duodenal crypts 180 minutes after intragastric administration of T-2 toxin (2 mg/kg).

- A. Control, x 300.  
Note the mitotic figures (arrows).
- B. T-2 toxin-treated, x 300.  
Mitotic activity is no longer visible.  
Many dense round bodies of various sizes are present in the epithelial cells at the base of crypts (arrows).
- C. T-2 toxin-treated, x 1200.  
Large numbers of intracellular inclusions are illustrated.  
Note the variable structure and density of staining.  
Some are less than 1  $\mu$ m and are weakly stained (short arrow), whereas larger bodies (long arrows) contain more densely stained material.  
Note the pyknosis (P) and karyorrhexis (K) of cells in the lamina propria.

Toluidine blue; 1 micron, Epon-embedded sections.

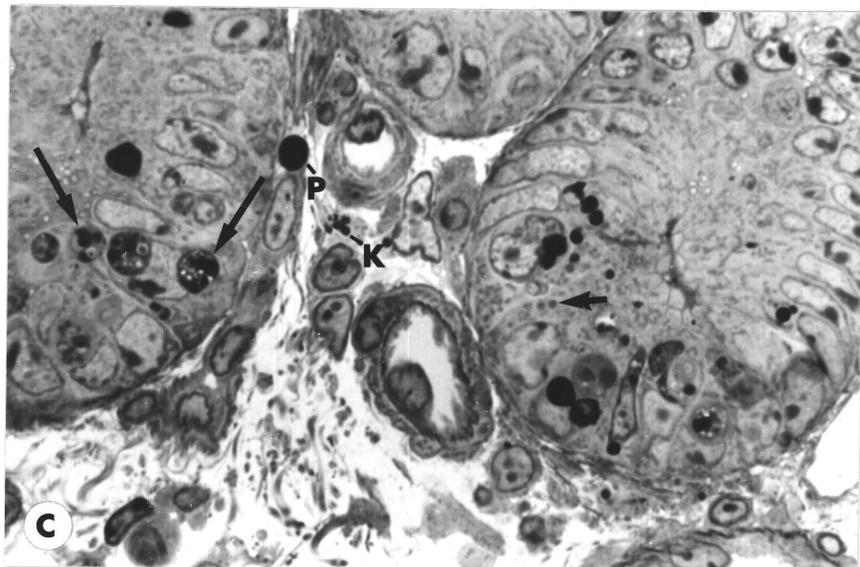
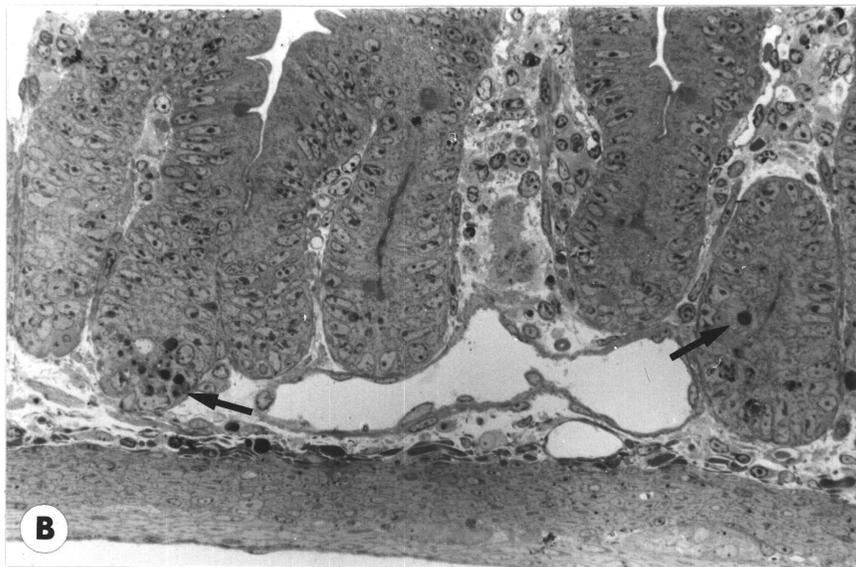
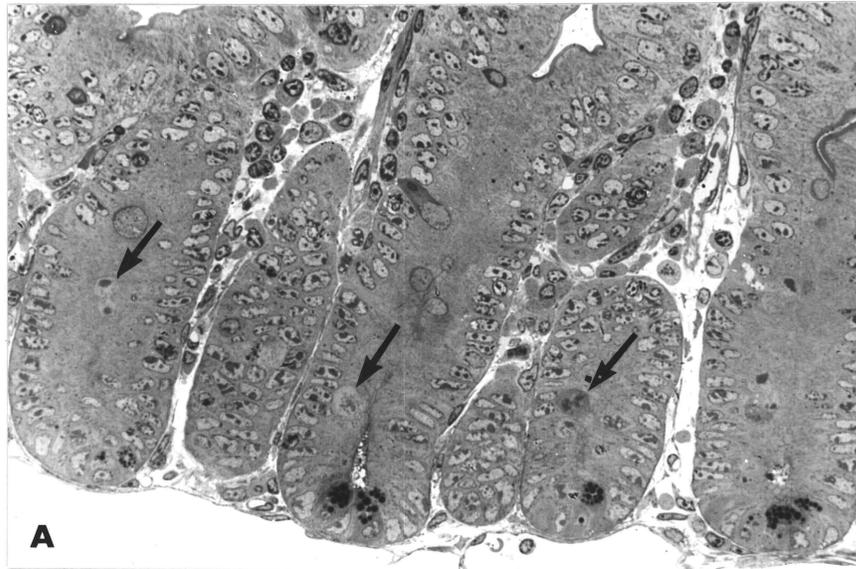


Fig. 21

were also observed. Small nuclear fragments were sometimes present within membrane-bound phagosomes in macrophages (Fig. 23 ). Round, dense fragments of cells, composed of dilated cisternae of rough endoplasmic reticulum and apparently derived from karyorrhectic plasma cells, were also found in phagosomes of some interstitial cells (Fig. 23). Newly formed phagosomes in macrophages were readily distinguishable from secondary lysosomes on the basis of double membrane enclosure and absence of lysosomal degradation (Fig. 23). Some interstitial cells exhibiting karyorrhexis had minimal amounts of cytoplasm and were identified as lymphocytes on this basis.

In the epithelium of the intestinal crypts, swelling of the interdigitating processes between the epithelial cells was a frequent early change (Fig. 24). Such processes may have been cytoplasmic extensions of intraepithelial lymphocytes, but this could not be established. Small, round, intracytoplasmic inclusions surrounded by double membranes, and containing cytoplasmic components or, occasionally, nuclear fragments, were present in undifferentiated epithelial cells at the base of the crypts (Fig. 25). Chromatin in nuclear fragments was clumped into a crescent against the nuclear membrane, but cytoplasmic components within inclusions were similar in structure to the cytoplasm of surrounding cells.

In the spleen, karyorrhectic fragments of nuclei were found in phagosomes of macrophages in the germinal centres of follicles, and in the red pulp (Fig. 26 ). Nuclear fragments exhibited similar crescent-shaped clumps of chromatin, and some had complete disruption of the nuclear envelope (Fig. 26). Smaller membrane-bound inclusions

Figure 22

Plasma cell in the lamina propria in a duodenal villus of a mouse treated 90 minutes previously with T-2 toxin (2 mg/kg).

Note the crescent-shaped clumping of chromatin (C) against the nuclear envelope. This nucleus is in the early stages of pyknosis.

Osmium fixed, x 17000.

Figure 23

Lamina propria of a duodenal villus in a mouse treated 90 minutes previously with T-2 toxin (2 mg/kg).

Various stages of pyknosis and karyorrhexis of lymphocytes and plasma cells are illustrated.

- M. Macrophage.
- L1. Lymphocytes with crescents of pyknotic nuclear chromatin. Note the rupture of the nuclear membrane (arrow).
- L2. Phagocytosed pyknotic fragment of a nucleus.
- P. Phagosomes containing dense cell fragments that are composed of dilated cisternae of rough endoplasmic reticulum; these are probably fragments of plasma cells.

Osmium fixed, x 5700.

Fig. 22

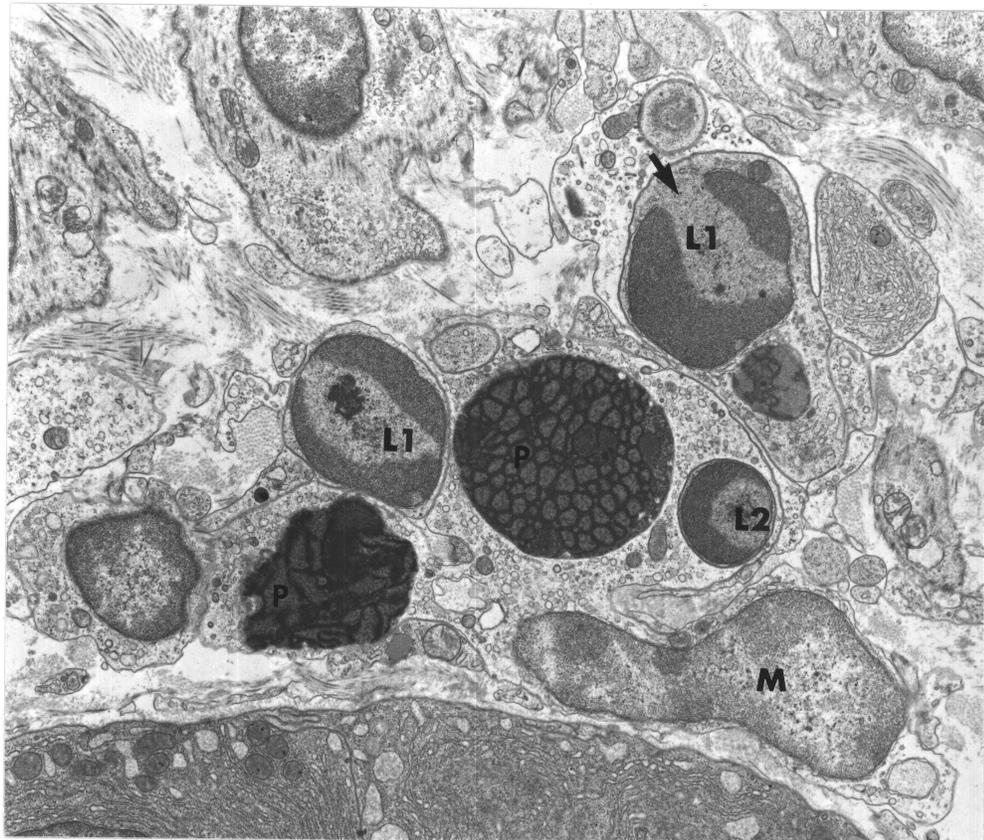
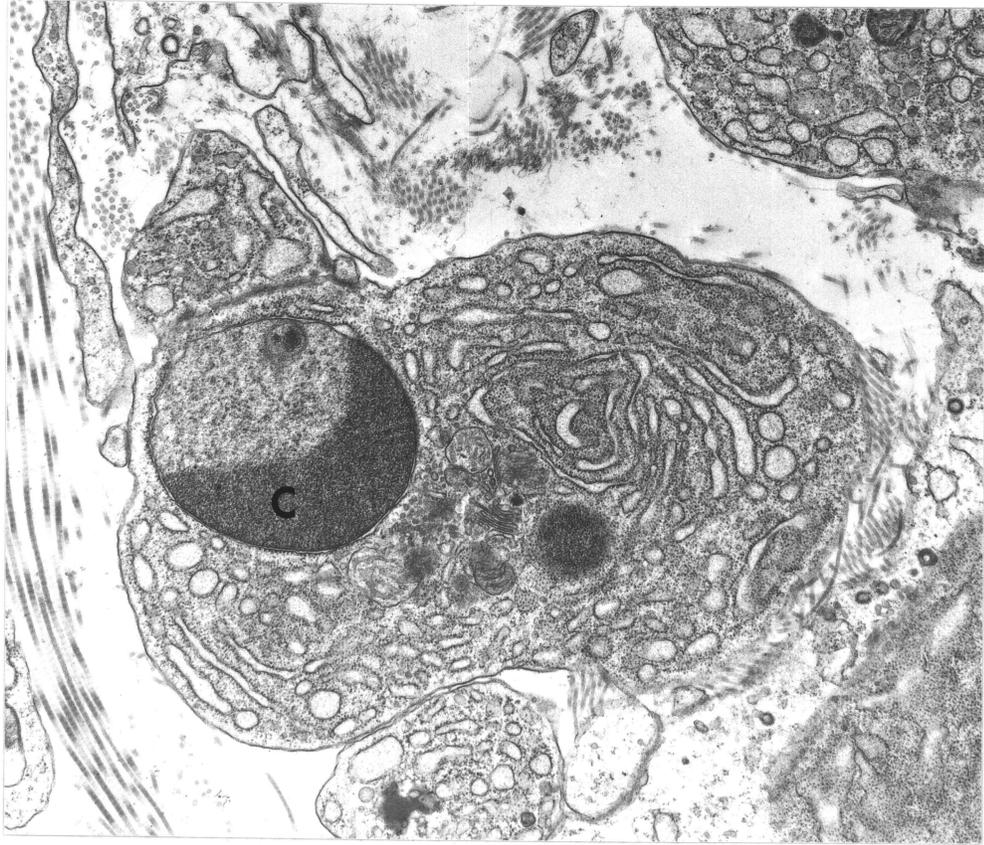


Fig. 23

Figure 24

Ultrastructural appearance of the basal aspect of intestinal crypt epithelial cells of a mouse treated 90 minutes previously with T-2 toxin (2 mg/kg)

Note the swollen intercellular cytoplasmic processes (asterisks). The crypt on the left is not affected.

- I. Interstitial lamina propria.
- BL. Basal lamina.

Osmium fixed; x 5700.

Figure 25

Ultrastructural appearance of apical region of intestinal crypt epithelial cells of a mouse treated 90 minutes previously with T-2 toxin.

- P1. Intracellular phagosomes lined by double membranes and containing normal cytoplasm.
- P2. Phagosome containing pyknotic nuclear fragment.
- ED. Epithelial cell with condensed cytoplasmic organelles indicating degeneration.
- ES. Epithelial cell with swollen cytoplasm and swollen microvillous pole (arrow).
- PC. Paneth cell.
- S. Immature secretory cells.

Osmium fixed; x 5700.

Fig. 24

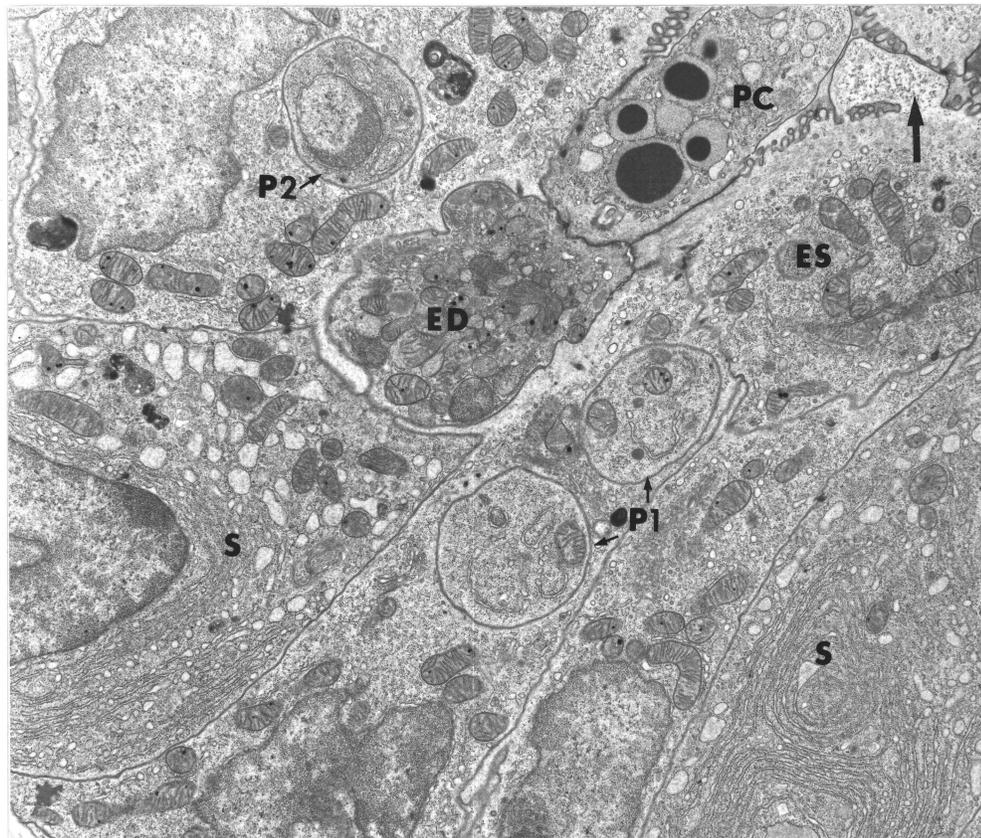
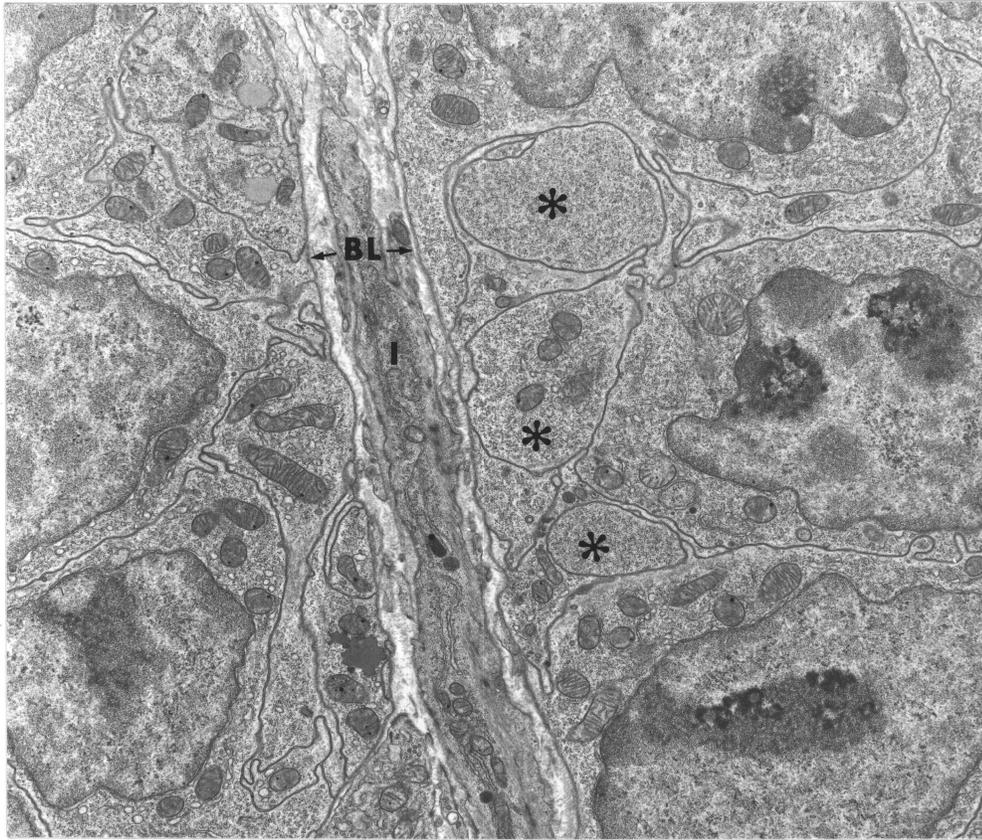


Fig. 25

Figure 26

Electron micrograph of the germinal centre of a lymphoid follicle in the spleen of a mouse treated 90 minutes previously with T-2 toxin (2 mg/kg).

- L. Normal lymphocytes.
- M. Cytoplasmic process of a reticular macrophage.
- P. Phagosomes in various stages of degradation.
  - P1. Fragment of a lymphocyte with clumped chromatin (asterisk), and loose plasma membrane (arrow).
  - P2. Small, round phagosome containing cytoplasm, and surrounded by double membranes.
  - P3. Fragment of nuclear material undergoing early lysosomal degradation.
  - P4. Fragment of cytoplasm and nucleus exhibiting degenerative and degradative changes.
  - P5. Fragments of nucleus in advanced stages of lysosomal degradation.
- A. Artifact from poor fixation.

Osmium fixed; x 11000.

Figure 27

Electron micrograph of a reticular macrophage (M) containing many fragments of lymphocytes at 90 minutes after treatment with T-2 toxin.

Most fragments of nuclear material have become osmiophilic and are being broken down into fragments by lysosomal action (arrows).

Refer to Figure 26 above for identification of labels.

Osmium fixed; x 11000.

Fig. 26

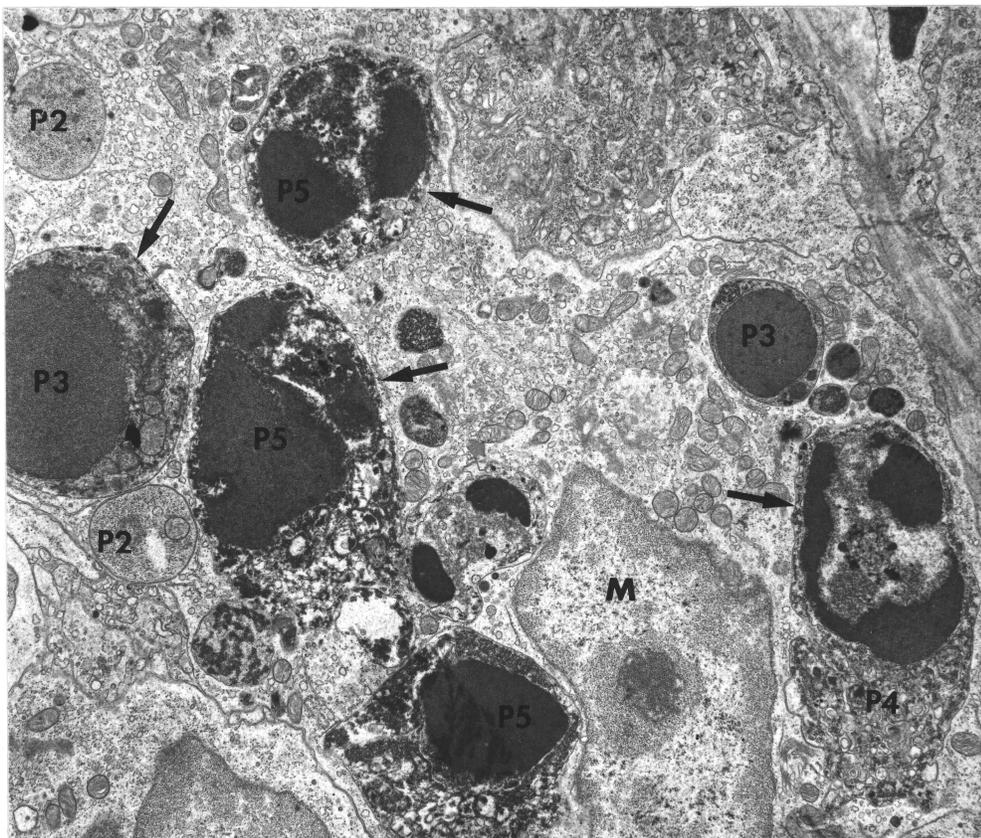
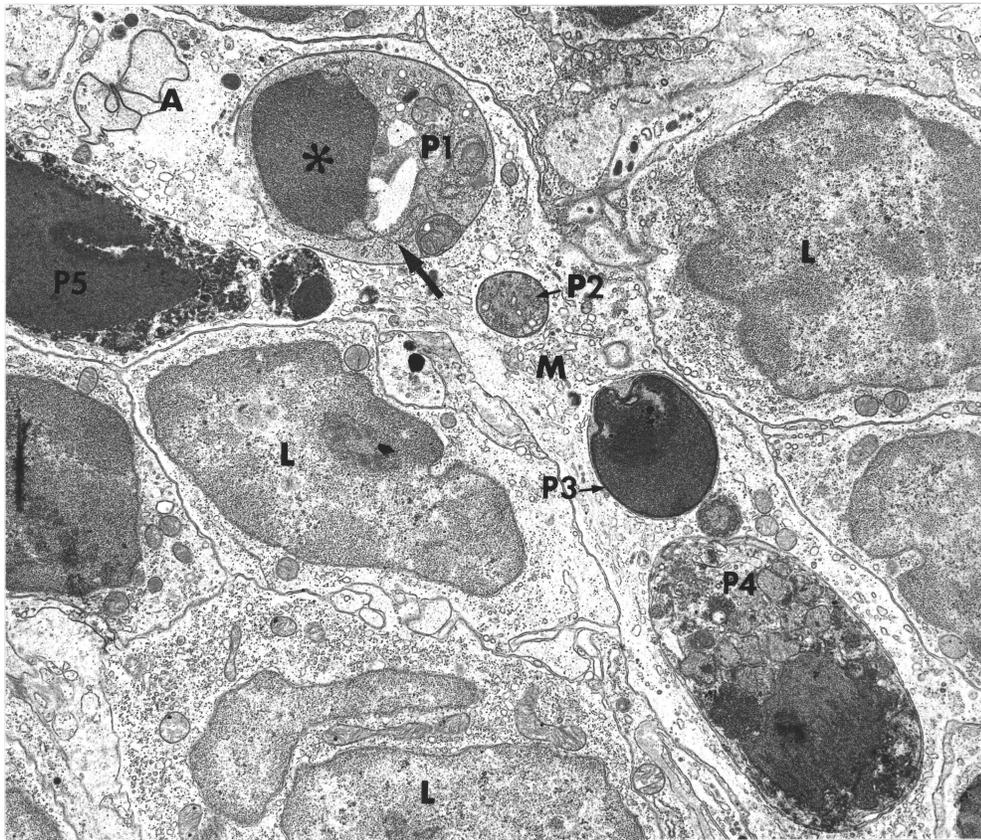


Fig. 27

in macrophages were more osmiophilic and had undergone partial lysosomal degradation (Fig. 27). Small, dense, secondary lysosomes and residual bodies were found in splenic macrophages both of treated and control mice.

Observations at 180 minutes: In the lamina propria of the intestine, many more pyknotic nuclei were found than at 90 minutes. Some of these were plasma cells, but many did not possess differentiated cytoplasm, nor an eccentric nucleus typical of plasma cells. A few cells had undergone complete disintegration into multiple round bodies of cytoplasmic or nuclear material, some of which were free within the interstitium, and some of which were located within heterophagosomes in interstitial macrophages (Fig. 28). Fragments of cells in heterophagosomes exhibited various degrees of lysosomal degradation, ranging from peripheral lysis and fragmentation of membranous organelles, through to complete breakdown of cell components, with shrinkage and increased electron density (Fig. 28).

In intestinal crypts, intracellular inclusions were numerous (Fig. 29), although the number in a crypt varied widely. Many inclusions were identified as heterophagosomes because they contained nuclear debris and were surrounded by double membranes. However, some smaller bodies containing cytoplasmic organelles may have been autophagosomes. Inclusions of each type were often multiple within an otherwise healthy crypt epithelial cell (Fig. 29, 30, 31). Heterophagosomes contained various cell components, including multiple small round particles of nuclear material enclosed within portions of the nuclear envelope

Figure 28

Electron micrograph of the lamina propria of a duodenal villus of a mouse treated 180 minutes previously with T-2 toxin (2 mg/kg).

There is marked karyorrhexis (K) of cells into numerous fragments (arrows).

- K. Karyorrhectic cells, type unidentified.
- PC. Plasma cell.
- L. Lymphocytes.
- C. Collagen fibrils.
- 1. Cell fragments in phagosomes. One fragment contains clumped nuclear chromatin (arrowhead), so is definitely a heterophagosome.
- 2. Cell fragments in secondary lysosomes undergoing degradation.

Osmium fixed; x 5700.



(Fig. 29 ), dense masses of membranous organelles such as mitochondria and endoplasmic reticulum (Fig. 29), or cytoplasmic ground substance. Cells containing inclusions were sometimes swollen, with stretched plasma membranes, and swollen, intercellular processes (Fig. 30 ). Larger inclusions were located towards the apical pole, causing indentation of the nucleus (Fig. 29 ). Some inclusions had been extruded into the lumen of the crypt, especially in severely damaged crypts (Fig. 29). At this stage, phagosomes in crypts had a similarly wide range of secondary degradative changes due to lysosomal hydrolase activity, as was observed in the interstitial macrophages of the lamina propria. Phagosomes in the crypts were mostly located within intact undifferentiated columnar epithelial cells, although some were seen within immature Paneth cells. Phagosomes were also found in absorptive columnar epithelial cells over the villi, but in such cases, they were infrequent, usually small, and more densely stained.

In the spleen, phagosomes were found in increased numbers after 180 minutes in macrophages in the germinal centres of follicles, and also in the red pulp. In general, similar degradative changes were observed in splenic phagosomes as were described for the intestinal locations, but breakdown of contents appeared to be more advanced (Fig. 32 ). Fragmentation and phagocytosis of erythrocytes at the rubricyte stage could be recognized in the red pulp (Fig. 33).

Figure 29

Ultrastructural appearance of phagosomes in duodenal crypt epithelial cells 180 minutes after treatment with T-2 toxin.

- BL. Basal lamina.
- E. Swollen crypt epithelial cells.
- 1. Phagosome containing cytoplasmic fragments.
- 2. Phagosomes containing nuclear fragments.  
Note the homogeneous dense chromatin in fragments of karyorrhectic nuclei (arrows).
- L. Primary lysosome. Secondary lysosomes and degradation of cell components in phagosomes have not yet begun.
- MV. Microvillous border at the lumen of a crypt.  
Note the flattening of the apical cell membrane and presence of necrotic cell debris in the lumen.

Glutaraldehyde fixed, x 9000.

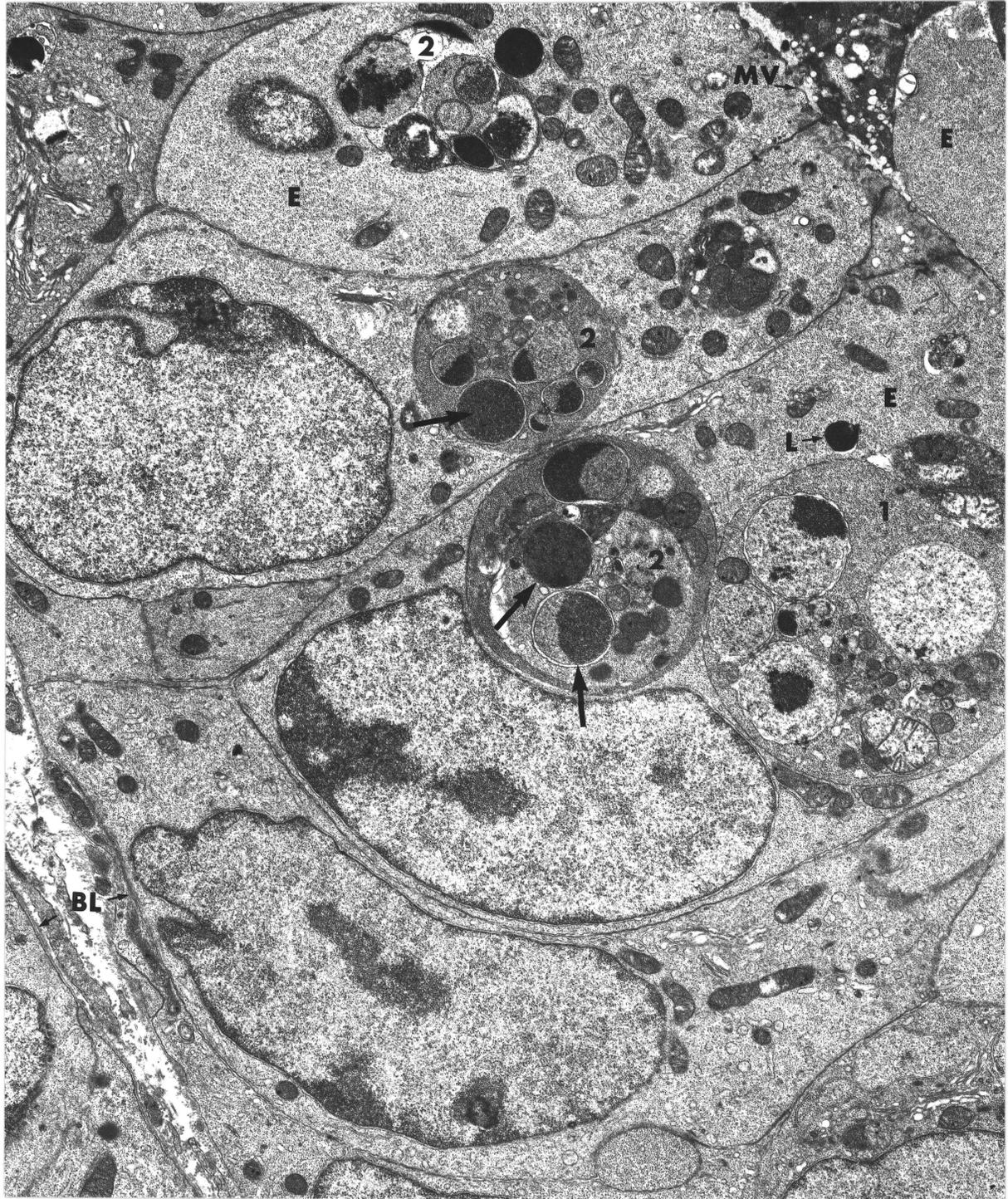


Fig. 29

Figure 30

Ultrastructural appearance of the basal aspect of columnar epithelial cells in duodenal crypts 180 minutes after treatment with T-2 toxin (2 mg/kg).

- BL. Basal lamina.
- E. Swollen epithelial cell.
- I. Swollen intercellular processes.  
Smaller membrane-bound portions of cytoplasm (asterisks) could be autophagosomes or heterophagosomes.
- H. Heterophagosome containing nuclear debris.  
Note the extreme indentation of nucleus.
- P. Phagosome containing cytoplasmic substance.
- L. Primary lysosome.

Glutaraldehyde fixed; x 7000.

Figure 31

Higher magnification of swollen intercellular process shown in Figure 30.

Note the round, double-membrane-bound phagosomes that contain similar components as the surrounding cytoplasm (arrows). It seems probable that such small phagosomes are autophagosomes.

Glutaraldehyde fixed; x 20000.

Fig. 30

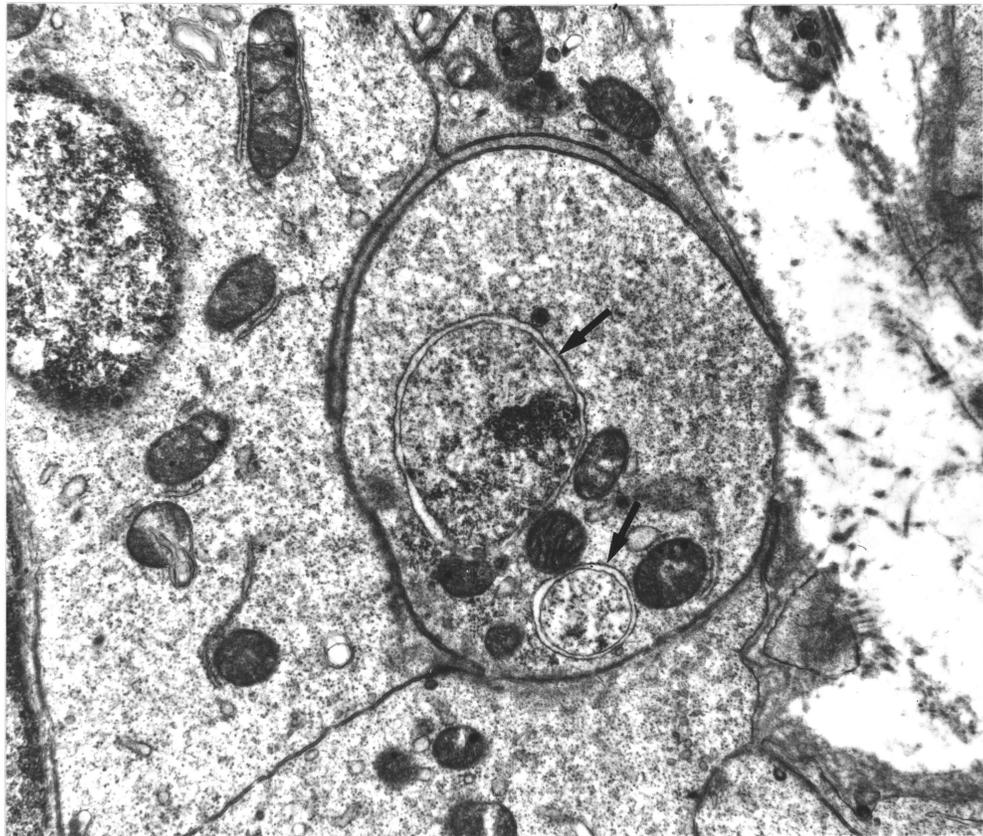
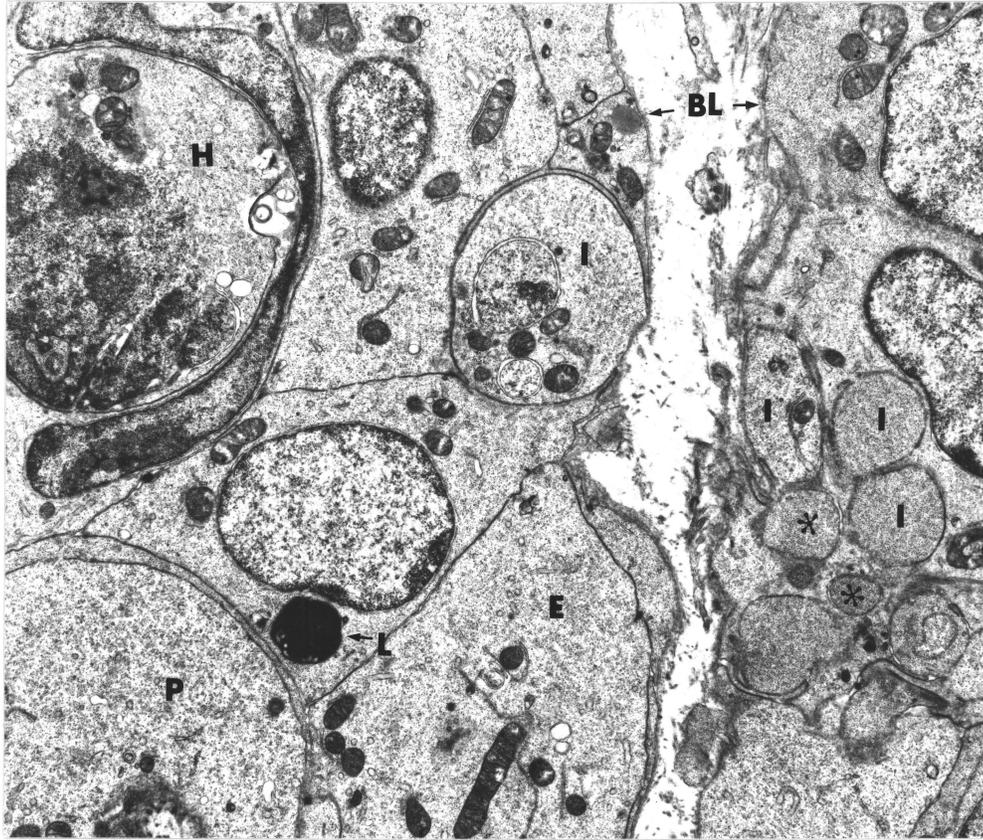


Fig. 31

Figure 32

Ultrastructural appearance of phagosomes in a splenic follicular reticular macrophage at 180 minutes after treatment with T-2 toxin (2 mg/kg).

Note the advanced stage of lysosomal degradation of phagocytosed fragments of karyorrhectic cells.

The osmiophilic, dense material has partly been converted into relatively unstained substances.

Similar, but fewer, phagosomes were found in follicles of control mice.

Osmium fixed; x 11,000.

Figure 33

Ultrastructural appearance of erythropoietic island in the spleen of a mouse treated 180 minutes previously with T-2 toxin (2 mg/kg).

Note the pyknosis and crescent formation of nuclear chromatin (arrow) and the phagocytosis of fragments of rubricytes (asterisk).

Similar changes were found to a much lesser extent in the splenic red pulp of control mice.

Osmium fixed; x 11,000.

Fig. 32

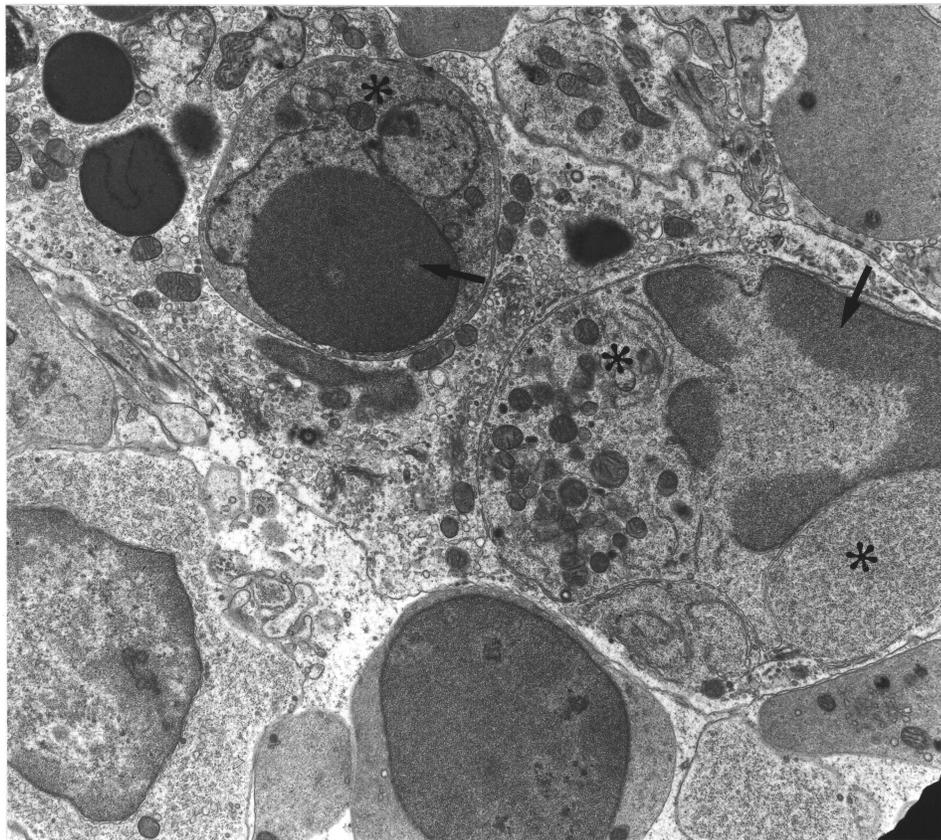
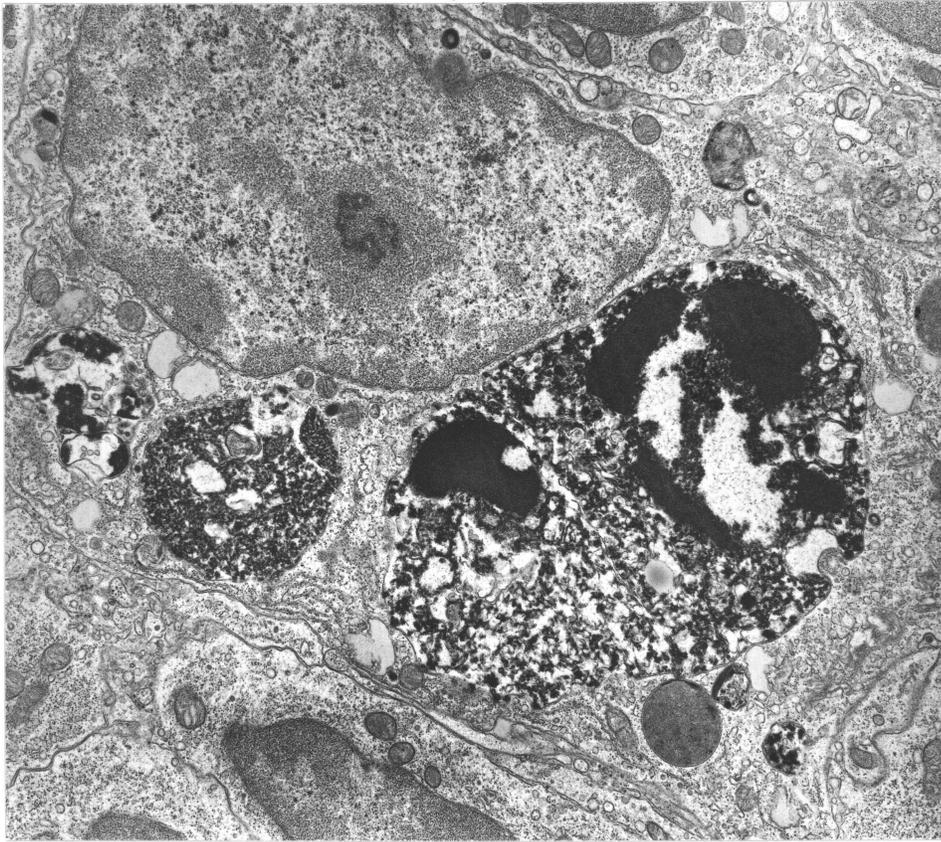


Fig. 33

## 7.5 Discussion

The intracellular inclusions in the intestinal crypts of mice treated with T-2 toxin were readily identified as phagosomes containing cell fragments. The possibility that these inclusions were interdigitations of neighboring cells was excluded because all bodies had round profiles in sections, and because lysosomal degradation occurred soon after the bodies were formed. Most phagosomes contained nuclear fragments and were identified as heterophagosomes because they were located in cells with intact nuclei. The smaller bodies containing only cytoplasmic components might have been autophagosomes, although early stages of enclosure of cytoplasm by membranes as described in autophagocytosis (deDuve and Wattiaux, 1966; Ericsson, 1969; Ghadially, 1975), were not observed. Most phagosomes probably originated from disintegrating cells adjacent to the columnar epithelial cells of the crypt because several heterophagosomes were frequently present within a single cell.

The identity of the cells from which the intraepithelial phagosomes originated could not be established. At the undegraded stage, cytoplasmic components within phagosomes appeared identical to the surrounding cytoplasm of the viable columnar epithelial cells (Fig. 25, 30, 31), suggesting that phagosomes may have been derived from undifferentiated crypt epithelial cells. The restriction of heterophagosomes to the germinal epithelium of the crypts in the absence of mitotic figures further suggests such an origin.

Similar intracellular inclusions have been observed in various

experimental animals treated with ionizing radiation or antineoplastic drugs. They were first described in mice exposed to X-irradiation by Montagna and Wilson (1955), and were examined electronmicroscopically by Hugon et al. (1965) and by Hugon and Borgers (1965). These investigators termed them "karyolytic bodies" and considered that they were phagosomes containing dead leukocytes and stem cells. Degenerating cells considered to be lymphocytes were observed in the basal part of the crypt between stem cells soon after irradiation. These were subsequently engulfed by neighbouring epithelial cells. However, the identity of the degenerating cells was not established. Karyolytic bodies have been observed in other species, including rhesus monkeys exposed to proton irradiation (Ghidoni and Campbell, 1969), and in rats treated with X-irradiation (Lieberman et al., 1970). They have also been observed in animals treated with antineoplastic agents including methotrexate (Trier, 1962; Altmann, 1974), hydroxyurea (Philips et al., 1967), cycloheximide (Verbin et al., 1971a), cytosine arabinoside (Lieberman et al., 1970; Verbin et al., 1971; Searle et al., 1975), nitrogen mustard (Lieberman et al., 1970; Verbin et al., 1974), actinomycin D (Philips et al., 1975) and colchicine (Dinsdale, 1975). Since the work of deDuve and Wattiaux (1965) describing lysosomal function, karyolytic bodies have been recognized to be phagosomes and secondary lysosomes, but the mechanism by which they form, and the cells from which they arise, have still not been determined.

Pathogenesis of phagosomes in response to these agents has been considered to be due to an apparently distinct mode of cell death termed "apoptosis" in which injured cells fragment soon after an insult,

without exhibiting the cytoplasmic degenerative changes seen in coagulation necrosis (Kerr et al., 1972). This phenomenon may be an exaggeration of normal cell turnover, because it is morphologically similar to karyorrhexis and phagocytosis of resulting fragments normally observed at a low rate in actively proliferating tissues such as bone marrow, lymphoid follicles and juvenile thymic cortex (Kerr et al., 1972; Searle et al., 1975), and in the intestinal crypts of normal mice (Cheng and Leblond, 1974). Under nutritional stress, apoptosis in the intestinal crypts of rats was enhanced (Elmes, 1977), supporting the concept that apoptosis is a nonspecific process of cell disintegration that can be induced by a variety of stimuli. A notable feature of this type of cell injury is that scattered individual cells are destroyed, leaving unharmed adjacent cells that engulf the particles (see Fig. 28). In the enteric epithelium, both villous and columnar epithelial cells of the crypts possess phagocytic capabilities; such an ability to remove dead cells is considered crucial to maintenance of the integrity of the enteric lining (Cheng and Leblond, 1974; Philips et al., 1975).

Although nuclear karyorrhexis with heterophagocytosis by surviving neighbour cells thus appears to be a well-characterized cytopathological event, there are divergent opinions on the mechanisms leading to it, and on the cell types that are susceptible. Because some agents that cause karyorrhexis are lethal to cells committed to DNA synthesis, DNA replication is considered to be the target for the initiating chemical injury (Lieberman et al., 1970; Philips et al., 1975). Agents such as hydroxyurea and cytosine arabinoside block DNA synthesis

without affecting synthesis of RNA or protein and each agent causes karyorrhexis in intestinal crypts (Philips et al., 1975; Verbin et al., 1973). Such injury can be prevented by cycloheximide and tenuazonic acid, both of which are potent inhibitors of eukaryotic synthesis of protein (Verbin et al., 1971b; Lieberman et al., 1970). This observation led to an hypothesis that injury to DNA results in synthesis of proteins which are responsible for the lethal events (Lieberman et al., 1970; Verbin et al., 1973). However, cycloheximide itself at high doses also causes karyorrhexis in intestinal crypts (Verbin et al., 1971a) and actinomycin D, an inhibitor of RNA synthesis, causes similar lesions without detectable inhibition of synthesis of DNA (Schwartz et al., 1963; Philips et al., 1975). Accordingly, the role of protein synthesis in this karyorrhectic injury has not yet been determined.

T-2 toxin, which is a potent inhibitor of eukaryotic protein synthesis (Ueno et al., 1973b; Cundliffe et al., 1974), apparently impairs DNA synthesis only as a secondary event (Ueno, 1977a). The fact that T-2 toxin causes karyorrhexis in intestinal crypts therefore appears inconsistent with the idea that karyorrhexis is a lethal sequel to impairment of DNA synthesis. However, the effects of T-2 toxin on synthesis of DNA and protein during acute toxic injury to germinal tissues have not been determined.

The observation that T-2 toxin causes pyknosis of plasma cell nuclei (Fig. 22) and lymphocytes in the intestinal lamina propria suggests

that cells need not necessarily be in S phase to be susceptible to this pattern of injury. Karyorrhexis of lymphocytes in the lamina propria has previously been observed by Verbin et al., (1973) in rats given cytosine arabinoside, and by Ghidoni and Campbell (1969) in monkeys after proton irradiation. Verbin et al. (1973) concluded that phagosomes in the intestinal crypts resulted from injured intraepithelial lymphocytes rather than from columnar epithelial cells. However, various other studies with irradiation or antineoplastic drugs failed to detect karyorrhexis in the lamina propria (Hugon and Borgers, 1966; Philips et al., 1967; Lieberman et al., 1970; Verbin et al., 1974; Cohen, 1978). Philips et al. (1975) concluded from such an observation in mice given cytosine arabinoside that intraepithelial lymphocytes, which are infrequent in mice, were not the target cell.

Searle et al., (1975) described apoptosis of plasma cells in the intestinal lamina propria of mice given cycloheximide. It is notable that cycloheximide, like T-2 toxin, is an inhibitor of eukaryotic protein synthesis, so the fact that plasma cells, which actively synthesize protein, disintegrate in response to two different inhibitors of protein synthesis suggests that this inhibitory activity may be involved in injury to plasma cells. Furthermore, the damage by T-2 toxin to splenic rubricytes (Fig. 33) that are actively synthesizing hemoglobin might be initiated by a similar mechanism.

The variable occurrence of necrosis of lymphoid cells in the lamina propria may be due to differences in dose of injurious agents used. However, observations on dose-response relationship of T-2 toxin-induced acute cellular injury in the previous experiment (Section

6.4.3) indicated that intestinal lymphoid cells are at least as sensitive to T-2 toxin as are cells of the intestinal crypts. Destruction of lymphoid cells may have been mediated by cortisone released under stress of intoxication. Pyknosis, but not karyorrhexis, has been observed in plasma cells in rats treated with corticosteroids (La Pushin and DeHarven, 1971). However, lymphoid cells were similarly susceptible to cycloheximide in both normal and adrenalectomized rats (Verbin et al., 1971b), suggesting that lymphoid karyorrhexis in the lamina propria is due to direct toxic injury. The effects of trichothecenes in adrenalectomized animals have not been examined.

T-2 toxin may have injured lymphoid cells in the lamina propria by a mechanism different from that leading to the epithelial lesions. Ochratoxin A, a nephrotoxic mycotoxin, does not injure crypt epithelial cells but causes karyorrhexis of mononuclear leukocytes in the intestinal lamina propria in dogs (Szczuch et al., 1973) and in guinea-pigs (Thacker and Carlton, 1977). Ochratoxin also causes necrosis in other lymphoid tissues (Szczuch et al., 1973). Because T-2 toxin, a potent topical irritant, was administered by the intragastric route, the lymphoid cells in the lamina propria may have been injured directly by T-2 toxin, or by mediators of the inflammatory response. However, similar lesions occurred after parenteral administration.\*

The mechanisms involved in the types of cell injury produced by T-2 toxin in this experiment are presently poorly understood. Biochemical and autoradiographical examination of alterations in nucleic acid and

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\* Five mice were given 2.5 mg/kg of T-2 toxin intraperitoneally and all had karyorrhexis of intestinal mucosal lymphocytes when examined at 6 hours.

protein metabolism preceding karyorrhexis might lead to a suitable hypothesis. Because of the apparent differences between the effects of T-2 toxin and other cytotoxic chemicals on the intestinal mucosa, trichothecene toxins may be useful chemical probes in the examination of this karyorrhectic or "apoptotic" mode of cell death.

## 8.0 EFFECTS OF SUBACUTE DIETARY T-2 TOXICOSIS IN MICE

### 8.1 Abstract

T-2 toxin (20 ppm), incorporated in balanced, semipurified diets containing 8%, 12% or 16% protein, was fed to three groups of 20 young male white Swiss mice weighing  $16.0 \pm 1.2$  g. After intervals of 1, 2, 3 or 4 weeks on the experimental diets, groups of four mice on each test diet, and control groups of four mice fed the corresponding toxin-free diet at a similar rate of intake, were examined hematologically and by necropsy. Mice on the 16%-protein diet were also examined after 6 weeks. Toxin-free diets were also fed ad lib to control mice examined at 4 weeks.

Dietary T-2 toxin consistently caused reduced growth, reduced food consumption, aplastic anemia, reticulocytopenia, lymphopenia, eosinopenia and neutrophilia. Affected mice developed characteristic lesions including perioral dermatitis, proliferative hyperkeratosis and ulceration of the squamous gastric mucosa, hepatomegaly, and atrophy of all lymphoid tissues. Hematopoiesis, especially erythropoiesis, in bone marrow and spleen were suppressed. In general, the dietary protein level did not influence the toxicity of dietary T-2 toxin during the first three weeks of the trial, except that food consumption and growth rates of mice were directly related to the protein level in the toxic diets.

During the latter half of the 6-week experiment, suppression of hematopoiesis in bone marrow and splenic red pulp was overcome. These atrophic tissues regenerated, resulting in splenomegaly and hypercellularity of bone marrow. Erythroid maturation was megaloblastic during the regenerative phase, whereas thrombopoiesis and granulopoiesis resumed

before erythropoiesis, and appeared normal. Regenerative changes in hematopoietic tissues were much less developed in mice consuming T-2 toxin in diets containing 8% or 12% protein, in comparison with mice on the nutritionally adequate 16%-protein diet. The effects of T-2 toxin on lymphoid tissues and stomach did not abate during the period of continuous exposure to T-2 toxin and these effects were similarly severe in each of the three diet groups. The degree of hepatomegaly was correlated with dietary protein level and with the onset of erythroid regeneration. Hematopoietic cells in bone marrow, spleen and lymphoid tissues exhibited pronounced regenerative responses one week after withdrawal of T-2 toxin from the diet, even though food intake was maintained at the same level. The results of this experiment demonstrated that mice are susceptible to both the hematopoietic-suppressive and the topical-irritant effects of T-2 toxin consumed in the diet. The effects on hematopoiesis were transient and the ability to recover depended on the nutritional composition of the toxic diet.

## 8.2 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 (Bamburg and Strong, 1971; Wogan, 1975; Ueno, 1977a). This view has arisen from the knowledge that T-2 toxin and other trichothecenes may be produced by Fusarium and other fungi growing on cereal products under cold, moist conditions (Burmeister, 1971; Ueno et al., 1972c; Joffe, 1974b). Trichothecenes, especially T-2 toxin, have been associated with lethal mycotoxicoses, such as alimentary toxic aleukia of man (Ueno, et al., 1972c; Mirocha and Pathre, 1973;

Joffe and Yagen, 1977; Lutsky et al., 1978), and mouldy-corn poisoning of cattle (Hsu et al., 1972). These diseases are believed to be partly due to depression of hematopoiesis by trichothecenes in Fusarium-contaminated diets (Lutsky et al., 1978; Joffe, 1978).

At present, there is little evidence to indicate that trichothecenes cause hematopoietic failure in animals ingesting them in the diet. Impaired hematopoiesis has not been reported in swine (Greenway and Puls, 1976), nor in poultry (Wyatt et al., 1972b; Greenway and Puls, 1976) with fusariotoxicosis, a naturally occurring mycotoxicosis caused by T-2 toxin and other trichothecenes in Fusarium-contaminated grain. Similarly, hematological abnormalities were not detected in chickens (Chi et al., 1977b; Speers et al., 1977) nor in swine (Weaver et al., 1978c; 1978d) fed rations containing T-2 toxin. Mild leukopenias in chickens consuming dietary T-2 toxin (Wyatt et al., 1975) and in mice consuming T-2 toxin or fusarenon-X (Ohtsubo and Sato, 1977), have been reported.

Support for the view that trichothecenes may cause hematopoietic failure is derived from observations of suppression of hematopoiesis in various animals given repeated doses of T-2 toxin (Grove et al., 1970; Lutsky et al., 1978) or other trichothecenes (Rüsch and Stähelin, 1965; Stähelin et al., 1968). Because trichothecenes are potent inhibitors of voluntary food consumption (Marasas et al., 1969; Bamberg and Strong, 1971; Kotsonis et al., 1975b), the toxicological significance of effects produced by such non-dietary exposure appears questionable.

The objectives of the following study were to determine the toxic effects of T-2 toxin during dietary exposure, and to identify conditions under which this toxin might cause hematopoietic failure. Because mice

are the most ideal animals in which to evaluate impairment both of the hematopoietic and the immune systems, T-2 toxin was administered to young Swiss mice by the natural dietary route. Natural mycotoxicoses are sometimes more severe when the nutritional quality of the diet is poor (Newberne, 1974; Hamilton, 1977), so the effects of T-2 toxin in diets of various protein levels were examined, in case optimal nutrition should mask the effects on hematopoiesis.

### 8.3 Materials and Methods

A total of 192 male weanling outbred Swiss mice (Canadian Breeding Farms and Laboratories Ltd., St. Constant-Laprairie, Québec), weighing  $16.0 \pm 1.2$  g were randomly assigned to groups of four and housed in plastic screen-top cages on softwood shavings. Tap water was supplied ad lib. The room was maintained at  $21^{\circ}\text{C}$  with 12 hours of fluorescent lighting per day. All management procedures conformed to the guidelines of the Canadian Council on Animal Care.

#### 8.3.1 Experimental design

Three semipurified diets, formulated as described in Table 65, Appendix B, and containing 8%, 12% or 16% protein, were used. Four experimental groups (A, B, C and D) were used for each of the three diets, as indicated in Table 27. The principal groups (A), of 20 mice each, were supplied ad lib with the semipurified diets containing T-2 toxin (Makor Chemicals, Jerusalem, Israel) at the rate of  $20 \mu\text{g/g}$  of dry diet (20 ppm). The main control groups (B), also consisting of 20 mice each, were supplied with the respective diets without T-2 toxin, at a restricted rate such that the rate of consumption of each of the three control diets, per gram of body weight, was similar to the rate at

Table 27 - Design of Experiment, Section 8.

Group <sup>a</sup>	Treatment	Number of mice examined					
		Day 0	Day 7	Day 14	Day 21	Day 28	Day 41
A	Semipurified diet containing T-2 toxin (20 ppm)		3x4 <sup>b</sup>	3x4	3x4	3x4	4 <sup>c</sup>
B	Semipurified control diets - restricted intake <sup>d</sup>		3x4	3x4	3x4	3x4	4 <sup>c</sup>
C	As for group A for 3 weeks, then as for group B					3x4	
D	Semipurified control diets - <u>ad lib</u>					3x8	
E	Pelleted natural-ingredient mouse diet <sup>e</sup>	12				8	

<sup>a</sup> Groups A, B, C and D were repeated for each of the 3 dietary protein levels, 8%, 12% and 16%. Formulae for diets are described in Appendix B, Table 65.

<sup>b</sup> 4 mice from each of the 3 diets.

<sup>c</sup> 4 mice from the 16% diet. Because no mortality occurred among mice consuming T-2 toxin in the 16% diet, extra mice remaining after day 28 were kept on their diets for a further 2 weeks.

<sup>d</sup> These control diets were fed at the rate at which the corresponding toxic diets were consumed by mice in group A.

<sup>e</sup> Formulation described in Appendix B, Table 64.

which the corresponding toxic diet was consumed. To evaluate the effect of withdrawal of T-2 toxin, groups of four mice (C) were transferred to the control diets after having been on the toxic diets for 3 weeks. After transfer, the respective control diets were supplied for one week at the rate at which the toxic diets had been consumed during the week prior to transfer. In order to monitor the effect of reduced dietary intake in groups A and B, further control groups (D) of eight mice each, were supplied ad lib with the semipurified diets for 4 weeks. Another group (E), consisting of mice fed on regular laboratory chow (see Table 64; Appendix B) was included in the experiment to identify effects that might be due to the semipurified diets. Twelve of these mice in group E were examined on day 0 and a further eight on day 28.

The experiment was designed to be conducted over a 4-week period, with 12 mice (4 from each of the 3 diets) necropsied from both groups A and B at weekly intervals. The extra mice were included in these two groups to ensure that four mice on each treatment were available for examination at each selected time. Because mortality did not occur in group A on the 16%-protein diet, the four mice remaining on this toxic diet at the end of the 4-week experiment were kept on the diet for a further 2 weeks, along with four on the corresponding control diet (B). The few remaining mice in group A for the other two diets, along with those remaining in the corresponding control group B, were discarded after 4 weeks.

### 8.3.2 Experimental observations

All mice 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 the average weekly food consumption rates for mice in each cage were determined. At weekly intervals, according to the schedule in Table 27, mice were randomly selected for hematological and necropsy examinations.

Between 9 and 11 a.m. on the day of termination, blood samples were collected from the tail of each mouse into heparinized calibrated pipets and diluted in normal saline for autocytometric (Coulter-S, Coulter Electronics Inc., Hialeah, Florida) determination of packed cell volume (PCV), erythrocyte count (RBC), hemoglobin concentration (HGB), mean corpuscular hemoglobin concentration (MCHC), and total leukocyte count (WBC). 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. After blood was collected, mice were killed by decapitation and necropsied. 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. After 24 hours, tissues were transferred to 70% ethanol for storage until they were embedded in paraffin, sectioned at 6  $\mu$ m and stained with hematoxylin and eosin.

### 8.3.3 Analysis of data

Continuous variables from groups A and B in the 4-week section of the study were analysed in a 3-way analysis of variance with toxin, dietary protein level, and time as factors. This enabled identification of significant changes due to dietary T-2 toxin, as well as significant changes with time, and with dietary protein level. The influence of dietary protein level on toxic effects was then assessed in a 2-way analysis of variance of data from those mice in group A. Inter-group comparisons among means of groups A, B, C, D and E at day 28 were made using a 1-way analysis of variance and Student-Newman-Keuls' (SNK) multiple range tests. Comparisons among the three diets for groups A, B, C and D at day 28 were also made in this way.

Comparisons between means of toxin-treated groups (A) and the corresponding control groups (B) were made using two-tailed Student t tests. The 95%-probability (p) level was used to indicate significance of factor effects and differences between means. All analyses were computed using methods and programs described by Nie et al. (1975).

## 8.4 Results

### 8.4.1 General observations

Mice consuming T-2 toxin in the diet appeared small and lethargic with dry ruffled fur, in contrast to the control mice in group B which were active and had normal fur gloss. By day 14, several mice consuming T-2 toxin exhibited dermatitis of the skin around the mouth, less frequently on the feet, and some developed scaly skin on their tails. Mice on the toxic diets frequently exhibited pallor of the skin during the second and third weeks. The frequencies of these physical changes in

general increased by the third week and were higher in mice on the lower protein diets (Table 28).

No deaths occurred in mice consuming T-2 toxin in the 16%-protein diet, nor in any of the control groups, but two mice consuming T-2 toxin in each of the two lower protein diets died during the 4-week experiment. One mouse on the 12% diet died on day 7 with intestinal hemorrhage (Fig. 34A), pulmonary hemorrhage, perioral dermatitis, and stomatitis. Two others from the 8%-protein diet died with severe perioral dermatitis (Fig. 34B) on days 19 and 25. One other mouse on the 12%-protein diet died on day 24 from the effects of severe anemia.

After mice in groups C were transferred from the toxic diets to the control diets, their physical condition improved.

Dietary T-2 toxin caused marked reductions in growth of mice on each of the three diets (Fig. 35). The degree of growth depression was greater at the lower levels of dietary protein ( $p = 0.004$ ; Table 66, 67, Appendix C). Growth rates of mice fed the 16%-protein control diet ad lib matched those of mice fed the natural-ingredient chow (E), indicating that the semipurified diet was adequate for normal growth of young mice (Fig. 35). Growth rates of control mice in group D were directly proportional to the protein level (Fig. 35).

Voluntary consumption rates of toxic diets were approximately half those of mice fed the control diets ad lib (Table 68, Appendix C). Consumption rates (per gram of body weight) were not significantly different ( $p = 0.80$ ) between groups A and B (Table 69, Appendix C).

Table 28 : Frequencies<sup>a</sup> of mortality and skin abnormalities observed in mice during consumption of semipurified diets containing T-2 toxin and different levels of protein.

	Week	Dietary protein level		
		8%	12%	16%
Mortality	1	0/24	1/24	0/24
	2	0/20	0/19	0/20
	3	1/15	0/15	0/16
	4	1/6	1/6	0/8
Perioral dermatitis	2	5/20	4/19	1/20
	3	9/15	11/15	4/16
Scaliness of tail skin	2	5/20	6/19	2/20
	3	11/15	10/15	10/16

<sup>a</sup> Values are ratios of number affected during each week to number on each diet at the beginning of each week.

Figure 34

Macroscopic lesions observed in mice fed semipurified diets containing 20 ppm of T-2 toxin.

- A. Intestinal hemorrhage in a mouse that died after 7 days on the toxic 12%-protein diet.
  
- B. Severe perioral dermatitis of a mouse that died after 19 days on the toxic 8%-protein diet.
  
- C. Enlarged stomach of a mouse killed after having consumed T-2 toxin for 28 days.  
Note the enlarged esophageal portion (E).
  
- D. Mucosal surface of the stomach illustrated in C.  
Note the greatly thickened stratified squamous mucosa over the esophageal region (E).  
The fundic mucosa (F) is not affected.

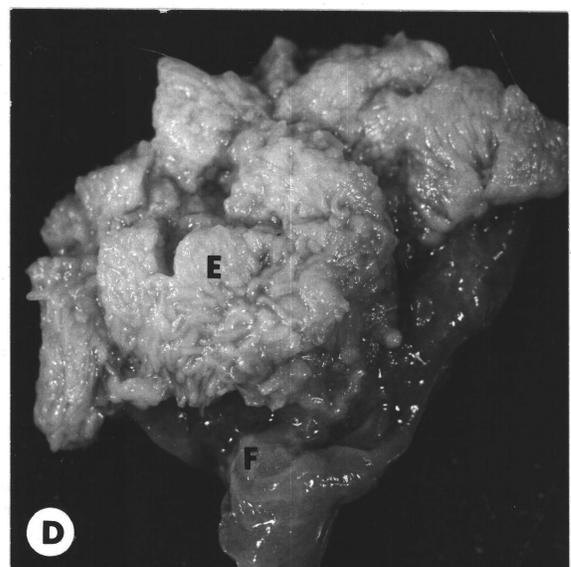
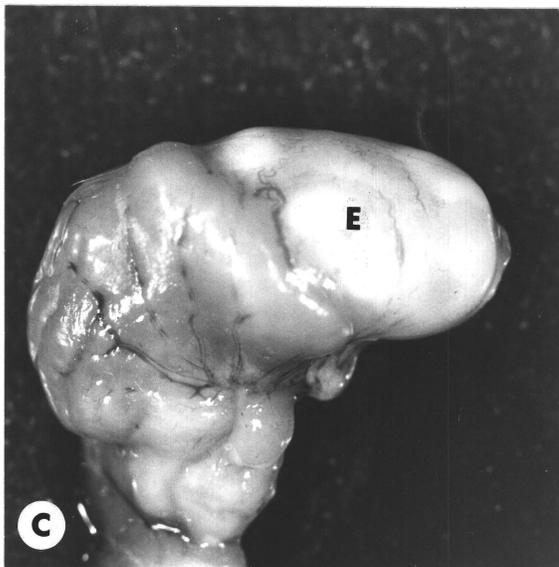
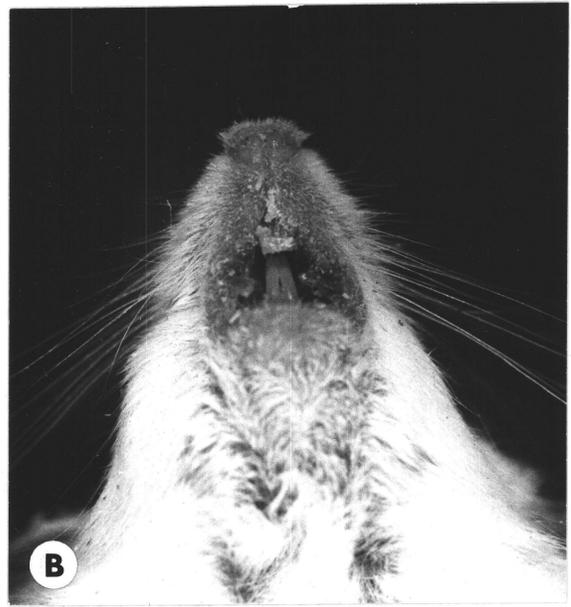


Fig. 34

## 8.4.2 Hematology

### 8.4.2.1 Erythron

Mice consuming T-2 toxin in the 16%-protein diet developed normochromic, normocytic anemia during the 6-week trial period (Table 29). Initially, erythropoiesis was markedly depressed, with circulating reticulocyte counts consistently below 0.1%, but after 6 weeks, reticulocytes were again present in circulation (Fig. 36B). During the period when circulating reticulocyte numbers were very low, the MCV was slightly reduced ( $p < 0.001$ ), but it returned to the control level after 6 weeks (Table 70). There were no significant differences due to diet or T-2 toxin on values of MCH and MCHC, although these values did vary consistently in all groups with time (Table 70, Appendix C).

The dietary protein level did not significantly influence decreases in hemoglobin concentration ( $p = 0.052$ ; Table 71, Appendix C), or PCV ( $p = 0.14$ ; Fig. 37). However, several mice consuming T-2 toxin in the 12%- and the 8%-protein diets developed more severe anemia than others in the same group (Fig. 37).

During the period of erythroid aplasia in treated mice (Group A), erythrocytes were morphologically normal, although polychromatophilic erythrocytes were infrequently seen (Fig. 38A, B, C). During the regenerative phase treated mice had abnormal erythrocytes in circulation, including large polychromatophilic macrocytes, microcytes, basophilic stippled erythrocytes, leptocytes, and poikilocytes (Fig. 38D).

After transfer from the toxic diets, mice had marked elevations in reticulocytes in circulation (Fig. 36), and red cell values returned to near normal levels (Fig. 37 and Table 31).

Table 29: Erythrocyte values\* of young Swiss mice after 28 days on semipurified diets containing T-2 toxin (20 ppm) and different levels of protein.

Variable	Dietary protein level	A	B	Group C	D	E
		20 ppm T-2 toxin	Restricted control	Toxin withdrawn	Ad lib control	Pellet control
Hemoglobin concentration (HGB) g/dl	8%	13.0 $\pm$ 1.8	16.1 $\pm$ 0.3	14.6 $\pm$ 0.8	15.4 $\pm$ 0.4	15.3
	12%	10.0 $\pm$ 0.7 a	15.7 $\pm$ 0.8 b	12.0 $\pm$ 2.5 ab	15.5 $\pm$ 0.4 b	15.3 b
	16%	9.8 $\pm$ 0.7 a	16.4 $\pm$ 0.7 b	15.0 $\pm$ 1.1 b	15.8 $\pm$ 0.3 b	15.3 b
Diet groups combined		10.9 $\pm$ 0.7 a	16.0 $\pm$ 0.3 b	14.0 $\pm$ 0.8 b	15.3 $\pm$ 0.1	15.3 $\pm$ 0.1 b
Erythrocyte count (RCC) $\times 10^6/\mu\text{l}$	8%	7.1 $\pm$ 0.7 a	9.2 $\pm$ 0.3 b	7.9 $\pm$ 0.5 ab	8.3 $\pm$ 0.2 ab	8.6 ab
	12%	5.9 $\pm$ 0.4 a	8.4 $\pm$ 0.6 b	6.2 $\pm$ 1.1 a	8.0 $\pm$ 0.2 b	8.6 b
	16%	5.5 $\pm$ 0.5 a	8.7 $\pm$ 0.5 b	8.4 $\pm$ 0.6 b	8.2 $\pm$ 0.3 b	8.6 b
Diet groups combined		6.2 $\pm$ 0.4 a	8.8 $\pm$ 0.3 b	7.6 $\pm$ 0.5 b	8.2 $\pm$ 0.1 b	8.6 $\pm$ 0.4 b
Packed cell volume (PCV) percent	8%	33.2 $\pm$ 3.7 a	41.2 $\pm$ 1.1 b	38.7 $\pm$ 2.6 ab	42.2 $\pm$ 0.9 b	42.7 b
	12%	27.8 $\pm$ 1.8 a	42.0 $\pm$ 2.3 b	30.6 $\pm$ 6.1 a	40.7 $\pm$ 0.9 b	42.7 b
	16%	25.7 $\pm$ 2.2 a	42.4 $\pm$ 2.2 b	41.2 $\pm$ 3.3 b	41.5 $\pm$ 1.0 b	42.7 b
Diet groups combined		28.9 $\pm$ 1.7 a	42.5 $\pm$ 1.0 b	37.5 $\pm$ 2.4 b	41.4 $\pm$ 0.4 b	42.7 $\pm$ 1.2 b

\* Values are means ( $\pm$ SEM) of separate groups of 4 mice.

a, b In any horizontal row, means followed by the same letter, or by no letter, do not differ at  $p = 0.05$ , by SNK multiple range test.

Influence of Dietary T-2 toxin (20ppm) in Mice

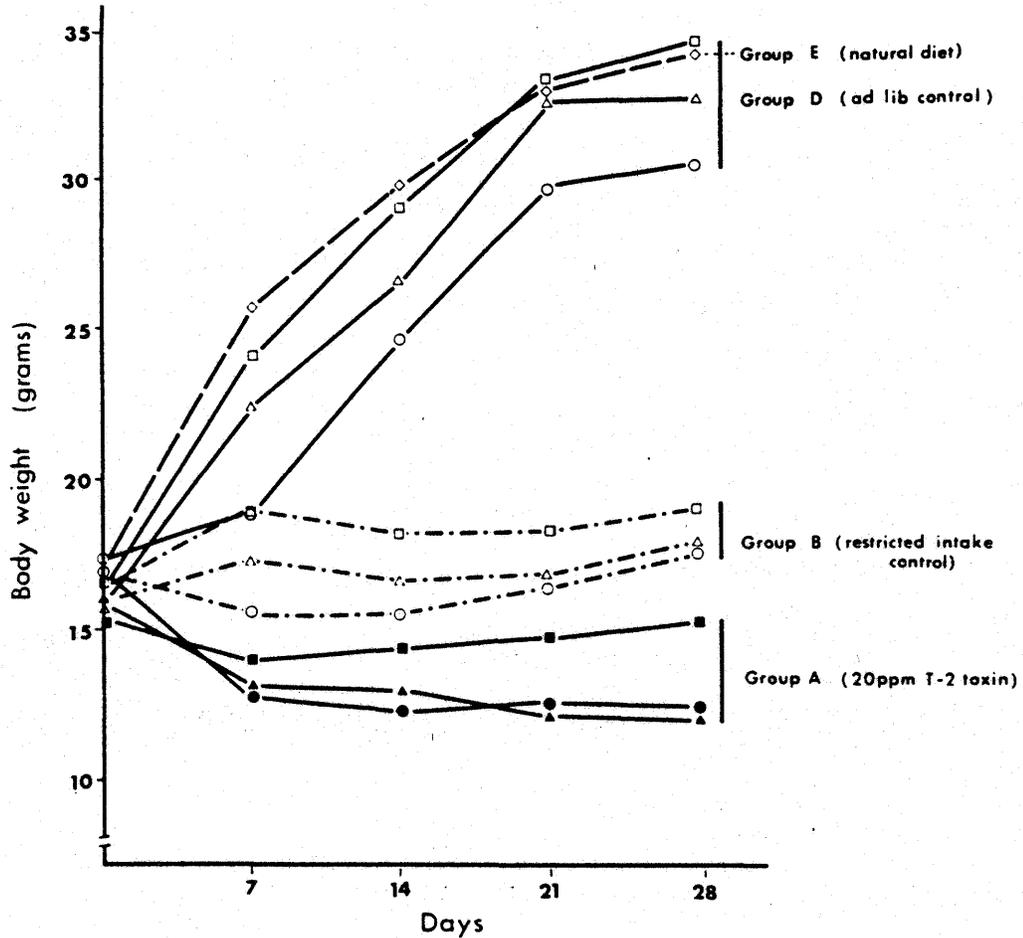


Fig. 35 : Growth curves of mice in group A fed diets containing T-2 toxin and different levels of protein (circles = 8%, triangles = 12%, squares = 16%), in comparison with mice fed corresponding control diets at a similar rate of intake (---) or ad lib (—). Growth rates of mice (Group E) fed a natural-ingredient diet are shown.

Figure 36

Sequential changes in numbers of circulating reticulocytes in mice consuming T-2 toxin (20 ppm) for up to 6 weeks.

- A. Comparison of effects of T-2 toxin in reticulocyte counts of mice on 3 different levels of dietary protein.

Note the effect of withdrawal of T-2 toxin from the diet (group C). Control group B were fed at the same rate as group A.

- B. Sequential change in reticulocyte counts of mice fed 20 ppm of T-2 toxin for 6 weeks in the 16%-protein diet (group A).

Note the return of reticulocytes by day 42.

Each point is the mean (±SEM) of separate groups of 4 mice. Control group B was fed at the same rate as group A.

Influence of Dietary T-2 toxin (20ppm) in Mice

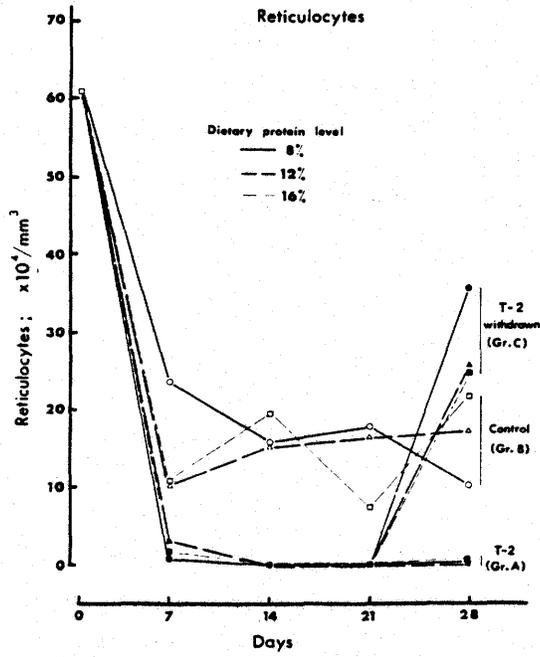


Fig. 36A

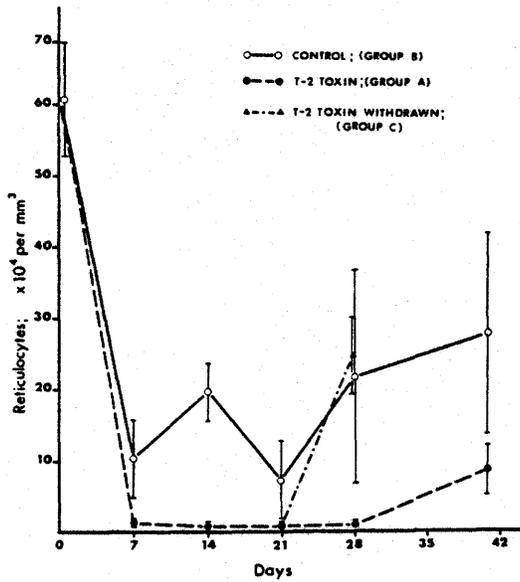


Fig. 36B

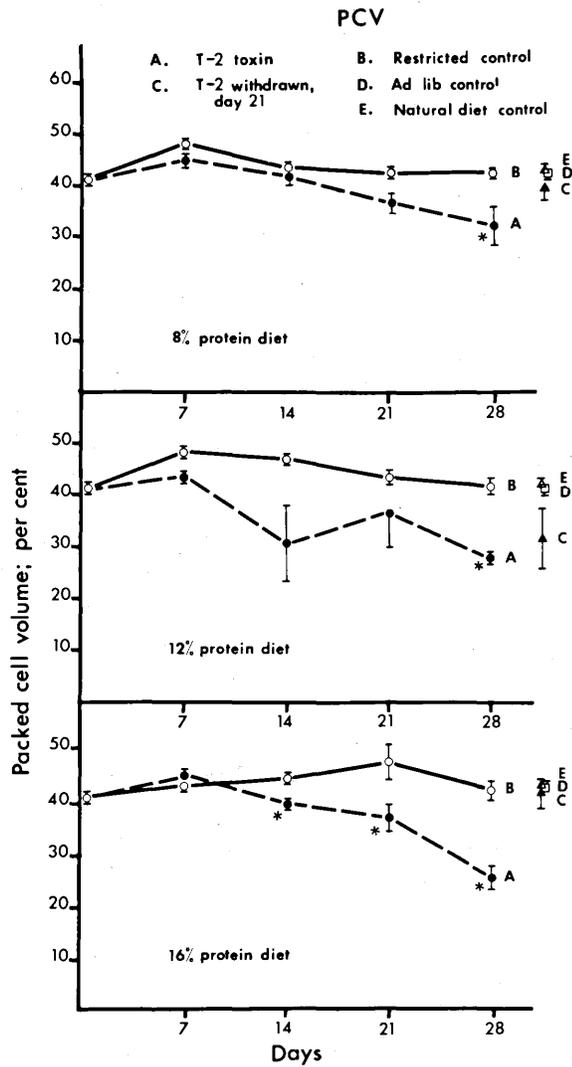


Fig. 37: Development of anemia in mice consuming T-2 toxin (A) in comparison with restricted intake groups (B). For comparison, 28-day values after toxin withdrawal (C) for ad lib-fed controls (D) and for natural-diet-fed controls (E) are shown. Points are means (+SEM) of separate groups of 4 mice. Wide deviations at 14 and 21 days for group A on the 12% diet are due to severe blood-loss by 1 mouse in each group.

Figure 38

Effect of dietary T-2 toxin (20 ppm) on the morphology of circulating erythrocytes.

- A. Control mouse; day 21; group B; x 300.  
Note the polychromatophilic erythrocytes in circulation (arrows).
  
- B. Mouse on T-2 toxin; day 21; group A; x 300.  
Polychromatophilic erythrocytes are absent.
  
- C. Mouse on T-2 toxin; day 21; group A; x 750.  
At this stage all erythrocytes are normal, but immature stages (polychromatophils) are absent.
  
- D. Mouse on T-2 toxin; day 41; group A; x 750.  
Note the reappearance of polychromatophilic erythrocytes (arrows).  
Some polychromatophilic cells have basophilic stippling (S) during the regenerative phase. Many erythrocytes have morphological abnormalities. Leptocytes (L), microcytes (M), and poikilocytes (P) are shown.

Wright's-Giemsa-stained peripheral blood smears.  
Magnification factors are given in each legend.

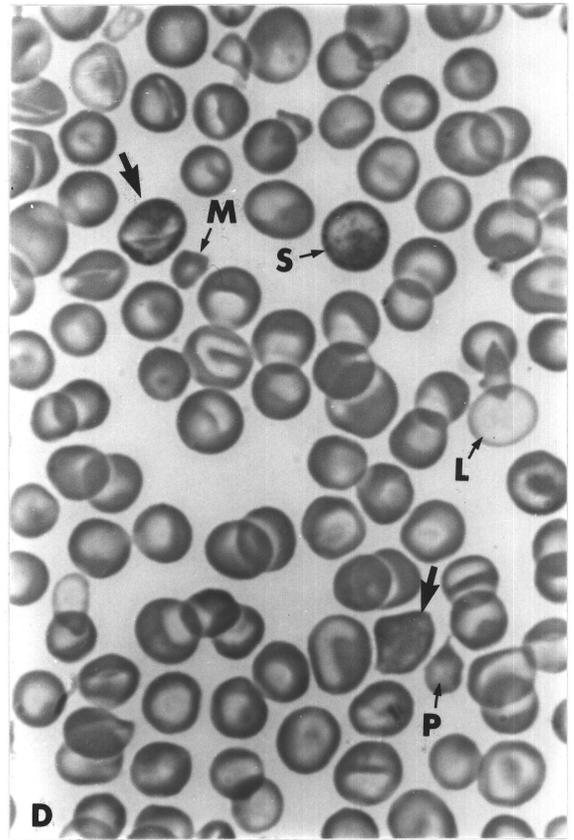
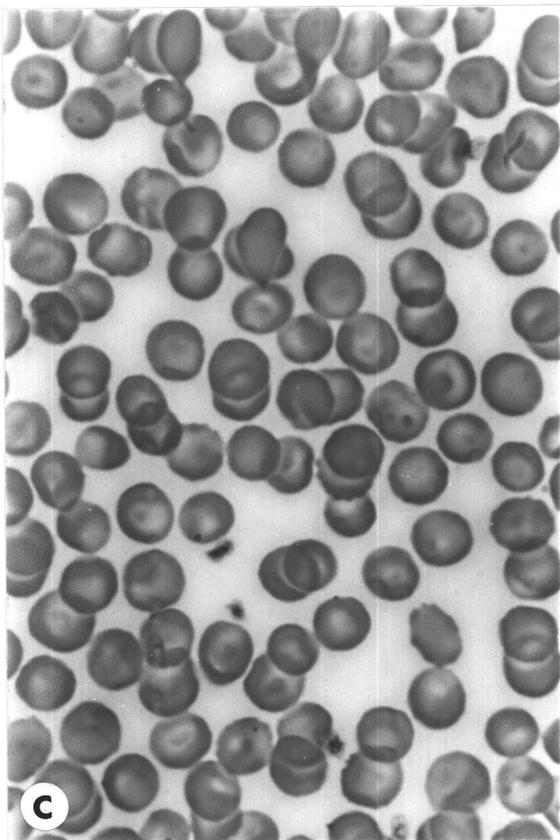
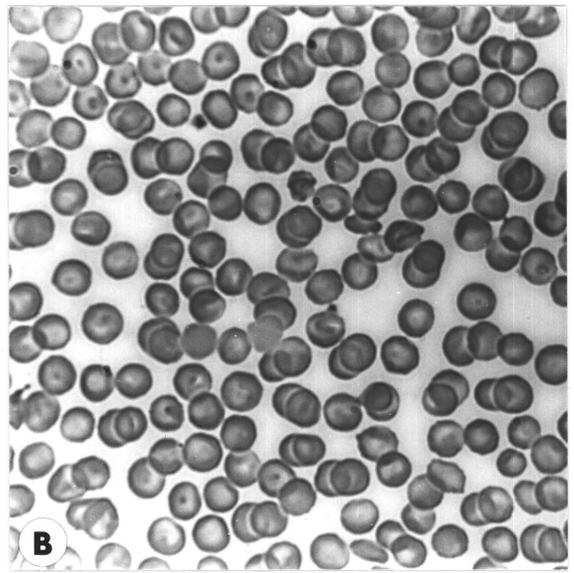
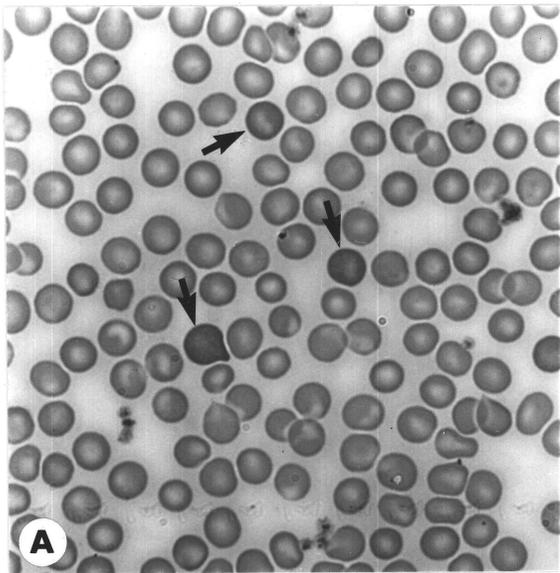


Fig. 38

#### 8.4.2.2 Leukon

Total leukocyte values were lower in mice consuming T-2 toxin, in comparison with mice in group B fed at the same rate (Table 72, Appendix C ), However, counts in control groups B were also lower than in mice fed ad lib on the corresponding semipurified diets or the natural diet. Lymphocyte counts declined precipitously in mice consuming the toxic diets (Fig. 39). Lymphocyte counts of ad lib fed control mice were significantly higher than for mice fed the control diets at the restricted rate (Fig. 39). Eosinophils disappeared from circulation of most mice on the toxic diets (Table 30).

Neutrophil counts of control mice in groups B, D and E were within normal limits at all stages, whereas in mice on the toxic diets, they progressively increased over the 4-or 6-week periods (Fig. 40). Changes in lymphocyte and neutrophil numbers were similar in mice on T-2 toxin, irrespective of the dietary protein level. One week after withdrawal of mice in group C from the toxic diets, lymphocyte counts remained low (Fig. 39), and neutrophil counts remained elevated (Fig. 40).

Thrombocyte numbers on peripheral blood smears of mice consuming T-2 toxin appeared reduced during the second and third week. Because most thrombocytes were clumped due to collection of blood in heparinized tubes, estimating their numbers was not possible. No morphological abnormalities in circulating leukocytes were observed in any mice.

#### 8.4.3 Necropsy findings

##### 8.4.3.1 Macroscopic findings

Mice consuming the diet containing T-2 toxin developed marked atrophy of the thymus and Peyer's patches. By day 7, and throughout

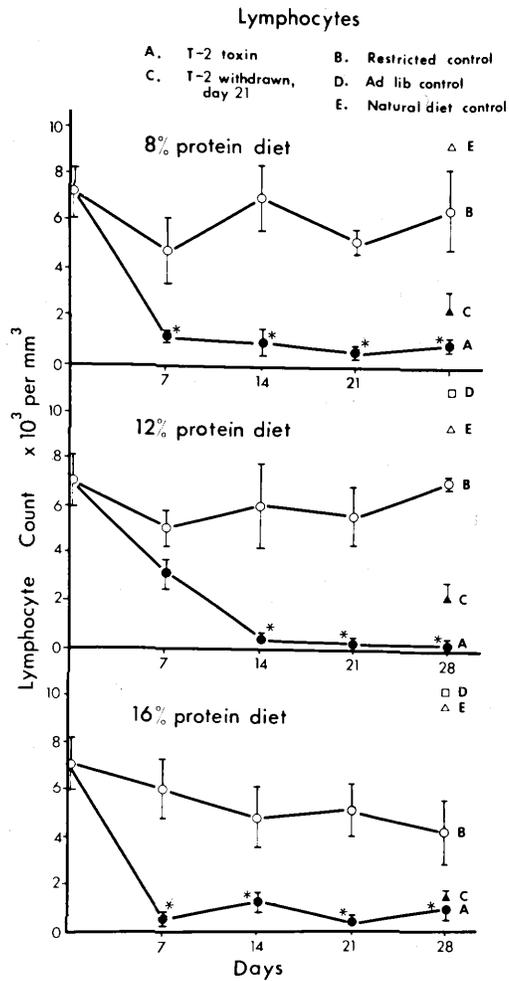


Fig. 39

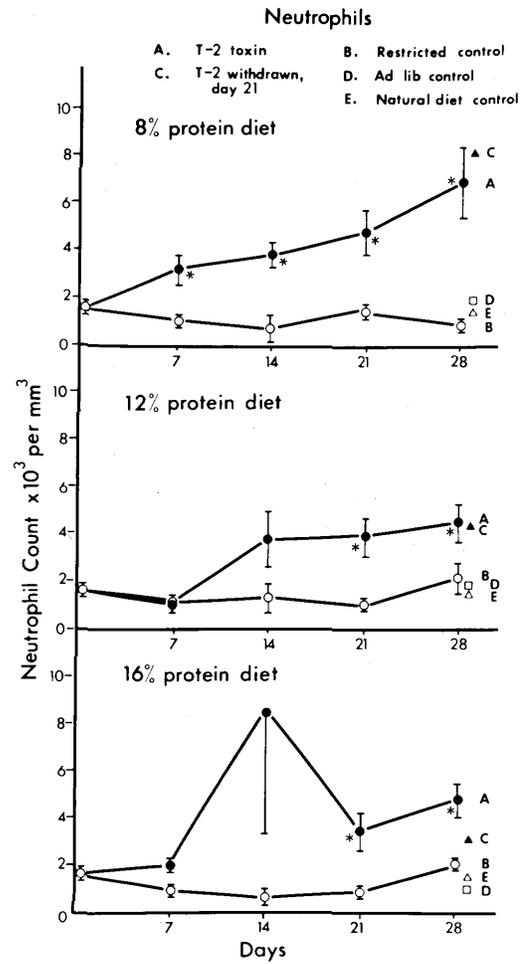


Fig. 40

Fig. 39: Sequential changes in lymphocyte counts of mice fed T-2 toxin (20 ppm).

Fig. 40: Sequential changes in neutrophil counts of mice fed T-2 toxin (20 ppm).

Values are means (+SEM) of separate groups of 4 mice. Means in group A which differ at  $p = 0.05$  from the corresponding mean in group B are indicated by asterisks.

Table 30 : Sequential changes in eosinophil counts per  $\mu$ l of peripheral blood of mice consuming T-2 toxin (20 ppm) for 6 weeks in a semipurified diet containing 16% protein.

Weeks on diet	Group				
	A 20 ppm T-2 toxin	B Restricted control	C Toxin withdrawn	D Ad lib control	E Pellet control
0	--	--	--	--	110 $\pm$ 40
1	60 $\pm$ 40 <sup>a</sup>	260 $\pm$ 110 <sup>a</sup>	--	--	--
2	0 <sup>a</sup>	160 $\pm$ 210 <sup>b</sup>	--	--	--
3	0 <sup>a</sup>	210 $\pm$ 90 <sup>b</sup>	--	--	--
4	10 $\pm$ 10 <sup>a</sup>	90 $\pm$ 60 <sup>a</sup>	40 $\pm$ 30 <sup>a</sup>	110 $\pm$ 50 <sup>a</sup>	110 $\pm$ 40 <sup>a</sup>
6	0 <sup>a</sup>	480 $\pm$ 280 <sup>b</sup>	--	--	--

<sup>a,b</sup> Values are means ( $\pm$  SEM) of separate groups of 4 mice. At each week, means followed by the same letter are not significantly different ( $p > 0.05$ ).

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 had atrophied during the first 7 days, such that on days 7 and 14, it was pale, tan, and greatly reduced in size. By day 28, two mice on the 16% protein diet had enlarged spleens due to proliferation of greyish-red, homogeneous tissue throughout the red pulp, and by day 41, extreme splenomegaly was found 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 consuming T-2 toxin was dark red on days 7 and 14, but as mice became visibly anemic, the marrow cavities appeared pale tan-yellow. Progressively increasing, papillary 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 (Fig. 34). Ulcerated areas, 1-2 mm in diameter, were sometimes present on the hyperplastic mucosa.

After withdrawal of the T-2 toxin from the diets of mice in group C, the thymus and spleen enlarged, with the latter becoming dark red and focally nodular, and bone marrow became distinctly red and cellular.

#### 8.4.3.2 Organ weights

The stomach progressively enlarged due to hyperkeratosis in all mice exposed to T-2 toxin. The degree of enlargement was not related to the dietary protein level (Table 73, Appendix C). The liver was enlarged, both absolutely ( $p < 0.001$ ), and relatively ( $p < 0.001$ ), in mice consuming T-2 toxin, in comparison with mice in group B. The degree of hepatic

enlargement on day 28 was significantly greater for the higher levels of dietary protein (Fig. 41; Table 74, Appendix C ).

The spleen in all mice on T-2 toxin was initially smaller than in mice on the corresponding restricted control diets, but by day 28, splenic weights of mice on the 16%-protein diet had increased (Fig. 42A,B). By day 41, the spleens of mice consuming T-2 toxin were greatly enlarged. Splenic weights were higher in mice in group C for each of the three diets 7 days after withdrawal from dietary T-2 toxin (Fig.42A). Thymic weights were greatly reduced by day 7 in mice exposed to T-2 toxin, and remained small throughout the experiment (Table 31). In mice fed on the control diets at the restricted rate (group B), there was a less severe and more gradual thymic atrophy. By day 28 the thymus was more severely atrophic in mice on the 16%-protein control diet than in those on the lower protein control diets (Table 31). By comparison, thymic weights in the ad lib fed control groups increased during the trial (Table 31). Testicular weights varied in proportion with body weights both in the controls and the treated mice, with no significant effects of toxin or diet on relative testicular weight (Table 74, Appendix C ). Mean values of all organ weights are given in Tables 73 to 75 in Appendix C ).

#### 8.4.3.3 Microscopic findings

During the first 3 weeks, similar histological and cytological changes were observed in mice consuming T-2 toxin in each of the three diets. Subsequently, there were differences among the diet groups in the rates of regeneration of tissues depleted by T-2 toxin. The changes observed in response to T-2 toxin in the 16%-protein diet will be described first, followed by the differences among the diet groups.

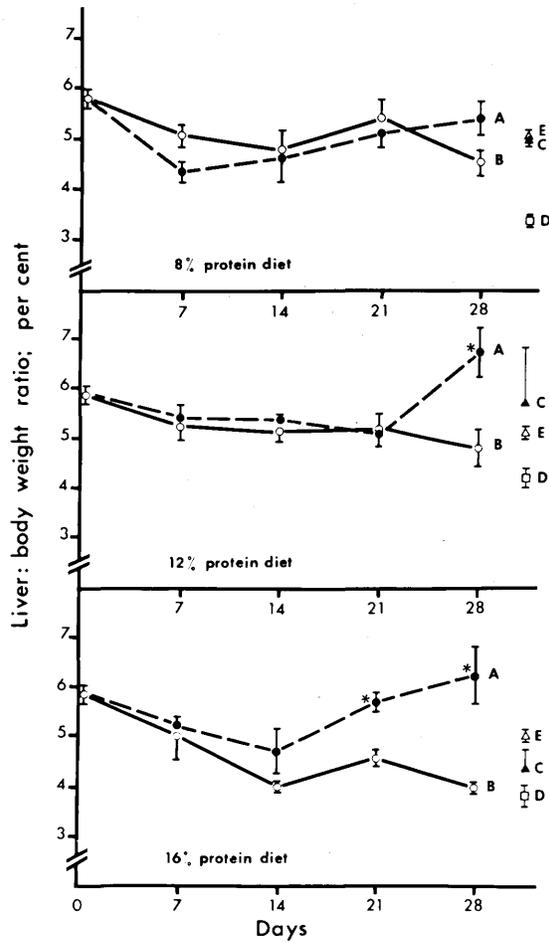


Fig. 41: Sequential changes in relative hepatic weights of mice fed different diets containing 20 ppm of T-2 toxin (A). Control groups (B) were fed toxin-free diets at the same rate. For comparison, 28-day values for ad lib-fed controls (D), natural-ingredient diet fed controls (E), and mice removed from the toxic diets on day 21 (C) are shown. Each point is the mean (+SEM) of a separate group of 4 mice. Values in group A that are significantly greater than the corresponding control group are indicated by asterisks.

Figure 42

- A. Sequential changes in relative splenic weights of mice fed different diets containing 20 ppm T-2 toxin (A). Control groups (B) were fed toxin-free diets at the same rate. For comparison, 28-day values for ad lib fed controls (D), natural-ingredient-diet fed controls (E), and mice removed from the toxic diet on day 21 (C) are shown. Each point is the mean (+SEM) of a separate group of 4 mice. Values in group A significantly different from the corresponding group are indicated by asterisks.

- B. Sequential changes in relative splenic weight of mice on the 16% protein diet. Note the extreme splenomegaly by day 42 in mice on T-2 toxin.

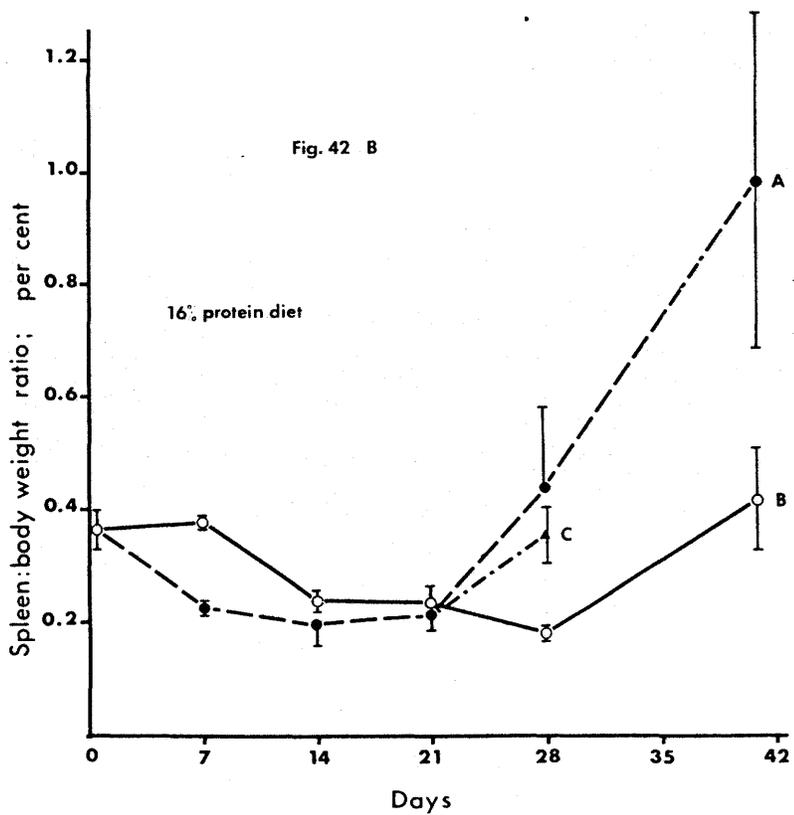
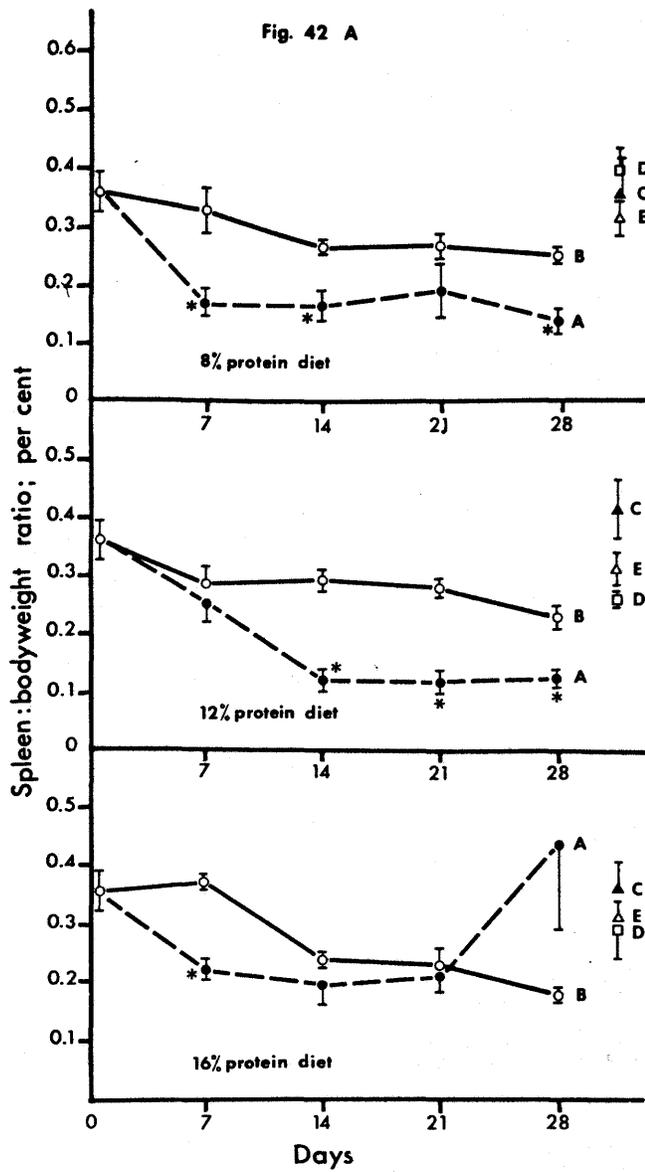


Table 31 : Relative and absolute thymic weights of young Swiss mice fed semipurified diets containing T-2 toxin (20 ppm) and different levels of protein.

Variable	Dietary protein level	Group				
		A 20 ppm T-2 toxin	B Restricted control	C Toxin withdrawn	D Ad lib control	E Pellet control
Relative thymic weight; % of body weight	8%	0.04±0.03 <sup>*a</sup>	0.13±0.02 <sup>b</sup>	0.05±0.02 <sup>a</sup>	0.19±0.02 <sup>b</sup>	0.17 <sup>b</sup>
	12%	0.03±0.02 <sup>a</sup>	0.08±0.01 <sup>a</sup>	0.01±0.00 <sup>a</sup>	0.26±0.02 <sup>b</sup>	0.17 <sup>c</sup>
	16%	0.05±0.01 <sup>a</sup>	0.03±0.01 <sup>a</sup>	0.09±0.03 <sup>a</sup>	0.20±0.02 <sup>b</sup>	0.17 <sup>b</sup>
Diet groups combined		0.04±0.01 <sup>a</sup>	0.08±0.01 <sup>b</sup>	0.06±0.01 <sup>ab</sup>	0.22±0.01 <sup>c</sup>	0.17±0.02 <sup>d</sup>
Absolute thymic weight; mg.	8%	4.0±2.5 <sup>a</sup>	22.5±4.0 <sup>b e</sup>	6.5±1.9 <sup>a</sup>	64.7±9.6 <sup>c</sup>	58.8 <sup>c</sup>
	12%	3.5±2.5 <sup>a</sup>	14.0±2.4 <sup>b e f</sup>	1.7±0.3 <sup>a</sup>	91.0±10.1 <sup>c</sup>	58.8 <sup>c</sup>
	16%	6.5±1.5 <sup>a</sup>	6.0±2.0 <sup>a f</sup>	13.3±2.7 <sup>a</sup>	70.0±12.3 <sup>b</sup>	58.8 <sup>b</sup>
Diet groups combined		4.7±1.2 <sup>a</sup>	14.2±2.6 <sup>b</sup>	7.6±1.8 <sup>a</sup>	75.3±6.6 <sup>c</sup>	58.8±8.5 <sup>c</sup>

\* Values are means (±SEM) of separate groups of 4 mice.

a,b,c,d In any horizontal row, means followed by the same letter, or by no letter, do not differ at p = 0.05, by SNK multiple range test.

e,f In any vertical column of 3 means, those followed by the same letter, or by no letter in this position, do not differ at p = 0.05 by SNK multiple range test.

The thymus of mice in the ad lib fed control groups contained normal dense populations of lymphocytes, and there were no differences between mice on the semipurified and the natural ingredient diets. The thymus of control mice on restricted intake (Group B) appeared normal on day 7 (Fig. 43A, 43B), but after 3 and 4 weeks, moderate depletion of cortical lymphocytes without granulocytic infiltration was observed in these control mice. Thymic atrophy did not occur in control mice in groups D and E. In mice fed T-2 toxin, lymphocytes disappeared from the thymic cortex within 7 days (Fig. 43C, D), at which stage neutrophils and eosinophils had infiltrated the medulla and some of the depleted regions of the cortex (Fig. 43D). The thymic cortex was reduced to a small residuum of reticular cells and epithelial cells (Fig. 43E, F). Granulocytes were not observed in the atrophic thymic cortices or medulla after day 14 (Fig. 43F), although the thymus remained atrophic throughout the 6-week trial.

Seven days after withdrawal of T-2 toxin from the diet, lymphoblastic proliferation was evident in the thymic cortex and medulla (Fig. 44A, B, C).

Thymic-dependent peripheral lymphoid populations, including periarteriolar sheaths in the spleen (Fig. 45A, B), paracortical regions of lymph nodes (Fig. 45C-F), and intraepithelial lymphocytes of the small intestine (theliolymphocytes) (Fig. 46A-D), appeared normal in control group B, but became depleted during the first two weeks on the toxic diets and remained atrophic throughout the trial.

Follicular activity in lymph nodes and spleen was not affected in the restricted-intake control group (B) (Fig. 45C, D), but it diminished in mice consuming T-2 toxin. By day 28, follicular activity was still

Figure 43

Effect of dietary T-2 toxin (20 ppm) on the thymus of young mice fed a 16%-protein diet.

- A. Low power microscopic appearance of a control mouse fed the 16% protein diet at the restricted rate for 14 days (group B). The cortex is densely cellular. x 30.
- B. Higher magnification of the thymic cortex and medulla of the mouse in A. x 120.
- C. Appearance of atrophic thymus with hypocellular, poorly demarkated cortex of a mouse fed T-2 toxin for 7 days. x 30
- D. High magnification of hypocellular thymic cortex from the mouse in C above. The cortex is populated by epithelial cells, vascular stromal cells, and neutrophils (arrows). Very few mature lymphocytes are evident. x 300.
- E. Appearance of the thymus of a mouse fed T-2 toxin for 14 days. The medulla contains decreased numbers of lymphocytes, whereas the cortex is totally depleted. Compare with B above. x 120.
- F. High magnification of completely atrophic thymic cortex of a mouse after 14 days on T-2 toxin. Note the enlarged thymic epithelial cells (arrows), and the absence of lymphocytes and granulocytes. x 300.

Hematoxylin and eosin. Magnification factors are indicated in each legend.

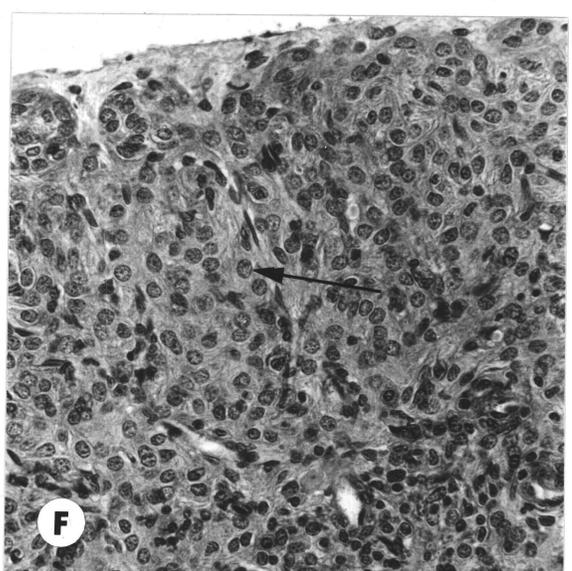
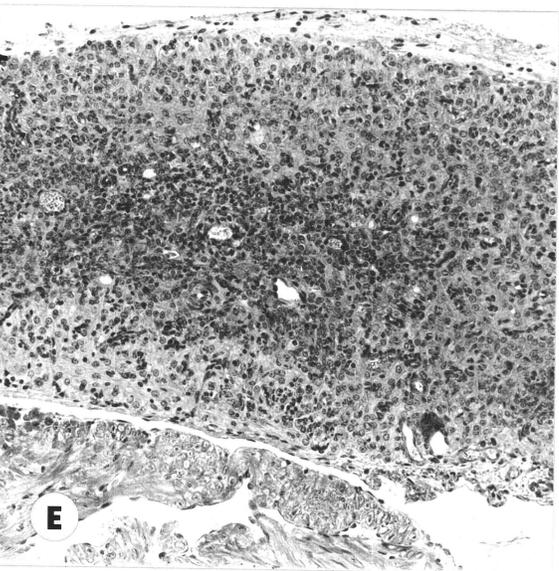
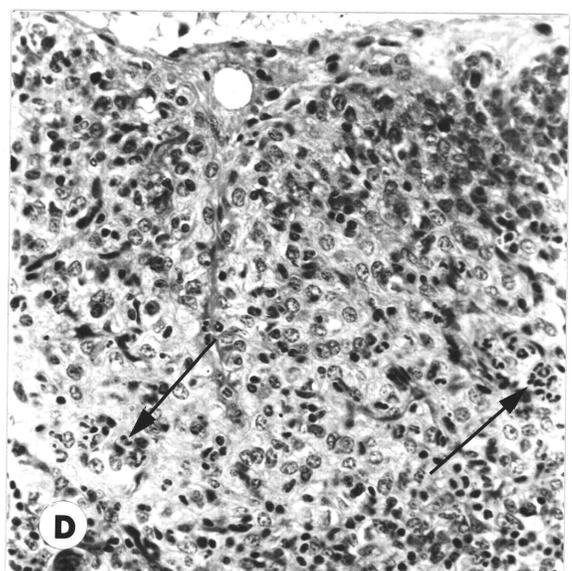
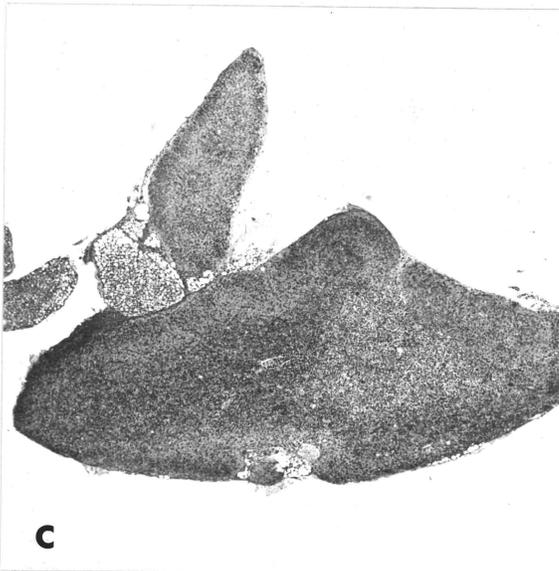
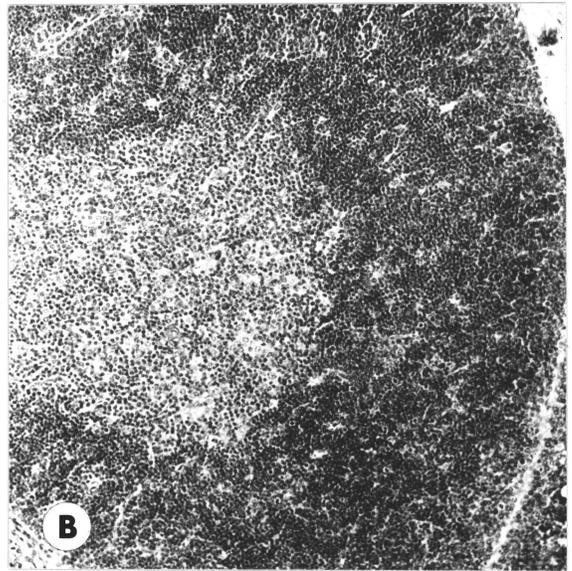


Fig. 43



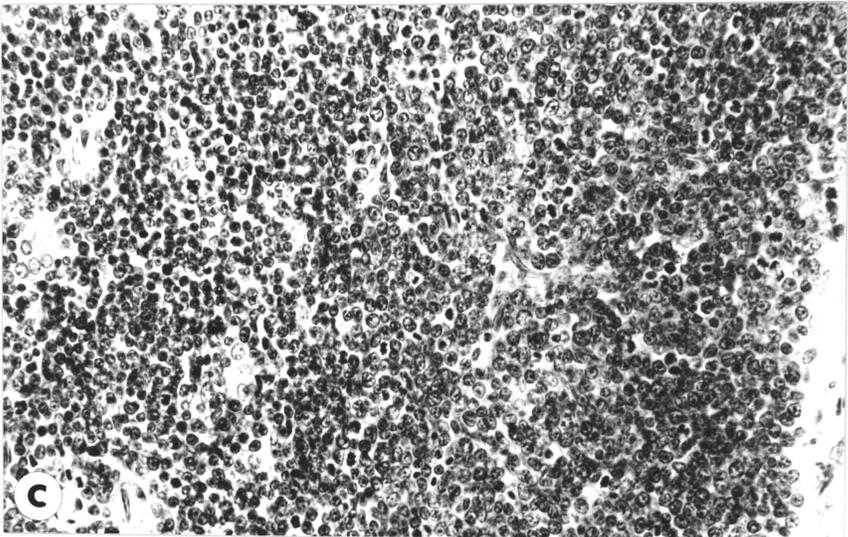
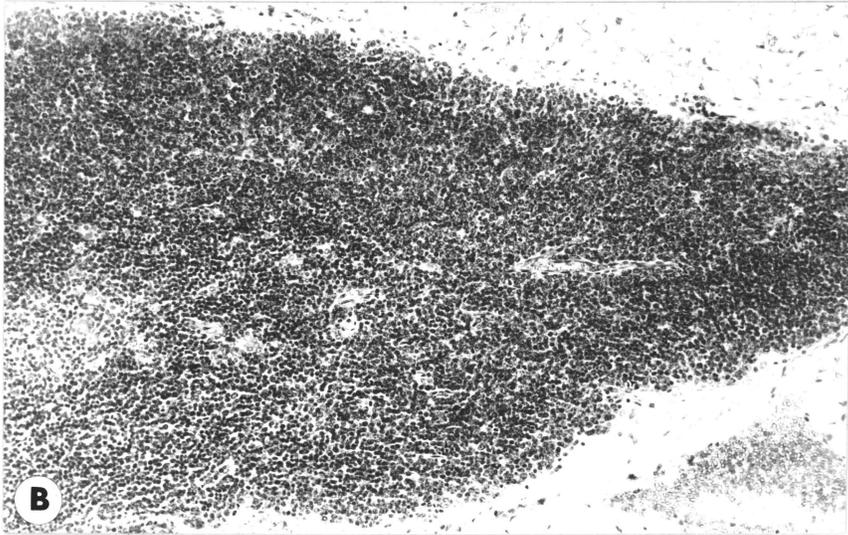
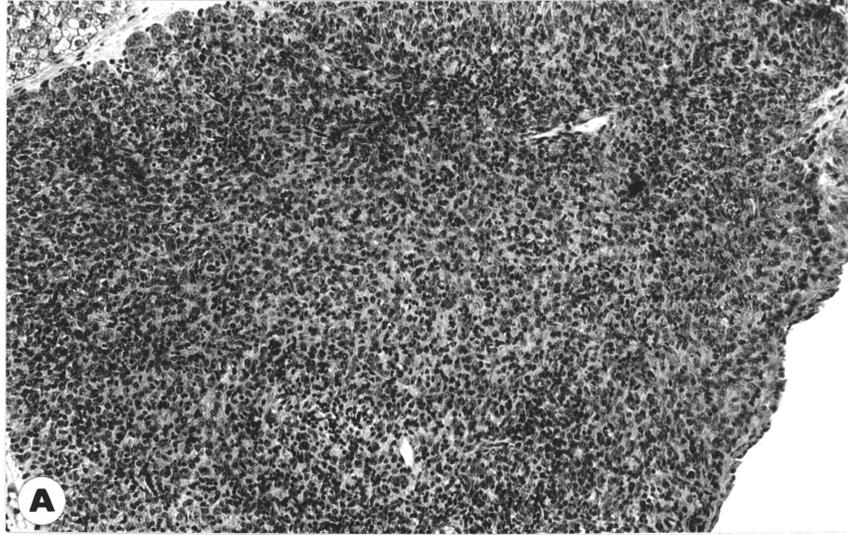


Fig. 44

Figure 45

Effect of dietary T-2 toxin (20 ppm) on lymphoid tissues of mice fed a 16%-protein diet.

- A. Normally populated periarteriolar lymphoid sheath in the spleen of a mouse fed the control diet at a restricted rate for 6 weeks. x 190.
  
- B. Depleted splenic lymphoid sheath after 6 weeks on the toxic diet. x 480.
  
- C. Normal mesenteric lymph node from control mouse in group B. Day 28. x 120.
  
- D. Normal germinal centre in the lymph node illustrated in Fig. 45C. x 300.
  
- E. Atrophic mesenteric lymph node from a mouse fed T-2 toxin for 4 weeks. Note the greatly reduced populations in the medullary cords and paracortex. x 120.
  
- F. Higher magnification of the cortex of the atrophic lymph node illustrated in Fig. 45E. Note the germinal centre with mitotic figures (arrow). Perifollicular and paracortical lymphoid populations are reduced. Compare with Fig. 45D. x 300.

Hematoxylin and eosin. Magnification factors indicated in each legend.

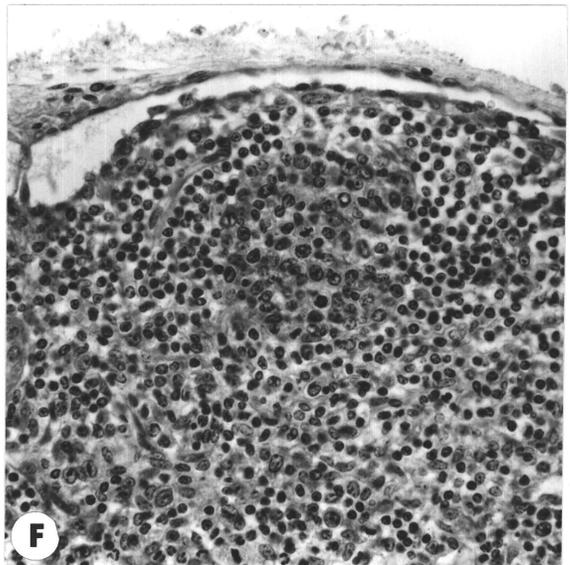
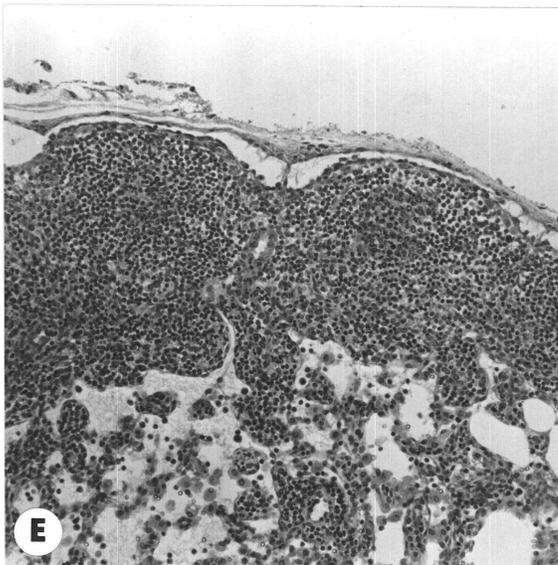
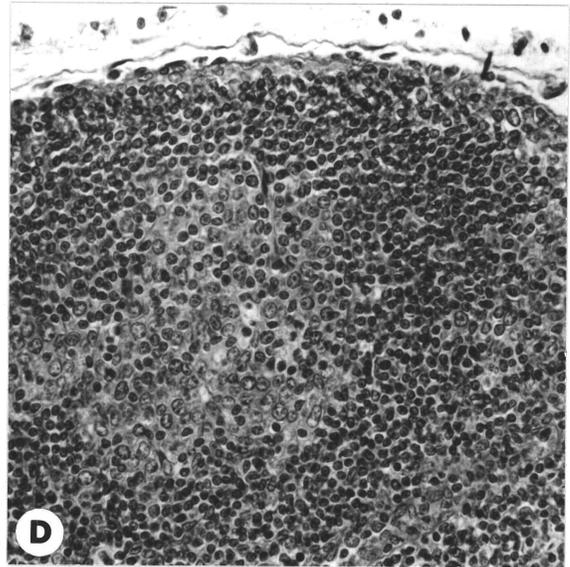
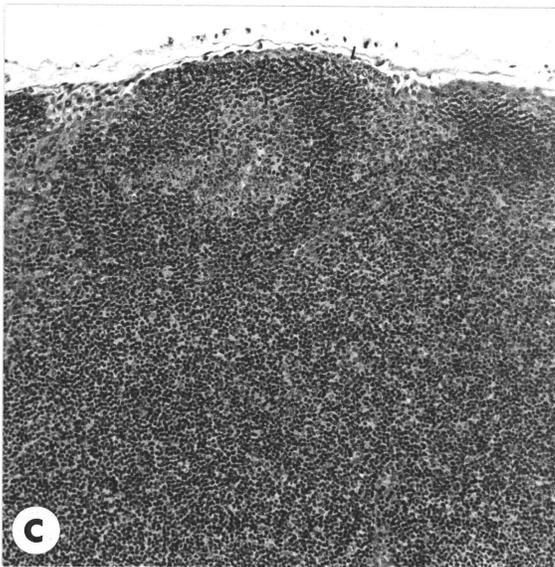
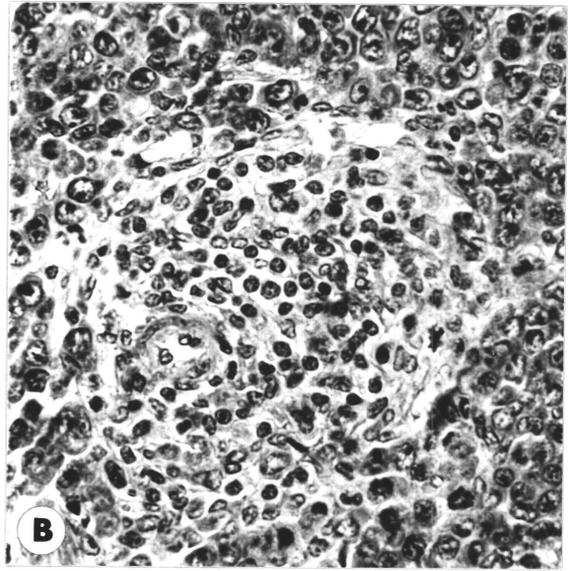
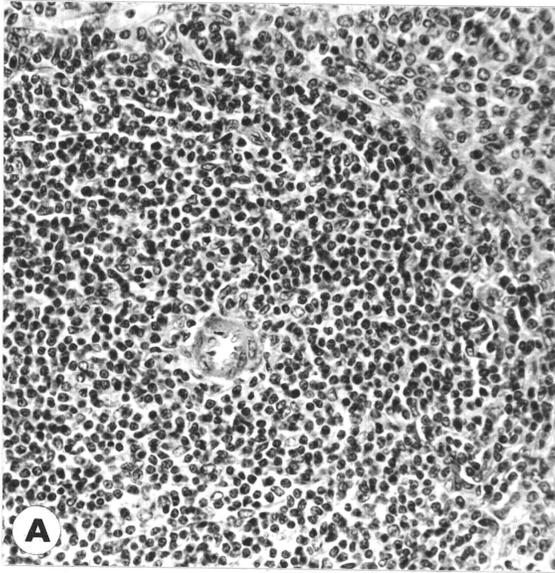


Fig. 45

Figure 46

Effect of dietary T-2 toxin (20 ppm) on populations of lymphoid cells in the intestinal lamina propria of mice fed on a 16%-protein diet.

- A. Normal jejunal mucosa of a mouse after 21 days on the control diet fed at a restricted rate (group B). Note the presence of intra-epithelial theliolymphocytes (arrows) and mononuclear cells in the lamina propria of the villi.  
x 190.
  
- B. Jejunal mucosa of a mouse after 21 days on a diet containing T-2 toxin. Note the absence of mononuclear cells in the lamina propria, and in the epithelium. The epithelium is hyperplastic. Cytolysosomes are present in the crypt epithelial cells (arrows).  
x 190.
  
- C. High magnification appearance of villi from the duodenum of a mouse after 6 weeks on a diet containing 20 ppm T-2 toxin. Few intraepithelial lymphocytes are present (arrows).  
x 300.
  
- D. Mitotic activity (arrow) in epithelial lymphocytes 7 days after withdrawal of T-2 toxin from the diet (group C).  
x 190.
  
- E. Mitotic activity (arrow) in the intestinal lamina propria of a mouse 7 days after withdrawal of T-2 toxin from the diet (group C).  
x 190.

Hematoxylin and eosin; magnification factors indicated in each legend.

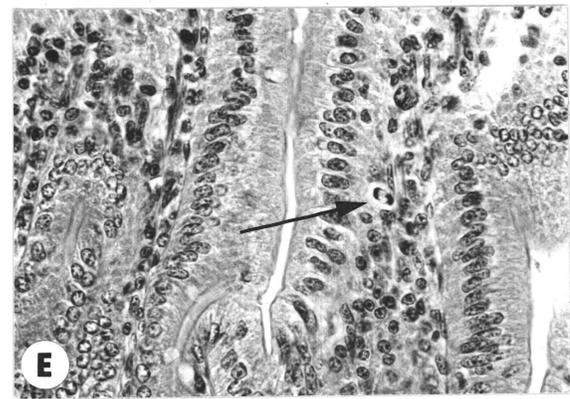
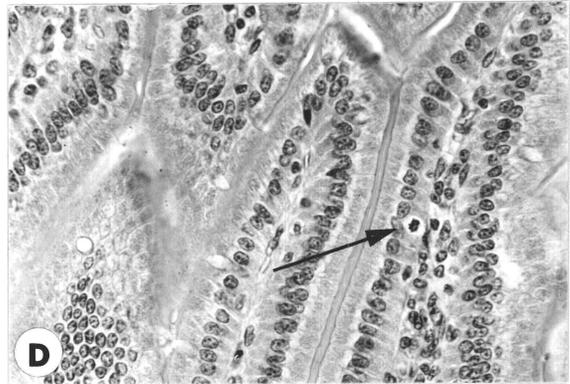
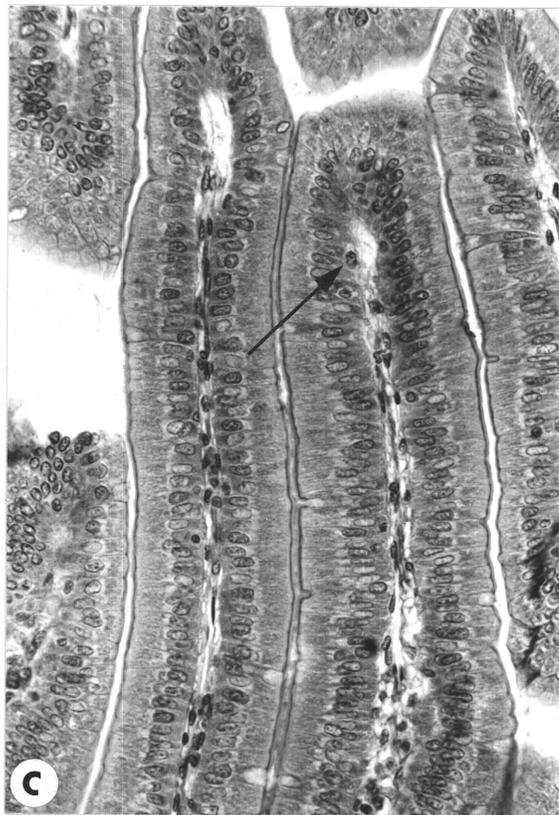
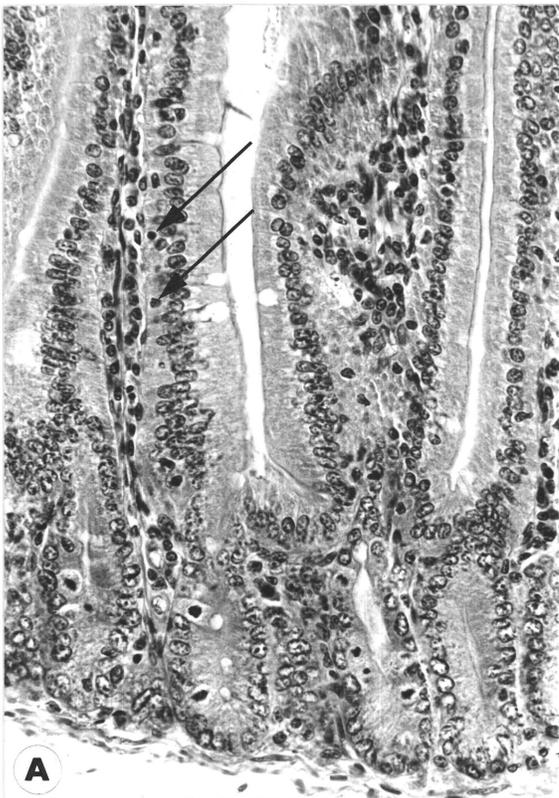


Fig. 46

evident in lymph nodes of most treated mice (Fig. 45E, F). Numbers of lymphocytes and plasma cells in the intestinal lamina propria (Fig. 46B), medullary cords of lymph nodes (Fig. 45E), and splenic cords were decreased in all mice fed T-2 toxin. Seven days after withdrawal of T-2 toxin from the diet, lymphoblastic proliferation was observed in the lymphoid follicles, medullary regions of the lymph nodes, intestinal epithelium (Fig. 46D), and intestinal lamina propria (Fig. 46E).

The splenic red pulp of mice in control group B appeared normally active (Fig. 47A, B), but mice consuming T-2 toxin were devoid of erythropoietic islands by day 14 (Fig. 47C, D). The red pulp was then reduced in size (Fig. 47C) and depleted of other hematopoietic activity (Fig. 48A). Hemosiderin in macrophages in the red pulp was visible when hematopoietic cells had disappeared, but amounts increased after day 14 (Fig. 48A). 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, many of which were in mitosis, and with limited amounts of pale-staining poorly demarkated cytoplasm. In some areas, a progressive sequence of nuclear indentation and elongation, indicating myelocytic differentiation, was observed (Fig. 48C). Large numbers of mature neutrophils were found in the red pulp of most mice on the toxic diets for more than 2 weeks (Fig. 48D). Megakaryocytes were also present in spleens in which myelopoiesis was active (Fig. 48C, D).

In mice with splenomegaly on days 28 and 41, undifferentiated cells were densely packed throughout the entire red pulp and many had round

Figure 47

Atrophy of splenic red pulp in mice fed T-2 toxin (20 ppm)  
in a 16%-protein diet for 14 days.

- A. Control mouse, group B.  
x 48 .
  
- B. Control mouse, group B.  
x 300.
  
- C. Treated mouse, group A.  
Note the marked atrophy of both red and white pulp.  
Lymphoid atrophy is less extreme at this stage.  
x 48.
  
- D. Treated mouse, group A.  
Note the depleted red pulp (R) and white pulp (W).  
Hematopoietic activity has disappeared from the red pulp, but  
there are some large undifferentiated cells present (arrows),  
some of which are in mitosis.  
x 300.

Hematoxylin and eosin; magnification factors are given in  
each legend.

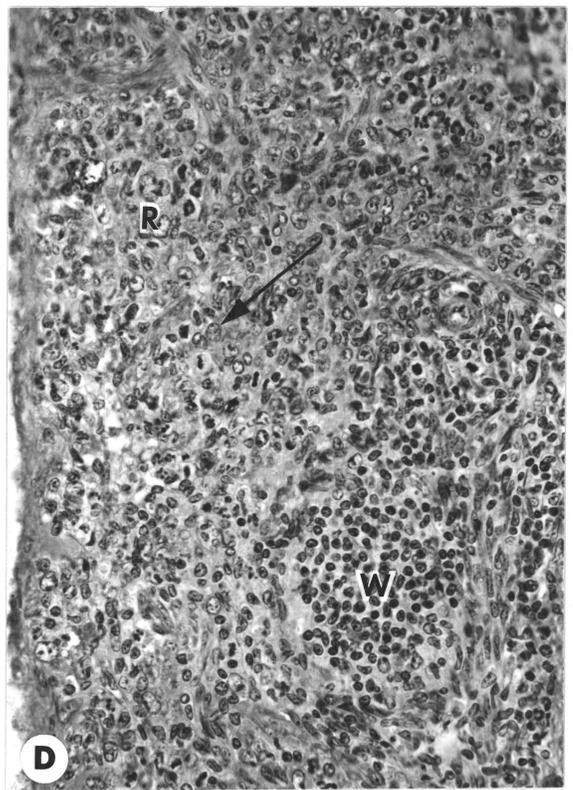
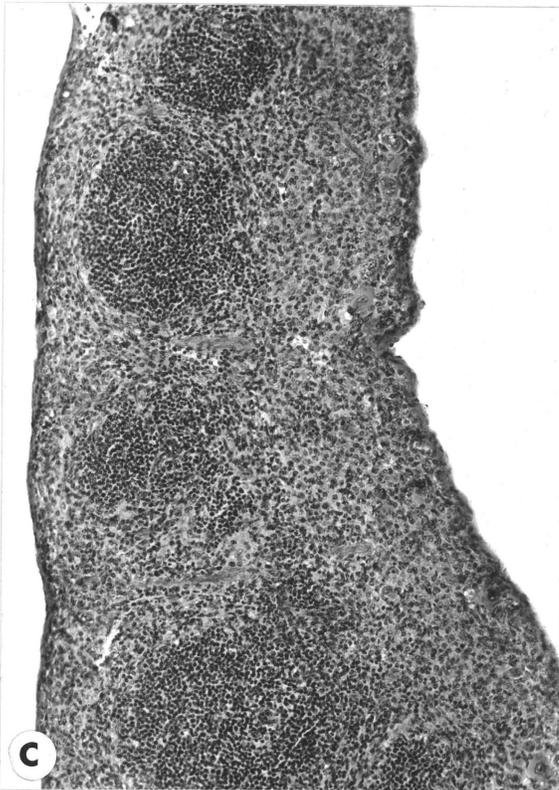
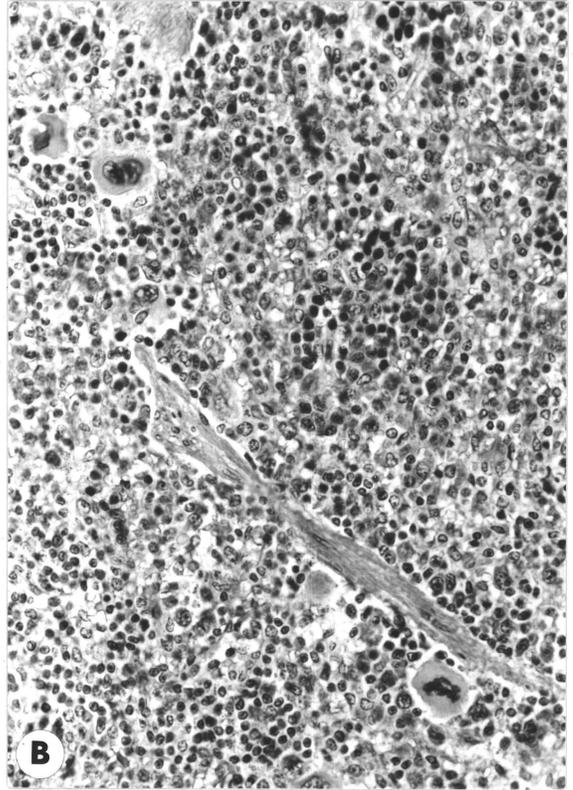
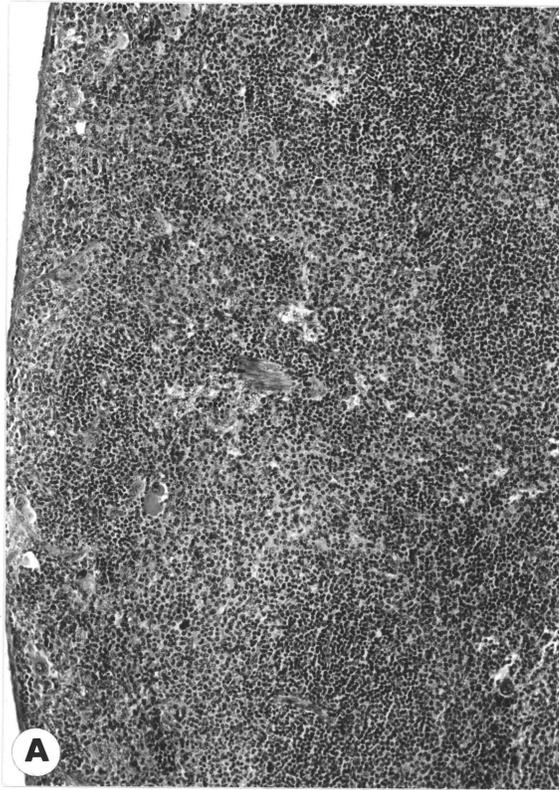


Fig. 47

nuclei and somewhat eosinophilic cytoplasm (Fig. 48E). These were identified as immature erythroid cells because they were accompanied by later stages with pyknotic nuclei (Fig. 48E). Numerous normal erythroblastic islands were observed in the splenic red pulp of mice in groups C (Fig. 48F).

The bone marrow in all control groups appeared normal, both histologically (Fig. 49A, 49B) and cytologically (Fig. 50A, 51A). During exposure to T-2 toxin, bone marrow initially became hypocellular (Fig. 49C), but then became repopulated during the latter half of the 6 weeks (Fig. 49D, 49E). Erythroblastic islands were not recognizable in sections of bone marrow from mice on T-2 toxin, but reappeared 7 days after toxin was withdrawn (Fig. 49F). On days 14 and 21, few red cell precursors were observed on smears of bone marrow from treated mice (Fig. 50B-D), and those remaining were abnormal (Fig. 50D). As the marrow repopulated during the last 3 weeks, erythroid cells became more frequent, but maturation remained abnormal. By day 28, immature stages (rubriblasts and prorubricytes) predominated (Fig. 50E, 50F). By day 41, erythropoiesis had resumed in all four mice still on T-2 toxin, but nuclei of some metarubricytes were fragmented (Fig. 51C). Both rubricytes and metarubricytes exhibited megaloblastic change (Fig. 51C, 51D), and less frequently, basophilic stippling of cytoplasm (Fig. 51D). By comparison, most regenerating erythroid cells were normal 7 days after withdrawal of T-2 toxin from the diet (Fig. 51E, 51F).

Myeloid populations in hypocellular bone marrow regenerated more rapidly than did erythroid populations. Between 2 and 4 weeks, mice on

Figure 48

Regeneration of hematopoietic populations in atrophic splenic red pulp during continuous exposure to dietary T-2 toxin (20 ppm).

- A. Atrophic red pulp on day 41. The red pulp (R) is devoid of hematopoietic activity. Some macrophages contain hemosiderin (arrow). Note the depleted periarteriolar lymphoid cuff (W).
  
- B. Clone of undifferentiated cells with mitotic activity (arrow) in the atrophic red pulp. Day 14.
  
- C. Proliferating undifferentiated cells in the red pulp. At this stage, both megokaryacytic (arrow) and myeloid (arrowhead) differentiation are evident. Day 21.
  
- D. Extreme infiltration of splenic red pulp by undifferentiated cells. Most of these are probably myeloblasts because they have pale cytoplasm. Many metamyelocytes (arrow) and segmented stages are present (arrowhead). Day 28.
  
- E. Infiltration of splenic red pulp by undifferentiated rubriblasts. These cells are responsible for splenomegaly on days 28 and 41. Many small, densely nucleated cells (arrows) are probably post-rubricyte stages. Note how the immature stages predominate. Day 41.
  
- F. Appearance of red pulp 7 days after removal of T-2 toxin from the diet of a mouse in group C. Note the colonies of differentiating erythroid cells with dense, small nuclei (circles). Some granulopoiesis is evident (arrow). Fewer immature stages are present compared with mice in C, D and E above. Day 28.

Hematoxylin and eosin; x 300.

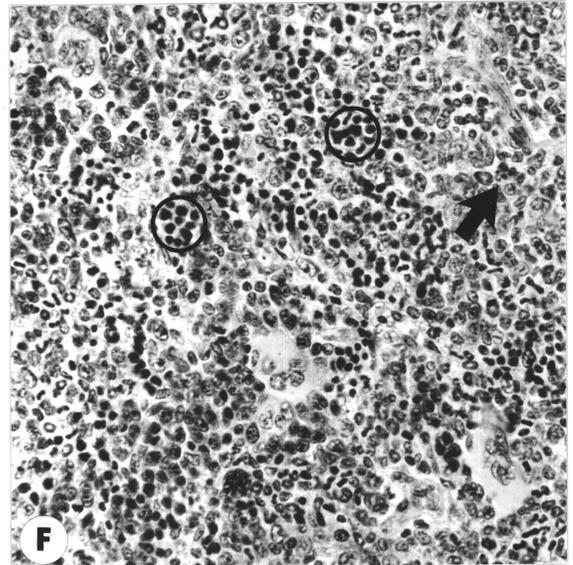
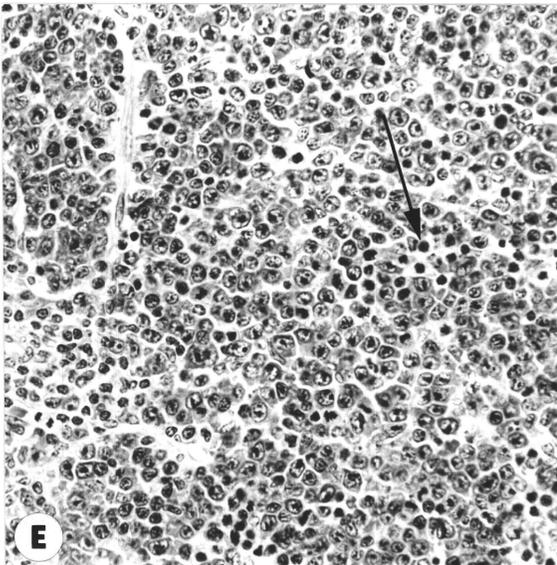
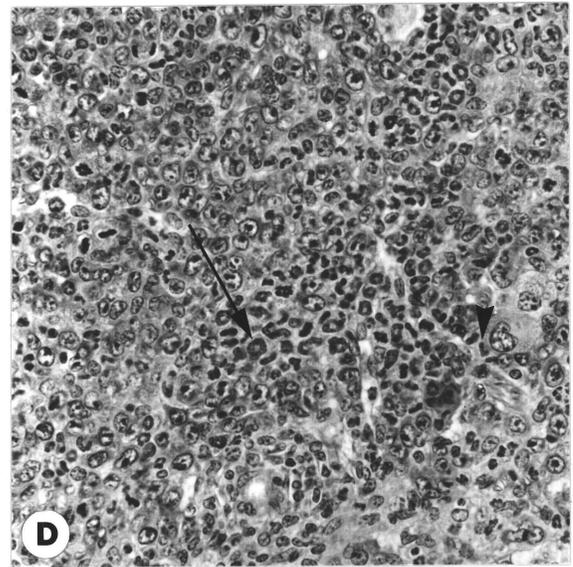
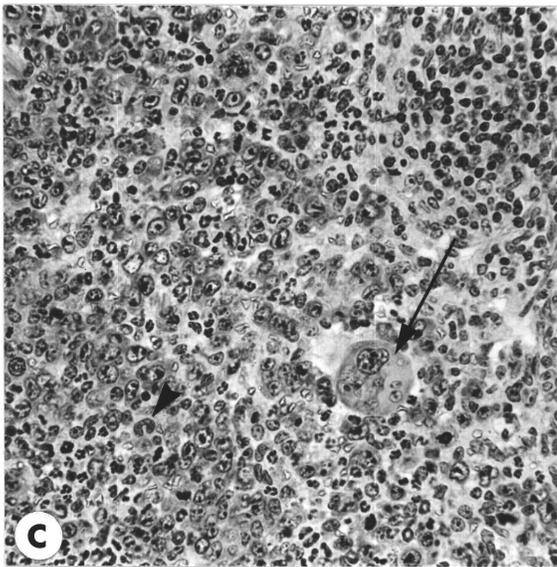
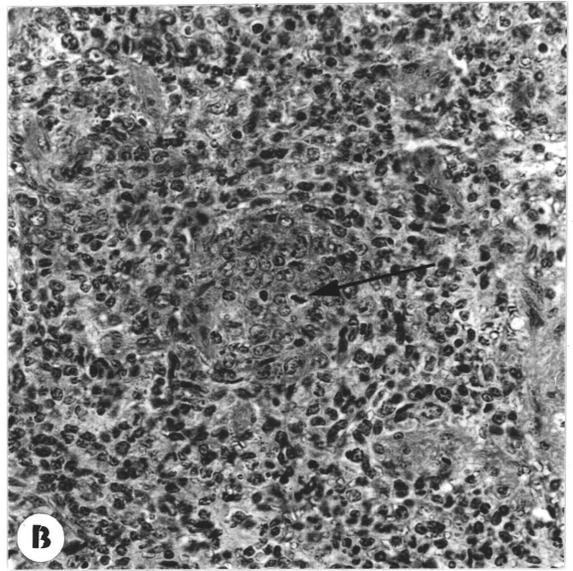
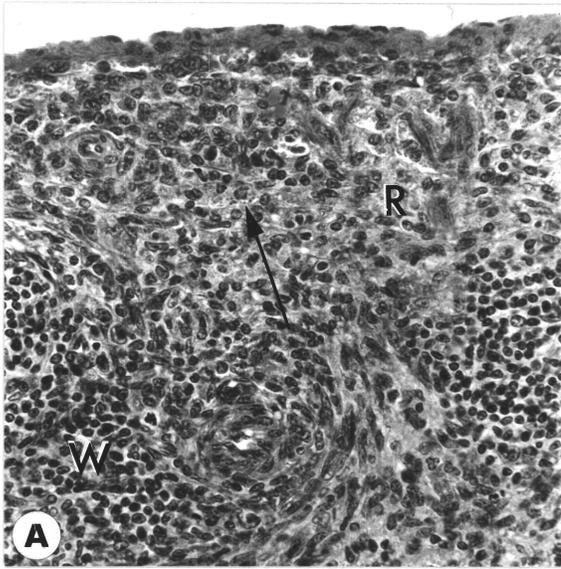


Fig. 48

Figure 49

Histological appearance of tibial bone marrow  
of mice after various periods of consumption of  
semipurified diets containing T-2 toxin (20 ppm).

- A. Normal appearance of marrow of a control mouse (group B) on a 16%-protein diet. Day 28.
  
- B. Normal appearance of marrow of a control mouse (group B) on an 8%-protein diet. Day 28.
  
- C. Atrophic bone marrow after 3 weeks on a 16%-protein diet containing T-2 toxin.  
Note the extreme hypocellularity.  
The remaining cells are members of the granulocytic and megakaryocytic series.
  
- D. Focal regeneration of bone marrow after 3 weeks on a 16%-protein diet containing T-2 toxin.  
Most of the regenerating foci are composed of members of the granulocytic series.
  
- E. Increased cellularity of regenerating bone marrow of a mouse after 6 weeks on a 16%-protein diet containing T-2 toxin.
  
- F. Cellular bone marrow of a mouse in group C that was removed from T-2 toxin on day 21 and placed on the control diet.  
Note the dense erythropoietic islands (circles).

Hematoxylin and eosin; x 120.

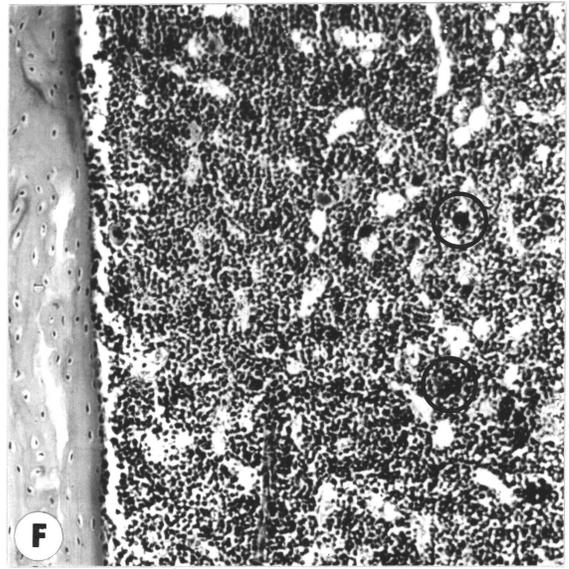
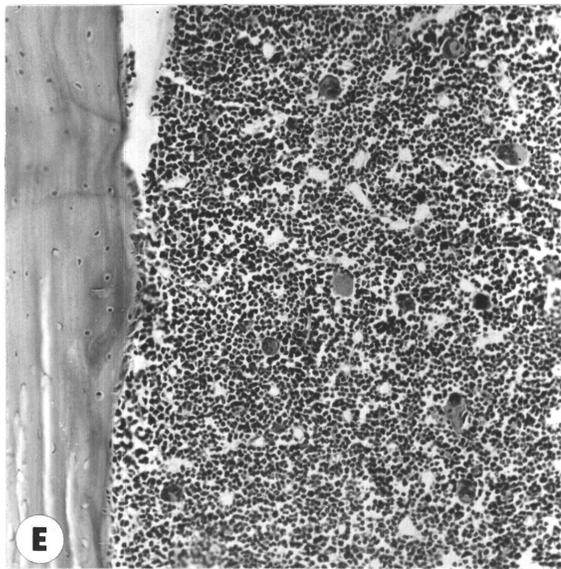
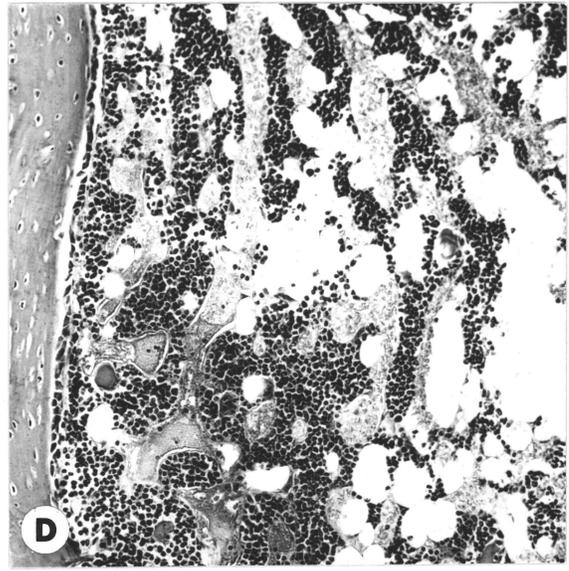
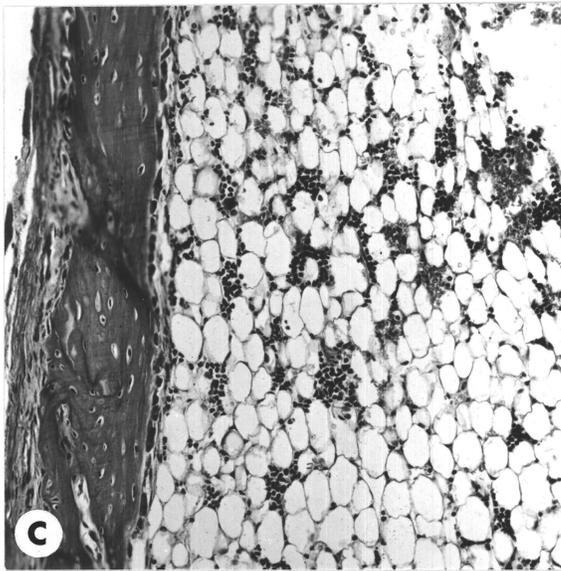
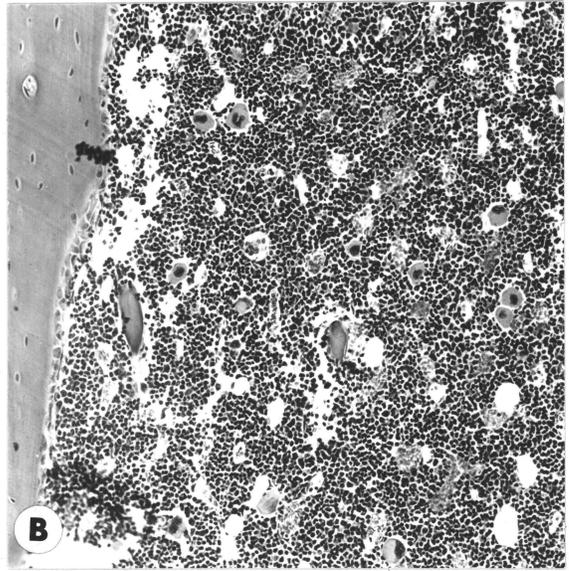
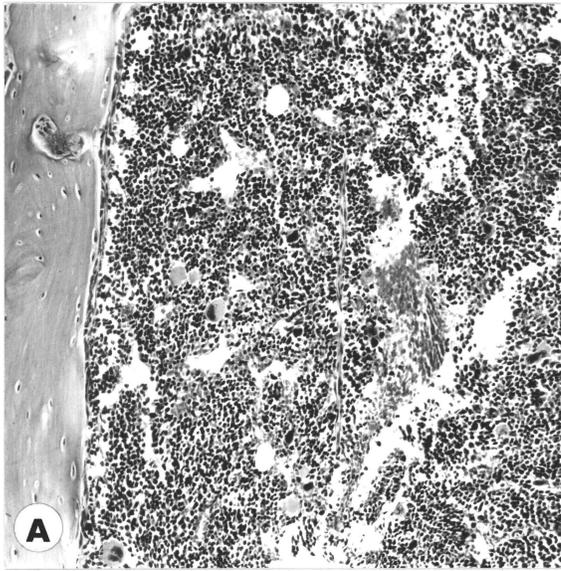


Fig. 49

T-2 toxin exhibited active, although reduced, granulopoiesis (Fig. 50C). However, the proportion of segmented stages was lower in treated mice than in the controls. Eosinophilic granulocytes were rarely present in treated mice. Numbers of megakaryocytes increased proportionally as neutrophilic myeloid populations regenerated.

Erythroid regeneration in bone marrow occurred less frequently in mice consuming T-2 toxin in diets of lower protein content. The degree of proliferation of immature erythroid cells in splenic red pulp was similarly diet-related. Seven days after mice in group C were placed on toxin-free diets, erythropoiesis in the spleen and bone marrow, and lymphopoiesis in the thymus, lymph nodes, spleen, and intestinal lamina propria had resumed at various degrees unrelated to the dietary protein level.

Perioral dermatitis caused by 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 at the tip and infiltrated by many neutrophils (Fig. 52). Histological changes in the lower intestinal tract were minimal except for the depletion of lymphoid cells in the lamina propria and intestinal epithelium (Fig. 46B). Villi of the small intestine were of normal length and conformation, and differentiation of epithelial cells in intestinal crypts appeared moderately hyperplastic. A few, round, intracytoplasmic bodies resembling cytolysosomes (Fig. 46B) were observed in the epithelial cells at the base of the crypts of some mice consuming T-2 toxin,

Figure 50

Effect of dietary T-2 toxin (20 ppm) in a 16%-protein semipurified diet on the cytological appearance of bone marrow of mice.

- A. Control mouse, group B, day 21.  
Note the numerous densely nucleated erythroid precursors (arrow).  
x 300.
- B. T-2 treated mouse, group A, day 21.  
The marrow is hypocellular and is composed mostly of fat (F), granulopoietic cells (G), and megakaryocytes (M).  
No members of the erythroid series are present.  
x 300.
- C. T-2 treated mouse, group A, day 14.  
This mouse has proliferating immature myeloid cells (arrows) and many segmented neutrophils, but no erythroid cells.  
x 300.
- D. T-2 treated mouse, group A, day 21.  
The bone marrow is populated mostly by myeloid cells, including progranulocytes (P), myelocytes (M), metamyelocytes (T) and mature polymorphs.  
One abnormal immature rubricyte with excessively hemoglobinized, stippled cytoplasm is present (arrow).  
x 750.
- E. T-2 treated mouse, group A, day 28.  
Myelopoiesis is active, but at this stage, many immature rubriblasts and prorubricytes are present (arrows).  
Few later stages are visible.  
x 300.
- F. Higher magnification of the immature prorubricytes in bone marrow shown in E above.  
x 1200.

Wright's-Giemsa stained impression smears of tibial bone marrow. Magnification factors are indicated in each legend.

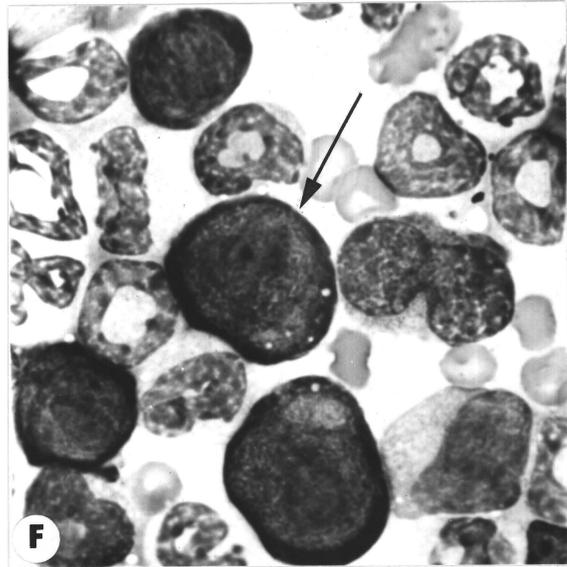
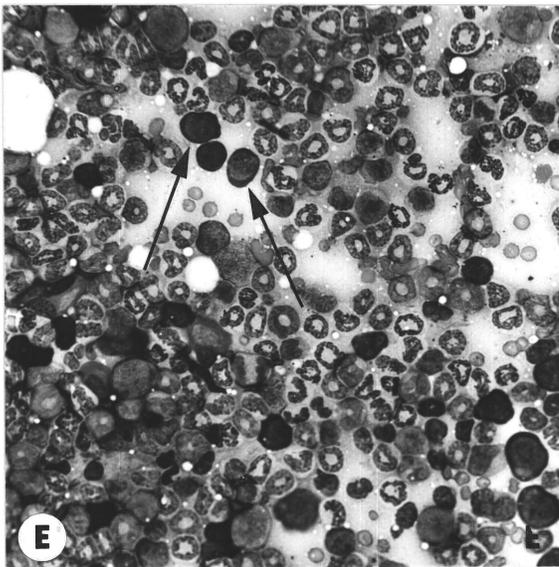
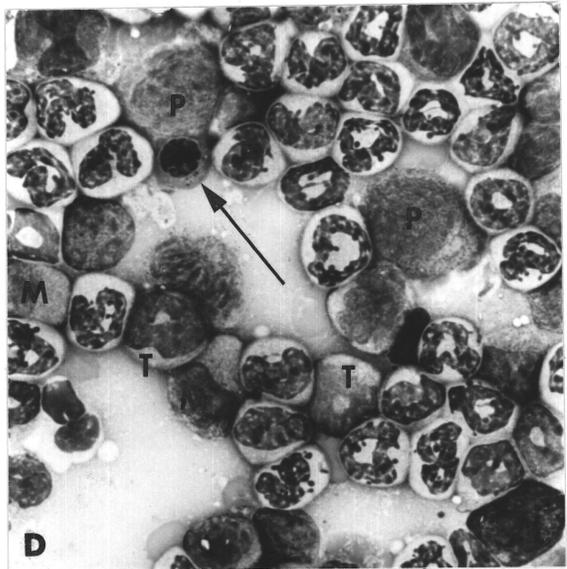
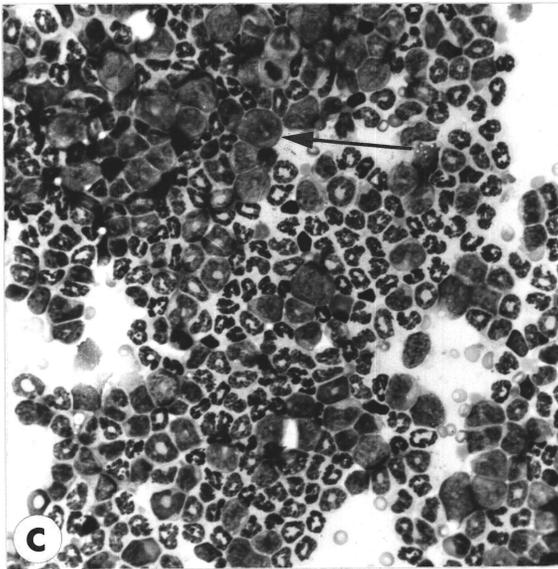
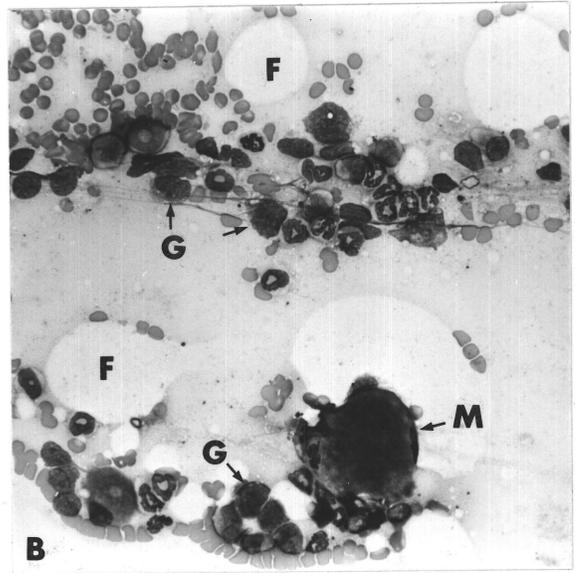
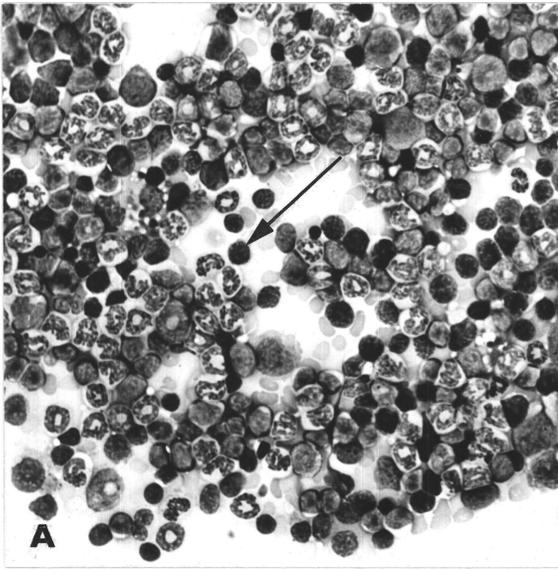


Fig. 50

Figure 51

Cytological appearance of erythroid regeneration in mice consuming dietary T-2 toxin (20 ppm) in a semipurified diet containing 16% protein.

- A. Control mouse, group B, day 41.  
Note the large number of normal immature erythroid cells at various stages of development (arrows).  
x 300.
  
- B. T-2-treated mouse, group B, day 41.  
Erythropoiesis is evident at this stage, but some immature erythrocytes have megaloblastic development (arrows).  
x 300.
  
- C. T-2-treated mouse, group B, day 41.  
Several abnormal rubricytes are shown. One has abnormal division of the nucleus (1), and another has megaloblastic development and stippled cytoplasm (2).  
x 750.
  
- D. Another field of the smear from the same mouse in C above.  
Other abnormalities of erythropoiesis are illustrated including megaloblastic development (1), and stippling of hemoglobinized cytoplasm of rubricytes (2).  
x 750.
  
- E. T-2-treated mouse, group C, day 28.  
This mouse was removed from the toxic diet 7 days previously. Note the presence of many erythroid cells at various stages of development (arrows).  
x 300.
  
- F. Higher magnification of smear in E above.  
Note the normal differentiation of immature erythroid cells. Normal mouse rubricytes and metarubricytes (arrows) have small quantities of cytoplasm. Compare with B, C and D above.  
x 750.

Wright's-Giemsa stained impression smears of bone marrow from the tibia. Magnification factors are given in each legend.

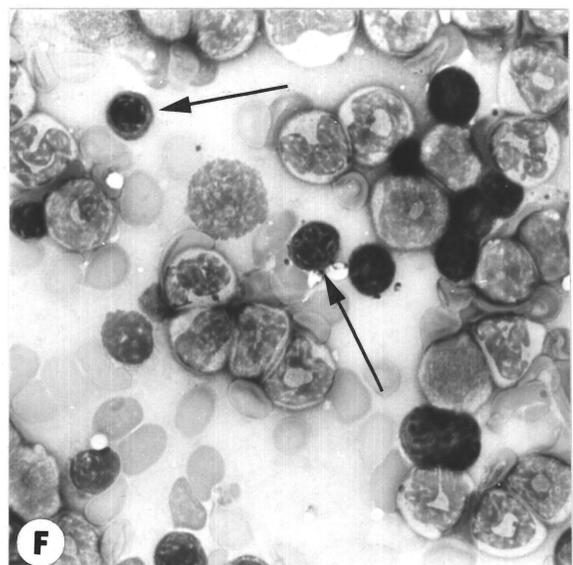
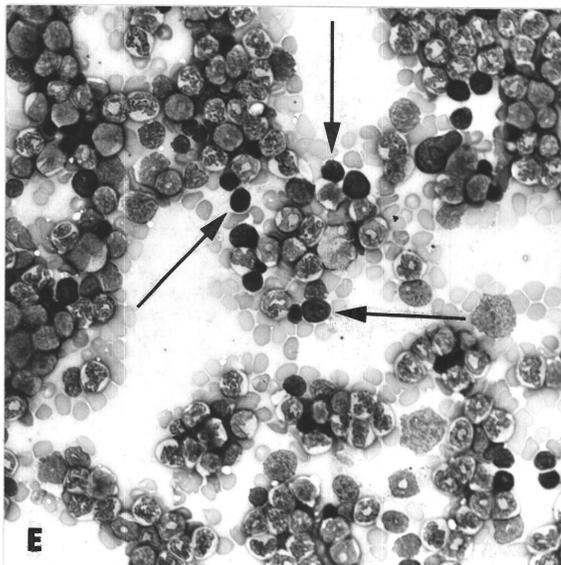
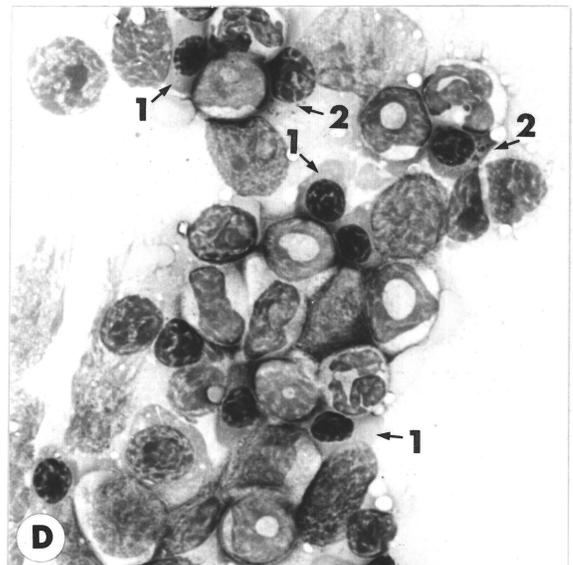
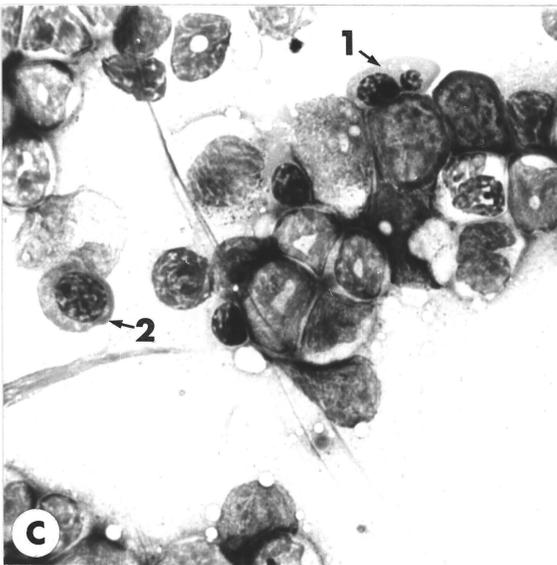
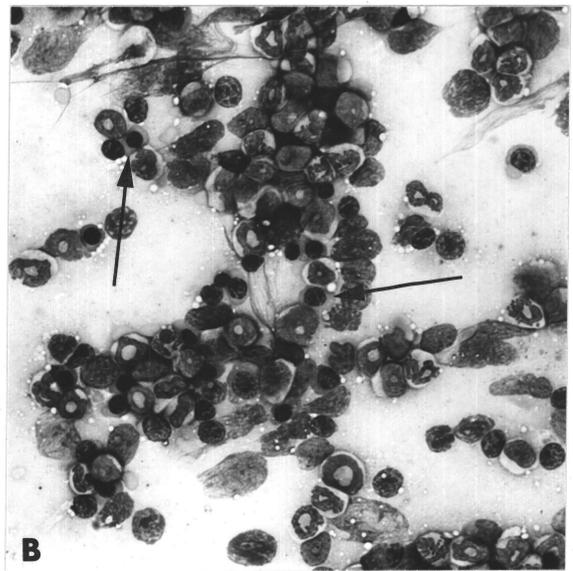
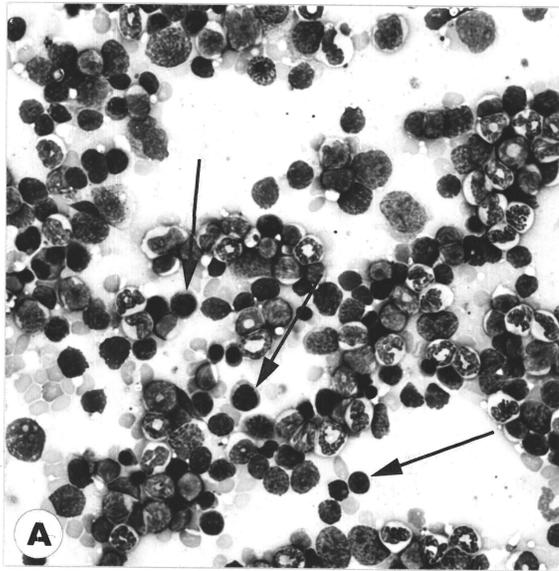


Fig. 51

Figure 52

Effect of dietary T-2 toxin (20 ppm) on the histological appearance of the squamous mucosa of the esophageal region of the stomach of mice.

- A. Normal mucosa; control mouse, group B, day 21.  
x 120.
  
- B. Normal mucosa. Higher magnification of Fig.52A above.  
Note the thin keratinized layer (arrow).  
x 300.
  
- C. T-2-treated mouse, group A, day 21.  
Note the thick keratin layer (K).  
x 120.
  
- D. Higher magnification of hyperplastic stratified squamous epithelium, showing papillary foldings of the epithelium and the thick layer of keratin (K). Compare with Fig. 52B above.  
x 300.
  
- E. Greatly thickened squamous mucosa near the margin of the fundic mucosa. The epithelium is extremely hyperplastic, with many papillary projections (P), and a thick layer of keratin (K).  
Note the ulceration with exudation of neutrophils (arrow), and submucosal edema (E).  
x 120.

Hematoxylin and eosin; magnification factors are indicated in each legend.

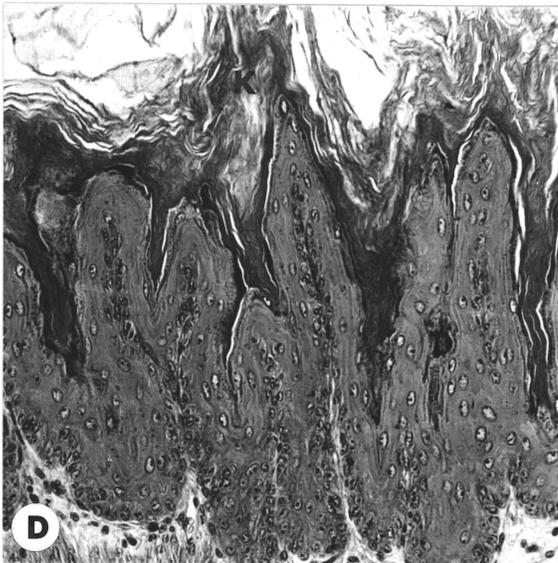
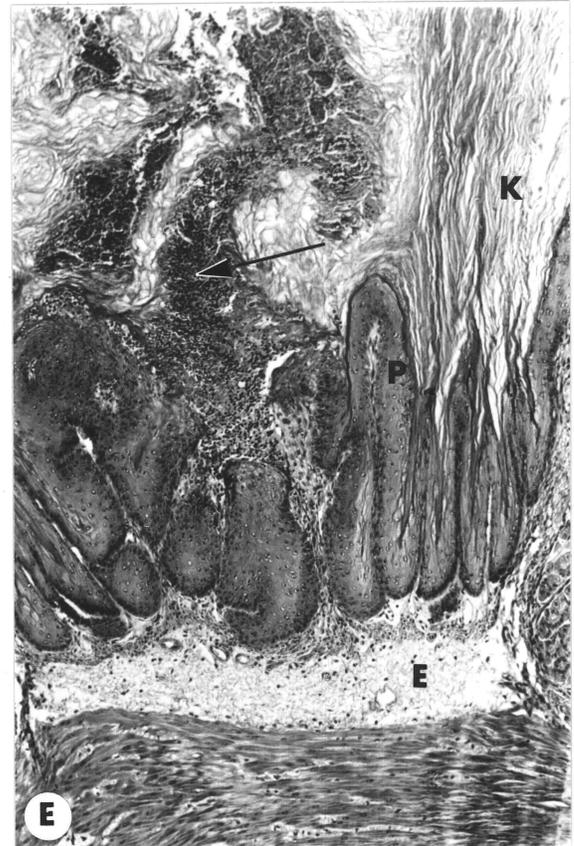
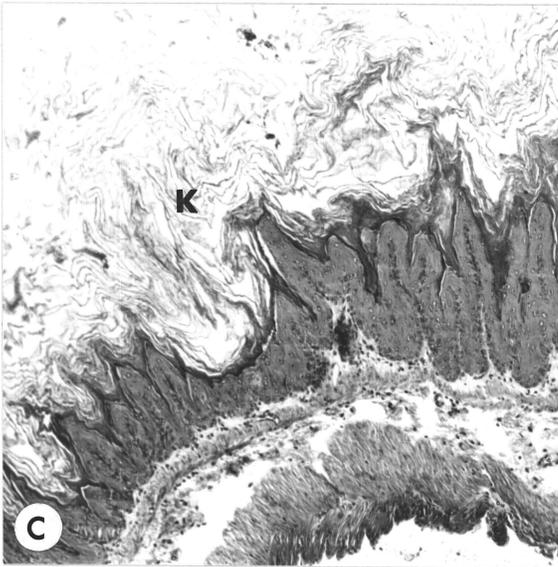
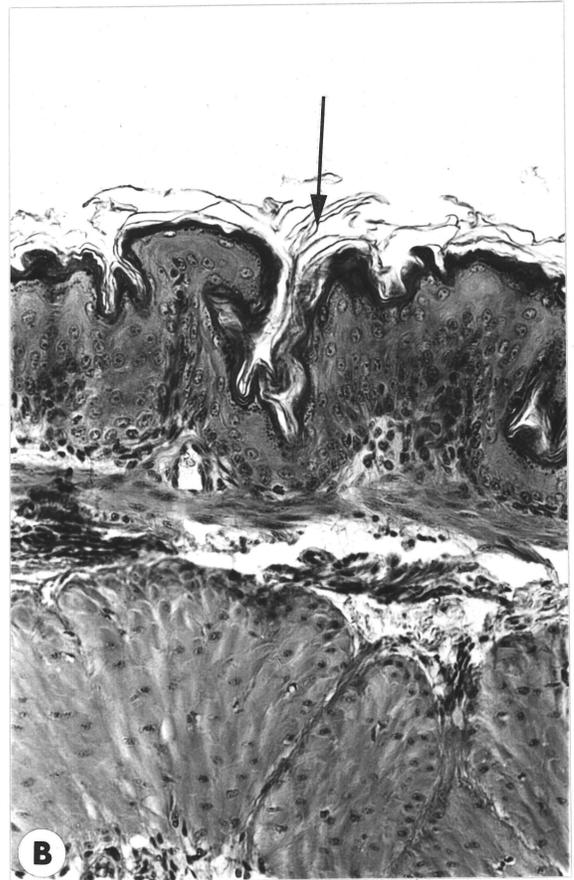
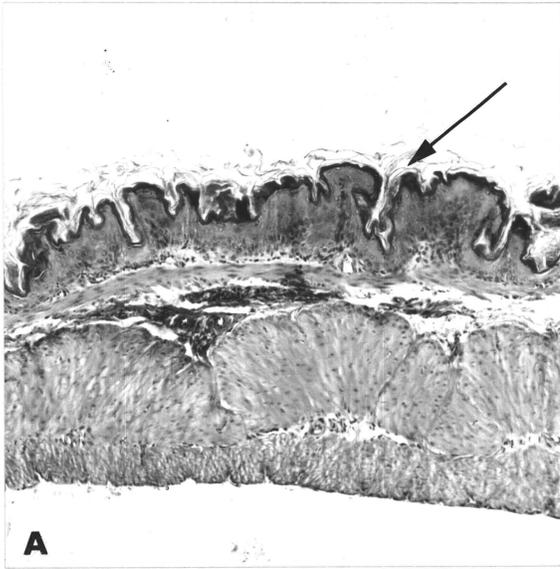


Fig. 52

whereas these were rarely observed in control mice. The degree of irritation of the perioral skin, stomach, or duodenum was similar among the different diet groups. Both treated and control mice infrequently exhibited a few typical adenoviral intranuclear inclusion bodies in jejunal epithelium.

## 8.5 Discussion

### 8.5.1 Toxic effects of dietary T-2 toxin

Mice fed T-2 toxin developed a subacute toxicity syndrome of reduced growth, hypoplastic anemia, lymphoid atrophy, gastric mucosal hyperkeratosis and perioral dermatitis. At the level used, T-2 toxin caused a substantial reduction in voluntary food intake, as has been recognized previously in other species including rats (Marasas et al., 1969; Kotsonis et al., 1975b), poultry (Wyatt et al., 1975; Chi and Mirocha, 1978), and swine (Weaver et al., 1978c). Such a reduction in intake indicates that mice fed T-2 toxin were also undernourished. However, the major changes observed can be considered to have been due to the toxicity of T-2 toxin because they began to resolve rapidly after withdrawal of toxin from the diets fed at the same rate, and they did not develop in mice fed the control diets at the same reduced intake.

Perioral dermatitis has been observed in experimentally induced T-2 toxicosis of rats (Marasas et al., 1969; Ohtsubo and Saito, 1977) and poultry (Wyatt et al., 1972b; Palyusik and Koplíc-Kovács, 1975; Chi and Mirocha, 1978), and it has similarly been recognized in naturally occurring trichothecene mycotoxicoses of poultry (Wyatt et al., 1972a;

Greenway and Puls, 1976), and swine (Pier, 1978, personal communication). Stomatitis and dermatitis occur in the early stages of alimentary toxic aleukia of man (Joffe, 1971; 1978; Mayer, 1953a), and also in stachybotryotoxicosis of horses (Forgacs and Carll, 1962; Rodricks and Eppley, 1974), each of which is thought to be caused by trichothecenes.

Hyperkeratosis and ulceration of the esophageal region of the stomach of mice consuming T-2 toxin is consistent with observations of Ohtsubo and Saito (1977) who described similar lesions in mice and rats consuming 15 ppm of T-2 toxin in the diet. Similar lesions have also been produced in rats by intragastric administration of crude extracts of Fusarium poae and F. sporotrichioides (Schoental and Joffe, 1974). Ulcerative proventriculitis and hyperkeratosis of the ventriculus have been observed in geese (Palyusik and Koplík-Kovács, 1975; Greenway and Puls, 1976) and turkey poults (Richard et al., 1978) after consumption of diets containing T-2 toxin. The location of the lesions in the upper alimentary tract suggests that they are caused by the topical irritant activity of T-2 toxin, as suggested by Wyatt et al. (1972b). Hyperplasia of the duodenal mucosa observed in mice in the present experiment appears likely to have been caused by the same activity.

The intestinal crypts were not atrophic in mice consuming T-2 toxin at dietary levels sufficient to cause impairment of hematopoiesis. This is consistent with the apparent absence of crypt necrosis and villus atrophy, both in the naturally occurring trichothecene mycotoxicoses (Mayer, 1952a; Smalley, 1973; Joffe, 1978), and during experimental consumption of trichothecenes in the diet (Chi et al., 1977b; Ohtsubo and Saito, 1977; Speers et al., 1977; Richard et al., 1978; Weaver et al.,

1978c; 1978d). Acute intoxication of mice with trichothecene mycotoxins, including T-2 toxin, results in rapid cytolytic injury to the germinal cells of the intestinal crypts, lymphoid follicles, bone marrow and thymic cortex (Saito, 1969; Saito and Ohtsubo, 1970; Ueno et al., 1971b; DeNicola et al., 1978) as described in Sections 6.4 and 7.4. In this respect, T-2 toxin resembles many other cytotoxic anti-neoplastic chemicals which cause similar acute injury (Graef et al., 1948; Lieberman et al., 1970; Altmann, 1974; Dinsdale, 1975). Crypt epithelial necrosis associated with enteritis has been produced in cats by repeated intragastric administration of T-2 toxin (Lutsky et al., 1978), but peak body levels of T-2 toxin after such administration would be higher than would occur if the same dose of T-2 toxin was gradually consumed in the diet. It would appear that T-2 toxin in the diet does not cause atrophic changes in the intestinal mucosa under most circumstances.

Diets containing T-2 toxin caused generalized lymphoid hypoplasia and lymphopenia in mice in the present experiment. 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. Lymphopenia and atrophy of the thymus and thymus-dependent lymphoid tissue occurring during the dietary protein deficiency are mediated partly by corticosteroid-induced lympholysis, and partly by undernutrition (Bell et al., 1976). The marked reduction of intake of diets containing T-2 toxin must therefore have contributed to the degree of atrophy, but because proliferation of lymphoblasts in the thymic cortex, lymph nodes, spleen, and intestinal mucosa was observed in mice after withdrawal of T-2 toxin from the diet supplied at the same

restricted intake, the T-2 toxin must have been partly responsible for the severe atrophy observed in treated mice. Atrophy of the thymus has been reported in turkey poults after consumption of T-2 toxin (Richard et al., 1978), and in mice given repeated doses of T-2 toxin (Rosenstein et al., 1979; Lafarge-Frayssinet et al., 1979). At present, the functional significance of such depletion has received limited investigation, but there is evidence that mice have depressed humoral and cellular immune responses (Rosenstein et al., 1979; Lafarge-Frayssinet et al., 1979).

The pattern of hematological alteration in mice consuming T-2 toxin in this study appears different from the hematopoietic failure observed in natural and experimental trichothecene mycotoxicoses of other species. Mice in this experiment developed aplastic anemia without granulocytopenia or thrombocytopenia, whereas in cases of alimentary toxic aleukia of man (Joffe, 1978), and stachybotryotoxicosis of horses (Rodricks and Eppley, 1974) affected individuals become neutropenic, with increased susceptibility to bacterial sepsis, and thrombocytopenic, with a tendency to develop hemorrhagic diathesis. In these diseases, erythropoiesis is also impaired, but affected individuals probably do not often survive long enough to become severely anemic. Similar patterns of pancytopenia have been experimentally reproduced in cats with repeated subcutaneous (Sato et al., 1975) or intragastric (Lutsky et al., 1978) doses of T-2 toxin, and in dogs, rats and monkeys with repeated parenteral doses of a closely related trichothecene, diacetoxyscirpenol (Stähelin et al., 1968). Mild anemia occurred in guinea-pigs given multiple intragastric doses of T-2 toxin for 27 days (DeNicola et al., 1978). Neutrophil counts were not reduced, but the guinea-pigs were lymphopenic. The pattern of

response of mice to dietary T-2 toxin appears to resemble that of guinea-pigs in that erythropoiesis is more susceptible to T-2 toxin than is myelopoiesis. The pattern of response observed in mice consuming T-2 toxin could be partly explained by the greater tendency of mice to become anemic during toxic suppression of hematopoiesis due to the short life span of erythrocytes in the order of 20 to 65 days (Schalm et al., 1975). In man, horses and cattle anemia develops slowly because the red blood cells generally survive for more than 100 days (Schalm et al., 1975). Neutrophil counts may be an imprecise measure of the degree of granulopoiesis in the mouse, particularly when inflammatory reactions such as the perioral dermatitis and gastritis are present, because mice normally have few neutrophils in circulation; large numbers of post-mitotic neutrophils are stored in bone marrow and these could be released due to stress or inflammatory reactions (Dunn, 1954). Cytological and histological examination of extremely hypocellular bone marrow of anemic mice revealed persistence of granulopoiesis in reduced amounts at a stage when erythropoiesis was totally suppressed, further suggesting that myeloid cells were less susceptible to T-2 toxin. Furthermore, during the 6-week experimental period, splenic and marrow myelopoiesis resumed early (within 2 to 3 weeks), whereas erythropoiesis did not resume until 4 to 6 weeks. Even when erythropoiesis did resume, maturation was megaloblastic and dysplastic, indicating continued interference with erythroid maturation (Beck, 1977). 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 at this stage, it is not known if T-2 toxin is selectively toxic to erythropoietic cells

in those species susceptible to T-2 mycotoxicosis in natural circumstances.

The mechanism by which the trichothecenes inhibit proliferation of germinal cells has received detailed investigation. Cytotoxicity has mostly been ascribed to the activity of these mycotoxins in inhibiting peptidyl transferase activity of eukaryotic ribosomes (Cundliffe et al., 1974; Wei and McLaughlin, 1974). However, according to some investigators, inhibition of protein synthesis alone would not lead to cell death of rapidly dividing cells (Ben-Ishay and Farber, 1975). The megaloblastic and dysplastic development of erythropoiesis in mice consuming T-2 toxin suggests that the toxin may have interfered with DNA synthesis and cell division, with less interference with synthesis of hemoglobin. Various cytotoxic, antineoplastic chemicals which 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) will produce megaloblastic anemia (Beck, 1977). Proliferation of hematopoietic precursors in splenic red pulp, with predominance of undifferentiated cells similar to those observed in response to T-2 toxin in this study, has been observed in mice exposed to repeated doses of triethylene melamine, azathioprine (a purine analogue), or methotrexate, each of which interferes with DNA synthesis (Krueger, 1972). Some trichothecenes have been found to inhibit DNA synthesis in addition to their effects on protein synthesis (Ueno, 1977a). The mechanism of such inhibition has not been determined, but the observed inhibition could be secondary to inhibition of protein synthesis (Chu, 1977).

The transition from erythroid aplasia in the initial 3 to 4 weeks

of continuous dietary exposure to T-2 toxin, through to a stage of regeneration, with splenomegaly, erythroid hyperplasia and reticulocytosis between 4 to 6 weeks, suggests that mice became less susceptible to the toxic effects on hematopoiesis. Further evidence for such resistance was provided by observations on changes in myelopoiesis which was initially reduced, but rapidly became hyperplastic by 3 to 4 weeks of exposure. Because hematopoietic regeneration occurred in mice that were continuously consuming T-2 toxin in the diet, either the hematopoietic cells became less sensitive to toxic impairment of multiplication, which seems improbable, or the amount of the active form of T-2 toxin decreased in the susceptible hematopoietic tissues.

Hematopoietic regeneration could be explained by an acquired ability of the liver to biotransform T-2 toxin into a metabolite that did not impair hematopoiesis. In this study, hepatic enlargement without histological changes occurred in mice consuming T-2 toxin in both the 12%-and 16%-protein diets, and was correlated with the occurrence of regeneration of hematopoietic tissues. Diets low in protein are known to reduce production of hepatic microsomal mixed-function oxidases (Campbell and Hayes, 1976) which are responsible for most biotransformations of xenobiotics (Parke and Williams, 1969). Many xenobiotics, such as DDT (Street, 1969), and phenobarbitone (Schulte-Hermann, 1974) 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 (Boyd, 1972). T-2 toxin is

deacetylated to HT-2 toxin by human, bovine, rat, and mouse hepatic microsomal enzymes (Ellison and Kotsonis, 1974; Ohta et al., 1977), but although HT-2 toxin is only slightly less acutely toxic than T-2 toxin (Ueno, 1977; Ohta et al., 1977), its metabolic fate and subacute toxic effects are not known. Variation between species' ability to biotransform xenobiotics is a major reason for differences in susceptibility to intoxication (Parke and Williams, 1969), and this may be the reason for apparent failure of dietary T-2 toxin to cause hematopoietic failure in some species such as poultry (Speers et al., 1977; Chi et al., 1977c) and swine (Weaver et al., 1978c; 1978d).

#### 8.5.2 Influence of dietary protein on toxicity

In addition to the influence of dietary protein level on the degree of hepatomegaly caused by T-2 toxin, several other observations in this study indicated that the dietary protein level influenced the rate of spontaneous recovery from toxic inhibition of erythropoiesis and myelopoiesis. Initially in the trial, all mice consuming T-2 toxin developed aplasia of erythroid tissues, but subsequently, immature myeloid and erythroid cells regenerated in the spleen and bone marrow of some mice. The onset of recovery of erythropoiesis, indicated by a transition from complete aplasia to megaloblastic erythroid maturation, was more frequently detected in mice consuming T-2 toxin in the 16% protein diet. Megaloblastic change in developing erythrocytes may be produced by many agents which impair DNA synthesis and is regarded to be a less severe form of impairment of erythropoiesis than is complete aplasia (Beck, 1977). Furthermore, the undifferentiated hematopoietic cells which proliferated in the red pulp of the spleen after initial

depletion, were present in greater numbers in mice consuming T-2 toxin in the 16%-protein diet, further supporting the suggestion that the dietary protein level influenced recovery from hematopoietic suppression. It is unlikely that the observed regeneration was due to transient suppression of hematopoiesis from 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 (Anagnostou et al., 1977; Fried et al., 1978).

One toxic effect observed only in mice consuming 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 declined precipitously, without hemosiderosis or icterus being evident, but in these mice, the site of hemorrhage was not determined. Intestinal hemorrhage, associated with prolonged prothrombin times, has been reported in calves given 30 daily intraruminal doses of T-2 toxin (Pier et al., 1976) and in one steer given 65 daily intramuscular doses of T-2 toxin (Grove et al., 1970). Because hemorrhagic enteritis occurred in some of the calves before prothrombin times were increased, the pathogenesis of such hemorrhage is unknown, but direct injury to the alimentary mucosa by T-2 toxin may have been involved (Pier et al., 1976). Hemorrhagic syndromes have been reported in cattle in association with rations containing T-2 toxin, but the nature of the hemostatic disorder in these cases was not determined (Hsu et al., 1972).

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, so it would not be influenced by hepatic detoxification of T-2 toxin.

### 8.5.3 Conclusions

This study demonstrated that mice are susceptible to T-2 toxin in their diet. The syndrome produced resembled naturally occurring putative trichothecene mycotoxicoses of other species in that food refusal and upper alimentary tract irritation were prominent effects. In addition, inhibition of hematopoiesis was demonstrated, but the pattern of suppression differed from the pancytopenias induced by non-dietary administration of T-2 toxin to other species. The severe effects of T-2 toxin on the lymphoid system suggest that this toxin might impair immune function of other species.

Although it has not been established that the responses of mice to T-2 toxin resemble those of other species, mice appear to be useful experimental animals for evaluation of factors influencing the toxicity of trichothecenes. With a suitable experimental animal it may be possible to identify dietary conditions that decrease or potentiate systemic toxicity of trichothecenes to mice, thereby minimizing the number of expensive and potentially hazardous feeding trials needed to determine the toxicity of trichothecenes to various livestock. The observation of recovery from hematopoietic inhibition during subacute exposure to T-2 toxin may explain the sporadic occurrence of hematopoietic failure in animals exposed to trichothecenes in the diet. Determination of factors influencing the development of such resistance may clarify our understanding of the role of these toxins in mycotoxic diseases. Because of the

influence of diet on the hematopoietic suppressive effects of T-2 toxin, such an understanding appears dependent on knowledge of the influence of nutrition 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.

## 9.0 DIETARY INFLUENCES ON THE SUBACUTE TOXICITY OF DIETARY T-2 TOXIN IN MICE

### 9.1 Abstract

Young weanling male Swiss mice weighing  $16.4 \pm 1.2$  g were fed diets containing T-2 toxin at levels of 0 ppm, 10 ppm or 20 ppm. Three diets were used; two were balanced semipurified diets containing either 8% or 16% protein, and the third was a regular natural-ingredient based laboratory mouse chow. Nine experimental groups (three diets, three levels of toxin) of 20 mice were used, and five mice from each were examined hematologically and by necropsy after 2, 4, 6 and 8 weeks on the diets.

Effects of T-2 toxin depended both on the dietary level of toxin and on the diet. All mice fed T-2 toxin at the 20-ppm level developed typical signs of toxicity, including reduced growth rates, reduced food consumption, perioral dermatitis, hyperkeratosis and ulcerative gastritis of the pars esophagea, hypoplasia of bone marrow and splenic red pulp, and generalized lymphoid depletion. Mortality was greatest in the mice fed T-2 toxin in the 8%-protein diet; none in this group survived beyond the 28-day observations. Mice surviving beyond 28 days on the other two diets exhibited regenerating hematopoietic tissue in the spleen and bone marrow, with abnormal erythroid maturation.

At the 10-ppm level, T-2 toxin caused anemia in mice on the 8%-protein diet, but not in mice on the 16%-protein diet, nor in mice on the natural-ingredient diet. In mice that became anemic, erythropoiesis was

hypoplastic, dysplastic, and megaloblastic during the first four weeks, but became hyperplastic by six and eight weeks.

T-2 toxin (10 ppm) also caused typical effects including hyperkeratosis of the squamous gastric mucosa, neutrophilia, and generalized lymphoid depletion. Lymphoid depletion was less severe in mice fed the 10-ppm level of toxin in the two optimal diets.

The method of housing also influenced toxicity; mice housed in box cages on softwood shavings had overcome the hematopoietic suppressive effects of the 20-ppm level of toxin by 56 days, whereas high mortality occurred in mice fed the same diet but housed in suspension cages.

## 9.2 Introduction

The toxicological significance of chemicals that may contaminate foodstuffs cannot be adequately evaluated unless the suspect chemicals are administered to experimental animals by the dietary route. Furthermore, food-borne mycotoxins, which may be present in diets of various nutritional qualities (Newberne, 1974; Hamilton, 1977), should not be evaluated solely in diets of optimal nutritional composition because nutrition is known to influence the toxicity of a wide range of toxic agents (McLean and McLean, 1969). These two considerations are particularly relevant to studies on the trichothecene group of mycotoxins,

because trichothecenes are potent inhibitors of voluntary food consumption (Kotsonis et al., 1975a), and have been associated with a mycotoxic disease of man that was more severe when the level of nutrition was poor (Joffe, 1971).

In the previous section (8), subacute toxic effects were observed in mice fed T-2 toxin in semipurified diets. The effects observed, including irritation of the upper alimentary tract, and suppression of proliferation of hematopoietic and lymphopoietic tissues resembled those observed in naturally occurring mycotoxicoses such as fusariotoxicosis (Wyatt et al., 1972a; Greenway and Puls, 1976), alimentary toxic aleukia (Joffe, 1971), and stachybotryotoxicosis (Forgacs and Carll, 1962), each of which is thought to be caused by trichothecene mycotoxins (Szathmary et al., 1976; Yagen and Joffe, 1976).

The observation that mice can overcome suppression of hematopoiesis after several weeks on toxic diets suggested a reason why apparently normal hematopoiesis has been found in other species fed diets containing T-2 toxin. Because the onset of regeneration of suppressed tissues was related to the level of dietary protein, the influence of nutrition on toxicity of T-2 toxin was further examined.

Several potential influences on the toxicity and subsequent recovery were considered in the experiment described in this section. A lower level of toxin (10 ppm) was used, in addition to the previously-used 20-ppm level, to determine if diet affected the toxicity at levels of toxin producing less severe effects than previously observed. Recovery from hematopoietic suppression was correlated with development of hepatomegaly in the previous experiment. Because mice were previously

housed on softwood shavings that might have modified the activity of hepatic microsomal enzymes (Wade et al., 1968; Schulte-Hermann, 1974), mice were housed in suspended cages to exclude the possibility that hepatomegaly and resistance to T-2 toxin were induced by this non-dietary factor. The semipurified diets used previously allowed optimal growth of control mice, but purified diets might lack certain unidentified trace ingredients present in natural-ingredient diets (Newberne et al., 1978). Accordingly, the toxicity of T-2 toxin in a regular, natural-ingredient stock diet was examined in comparison with the semipurified diets previously used. Because the hematopoietic regeneration phase was not complete by the end of 6 weeks on T-2 toxin in the previous experiment, an 8-week observation period was used in this experiment.

### 9.3 Materials and Methods

#### 9.3.1 Experimental design

Young weanling male Swiss mice (from Animal Resources Centre, University of Saskatchewan) of the same strain used in the previous experiment were assigned to groups of five at random, except that some groups were reorganized to minimize the variation among group weights. A total of 197 mice weighing  $16.4 \pm 1.2$  g was used. All groups were separately housed in stainless-steel suspension cages with wire mesh floors. Tap water was supplied ad lib; the room was maintained at  $21^{\circ}\text{C}$ , with a 12-hour fluorescent lighting cycle; and all management and procedures conformed to the guidelines of the Canadian Council on Animal Care.

Three experimental diets were used. Two were semipurified diets

containing 8% or 16% protein, as used in the previous experiments and described in Appendix B. The third ration was a regular stock mouse diet based on natural ingredients as described in Appendix B. This diet was finely ground and prepared into a moist gelatin-bound cake as described in Appendix B. Two levels (10 ppm and 20 ppm) of T-2 toxin (Makor Chemicals, Jerusalem, Israel) were used for each diet, and toxin-free diets were used as controls. All diets were prepared twice weekly, stored at 4°C, supplied freshly each day, and provided ad lib. Because it had been satisfactorily demonstrated in the previous experiment that the effects under consideration were not due solely to undernutrition, paired feeding of control diets was not done in this experiment.

Groups of 20 mice were fed on each of the nine experimental diets as described in Table 32. Five from each treatment were examined after 2, 4, 6 and 8 weeks, except that some mice in the 20-ppm groups did not survive until the 6- or 8-week stages. One additional group of 10 mice fed the natural diet containing 20 ppm T-2 toxin (Group N-20-A) was housed on softwood shavings for comparison with effects observed in the previous experiment.

Values for day 0 were obtained from 12 mice bled and necropsied on the day the experiment began. Because of the large number of mice used in this trial, each of the three diet groups were commenced separately over 3 consecutive days. Subsequently, all weekly procedures were conducted over the corresponding 3 days.

TABLE 32: Design of Experiment, Section 9.

Diet	Level of T-2 toxin (ppm)	Code	Number of mice				
			Weeks on diet				
			0	2	4	6	8
Semipurified; <sup>a</sup> 8% protein	0	L-0		5	5	5	5
	10	L-10		5	5	5	5
	20	L-20		5	5	(5) <sup>c</sup>	(5)
Semipurified; <sup>a</sup> 16% protein	0	H-0	12	5	5	5	5
	10	H-10		5	5	5	5
	20	H-20		5	5	(5)	(5)
Natural ingredient <sup>b</sup> 18.6% protein	0	N-0		5	5	5	5
	10	N-10		5	5	5	5
	20	N-20		5	5	(5)	(5)
	20	N-20-A <sup>d</sup>			5		5

<sup>a</sup> Formulation described in Appendix B, Table 65.

<sup>b</sup> Formulation described in Appendix B, Table 64.

<sup>c</sup> Groups in parentheses did not survive.

<sup>d</sup> This group was housed on softwood shavings.

### 9.3.2 Experimental observations

All mice were inspected at least once daily and were individually examined every week. Mice that became totally anorectic were killed and necropsied. Body weights were determined on day 0 and at weekly intervals thereafter. Rates of consumption of all diets were measured for each cage of mice. At each scheduled stage, mice for examination were bled between 9 and 11 a.m. for hematological examination as described in Section 8.3.2. The same afternoon, mice were killed and necropsied as described previously in Section 8.3.2. Organ weights of liver, spleen and thymus were determined. Impression smears of bone marrow from the right tibial shaft were stained with Wrights-Giemsa. Differential cell counts from 500 cells were determined on smears of bone marrow collected from controls and from the 10-ppm treated groups. Impression smears from the bone marrow, and from the cut surface of the spleen of some mice were stained with peroxidase reagent using the method of Rytömaa (1962). Samples of liver, spleen, thymus, mesenteric lymph node, stomach, duodenum, jejunum and bone marrow were collected from each mouse. Other tissues were collected only if they exhibited gross lesions. All tissues were processed for histological examination as described in Section 8.3.2.

### 9.3.3 Analysis of data

Effects due to diet were examined separately for each of the 3 levels of T-2 toxin using one-way analyses of variance at each of the 4 times. Similarly, effects due to toxin were examined for each diet using one-way analyses of variance at each of the 4 times. Significant differences between means were determined using Student-Newman-Keuls' (SNK) multiple

range tests (Winer, 1971). For each diet, sequential changes were identified in a 4 x 2 analysis of variance, considering the 4 times of examination, and the 0- and 10-ppm levels of T-2 toxin as non-metric variables. The 95%-probability level was used to identify significant effects and differences between means. All calculations were conducted on an IBM 370 computer using commercially prepared statistical programs (Nie et al., 1975).

#### 9.4 Results

In general, T-2 toxin produced similar effects as were described for the previous experiment (Section 8.4). In the following description, findings consistent with those previously recorded will be reported as such and will not be redescribed. Sections 9.4.1, 9.4.2 and 9.4.3 contain results for this experiment exclusive of those obtained for group N-20-A that was housed on softwood shavings. Since group N-20-A was managed similarly to mice in the previous experiment and thus acted as a control between the two experiments, findings for this group will be reported separately in Section 9.4.4.

##### 9.4.1 General observations

All mice consuming the control diets were normal throughout the trial. Similar growth rates occurred for each of the three control diets (Table 33), but mice fed diets containing T-2 toxin had dose-related reductions in growth and food consumption. Spillage of diets containing 20 ppm of T-2 toxin was marked, whereas diets containing 10 ppm were more palatable. Many mice fed diets containing 20 ppm did not grow, and after several weeks became emaciated and lethargic. Some of these

TABLE 33: Sequential changes in body weights of young mice fed T-2 toxin in different diets. Section 9.4.1.

Week	Level of T-2 toxin (ppm)	8% protein semipurified	16% protein semipurified	18.6% natural ingredient diet	
				without bedding	with bedding
0	0	17.0+0.3	16.9+0.5	15.3+0.8	--
	10	16.4+0.5	16.4+0.7	15.4+0.4	--
	20	15.6+0.1	15.7+0.8	15.2+0.3	16.1+0.6
1	0	20.3+0.6	24.9+0.5	23.6+0.9	--
	10	14.4+0.3	16.9+0.7	17.0+0.9	--
	20	12.1+0.7	12.8+0.5	12.5+0.5	12.7+0.4
2	0	25.4+0.6	31.8+0.5	28.9+0.6	-
	10	14.8+0.4	18.9+0.5	19.9+0.6	-
	20	11.4+0.8	13.6+0.3	13.0+0.4	NA
3	0	28.9+0.7	37.1+0.5	28.4+0.6	-
	10	14.8+0.5	20.6+0.7	19.0+0.8	-
	20	12.1+0.7	12.8+1.1	14.9+1.3	15.2+0.5
4	0	33.8+0.8	39.5+0.5	32.8+0.8	-
	10	15.4+0.6	21.4+0.6	22.3+0.9	-
	20	14.8	13.9+0.6	17.6+1.4	NA
5	0	35.9+1.4	41.5+0.7	37.7+0.7	-
	10	13.1+1.8	22.5+1.0	24.1+1.1	-
	20	X	14.3+0.9	24.4+2.7	21.7+1.5
6	0	37.5+1.6	43.3+1.1	38.6+0.9	-
	10	15.1+0.6	22.0+1.0	24.9+1.0	-
	20	X	X	25.1+2.8	23.1+1.8
7	0	39.2+1.6	43.4+1.5	38.1+1.0	-
	10	16.9+0.6	23.9+1.3	25.8+1.4	-
	20	X	X	X	25.3+2.5
8	0	40.5+2.2	45.3+1.4	39.8+1.8	-
	10	17.6+0.8	28.9+1.6	29.1+1.3	-
	20	X	X	X	26.6+2.9

<sup>a</sup> Values are means (+SEM) of all mice on the respective diets at the end of each week.

X No survivors.

TABLE 34: Frequencies of perioral dermatitis in young Swiss mice consuming T-2 toxin in different diets.

Group	Week							
	1	2	3	4	5	6	7	8
L-10 <sup>a</sup>	0/20 <sup>b</sup>	0/20	1/15	0/15	0/10	0/10	0/5	0/5
L-20	1/19	9/19	4/8	0/1	--	--	--	--
H-10	0/19	0/19	0/14	0/14	0/9	0/9	0/5	0/5
H-20	0/20	5/18	0/10	0/6	0/2	0/2	--	--
N-10	0/20	0/19	0/15	0/15	0/10	0/10	0/5	0/5
N-20	0/19	5/16	1/7	0/6	0/2	0/2	--	--

<sup>a</sup> Code: L = 8% protein semipurified diet; H = 16% protein semipurified diet; N = natural ingredient diet; -10 = 10 ppm T-2 toxin; -20 = 20 ppm T-2 toxin.

<sup>b</sup> Values are ratios of No. affected/No. on diet at each stage.

developed dermatitis around the muzzle and less frequently on the feet (Table 34), similar to mice described in Section 8.4.1 in the previous experiment.

Mortality was high among mice fed T-2 toxin at the 20-ppm level, irrespective of the diet. Most deaths occurred during weeks 3 and 4, and were more frequent in mice in the low-protein-diet group (Table 35). Deaths, either spontaneous or from euthanasia, were preceded by emaciation, lethargy and anorexia. Many mice exhibited skin pallor and several became severely anemic within 3 weeks on the toxic diets; in some of these mice, hemorrhagic diarrhea preceded the development of anemia and a few that deteriorated more rapidly were found, at post mortem, to have hemorrhagic enteritis.

All mice consuming T-2 toxin at the 10-ppm level survived the scheduled experimental observation periods, except two that died from peritonitis due to Pasteurella pneumotropica. Abnormalities produced by the 20-ppm level of toxin, such as perioral dermatitis, lethargy and anorexia, were not observed in the lower dose group, but mice in group L-10 had pale skin and ruffled, thin hair coats by day 28. On days 42 and 56, all mice in group L-10 appeared pale and mildly alopecic. The 10-ppm level of T-2 toxin in the diets H-10 and N-10 did not cause these physical effects, other than coarse dry fur at day 14.

#### 9.4.2 Hematology

##### 9.4.2.1 Erythron

All mice consuming T-2 toxin at 20 ppm developed aplastic anemia (Fig 53) and reticulocytopenia by day 28 (Fig. 54; Tables 36-38 ). At the high level of toxin, the degree of anemia was similar in each of the 3 diet

TABLE 35: Mortality rates of young Swiss mice consuming T-2 toxin in different diets.

Group	Week							
	1	2	3	4	5	6	7	8
L-0 <sup>a</sup>	0/20 <sup>b</sup>	0/20	0/15	0/15	0/10	0/10	0/5	0/5
L-10	0/20	0/20	0/15	0/15	0/10	0/10	0/5	0/5
L-20	0/20	1/20	6/14	7/8	--	--	--	--
H-0	0/20	0/20	0/15	0/15	0/10	0/10	0/5	0/5
H-10	0/20	1/20	0/14	0/14	0/9	0/9	0/5	0/5
H-20	0/20	2/20	3/13	4/10	0/2	2/2	--	--
N-0	0/20	0/20	0/15	0/15	0/10	0/10	0/5	0/5
N-10	0/20	0/20	0/15	0/15	0/10	0/10	0/5	0/5
N-20	1/20	3/19	5/12	0/7	1/3	0/2	--	--
N-20A <sup>c</sup>	0/10	0/10	0/10	0/10	0/5	0/5	0/5	0/5

<sup>a</sup> Code: L = 8%-protein semipurified diet; H = 16%-protein semipurified diet; N = natural-ingredient diet; -0 = control; -10 = 10 ppm T-2 toxin; -20 = 20 ppm T-2 toxin.

<sup>b</sup> Values are expressed as No. of deaths/No. on diet at the beginning of that week.

<sup>c</sup> This group was housed on softwood shavings, whereas others were kept in suspended cages.

groups. However, for the 10-ppm level, anemia developed only in mice on the 8% diet (group L-10) (Fig. 53; Table 36), and was less severe than the anemia produced by the 20-ppm level (Fig. 55). The 10-ppm level of T-2 toxin caused depression of reticulocyte numbers by day 14 in all three diet groups, but only in the 8%-diet group (L-10) were reticulocyte numbers less than 0.1%. After anemia had developed in group L-10, reticulocytes returned into circulation (Fig. 54) and by day 56, the erythrocyte count had begun to rise (Fig. 53).

Alterations in MCV (Table 39) in general were correlated directly with changes in reticulocytes. By day 56, mice in group L-10 had significant macrocytosis compared to the other groups on the 0-ppm or 10-ppm levels of toxin. At this time, and also on day 42, mice on the 10-ppm diets had significantly higher MCV values than the corresponding control groups (Table 39). The MCV of mice on the 20-ppm levels were low in all three diet groups on day 28 (Table 39). No consistent trends were found in the values of MCH and MCHC. MCH values were correlated closely with MCV values. An unusually high value was obtained for the MCHC value in group L-20 on day 28 (Table 39); this was probably an anomaly due to one mouse having erythrocytes that were heavily parasitized by Eperythroozoon coccoides. This parasite was not observed in any other mice.

Direct blood smears from mice in control groups were morphologically normal (Fig. 56A). In all mice on 20-ppm T-2 toxin, polychromatophilic erythrocytes had virtually disappeared. In the 10-ppm groups, polychromatophils were infrequently found in all three diet groups on day 14 (Fig. 56B). Subsequently, mice in group L-10 entered a regenerative phase with release of polychromatophilic macrocytes (Fig. 56C) and erythrocytes with basophilic stippling of cytoplasm (Fig. 56D)

Figure 53

Sequential changes in erythrocyte counts of mice fed T-2 toxin (10 ppm) in 3 different diets for 8 weeks.

L-10 Semipurified diet containing 8% protein.

H-10 Semipurified diet containing 16% protein.

NI-10 Natural-ingredient based diet.

Note that T-2 toxin caused anemia only in mice on the 8%-protein diet.

Points are means ( $\pm$  SEM) of separate groups of 5 mice.

Figure 54

Sequential changes in reticulocyte counts of mice fed T-2 toxin (10 ppm) in 3 different diets for 8 weeks.

Refer to the legend of Fig. 53 for description of codes.

Note that mice fed T-2 toxin in the 8%-protein diet became reticulocytopenic, but then developed a marked reticulocytosis.

Points are means ( $\pm$  SEM) of separate groups of 5 mice.

Fig. 53 Erythrocyte Counts

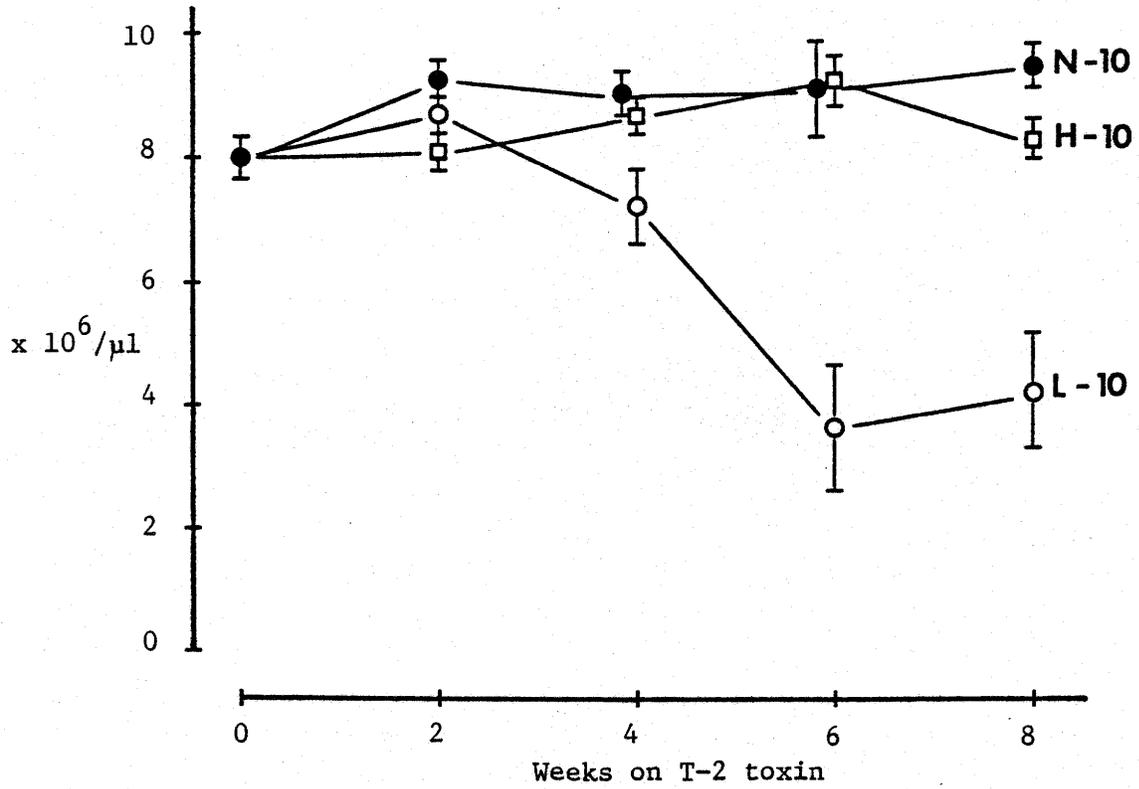


Fig. 54 Reticulocyte Counts

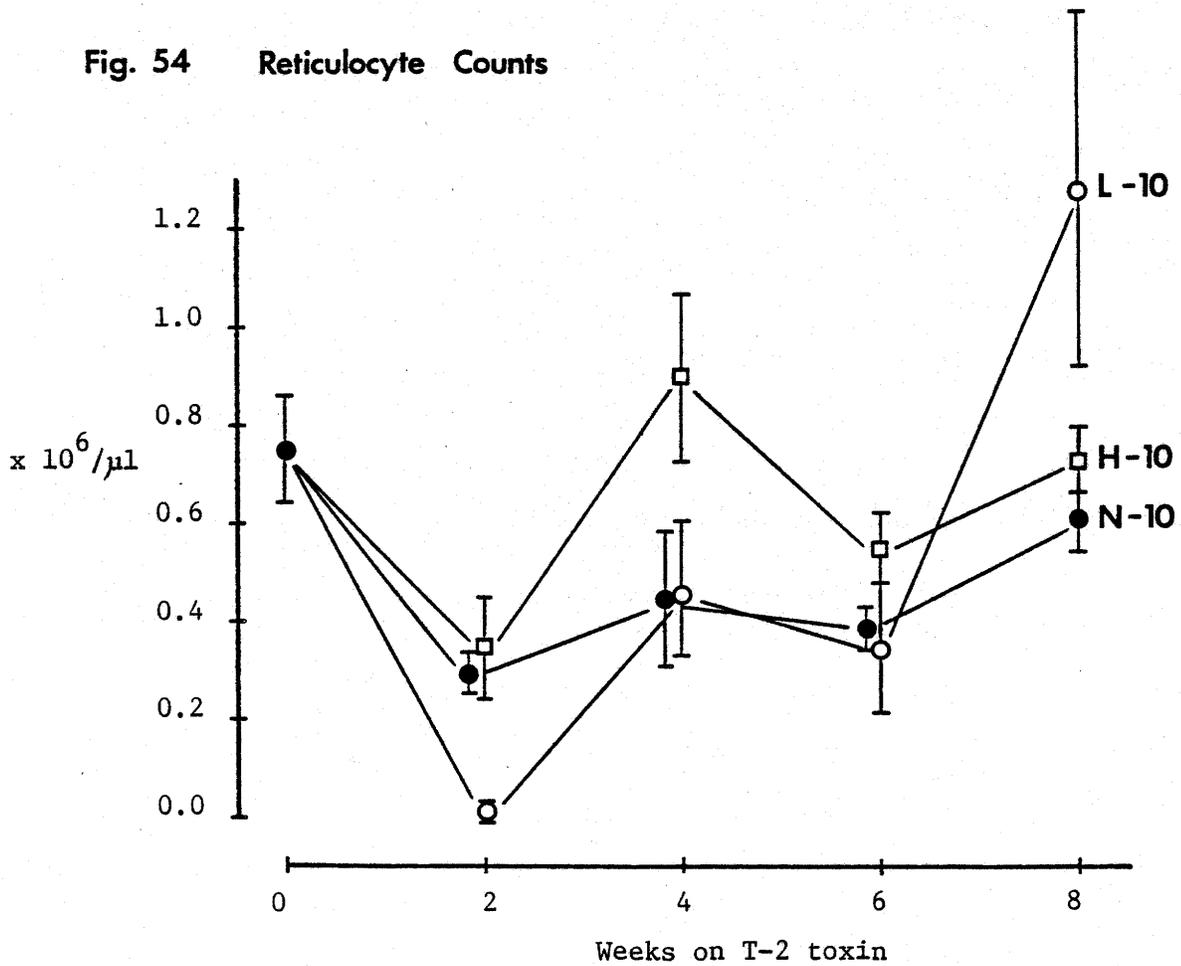


TABLE 36: Erythrocyte values ( $\bar{X} \pm \text{SEM}$ ) of young Swiss mice fed T-2 toxin in three different diets.

Variable	Week	T-2 toxin (ppm)	8% protein semipurified	16% protein semipurified	18.6% protein stock diet	P	
Erythrocyte count $\times 10^6/\mu\text{l}$	0	0	--	--	8.0 $\pm$ 0.2		
	2	0	8.9 $\pm$ 0.2	8.8 $\pm$ 0.3	9.0 $\pm$ 0.1	0.82	
		10	8.7 $\pm$ 0.4 <sup>ab</sup>	8.2 $\pm$ 0.3 <sup>a</sup>	9.4 $\pm$ 0.1 <sup>b</sup>	0.05	
		20	9.3 $\pm$ 0.1 <sup>a</sup>	8.5 $\pm$ 0.3 <sup>b</sup>	8.1 $\pm$ 0.3 <sup>b</sup>	0.02	
	4	0	9.4 $\pm$ 0.1 <sup>a</sup>	9.2 $\pm$ 0.2 <sup>a</sup>	10.3 $\pm$ 0.1 <sup>b</sup>	< 0.001	
		10	7.3 $\pm$ 0.6 <sup>a</sup>	8.7 $\pm$ 0.2 <sup>b</sup>	9.1 $\pm$ 0.3 <sup>b</sup>	0.02	
		20	5.0 $\pm$ 1.1	6.3 $\pm$ 0.3	6.2 $\pm$ 0.6	0.45	
	6	0	10.0 $\pm$ 0.1	10.1 $\pm$ 0.4	9.7 $\pm$ 0.2	0.17	
		10	3.6 $\pm$ 0.9 <sup>a</sup>	9.4 $\pm$ 0.4 <sup>b</sup>	9.3 $\pm$ 0.8 <sup>b</sup>	< 0.001	
		20	X	2.1 $\pm$ 0.1 <sup>d</sup>	8.2 $\pm$ 0.5		
	8	0	9.6 $\pm$ 0.1	9.8 $\pm$ 0.2	9.5 $\pm$ 0.2	0.63	
		10	4.3 $\pm$ 0.9 <sup>a</sup>	8.4 $\pm$ 0.2 <sup>b</sup>	9.6 $\pm$ 0.3 <sup>b</sup>	< 0.001	
		20	X	X	X		
	Mean Corpuscular Volume (MCV) $\mu^3$	0	0	--	--	50.4 $\pm$ 0.3	
		2	0	47.0 $\pm$ 0.3	46.6 $\pm$ 0.2	47.6 $\pm$ 0.5	0.21
10			44.2 $\pm$ 0.6 <sup>a</sup>	47.4 $\pm$ 0.9 <sup>b</sup>	47.4 $\pm$ 0.9 <sup>b</sup>	0.02	
20			44.4 $\pm$ 0.9	46.2 $\pm$ 1.5	44.8 $\pm$ 0.8	0.51	
4		0	45.2 $\pm$ 0.5 <sup>a</sup>	45.4 $\pm$ 0.5 <sup>a</sup>	43.6 $\pm$ 0.5 <sup>a</sup>	0.05	
		10	42.8 $\pm$ 1.2 <sup>a</sup>	47.4 $\pm$ 1.0 <sup>b</sup>	46.4 $\pm$ 1.0 <sup>b</sup>	0.02	
		20	39.0 $\pm$ 1.1	39.8 $\pm$ 0.6	39.7 $\pm$ 0.3	0.77	
6		0	45.2 $\pm$ 0.4	45.8 $\pm$ 0.9	44.4 $\pm$ 0.2	0.25	
		10	45.2 $\pm$ 2.3	49.3 $\pm$ 1.3	48.8 $\pm$ 0.7	0.19	
		20	X	40.0 $\pm$ 3.0 <sup>d</sup>	52.5 $\pm$ 0.5		
8		0	45.2 $\pm$ 0.2 <sup>ab</sup>	44.4 $\pm$ 0.5 <sup>a</sup>	45.8 $\pm$ 0.2 <sup>b</sup>	0.04	
		10	55.2 $\pm$ 1.9 <sup>a</sup>	50.4 $\pm$ 0.4 <sup>b</sup>	47.2 $\pm$ 1.2 <sup>b</sup>	0.004	
		20	X	X	X		

a,b,c Means followed by the same letter, or by no letter, do not differ at p = 0.05 by SNK multiple range tests.

X No survivors.

d At 39 days.

TABLE 37: Reticulocyte values ( $\bar{X} \pm \text{SEM}$ ) of young Swiss mice fed T-2 toxin in three different diets.

Variable	Week	T-2 toxin (ppm)	8% protein semipurified	16% protein semipurified	18.6% protein stock diet	P
Reticulocyte count percent	0	0	--	--	9.5 $\pm$ 1.3	
	2	0	7.4 $\pm$ 1.0	7.5 $\pm$ 1.5	4.7 $\pm$ 0.7	0.18
		10	0.2 $\pm$ 0.1 <sup>a</sup>	4.3 $\pm$ 1.2 <sup>b</sup>	3.0 $\pm$ 0.4 <sup>b</sup>	0.008
		20	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.62
	4	0	4.0 $\pm$ 0.6 <sup>a</sup>	10.0 $\pm$ 2.8 <sup>b</sup>	3.2 $\pm$ 0.4 <sup>a</sup>	0.03
		10	6.1 $\pm$ 1.6	10.5 $\pm$ 2.1	5.1 $\pm$ 1.7	0.13
		20	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.7 $\pm$ 0.7	0.41
	6	0	5.8 $\pm$ 0.8	6.2 $\pm$ 0.5	6.2 $\pm$ 1.4	0.95
		10	8.3 $\pm$ 3.1	5.9 $\pm$ 0.5	4.3 $\pm$ 0.4	0.35
		20	X	0.0 $\pm$ 0.0 <sup>d</sup>	6.6 $\pm$ 1.7	
	8	0	6.0 $\pm$ 0.8	8.2 $\pm$ 1.7	7.9 $\pm$ 1.1	0.44
		10	28.0 $\pm$ 4.7 <sup>a</sup>	8.7 $\pm$ 0.7 <sup>b</sup>	6.5 $\pm$ 1.2 <sup>b</sup>	<0.001
20		X	X	X		
Reticulocytes x10 <sup>6</sup> /μl	0	0	--	--	0.76 $\pm$ 0.11	
	2	0	0.66 $\pm$ 0.08	0.65 $\pm$ 0.12	0.42 $\pm$ 0.06	0.17
		10	0.02 $\pm$ 0.01 <sup>a</sup>	0.35 $\pm$ 0.10 <sup>b</sup>	0.28 $\pm$ 0.04 <sup>b</sup>	0.004
		20	0.00 $\pm$ 0.0	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	
	4	0	0.37 $\pm$ 0.06 <sup>a</sup>	0.92 $\pm$ 0.24 <sup>b</sup>	0.33 $\pm$ 0.04 <sup>a</sup>	0.03
		10	0.47 $\pm$ 0.14	0.90 $\pm$ 0.18	0.45 $\pm$ 0.14	0.11
		20	0.00 $\pm$ 0.0	0.00 $\pm$ 0.0	0.05 $\pm$ 0.05	0.41
	6	0	0.58 $\pm$ 0.07	0.63 $\pm$ 0.06	0.60 $\pm$ 0.14	0.94
		10	0.36 $\pm$ 0.18	0.55 $\pm$ 0.07	0.39 $\pm$ 0.04	0.52
		20	X	0.00 $\pm$ 0.00 <sup>d</sup>	0.54 $\pm$ 0.16	
	8	0	0.57 $\pm$ 0.08	0.79 $\pm$ 0.17	0.75 $\pm$ 0.10	0.41
		10	1.28 $\pm$ 0.38	0.73 $\pm$ 0.07	0.61 $\pm$ 0.09	0.13
20		X	X	X		

a,b,c Means followed by the same letter, or by no letter, do not differ at p = 0.05 by SNK multiple range tests.

X No survivors

NA Data not available

d At 39 days.

TABLE 38: Erythrocyte values ( $\bar{X} \pm \text{SEM}$ ) of young Swiss mice fed T-2 toxin in different diets.

Variable	Week	T-2 toxin (ppm)	8% protein semipurified	16% protein semipurified	18.6% protein stock diet	P	
Hemoglobin concentration (HGB) g/dl	0	0	--	--	13.6 $\pm$ 0.3		
	2	0	15.9 $\pm$ 0.5	15.9 $\pm$ 0.3	15.8 $\pm$ 0.1	0.99	
		10	14.0 $\pm$ 0.5 <sup>a</sup>	14.6 $\pm$ 0.5 <sup>a</sup>	16.0 $\pm$ 0.3 <sup>b</sup>	0.03	
		20	15.4 $\pm$ 0.3	14.1 $\pm$ 0.6	13.6 $\pm$ 0.6	0.07	
	4	0	16.2 $\pm$ 0.3 <sup>a</sup>	17.4 $\pm$ 0.3 <sup>b</sup>	16.2 $\pm$ 0.1 <sup>a</sup>	0.01	
		10	11.9 $\pm$ 1.2 <sup>a</sup>	16.1 $\pm$ 0.2 <sup>b</sup>	15.0 $\pm$ 0.5 <sup>b</sup>	0.005	
		20	7.9 $\pm$ 1.5	9.5 $\pm$ 0.5	9.3 $\pm$ 0.8	0.52	
	6	0	16.6 $\pm$ 0.1	17.0 $\pm$ 0.5	16.0 $\pm$ 0.2	0.13	
		10	6.3 $\pm$ 1.6 <sup>a</sup>	16.2 $\pm$ 0.5 <sup>b</sup>	16.0 $\pm$ 1.2 <sup>b</sup>	<0.001	
		20	X	2.6 $\pm$ 1.3 <sup>d</sup>	14.6 $\pm$ 1.0		
	8	0	16.2 $\pm$ 0.3	16.3 $\pm$ 0.3	16.7 $\pm$ 0.3	0.72	
		10	8.9 $\pm$ 1.6 <sup>a</sup>	15.6 $\pm$ 0.3 <sup>b</sup>	16.2 $\pm$ 0.2 <sup>b</sup>	<0.001	
		20	X	X	X		
	Packed cell volume (PCV) percent	0	0	--	--	38.3 $\pm$ 0.9	
		2	0	42.0 $\pm$ 0.8	41.0 $\pm$ 1.0	42.7 $\pm$ 1.1	0.48
			10	38.5 $\pm$ 1.8 <sup>a</sup>	39.0 $\pm$ 1.7 <sup>a</sup>	44.4 $\pm$ 1.1 <sup>a</sup>	0.04
20			41.2 $\pm$ 0.9 <sup>a</sup>	39.0 $\pm$ 1.2 <sup>ab</sup>	36.1 $\pm$ 1.6 <sup>b</sup>	0.04	
4		0	42.4 $\pm$ 0.7 <sup>a</sup>	41.9 $\pm$ 1.0 <sup>a</sup>	44.8 $\pm$ 0.5 <sup>a</sup>	0.04	
		10	31.5 $\pm$ 3.2 <sup>a</sup>	41.3 $\pm$ 0.4 <sup>b</sup>	42.0 $\pm$ 1.5 <sup>b</sup>	0.006	
		20	19.6 $\pm$ 4.7	25.2 $\pm$ 1.6	24.6 $\pm$ 2.2	0.44	
6		0	42.3 $\pm$ 0.4 <sup>a</sup>	46.1 $\pm$ 0.8 <sup>a</sup>	42.9 $\pm$ 0.6 <sup>b</sup>	0.01	
		10	17.1 $\pm$ 4.8 <sup>a</sup>	46.0 $\pm$ 1.9 <sup>b</sup>	45.2 $\pm$ 3.2 <sup>b</sup>	<0.001	
		20	X	8.4 $\pm$ 1.2 <sup>d</sup>	42.4 $\pm$ 1.8		
8		0	43.3 $\pm$ 0.4	43.4 $\pm$ 1.2	43.6 $\pm$ 1.2	0.98	
		10	23.6 $\pm$ 4.8 <sup>a</sup>	42.5 $\pm$ 1.0 <sup>b</sup>	45.0 $\pm$ 0.4	<0.001	
	20	X	X	X			

a,b,c Means followed by the same letter, or by no letter, do not differ at p = 0.05 by SNK multiple range tests

X No survivors

d At 39 days.

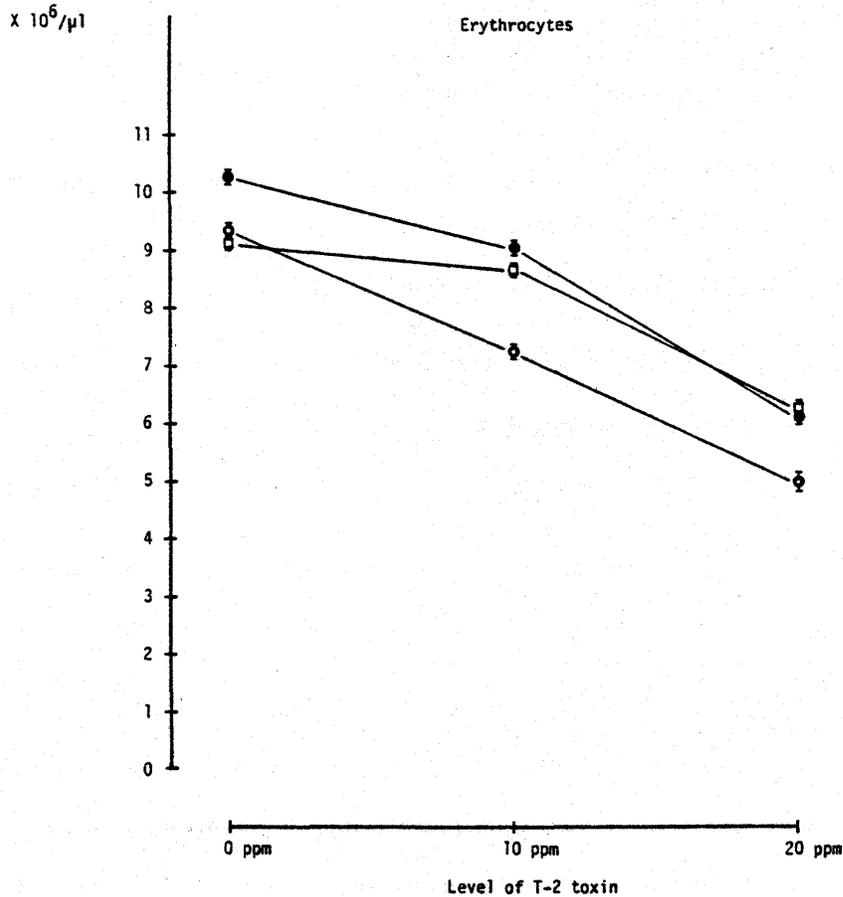


Fig. 55: Comparison of erythrocyte counts of young mice fed T-2 toxin at 3 levels in 3 different diets for 4 weeks.

Natural-ingredient diet (●); 16%-protein semipurified diet (□); 8%-protein semipurified diet (○).

Each point is the mean (±SEM) of separate groups of 4 or 5 mice.

All mice on the 20-ppm level became anemic, but only those on the 8%-SP diet became anemic from the 10-ppm level. The 0-ppm level did not cause anemia in any of the diet groups. However, erythrocyte counts were significantly higher in controls fed the natural-ingredient diet, but this difference existed only on day 28.

TABLE 39: Erythrocytic indices ( $\bar{X} \pm \text{SEM}$ ) of young Swiss mice fed T-2 toxin in three different diets.

Variable	Week	T-2 toxin (ppm)	8% protein semipurified	16% protein semipurified	18.6% protein stock diet	P
Mean corpuscular hemoglobin (MCH) pg	0	0	--	--	17.0 $\pm$ 0.1	
	2	0	17.8 $\pm$ 0.4	18.1 $\pm$ 0.2	17.7 $\pm$ 0.3	0.71
		10	16.1 $\pm$ 0.2 <sup>a</sup>	17.7 $\pm$ 0.6 <sup>b</sup>	17.1 $\pm$ 0.5 <sup>b</sup>	0.003
		20	16.6 $\pm$ 0.4	16.7 $\pm$ 0.7	16.8 $\pm$ 0.3	0.98
	4	0	17.3 $\pm$ 0.3 <sup>a</sup>	18.9 $\pm$ 0.1 <sup>b</sup>	15.8 $\pm$ 0.2 <sup>c</sup>	< 0.001
		10	16.2 $\pm$ 0.4 <sup>a</sup>	18.5 $\pm$ 0.4 <sup>b</sup>	16.5 $\pm$ 0.3 <sup>a</sup>	0.001
		20	16.6 $\pm$ 1.7	15.0 $\pm$ 0.2	15.1 $\pm$ 0.2	0.52
	6	0	16.5 $\pm$ 0.1	16.8 $\pm$ 0.4	16.6 $\pm$ 0.2	0.72
		10	17.2 $\pm$ 0.5	17.4 $\pm$ 0.3	17.3 $\pm$ 0.3	0.94
		20	X	17.1 $\pm$ 0.6 <sup>d</sup>	18.0 $\pm$ 0.4	
	8	0	16.9 $\pm$ 0.2	16.7 $\pm$ 0.2	17.4 $\pm$ 0.2	0.13
		10	21.2 $\pm$ 3.4 <sup>a</sup>	18.6 $\pm$ 0.4 <sup>ab</sup>	17.0 $\pm$ 0.4 <sup>b</sup>	0.02
20		X	X	X		
Mean corpuscular hemoglobin concentration (MCHC) percent	0	0	--	--	35.0 $\pm$ 0.2	
	2	0	37.9 $\pm$ 0.7	38.8 $\pm$ 0.3	37.2 $\pm$ 1.0	0.37
		10	36.5 $\pm$ 0.6	37.4 $\pm$ 0.5	36.2 $\pm$ 0.3	0.25
		20	37.4 $\pm$ 0.5	36.4 $\pm$ 2.3	37.5 $\pm$ 0.2	0.84
	4	0	38.2 $\pm$ 0.3 <sup>a</sup>	41.6 $\pm$ 0.4 <sup>b</sup>	36.2 $\pm$ 0.2 <sup>c</sup>	< 0.001
		10	37.9 $\pm$ 0.3 <sup>a</sup>	39.1 $\pm$ 0.6 <sup>a</sup>	35.7 $\pm$ 0.4 <sup>b</sup>	< 0.001
		20	42.8 $\pm$ 4.8	37.8 $\pm$ 0.8	38.0 $\pm$ 0.1	0.45
	6	0	36.6 $\pm$ 0.1	36.8 $\pm$ 0.6	37.3 $\pm$ 0.4	0.48
		10	38.3 $\pm$ 1.3	35.3 $\pm$ 0.4	35.4 $\pm$ 0.4	0.05
		20	X	41.8 $\pm$ 4.3 <sup>d</sup>	33.4 $\pm$ 0.8	
	8	0	37.5 $\pm$ 0.7	37.5 $\pm$ 0.7	38.0 $\pm$ 0.6	0.81
		10	38.4 $\pm$ 2.7	36.8 $\pm$ 0.3	36.0 $\pm$ 0.2	0.55
20		X	X	X		

a,b,c Means followed by the same letter, or by no letter, do not differ at p = 0.05 by SNK multiple range tests.

X No survivors.

<sup>d</sup> At 39 days.

Figure 56

Abnormalities in erythroid maturation in mice  
fed T-2 toxin for up to 8 weeks.

- A. Peripheral blood smear, day 56, group L-0.  
All erythrocytes are morphologically normal in control mice.
  
- B. Peripheral blood smear, day 14, group L-10.  
Erythrocytes are normal, but polychromatophils are absent.
  
- C. Peripheral blood smear, day 28, group L-10.  
Polychromatophils have returned to circulation (arrows).
  
- D. Peripheral blood smear, day 56, group L-10.  
Many large polychromatophilic erythrocytes are in  
circulation (R). Some have basophilic stippling (S).  
Various abnormalities of erythrocytic morphology are present,  
including leptocytes (L), poikilocytes (P), microcytes (M)  
and stomatocytes (T).
  
- E. Bone marrow smear, day 42, group L-10.  
Note the megaloblastic maturation of young erythrocytes  
(arrow) and the basophilic stippling of cytoplasm (S).  
Some abnormalities in nuclear shape in developing red cells  
are evident (arrowhead).
  
- F. Peripheral blood smear, day 56, N-20-A.  
Mice fed higher levels of T-2 toxin (20 ppm) for 8 weeks also  
have abnormalities of circulating erythrocytes, similar to  
those shown in Fig. 56D above.

Wright's-Giemsa stained smears, x 750.

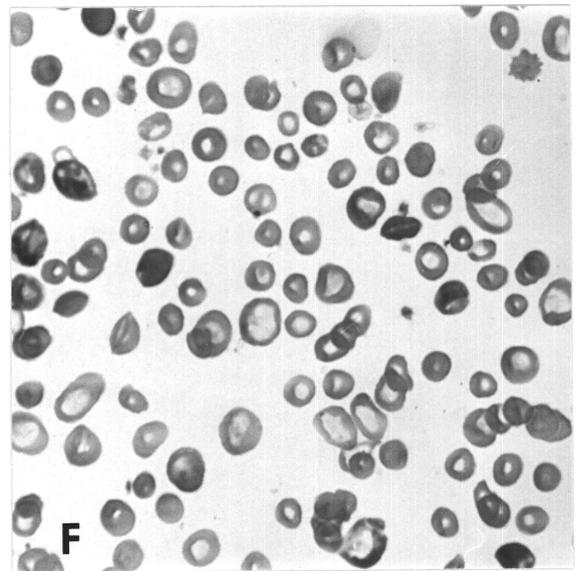
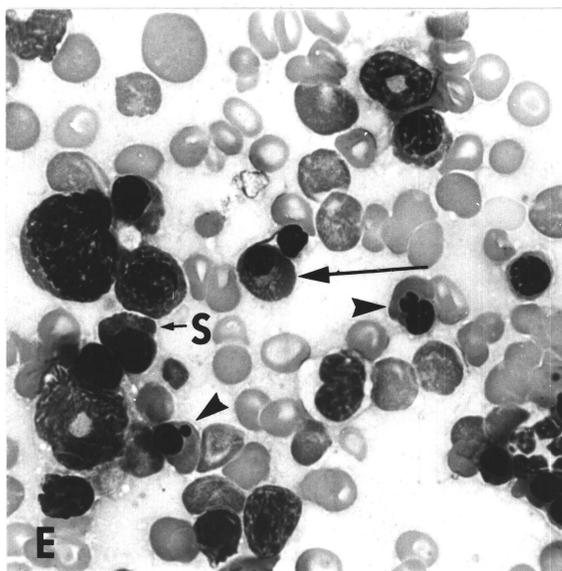
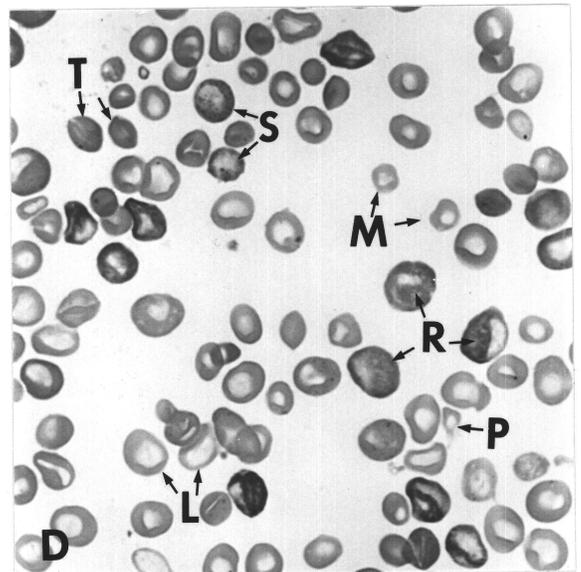
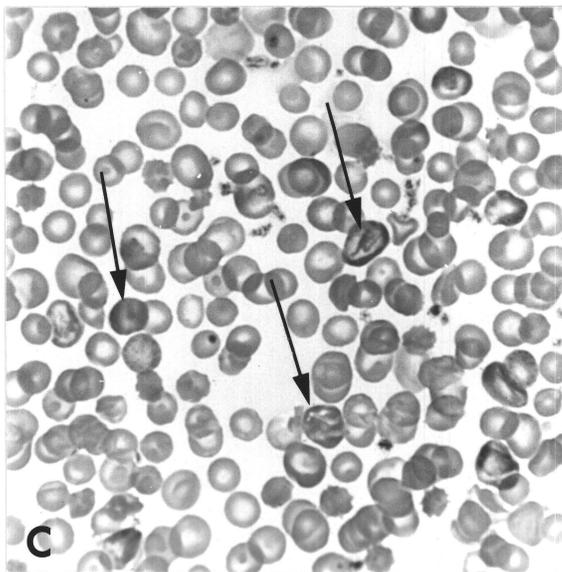
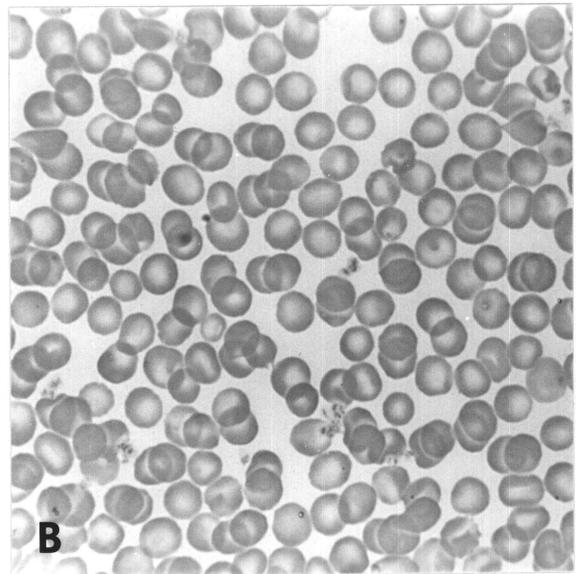
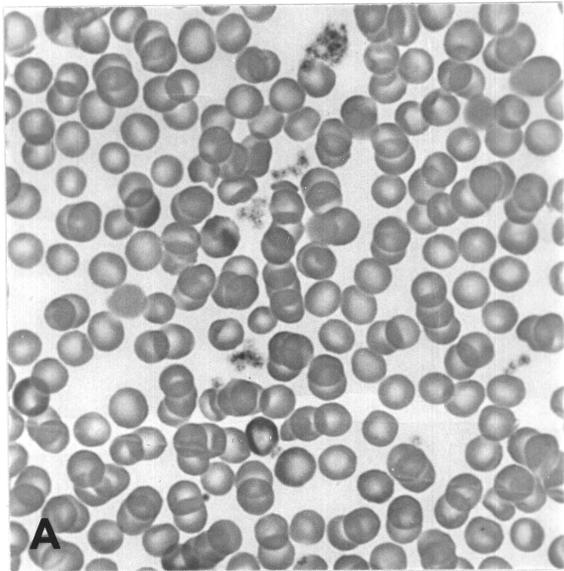


Fig. 56

into circulation. At 42 and 56 days, erythrocytes in these mice also exhibited marked anisocytosis with many poikilocytes, microspherocytes, macrocytes and leptocytes evident in circulation (Fig. 56D). Anemic mice had abnormalities of bone marrow (Fig. 56E) described in Section 9.4.3.3. No morphological abnormalities were observed in erythrocytes of mice on the other 2 diets containing the 10-ppm level of toxin. Some mice on the 20-ppm level in group N-20-A also exhibited morphological abnormalities of circulating erythrocytes at 8 weeks (Fig. 56F).

#### 9.4.2.2 Leukon

T-2 toxin caused a dose-related depression in total leukocyte counts, the degree of which did not appear to be diet-related (Fig. 57A; Table 40). Reductions were due mainly to dose-dependent decreases in numbers of circulating lymphocytes (Fig. 57B; Table 41). Neutrophil counts were elevated in mice receiving T-2 toxin (Fig. 57C), particularly in those on the 16%-protein diet (Fig. 58). However, the degree of neutrophilia was not dose-related, because the higher level of toxin resulted in lower neutrophil counts than did the 10-ppm level of toxin (Fig. 57C; Table 41). Lymphocyte counts in mice receiving the 10-ppm levels were similar in each of the 3 diet groups and were lowest on day 14 (Fig. 59).

Mice consuming T-2 toxin at both levels and in all diets had virtually absolute eosinopenia (Table 42). Eosinophils returned into circulation by weeks 6 and 8 in some mice on the 10-ppm level in the 2 normal protein diets (groups H-10 and N-10), but remained low in group L-10. Monocyte counts (Table 42) were low in mice on the 20-ppm levels in all diets, but were similar in the control and the 10-ppm groups (Table 42). On days 14 and 28, mice in group H-10 had significantly

TABLE 40: Total leukocyte values ( $\bar{X} \pm \text{SEM}$ ) of young Swiss mice fed T-2 toxin in three different diets.

Variable	Week	T-2 toxin (ppm)	8% protein semipurified	16% protein semipurified	18.6% protein stock diet	P
	0	0	--	--	8.4 $\pm$ 0.6	
Leukocyte count x 10 <sup>3</sup> / $\mu$ l	2	0	13.2 $\pm$ 1.5	13.8 $\pm$ 1.2	13.0 $\pm$ 1.0	0.89
		10	4.9 $\pm$ 0.7 <sup>a</sup>	10.4 $\pm$ 1.1 <sup>b</sup>	6.8 $\pm$ 0.6 <sup>a</sup>	0.002
		20	5.3 $\pm$ 0.8 <sup>a</sup>	9.3 $\pm$ 1.2 <sup>b</sup>	3.2 $\pm$ 0.6 <sup>a</sup>	0.003
	4	0	18.8 $\pm$ 1.4	19.4 $\pm$ 1.8	16.3 $\pm$ 1.5	0.39
		10	10.2 $\pm$ 1.9	14.6 $\pm$ 2.5	8.3 $\pm$ 0.5	0.09
		20	3.3 $\pm$ 1.6	3.4 $\pm$ 0.6	3.3 $\pm$ 0.3	1.0
	6	0	19.6 $\pm$ 1.5	16.3 $\pm$ 1.3	18.8 $\pm$ 1.3	0.26
		10	5.8 $\pm$ 1.4 <sup>a</sup>	11.2 $\pm$ 1.3 <sup>b</sup>	9.4 $\pm$ 0.6 <sup>b</sup>	0.02
		20	X	1.6 $\pm$ 0.5 <sup>d</sup>	3.5 $\pm$ 1.1	
	8	0	21.5 $\pm$ 1.7 <sup>a</sup>	14.9 $\pm$ 1.5 <sup>b</sup>	17.3 $\pm$ 1.9 <sup>ab</sup>	0.05
		10	6.3 $\pm$ 1.7 <sup>a</sup>	15.3 $\pm$ 1.4 <sup>b</sup>	7.8 $\pm$ 1.3 <sup>a</sup>	0.002
		20	X	X	X	

a,b,c Means followed by the same letter, or by no letter, do not differ at p = 0.05 by SNK multiple range tests.

X No survivors.

d At 39 days.

TABLE 41: Leukocyte values ( $\bar{X} \pm \text{SEM}$ ) of young Swiss mice fed T-2 toxin in three different diets.

Variable	Week	T-2 toxin (ppm)	8% protein semipurified	16% protein semipurified	18.6% protein stock diet	P	
Neutrophil count $\times 10^3/\mu\text{l}$	0	0	--	--	1.4 $\pm$ 0.1		
	2	0	2.0 $\pm$ 0.3	1.8 $\pm$ 0.4	1.5 $\pm$ 0.4	0.63	
		10	3.4 $\pm$ 0.4 <sup>a</sup>	7.3 $\pm$ 0.7 <sup>b</sup>	3.7 $\pm$ 0.7 <sup>a</sup>	<0.001	
		20	4.1 $\pm$ 0.5 <sup>a</sup>	8.3 $\pm$ 1.3 <sup>b</sup>	2.1 $\pm$ 0.7 <sup>a</sup>	0.002	
	4	0	1.2 $\pm$ 0.2	1.5 $\pm$ 0.2	2.3 $\pm$ 0.4	0.06	
		10	4.4 $\pm$ 0.8 <sup>ab</sup>	8.0 $\pm$ 1.8 <sup>b</sup>	3.2 $\pm$ 0.6 <sup>a</sup>	0.04	
		20	0.5 $\pm$ 0.0 <sup>a</sup>	3.6 $\pm$ 0.3 <sup>b</sup>	1.6 $\pm$ 0.1 <sup>a</sup>	0.003	
	6	0	1.2 $\pm$ 0.2	1.3 $\pm$ 0.1	1.2 $\pm$ 0.3	0.94	
		10	1.8 $\pm$ 0.2 <sup>a</sup>	7.6 $\pm$ 1.3 <sup>b</sup>	4.9 $\pm$ 0.7 <sup>c</sup>	0.001	
		20	X	0.0 $\pm$ 0.0 <sup>d</sup>	1.8 $\pm$ 0.3		
	8	0	2.1 $\pm$ 0.2	1.4 $\pm$ 0.1	1.9 $\pm$ 0.3	0.11	
		10	3.9 $\pm$ 1.0 <sup>a</sup>	10.9 $\pm$ 1.3 <sup>b</sup>	3.7 $\pm$ 0.7 <sup>a</sup>	<0.001	
		20	X	X	X		
	Lymphocyte count $\times 10^3/\mu\text{l}$	0	0	--	--	6.6 $\pm$ 0.6	
		2	0	11.2 $\pm$ 1.3	11.4 $\pm$ 1.5	11.3 $\pm$ 1.0	0.99
10			1.5 $\pm$ 0.5	2.8 $\pm$ 0.6	3.1 $\pm$ 0.3	0.08	
20			1.2 $\pm$ 0.3	0.9 $\pm$ 0.2	1.1 $\pm$ 0.2	0.79	
4		0	16.4 $\pm$ 1.5	16.4 $\pm$ 2.3	13.5 $\pm$ 1.4	0.41	
		10	5.2 $\pm$ 1.2	4.6 $\pm$ 0.6	4.9 $\pm$ 0.8	0.88	
		20	1.5 $\pm$ 0.0 <sup>ab</sup>	0.3 $\pm$ 0.1 <sup>a</sup>	1.7 $\pm$ 0.3 <sup>b</sup>	0.03	
6		0	17.6 $\pm$ 1.6	14.2 $\pm$ 1.3	16.4 $\pm$ 1.5	0.28	
		10	3.8 $\pm$ 1.1	3.3 $\pm$ 0.8	4.0 $\pm$ 0.5	0.84	
		20	X	1.7 $\pm$ 0.4 <sup>d</sup>	1.7 $\pm$ 0.8		
8		0	18.8 $\pm$ 1.4 <sup>a</sup>	12.9 $\pm$ 1.3 <sup>b</sup>	15.3 $\pm$ 1.6 <sup>ab</sup>	0.04	
		10	2.3 $\pm$ 0.8	4.2 $\pm$ 0.3	4.0 $\pm$ 0.8	0.17	
		20	X	X	X		

a,b,c Means followed by the same letter, or by no letter, do not differ at  $p = 0.05$  by SNK multiple range tests.

X No survivors.

d At 39 days.

TABLE 42: Eosinophils and monocytes ( $\bar{X} \pm \text{SEM}$ ) of young Swiss mice fed T-2 toxin in three different diets.

Variable	Week	T-2 toxin (ppm)	8% protein semipurified	16% protein semipurified	18.6% protein stock diet	P	
Eosinophil count $\times 10^3/\mu\text{l}$	0	0	--	--	0.13 $\pm$ 0.04		
	2	0	0.00 $\pm$ 0.00 <sup>a</sup>	0.16 $\pm$ 0.05 <sup>b</sup>	0.00 $\pm$ 0.00 <sup>a</sup>	0.005	
		10	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	1.00	
		20	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	1.00	
	4	0	0.31 $\pm$ 0.09	0.49 $\pm$ 0.20	0.20 $\pm$ 0.07	0.32	
		10	0.08 $\pm$ 0.04	0.00 $\pm$ 0.00	0.03 $\pm$ 0.03	0.20	
		20	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	1.00	
	6	0	0.37 $\pm$ 0.14	0.59 $\pm$ 0.10	0.36 $\pm$ 0.06	0.26	
		10	0.00 $\pm$ 0.00	0.10 $\pm$ 0.10	0.09 $\pm$ 0.04	0.38	
		20	X	0.00 $\pm$ 0.00 <sup>d</sup>	0.00 $\pm$ 0.00		
	8	0	0.41 $\pm$ 0.15	0.53 $\pm$ 0.39	0.08 $\pm$ 0.05	0.44	
		10	0.0 $\pm$ 0.0	0.24 $\pm$ 0.10	0.07 $\pm$ 0.03	0.05	
		20	X	X	X		
	Monocyte count $\times 10^3/\mu\text{l}$	0	0	--	--	0.24 $\pm$ 0.13	
		2	0	0.00 $\pm$ 0.00 <sup>a</sup>	0.29 $\pm$ 0.04 <sup>b</sup>	0.10 $\pm$ 0.04 <sup>a</sup>	<0.001
10			0.01 $\pm$ 0.01 <sup>a</sup>	0.19 $\pm$ 0.09 <sup>b</sup>	0.02 $\pm$ 0.02 <sup>a</sup>	0.05	
20			0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	1.00	
4		0	0.83 $\pm$ 0.20	0.69 $\pm$ 0.23	0.37 $\pm$ 0.05	0.22	
		10	0.63 $\pm$ 0.18 <sup>a</sup>	2.02 $\pm$ 0.59 <sup>b</sup>	0.17 $\pm$ 0.10 <sup>a</sup>	0.009	
		20	0.0 $\pm$ 0.0	0.07 $\pm$ 0.02	0.01 $\pm$ 0.01	0.08	
6		0	0.40 $\pm$ 0.19	0.23 $\pm$ 0.08	0.43 $\pm$ 0.08	0.47	
		10	0.07 $\pm$ 0.06	0.08 $\pm$ 0.03	0.34 $\pm$ 0.14	0.14	
		20	X	0.00 $\pm$ 0.00 <sup>d</sup>	0.00 $\pm$ 0.00		
8		0	0.04 $\pm$ 0.04	0.02 $\pm$ 0.02	0.0 $\pm$ 0.0	0.55	
		10	0.0 $\pm$ 0.0	0.04 $\pm$ 0.04	0.0 $\pm$ 0.0	0.44	
		20	X	X	X		

<sup>a,b,c</sup> Means followed by the same letter, or by no letter, do not differ at  $p = 0.05$  by SNK multiple range tests.

X No survivors.

<sup>d</sup> At day 39.

Figure 57

Comparison of effects of T-2 toxin at 3 levels in 3 different diets on leukocyte counts of young Swiss mice fed for 4 weeks.

- Natural-ingredient diet.
- 16%-protein semipurified diet.
- 8%-protein semipurified diet.

All points are means ( $\pm$ SEM) of separate groups of 4 or 5 mice, except that counts from only 1 mouse were obtained for the 20-ppm, 8% SP diet.

- A. Total leukocyte counts.  
Note the dose dependent leukopenia that does not depend on the diet.
- B. Total lymphocyte counts.  
T-2 toxin caused similarly severe dose-related lymphopenia irrespective of the diet used.  
Note that mice on the 10-ppm level were markedly leukopenic compared with the ad lib fed control groups fed 0 ppm.
- C. Total neutrophil counts.  
T-2 toxin caused neutrophilia that was more severe at the 10-ppm level than at the 20-ppm level. This is possibly due to a myelosuppressive effect of the high level. The neutrophilia occurs due to ulcerative gastritis in mice on toxic diets.

# Leukocyte Counts (Day 28)

○ 8% protein SP diet    □ 16% protein SP diet    ● Natural ingredient diet

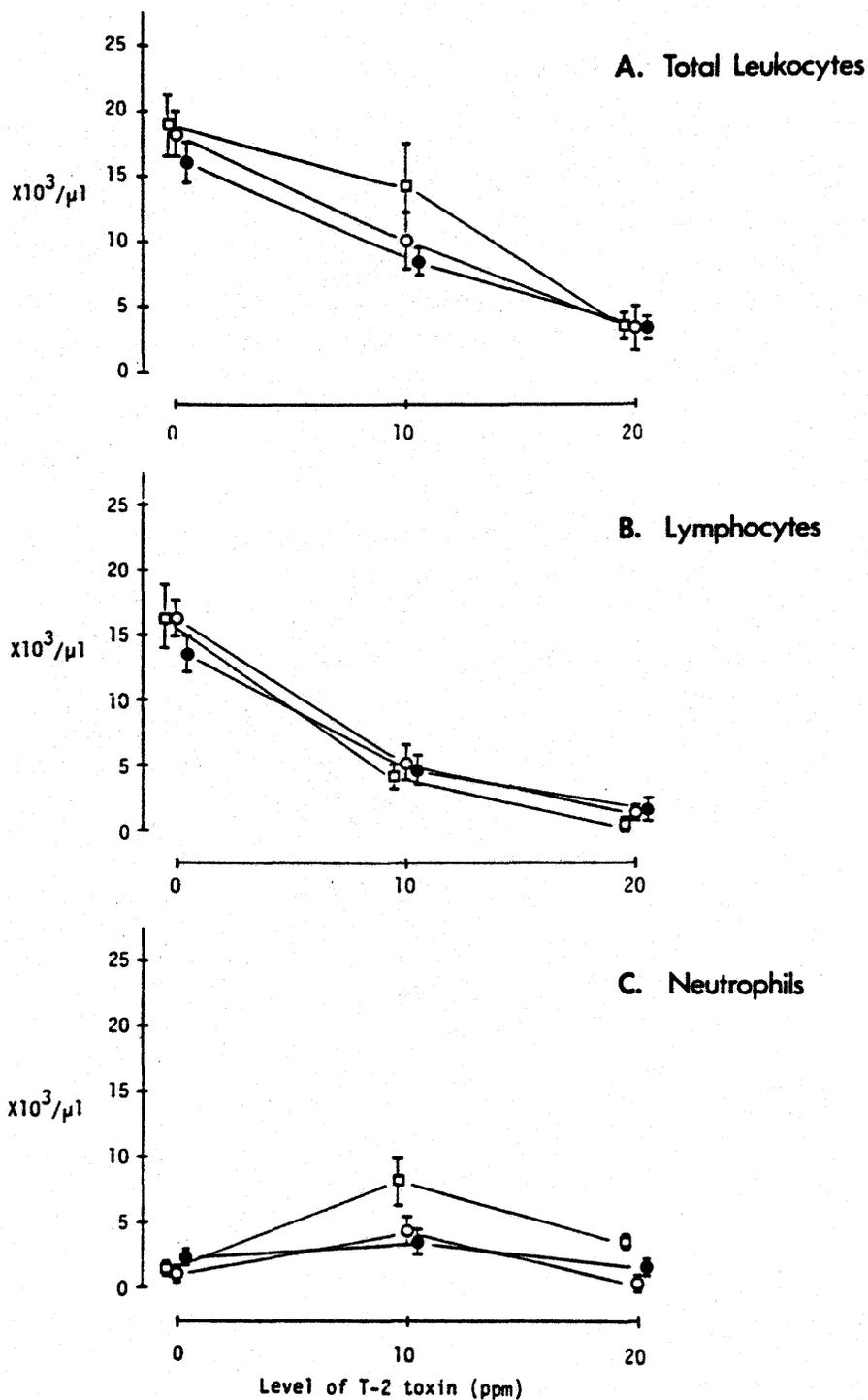


Figure 58

Sequential changes in neutrophil counts of mice fed T-2 toxin (10 ppm) in 3 different diets for 8 weeks.

All groups developed neutrophilia, but it is more severe in the 16% protein semipurified diet group.

Control range is 1.2 - 2.3 (Table 41).

Points are means (+SEM) of separate groups of 5 mice.

Figure 59

Sequential changes in lymphocyte counts of mice fed T-2 toxin (10 ppm) in 3 different diets for 8 weeks.

All groups developed a similarly marked degree of lymphopenia that was most severe at 2 weeks, especially in the 8% protein semipurified diet group.

Control range is 11.2 - 18.8 (Table 41).

Points are means (+SEM) of separate groups of 5 mice.

Fig. 58 Neutrophil Counts

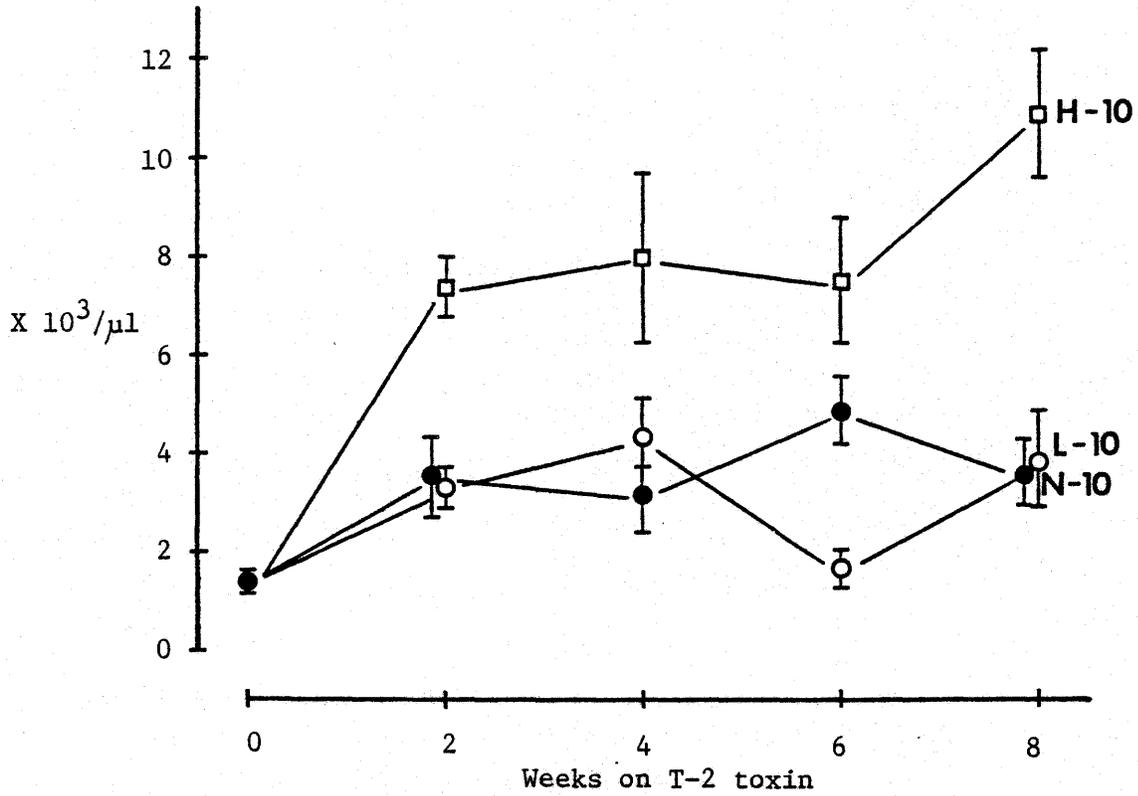
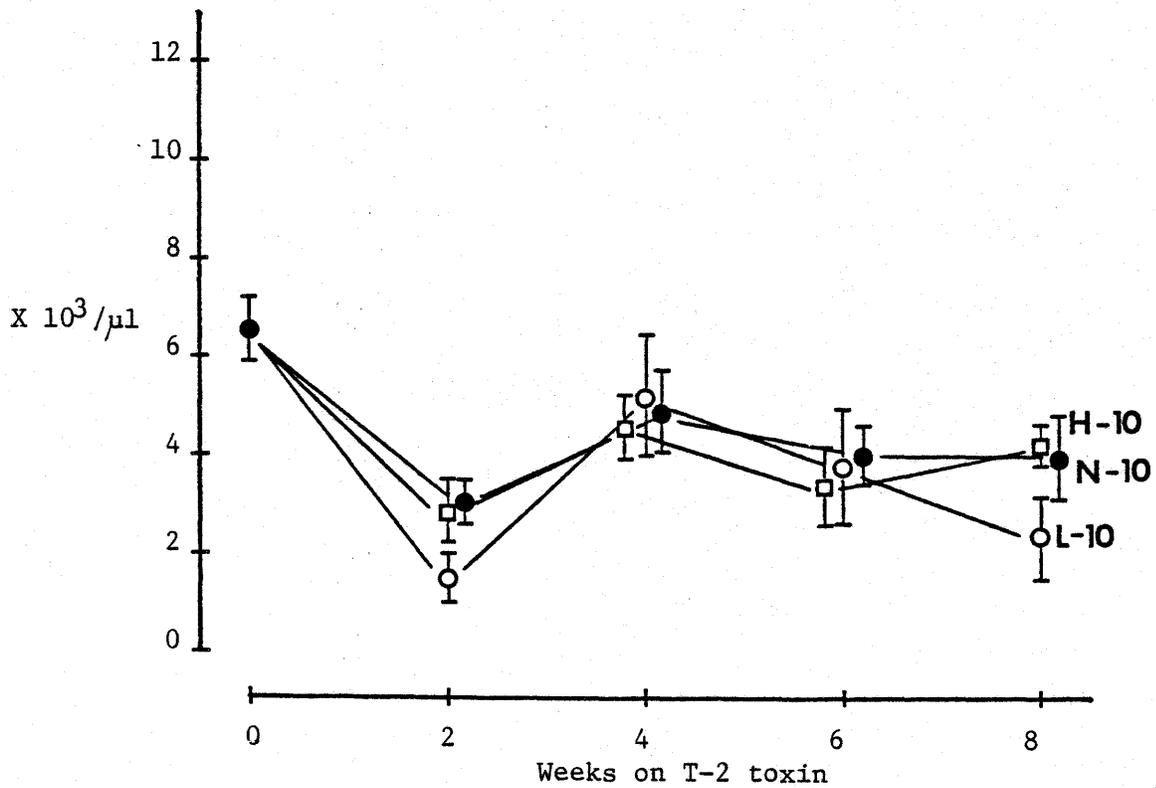


Fig. 59 Lymphocyte Counts



higher monocyte counts, which correlated with marked elevation in neutrophil counts in these 2 groups (Tables 41, 42).

Leukocytes were morphologically normal in all mice. Numbers of thrombocytes on blood smears were reduced in most mice on the 20-ppm level of toxin on days 14 and 28, irrespective of diet. However, numbers varied widely between mice in these groups, with a few having virtually no thrombocytes present on blood smears, particularly those mice that were killed in a moribund state.

#### 9.4.3 Necropsy findings

No consistent or significant gross or microscopic changes were found in any of the 3 control diet groups, except that the largest mice in groups L-0 and H-0 developed hepatic lipidosis by days 42 and 56. Mice consuming T-2 toxin at the 20-ppm level consistently developed aplastic anemia, gastric hyperkeratosis with ulceration, and generalized lymphoid atrophy similar to mice on this level in the previous experiment (Sections 8.4.3.1, 8.4.3.3). This high level produced similar effects in each of the 3 diet groups, and because they were similar to those described previously, they will not be described again. Similar, but less severe, effects were observed in mice consuming the 10-ppm level, with marked differences in severity of these effects and responses being observed among the different diet groups.

##### 9.4.3.1 Macroscopic findings

Mice that died, or that were euthanized in a moribund state, were extremely emaciated, anemic, and some had hemorrhage into the alimentary tract (Fig. 60A, 60B). All mice on the 20-ppm level of toxin developed complete atrophy of red and white pulp of the spleen (Fig. 61), and of



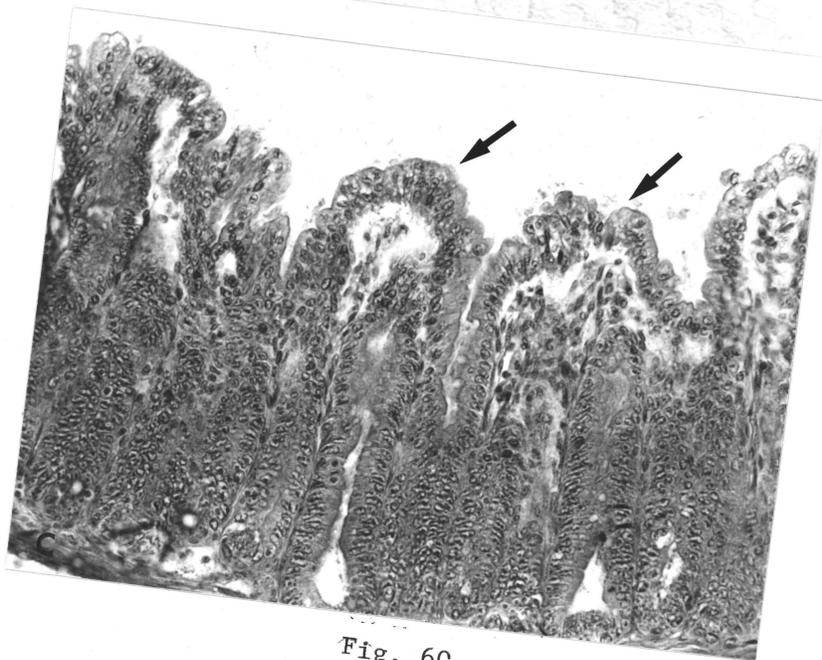
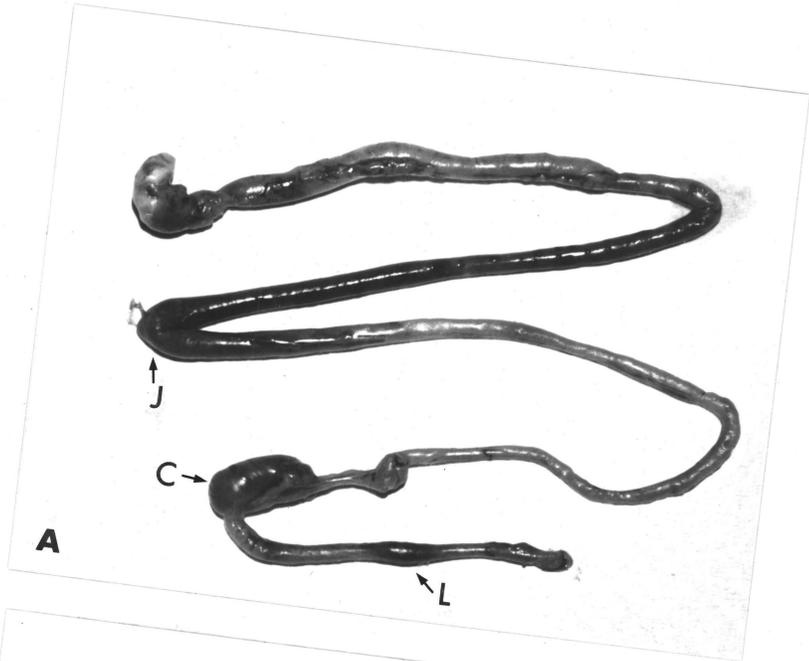


Fig. 60

other lymphoid organs. These also developed severe proliferative hyperkeratosis of the squamous gastric mucosa (Fig. 62), and perioral dermatitis as previously observed. Mice on the lower level of T-2 toxin (10 ppm) had different effects depending on the diet. Mice in group L-10 consistently had atrophic lymphoid tissues throughout the 8 week experimental period. The thymus was markedly reduced in size by 14 days, but the splenic white pulp, Peyer's patches, and mesenteric lymph nodes were atrophic by day 28. For the two other diets, the degree of thymic atrophy was more variable, and was generally less severe than for group L-10. For the natural-ingredient diet, there were few differences in the thymus between control (N-0) and treated (N-10) groups. In most mice in groups H-10 and N-10, there were no appreciable reductions in the size of the splenic white pulp, mesenteric lymph nodes or Peyer's patches, except for slight atrophy observed on days 14 and 28 in no more than 2 at each stage in each group. After 28 days, the 10-ppm level of toxin had no visible effects on the macroscopic appearance of these lymphoid tissues in mice on the 2 adequate diets (H-10 and N-10), in contrast to mice in group L-10 in which generalized lymphoid atrophy was consistently evident.

The splenic red pulp was moderately atrophic and tan-coloured by day 14 in most mice in group L-10, but subsequently, mice in this group underwent progressive and eventually extreme splenic enlargement (Fig. 63). By day 28, and thereafter, the enlarged spleens were homogeneously red, but not until days 42 and 56 did they develop the same deep dark red colour observed in the controls. In contrast, mice consuming the 10-ppm level in the 2 adequate diets did not exhibit abnormalities in the splenic

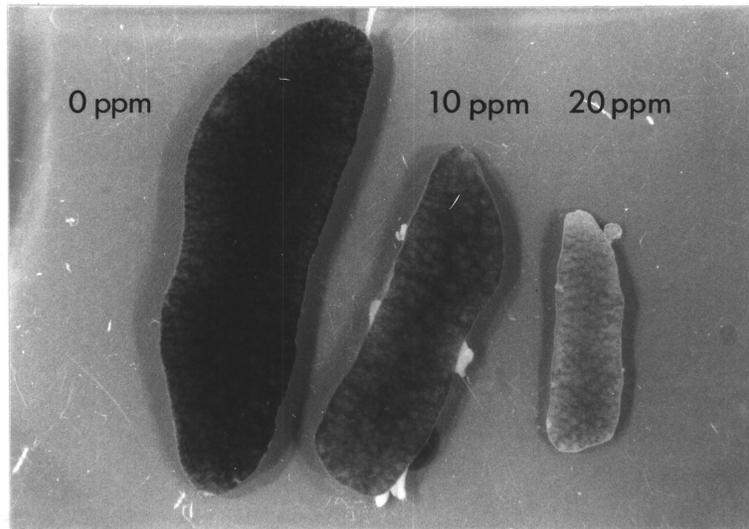


Fig. 61: Appearance of the spleen of mice fed T-2 toxin in the natural-ingredient diet for 14 days. Note the atrophy of the red pulp. White pulp is still distinguishable.

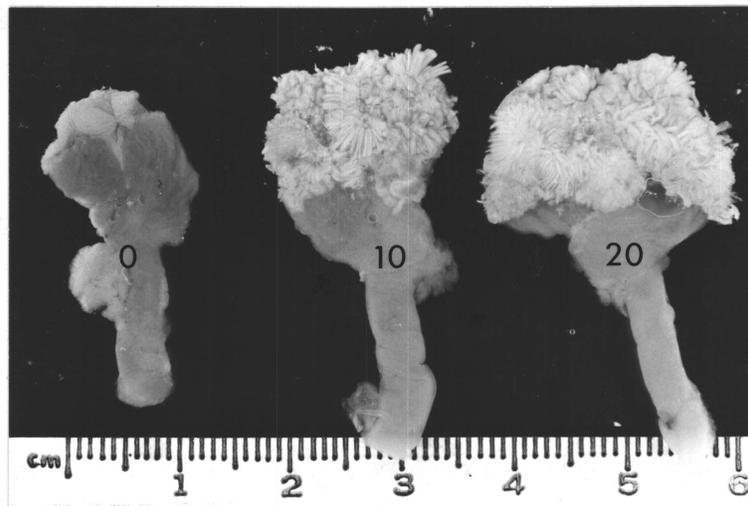


Fig. 62: Appearance of the stomach of mice fed T-2 toxin in the natural-ingredient diet for 4 weeks. Note the papillary proliferation of the squamous mucosa in each of the mice on the toxic diets.

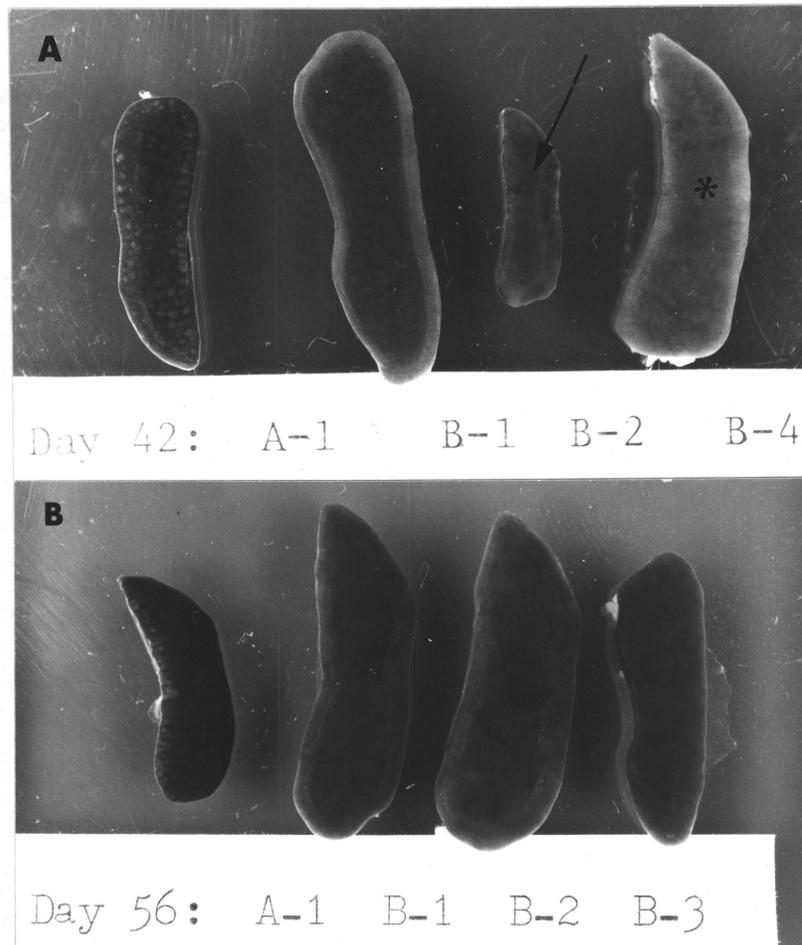


Fig. 63: Splenomegaly in mice fed T-2 toxin (10 ppm) in the 8%-protein semipurified diet for 6 and 8 weeks.

- A. At 6 weeks.  
The degree of splenic enlargement was variable; some mice still had atrophic spleens (arrow) at this stage. Moderately enlarged spleens had homogeneous pale red parenchyma (asterisk).
- B. At 8 weeks.  
All mice on 10 ppm T-2 toxin in this diet had extreme splenomegaly. At this stage the spleen was dark red and homogeneous. White pulp was not discernable (compare with control).

A-1 = Control; B-1 to B-4 = Treated.

red pulp. The macroscopic appearance of the bone marrow was variable in all groups on the 10-ppm level of toxin, except that on days 42 and 56, it was consistently dark red and cellular in all mice in group L-10.

The squamous gastric mucosa was consistently hyperkeratotic in all groups consuming the 10-ppm level of T-2 toxin. The degree of thickening increased with time, and a few mice developed small ulcers in the hyperplastic mucosa. The degree of hyperkeratosis was more severe in mice consuming the 20-ppm level of toxin (Fig. 62), but differences in severity of these changes were not apparent among the different diet groups.

#### 9.4.3.2 Organ weight changes

All mice consuming T-2 toxin had reduced thymic weights; this was dose-related but not diet-related in mice fed the 20-ppm level (Fig. 64A). However, for the 10-ppm level, thymic weights were lower in mice on the 8%-protein diet (group L-10) (Fig. 64A). The thymic weights of mice fed the 10-ppm level in the 16%-protein diet (group H-10) were lower than in the controls (Table 43). Mice in group N-10 had significantly lower thymic weights than control mice (N-0) after 4 weeks, but subsequently, the thymus was similar in groups N-0 and N-10 (Table 43).

The spleen also exhibited a dose-related reduction in size on days 14 and 28; this reduction was not diet-related. Subsequently, relative splenic weights of mice on the 10-ppm level were similar to the controls except in group L-10 in which progressively increasing, marked splenomegaly occurred (Fig. 64B and 64C; Table 44).

Among the control groups, mice on the natural-ingredient diets

TABLE 43: Thymic weights ( $\bar{X}$ +SEM) of young Swiss mice fed T-2 toxin in three different diets.

Variable	Week	T-2 toxin (ppm)	8% protein semipurified	16% protein semipurified	18.6% protein stock diet	P	
Thymic weight mg	0	0			NA		
	2	0	NA	NA	NA		
		10	NA	NA	NA		
		20	NA	NA	NA		
	4	0	91.0 $\pm$ 7.4	75.4 $\pm$ 11.2	72.8 $\pm$ 8.5	0.35	
		10	10.8 $\pm$ 2.7 <sup>a</sup>	24.6 $\pm$ 4.6 <sup>b</sup>	27.2 $\pm$ 2.9 <sup>b</sup>	0.01	
		20	4.0 $\pm$ 1.2 <sup>a</sup>	4.0 $\pm$ 0.4 <sup>a</sup>	8.5 $\pm$ 0.6 <sup>b</sup>	0.003	
	6	0	63.6 $\pm$ 11.1 <sup>a</sup>	62.2 $\pm$ 7.0 <sup>a</sup>	104.0 $\pm$ 9.0 <sup>b</sup>	0.01	
		10	7.8 $\pm$ 1.2 <sup>a</sup>	11.5 $\pm$ 1.8 <sup>a</sup>	53.6 $\pm$ 11.2 <sup>b</sup>	0.001	
		20	X	< 2.0 <sup>d</sup>	14.0 $\pm$ 4.0		
	8	0	101.8 $\pm$ 11.8 <sup>a</sup>	103.6 $\pm$ 9.5 <sup>a</sup>	60.0 $\pm$ 12.9 <sup>b</sup>	0.03	
		10	5.6 $\pm$ 1.6 <sup>a</sup>	26.8 $\pm$ 6.3 <sup>a</sup>	55.6 $\pm$ 13.5 <sup>b</sup>	0.005	
		20	X	X	X		
	Relative thymic weight percent of BW	0	0			NA	
		2	0	NA	NA	NA	
10			NA	NA	NA		
20			NA	NA	NA		
4		0	0.27 $\pm$ 0.02	0.20 $\pm$ 0.03	0.24 $\pm$ 0.03	0.25	
		10	0.07 $\pm$ 0.01 <sup>a</sup>	0.12 $\pm$ 0.02 <sup>b</sup>	0.12 $\pm$ 0.01 <sup>b</sup>	0.04	
		20	0.03 $\pm$ 0.01 <sup>a</sup>	0.03 $\pm$ 0.01 <sup>a</sup>	0.05 $\pm$ 0.00 <sup>b</sup>	0.02	
6		0	0.16 $\pm$ 0.02 <sup>a</sup>	0.14 $\pm$ 0.02 <sup>a</sup>	0.26 $\pm$ 0.02 <sup>b</sup>	0.004	
		10	0.05 $\pm$ 0.01 <sup>a</sup>	0.05 $\pm$ 0.01 <sup>a</sup>	0.20 $\pm$ 0.04 <sup>b</sup>	0.002	
		20	X	< 0.02 <sup>d</sup>	0.06 $\pm$ 0.02		
8		0	0.25 $\pm$ 0.02	0.24 $\pm$ 0.03	0.15 $\pm$ 0.04	0.06	
		10	0.03 $\pm$ 0.01 <sup>a</sup>	0.09 $\pm$ 0.02 <sup>a</sup>	0.18 $\pm$ 0.04 <sup>b</sup>	0.006	
		20	X	X	X		

a,b,c Means followed by the same letter, or by no letter, do not differ at p = 0.5 by SNK multiple range tests.

X No survivors.

NA Data not available.

<sup>d</sup> At 39 days.

TABLE 44: Splenic weights ( $\bar{X}$ +SEM) of young Swiss mice fed T-2 toxin in three different diets.

Variable	Week	T-2 toxin (ppm)	8% protein semipurified	16% protein semipurified	18.6% protein stock diet	P	
Splenic weight mg	0	0	--	--	119+12		
	2	0	138+11	171+16	176+19	0.23	
		10	50+32 <sup>a</sup>	125+19 <sup>b</sup>	80+23 <sup>a</sup>	0.002	
		20	33+3	28+3	24+2	0.09	
	4	0	254+22 <sup>a</sup>	336+17 <sup>b</sup>	215+4 <sup>a</sup>	<0.001	
		10	152+25	205+18	146+30	0.22	
		20	52+2 <sup>a</sup>	18+3 <sup>b</sup>	54+9 <sup>a</sup>	0.005	
	6	0	220+8 <sup>a</sup>	164+8 <sup>b</sup>	214+14 <sup>a</sup>	0.005	
		10	214+61	102+15	132+30	0.20	
		20	X	22+2 <sup>d</sup>	130+45		
	8	0	172.4+17.1 <sup>a</sup>	256.8+8.7 <sup>b</sup>	260.2+11.7 <sup>b</sup>	< 0.001	
		10	382.4+67.5 <sup>a</sup>	129.6+4.0 <sup>b</sup>	188.4+11.7 <sup>b</sup>	0.002	
		20	X	X	X		
	Relative splenic weight percent of BW	0	0	--	--	0.71+0.05	
		2	0	0.52+0.05	0.55+0.04	0.60+0.06	0.46
			10	0.35+0.08 <sup>a</sup>	0.69+0.05 <sup>b</sup>	0.43+0.05 <sup>a</sup>	0.005
			20	0.26+0.03	0.21+0.02	0.18+0.00	0.10
		4	0	0.75+0.07	0.87+0.05	0.72+0.01	0.09
10			1.00+0.11	0.98+0.08	0.66+0.12	0.07	
20			0.39+0.01 <sup>a</sup>	0.14+0.02 <sup>b</sup>	0.34+0.05 <sup>a</sup>	0.002	
6		0	0.56+0.04 <sup>a</sup>	0.37+0.02 <sup>b</sup>	0.53+0.03 <sup>a</sup>	0.003	
		10	1.35+0.32 <sup>a</sup>	0.44+0.04 <sup>b</sup>	0.52+0.15 <sup>b</sup>	0.02	
		20	X	0.17+0.02 <sup>d</sup>	0.50+0.12		
8		0	0.43+0.03 <sup>a</sup>	0.59+0.01 <sup>b</sup>	0.64+0.04 <sup>b</sup>	<0.001	
		10	2.21+0.40 <sup>a</sup>	0.46+0.02 <sup>b</sup>	0.65+0.06 <sup>b</sup>	<0.001	
	20	X	X	X			

a,b,c Means followed by the same letter, or by no letter, do not differ at p = 0.05 by SNK multiple range tests.

X No survivors.

d At 39 days.

TABLE 45: Hepatic weights ( $\bar{X}$ +SEM) of young Swiss mice fed T-2 toxin in three different diets.

Variable	Week	T-2 toxin (ppm)	8% protein semipurified	16% protein semipurified	18.6% protein stock diet	P	
Hepatic weight g	0	0			0.77±0.04		
	2	0	0.97±0.06 <sup>a</sup>	1.25±0.09 <sup>b</sup>	1.59±0.08 <sup>c</sup>	< 0.001	
		10	0.63±0.02 <sup>a</sup>	0.88±0.07 <sup>b</sup>	1.04±0.07 <sup>b</sup>	0.001	
		20	0.61±0.04	0.66±0.03	0.73±0.04	0.14	
	4	0	1.54±0.04 <sup>a</sup>	1.71±0.02 <sup>b</sup>	1.41±0.08 <sup>a</sup>	0.005	
		10	0.84±0.06 <sup>a</sup>	1.21±0.8 <sup>b</sup>	1.27±0.10 <sup>b</sup>	0.005	
		20	0.82±0.11	0.88±0.07	1.06±0.07	0.12	
	6	0	1.26±0.06 <sup>a</sup>	1.74±0.09 <sup>b</sup>	2.08±0.14 <sup>c</sup>	< 0.001	
		10	0.84±0.07 <sup>a</sup>	1.11±0.07 <sup>b</sup>	1.47±0.09 <sup>c</sup>	< 0.001	
		20	X	0.91±0.08 <sup>d</sup>	1.61±0.17		
	8	0	1.45±0.10 <sup>a</sup>	1.62±0.06 <sup>a</sup>	1.93±0.03 <sup>b</sup>	0.002	
		10	0.92±0.06 <sup>a</sup>	1.48±0.06 <sup>b</sup>	1.64±0.02 <sup>c</sup>	< 0.001	
		20	X	X	X		
	Relative hepatic weight percent of BW	0	0	--	--	4.69±0.12	
		2	0	3.58±0.14 <sup>a</sup>	4.05±0.15 <sup>a</sup>	5.47±0.24 <sup>b</sup>	< 0.001
10			4.50±0.07 <sup>a</sup>	4.78±0.16 <sup>a</sup>	5.62±0.18 <sup>b</sup>	< 0.001	
20			4.76±0.31	5.01±0.14	5.67±0.33	0.10	
4		0	4.50±0.09	4.46±0.05	4.68±0.20	0.46	
		10	5.66±0.13	5.72±0.12	5.77±0.13	0.83	
		20	6.04±0.50	6.66±0.69	6.76±0.26	0.39	
6		0	3.19±0.11 <sup>a</sup>	3.88±0.12 <sup>b</sup>	5.18±0.08 <sup>c</sup>	< 0.001	
		10	5.52±0.27	4.83±0.12	5.70±0.59	0.27	
		20	X	6.97±0.44 <sup>d</sup>	6.41±0.01		
8		0	3.59±0.18 <sup>a</sup>	3.73±0.14 <sup>a</sup>	4.73±0.12 <sup>b</sup>	< 0.001	
		10	5.30±0.40	5.21±0.14	5.68±0.28	0.51	
		20	X	X	X		

a,b,c Means followed by the same letter, or by no letter, do not differ at p = 0.05 by SNK multiple range tests.

X No survivors.

d At 39 days.

Figure 64

Effect of different levels of dietary T-2 toxin on the relative weights of thymus (A), spleen (B), and liver (D) of mice fed different diets for 4 weeks.

For comparison, the sequential changes in relative weights of spleen (C) and liver (E) of mice fed the three different diets containing 10 ppm of T-2 toxin are illustrated on the right side of the chart.

All points are means ( $\pm$ SEM) of separate groups of 4 or 5 mice. Relative organ weights are expressed as percentages of body weights.

- A. Thymus to body weight ratios. Day 28.  
Note the dose-related thymic atrophy. At the 10-ppm level, T-2 toxin caused more severe atrophy in mice fed the 8%-protein diet than in mice fed the other two diets.
- B. Spleen to body weight ratios. Day 28.  
The 20-ppm level of T-2 toxin caused splenic atrophy in all mice. The 10-ppm level of T-2 toxin caused splenomegaly in mice on the 16% and 8% protein semipurified diets by day 28 but not in mice fed the 10-ppm level of toxin in the natural-ingredient diet. The splenomegaly was due to a hyperplastic response in the red pulp.
- C. Spleen to body weight ratios. 10-ppm level of T-2 toxin.  
Note the progressive, marked splenomegaly in mice fed the 8%-protein diet containing 10 ppm of T-2 toxin. Splenomegaly at day 28 in mice on the 16%-protein diet is temporary (see Fig. 64D below).
- D. Liver to body weight ratios. Day 28.  
There is a dose-related increase in relative hepatic weight that is independent of the diet. Wide differences in body weights of mice on the different levels of toxin make this difference difficult to interpret.
- E. Liver to body weight ratios. 10-ppm level of T-2 toxin.  
The sequential plots of relative hepatic weights indicate no consistent differences among the different diet groups. However, on day 14, the livers of mice on the natural-ingredient diet were significantly larger than in mice on the other two diets.

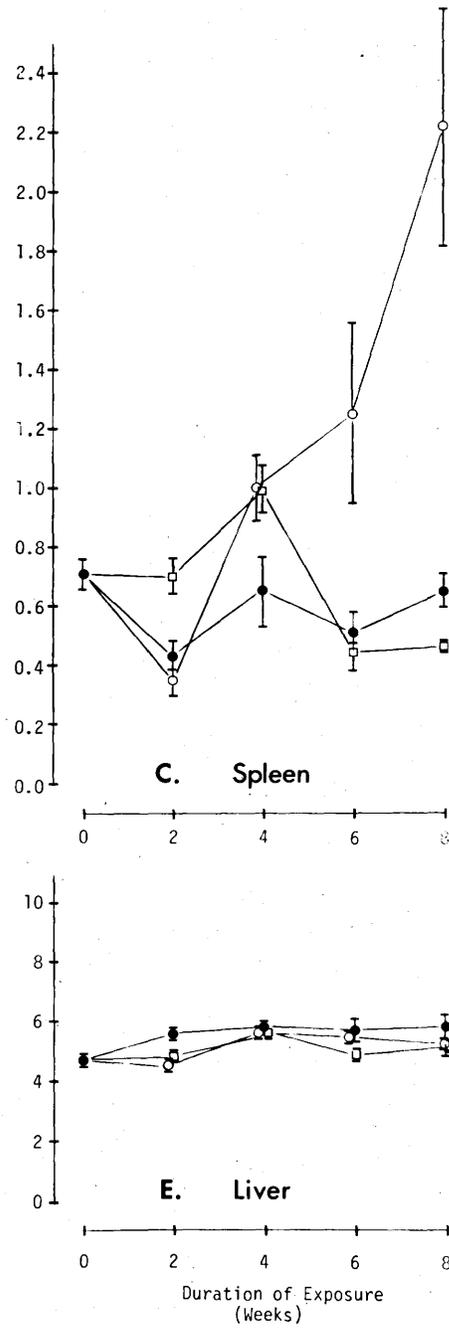
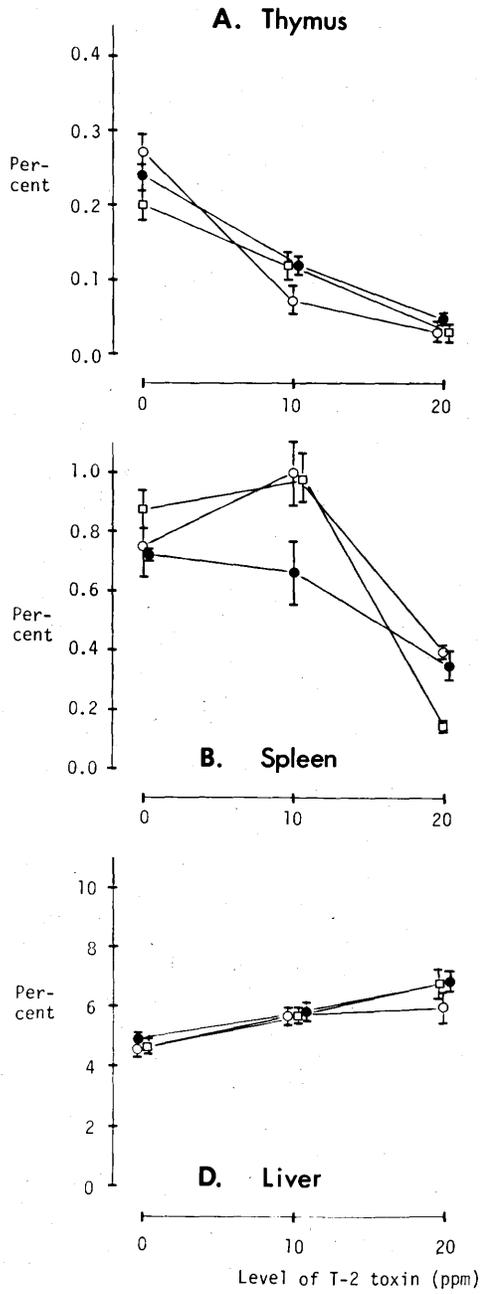
Fig. 64

Organ weight : Body weight Ratios

Dose-Response

Sequential Changes at 10ppm T-2 Toxin

○ 8% protein SP diet    □ 16% protein SP diet    ● Natural-ingredient diet



consistently had higher liver weights, both absolutely and relatively (Table 45). Livers of mice on the 8%-protein control diet (L-0) were consistently the smallest, but livers of mice in group H-0 were smaller than for group N-0 on days 14, 42 and 56. Although absolute liver weights of mice consuming T-2 toxin were smaller than controls, relative weights were generally higher; the degree of relative enlargement was dose-related, but not related to diet (Fig. 64D, 64E).

#### 9.4.3.3 Microscopic findings

In mice on the 20-ppm level of T-2 toxin, similar microscopic lesions were observed as previously described (Section 8.4.3.3). However, at 28 days, regeneration of hematopoietic tissues was less pronounced than was observed in the previous experiment. Small groups of regenerating immature myeloid cells were evident by 28 days in the splenic red pulp in only two of four mice in group N-20, and in only two of five mice in group H-20, whereas in the previous experiment, mice on the H-20 diet had marked regeneration by 3 or 4 weeks. However, in two mice remaining in group N-20 on day 42, large regenerative foci of erythroid and myeloid populations were found in the spleen and bone marrow.

The 10-ppm level of T-2 toxin produced different microscopic changes depending on the diet. Hematopoietic populations were consistently present in the splenic red pulp of control mice in group L-0 (Fig. 65A), but were absent at day 14 in three treated mice in group L-10 (Fig. 65B). By day 14, two mice in this group had small colonies of regenerating cells in the red pulp (Fig. 65C), but by day 28 regeneration of immature hematopoietic cells, mostly identifiable as erythroid, was evident throughout the red

pulp of all mice in this group (Fig. 65D). Subsequently, the regenerating population increased such that on days 42 and 56, erythroid maturation was visible, although immature stages appeared to predominate (Fig. 65E,F). By comparison, mice on the 10-ppm level in the optimal diets did not, at any stage, exhibit signs of atrophy or hyperplasia of hematopoietic populations in the splenic red pulp, except that a mouse in group H-10 had colonies of undifferentiated cells in the red pulp on day 14.

In group L-10, proportions of erythroid precursors in bone marrow declined by day 14, but then increased (Table 46). Abnormal erythropoiesis (Fig. 56F), in the form of megaloblastic development, basophilic stippling of cytoplasm of rubricytes, and fragmentation of nuclei of rubricytes and metarubricytes, was observed on smears of bone marrow on days 28 and 42 in group L-10. By day 56, such abnormalities were observed in only two mice in this group.

Hematopoiesis in mice in groups H-10 and N-10 usually appeared normal, except that numbers of eosinophils and their precursors were depleted at all stages in these groups (Table 47). On day 14, two mice in group H-10 had mild degrees of megaloblastic and dysplastic erythroid maturation similar to changes observed in group L-10.

Myeloid to erythroid ratios (ME) (Table 46) were generally increased in each of three groups on the 10-ppm level of toxin. On day 14, ME ratios were near normal in mice in groups H-10 and N-10, but were greatly elevated in group L-10 due to depression of erythropoiesis in this group (Table 46). Subsequently, ME ratios were elevated in each of these groups, due to an increase in the activity of neutrophilic myelopoiesis. Ratios of mature to immature stages in both the erythroid and myeloid populations

(Table 48) were consistent in all control groups, but on day 14, mice in groups H-10 and N-10 had significantly more mature erythroid cells than did the corresponding control groups, whereas in mice in group L-10 numbers of mature erythroid cells were lower than in the controls. On days 14, 42 and 56, all 3 groups on the 10-ppm toxic diets had similar increases in the proportions of mature to immature myeloid stages.

Microscopic changes in lymphoid tissues also varied depending on the diet. All mice on the 20-ppm level consistently displayed a similar severe degree of atrophy of thymus, splenic white pulp, lymph nodes and gut-associated lymphoid tissue as was previously observed (Section 8.4.2.3). The 10-ppm level induced thymic atrophy in all mice on the 8%-protein diet (group L-10) and this persisted throughout the 8-week experiment. However, for the 2 optimal diets, only some mice exhibited thymic atrophy, the degree of which was variable and rarely complete (Table 49). Other lymphoid tissues were less severely affected than the thymus in all groups, such that only those mice on the 8%-protein diet exhibited histological evidence of atrophy of the splenic white pulp and the paracortical zones of lymph nodes. Gut-associated lymphoid tissue in mice on the 10-ppm level of toxin did not appear depleted. Other than the overall reduction in the size of the Peyer's patches in many mice in these groups on the 10-ppm level of toxin, the composition of these lymphoid populations was not remarkable.

Few other microscopic lesions were found in mice on the 10-ppm level. All developed marked hyperkeratosis in the squamous gastric mucosa, often in association with small ulcerations of the mucosa, and focal edema with neutrophilic exudation. Mice on the natural-ingredient

TABLE 46: Ratios of myeloid to erythroid nucleated cells in bone marrow of mice consuming 10 ppm of T-2 toxin in 3 different diets. Section 9.4.3.3.

Variable	Week	Level of T-2 toxin (ppm)	8% protein semipurified	16% protein semipurified	18.6% protein natural-ingredient
Ratio of myeloid to erythroid cells in bone marrow	2	0	2.0+0.3*	2.2+0.2	2.4+0.6
		10	20.3+8.0 <sup>a</sup>	2.7+0.9 <sup>b</sup>	2.6+0.3 <sup>b</sup>
	4	0	3.2+1.5	3.1+0.5	2.6+0.7
		10	5.9+2.2	9.3+10.9	3.2+1.5
	6	0	2.0+0.4	2.2+0.3	2.0+0.3
		10	2.3+1.1	3.2+0.7	3.2+1.6
	8	0	2.5+0.5	2.6+0.4	2.5+0.3
		10	3.5+0.6	7.7+7.1	5.0+1.4

Analysis of Variance

<u>Time (wks.)</u>	<u>Effect</u>	<u>p value</u>
2	Toxin	< 0.001
	Toxin x Diet	< 0.001
4	Toxin	0.05
	Toxin x Diet	0.32
6	Toxin	0.02
	Toxin x Diet	0.49
8	Toxin	0.01
	Toxin x Diet	0.26
All times combined	Toxin	< 0.001
	Toxin x Diet	< 0.001

\* Values are means (+SD) of separate groups of 4 or 5 mice.  
a,b In any row, means followed by the same letter or by no letter do not differ at p=0.05 by SNK multiple range test.

TABLE 47: Effect of T-2 toxin in different diets on eosinophilic myelopoiesis in bone marrow of mice. Section 9.4.3.3.

Week	Level of T-2 toxin (ppm)	8% protein semipurified diet	16% protein semipurified diet	18.6% protein natural-ingredient diet
2	0	16.8 $\pm$ 3.3 <sup>a</sup>	15.4 $\pm$ 2.7	18.2 $\pm$ 2.2
	10	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.2 $\pm$ 0.4
4	0	27.3 $\pm$ 4.3	26.3 $\pm$ 19.3	17.8 $\pm$ 4.8
	10	0.4 $\pm$ 0.9	6.0 $\pm$ 10.7	1.7 $\pm$ 1.6
6	0	17.2 $\pm$ 4.3	22.6 $\pm$ 4.5	23.8 $\pm$ 5.1
	10	0.3 $\pm$ 0.5	4.5 $\pm$ 4.1	9.0 $\pm$ 9.0
8	0	16.0 $\pm$ 3.4	18.6 $\pm$ 5.8	22.8 $\pm$ 7.5
	10	1.0 $\pm$ 1.2	1.3 $\pm$ 1.5	6.6 $\pm$ 4.8

<sup>a</sup>Values are mean percentages of eosinophils among nucleated cells in smears of bone marrow ( $\pm$ SD).

TABLE 48: Effect of T-2 toxin (10 ppm) in different diets on the proportions of mature to immature stages in erythroid and myeloid populations of bone marrow of mice. Section 9.4.3.3.

Variable	Week	Level of T-2 toxin (ppm)	8% protein semipurified	16% protein semipurified	18.6% protein natural-ingredient	
Ratio of post-rubricyte to pre-rubricyte stages	2	0	14 ± 4 <sup>*</sup>	16 ± 5	13 ± 4	
		10	8 ± 8 <sup>a</sup>	38 ± 29 <sup>b</sup>	24 ± 6 <sup>ab</sup>	
	4	0	13 ± 8	19 ± 6	20 ± 2	
		10	15 ± 8	13 ± 7	28 ± 13	
	6	0	26 ± 6	31 ± 13	13 ± 4	
		10	13 ± 4	28 ± 10	34 ± 29	
	8	0	16 ± 5	17 ± 5	17 ± 6	
		10	11 ± 4	18 ± 6	18 ± 4	
	Ratio of post-myelocyte to pre-myelocyte stages	2	0	17 ± 4	15 ± 2	15 ± 2
			10	39 ± 13	23 ± 7	45 ± 28
4		0	13 ± 2	15 ± 4	14 ± 3	
		10	15 ± 2	17 ± 8	19 ± 5	
6		0	14 ± 2	15 ± 1	17 ± 5	
		10	28 ± 3	28 ± 4	25 ± 11	
8		0	18 ± 4	17 ± 1	16 ± 2	
		10	31 ± 11	21 ± 2	26 ± 7	

\* Values are means (±SD) of separate groups of 4 or 5 mice.

a,b,c Values in any row followed by the same letter or by no letter do not differ at p = 0.05 by SNK multiple range test.

TABLE 49: Effect of dietary T-2 toxin in different diets on the degree of thymic cortical cellularity of young Swiss mice. Section 9.4.3.3.

Weeks on diet	Level of T-2 toxin (ppm)	Diet		
		8% protein semipurified	16% protein semipurified	18.6% protein natural-ingredient
2	0	4,4,4,4,4 <sup>a</sup>	4,4,4,4,4	4,4,4,4,4
	10	0,0,0,0,0	2, <sup>b</sup> 3,2, <sup>b</sup> 4,4	4,4,4,4,4
	20	0,0,0,0,0	0,0,0,0,0	0,0,0,0,0
4	0	4,4,4,4,4	4,4,4,4,4	4,4,4,4,4
	10	0,0,2,0	2,3,4,0,1	3,4,4,4,4
	20	0	0,0,0,0,0	0,0,0,0
6	0	4,-,4,4,3	4,4,4,4,4	-,4,4,4,4
	10	-,0,0,1,0	2,1,4,2	3,3,4,4,0
	20	-	0,0 <sup>c</sup>	0,0
8	0	3,4,4,-,4	4,4,4,4,4	2,3,3,4,2
	10	1,-,0,0,0	4,3,2, <sup>b</sup> 2, <sup>b</sup> 1	4,-,1,4,3 <sup>b</sup>
	20	-	-	-
	20-A <sup>d</sup>	-	-	3 <sup>b</sup> ,4.0,1

<sup>a</sup> Numbers are scores for the degree of cellularity for each mouse examined in the group.

Scores were allocated as follows: 0 = complete depletion; 1 = residual pockets of lymphocytes in cortex; 2 = sparsely but evenly populated, thin cortex; 3 = reduced cortex with few mitoses; 4 = cellular, active cortex (normal).

<sup>b</sup> Active regeneration evident.

<sup>c</sup> These two mice were examined on day 39.

<sup>d</sup> Group housed on wood shavings.

Figure 65

Changes in the microscopic appearance of splenic red pulp in mice fed T-2 toxin (10 ppm) in an 8% protein, semipurified diet during an 8-week period.

- A. Normal red pulp, day 28, group L-0.
  
- B. Atrophic red pulp, day 14, group L-10.  
Note the absence of erythropoietic islands.  
Some immature hematopoietic cells are present (arrows).
  
- C. Atrophic red pulp, day 14, group L-10.  
This mouse has regenerating colonies of undifferentiated hematopoietic cells (arrows).
  
- D. Regenerating red pulp, day 28, group L-10.  
Regenerating, immature erythroid cells (arrows) have infiltrated the red pulp, but maturing stages are not present.
  
- E. Hyperplastic red pulp, day 42, group L-10.  
Note the extreme infiltration of undifferentiated hematopoietic cells that have dark cytoplasm, suggesting they are members of the erythroid series (arrows).
  
- F. Hyperplastic red pulp, day 56, group L-10.  
At this stage, erythroid maturation is evident. The cells with dense pyknotic nuclei are rubricyte stages (circles). However, the immature stages predominate.

Hematoxylin and eosin; x 300.

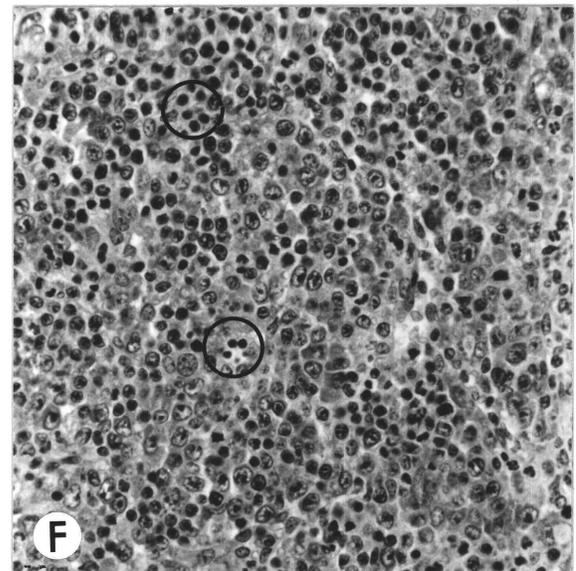
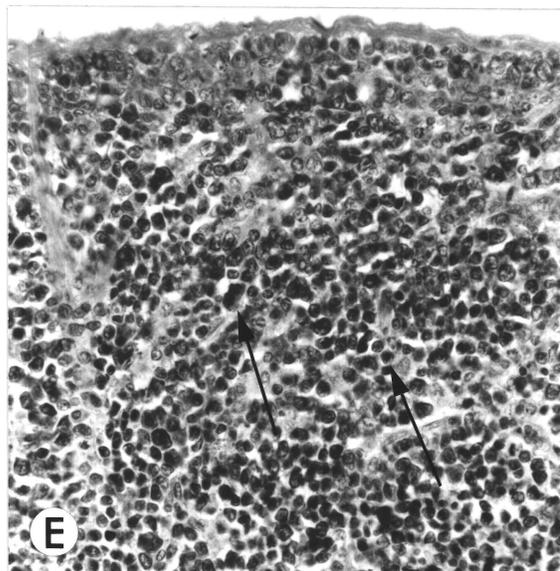
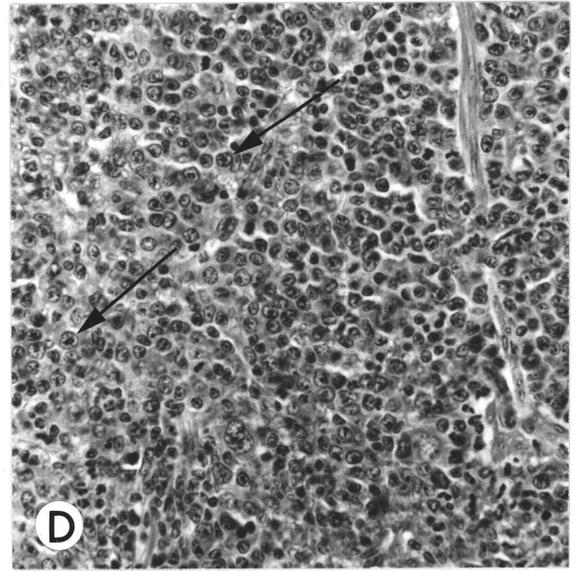
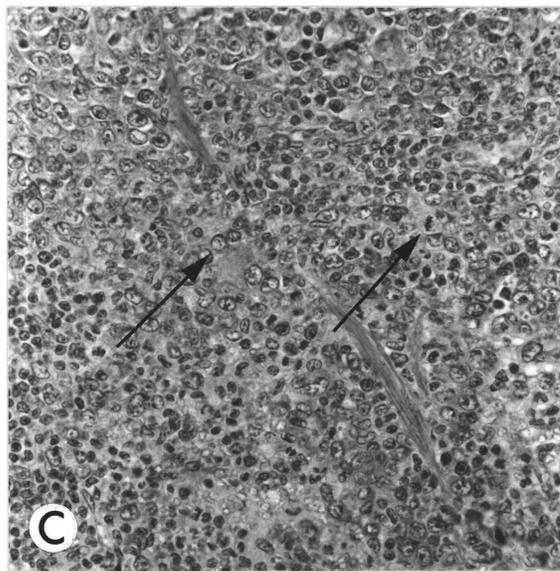
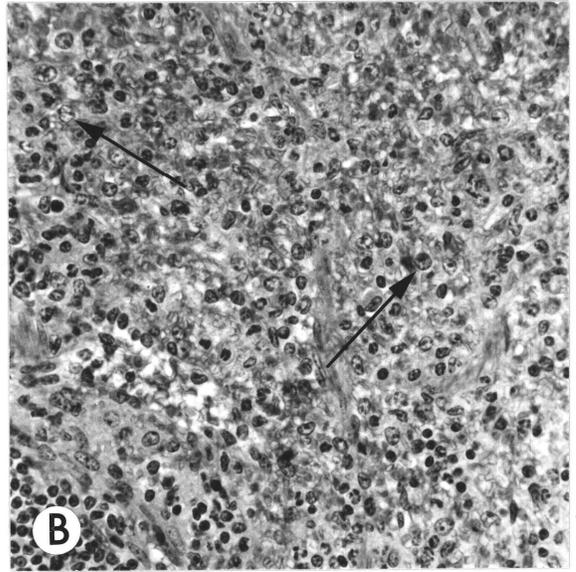
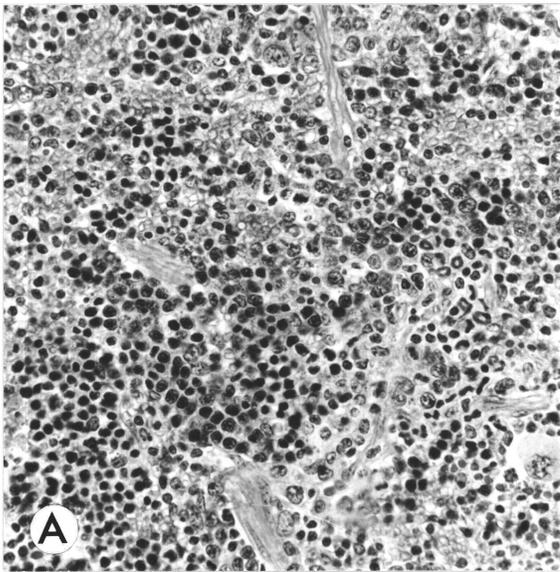


Fig. 65

diet appeared to have greater numbers of bacteria growing over the excess keratin. Two of each group of five mice from group L-10 examined on days 42 and 56 had marked proliferation of Kupffer's cells throughout the liver.

#### 9.4.4 Influence of housing on toxicity of dietary T-2 toxin

Mice in group N-20-A were given the same diet as mice in group N-20, but were housed on softwood bedding in shoebox cages, in the manner used in the previous experiment (Section 8.3). No deaths occurred in these mice during the 8-week period of observation, and growth rates were similar to those observed in group N-20 (Table 33). This was in contrast to the high mortality that occurred in mice in group N-20 (Table 35). Values for most variables were similar in group N-20 at 6 weeks and in N-20-A at 8 weeks, except that numbers of reticulocytes, eosinophils and monocytes, and weights of thymus and spleen were greater in group N-20-A (Table 50). Mice in each of these two groups had similar histological evidence of regeneration from previous anemia. One mouse in group N-20-A was much smaller than the others in the group on day 56, and had marked anemia ( $5.2 \times 10^6$  RBC/ $\mu$ l). However, this mouse had reticulocytosis and a greatly enlarged spleen due to erythroid regeneration. The others in group N-20-A had hematological values in the control range, except for leukopenia and lymphopenia. In two of five mice in this group, the thymus (Table 49), and all other lymphoid tissues were normally populated. However, the three others had generalized lymphoid depletion and in two of these the thymus was totally atrophic (Table 49).

All mice in group N-20-A had hepatomegaly, with relative hepatic weights of over 7% (Table 50), in contrast to control values in the range of 3.5 to 5%.

TABLE 50: Comparison of effects of T-2 toxin (20 ppm) in a natural-ingredient diet fed to young Swiss mice housed in suspension cages, and on softwood bedding.

Variable	Suspension cages (at 6 weeks)	Softwood bedding (at 8 weeks)
Hemoglobin (g/dl)	14.6 $\pm$ 1.0 <sup>a</sup>	12.7 $\pm$ 1.9
PCV (%)	42.4 $\pm$ 1.8	36.3 $\pm$ 5.6
RBC ( $\times 10^6/\mu\text{l}$ )	8.2 $\pm$ 0.5	7.3 $\pm$ 1.1
MCV ( $\mu^3$ )	52.5 $\pm$ 0.5	49.6 $\pm$ 0.7
MCH (pg)	18.0 $\pm$ 0.4	17.3 $\pm$ 0.2
MCHC (%)	33.4 $\pm$ 0.8	33.9 $\pm$ 0.5
Reticulocytes ( $\times 10^4/\mu\text{l}$ )	54.0 $\pm$ 16.0	68.0 $\pm$ 9.3
Reticulocytes (%)	6.6 $\pm$ 1.7	10.6 $\pm$ 2.1
WBC ( $\times 10^3/\mu\text{l}$ )	3.5 $\pm$ 1.1	4.4 $\pm$ 0.5
Neutrophils ( $\times 10^3/\mu\text{l}$ )	1.8 $\pm$ 0.3	1.46 $\pm$ 0.39
Lymphocytes ( $\times 10^3/\mu\text{l}$ )	1.7 $\pm$ 0.8	2.60 $\pm$ 0.42
Eosinophils ( $\times 10^3/\mu\text{l}$ )	0.0 $\pm$ 0.0	0.13 $\pm$ 0.08
Monocytes ( $\times 10^3/\mu\text{l}$ )	0.0 $\pm$ 0.0	0.20 $\pm$ 0.06
Liver weight (g)	1.61 $\pm$ 0.17	1.83 $\pm$ 0.14
Liver weight (% of BW)	6.41 $\pm$ 0.01	7.04 $\pm$ 0.42
Splenic weight (mg)	130 $\pm$ 45	209.0 $\pm$ 20.0
Splenic weight (% of BW)	0.50 $\pm$ 0.12	0.89 $\pm$ 0.24
Thymic weight (mg)	14.0 $\pm$ 4.0	24.6 $\pm$ 6.9
Thymic weight (% of BW)	0.06 $\pm$ 0.02	0.09 $\pm$ 0.02

<sup>a</sup>Values are means ( $\pm$ SEM)

## 9.5 Discussion

T-2 toxin at a level of 20 ppm in the three different diets produced aplastic anemia, lymphopenia, irritation of the upper alimentary tract, and weight loss similar to the effects observed in the previous experiment (Section 8.4). This demonstrated that these previously observed effects were not artifacts due to an unusual interaction of T-2 toxin with the semipurified diet used. The present observations that mice with these effects had reduced rates of food consumption due to food refusal and were in poor condition, or sometimes emaciated, were consistent with those previously described (Section 8.4.1), and further support the interpretation (Section 8.5.1) that both malnutrition and exposure to T-2 toxin contribute to cause these severe effects.

The toxicity of dietary T-2 toxin at 20 ppm was apparently higher in the present experiment than in the previous (Section 8), because high mortality occurred this time. Toxic effects and mortality were least severe in mice on the natural-ingredient diet (H-20). The greater severity of effects observed this time might have been due to greater energy requirements of mice in suspension cages compared with those in enclosed boxed on bedding. During the first week of the present experiment, mice spilled food containing the 20-ppm level of toxin through the mesh floors at such a rate that some groups occasionally were without food for short periods. This situation may also have resulted in a greater degree of undernourishment in mice in the present experiment compared with mice in the previous experiment that continuously had access to their spilled food.

Further evidence was obtained that nutritional composition of the diet influences the rate at which hematopoietic tissues regenerate after initially

being depleted by T-2 toxin. Regeneration of myeloid and erythroid tissues had begun in the spleen and bone marrow in mice after 4 and 6 weeks on the natural-ingredient diet containing 20 ppm of T-2 toxin (N-20). The degree of regeneration was, however, less than was previously observed at the same stages. Mice in group N-20-A, managed in the manner employed in the previous experiment, exhibited marked regeneration by 8 weeks, with splenomegaly similar to that observed in mice on the high-protein semipurified diet in the previous experiment. Thus, the differences in the rates of recovery of mice fed the 20-ppm level apparently depend on other factors such as the cage environment or the degree of general under-nourishment rather than on more subtle changes in dietary composition. However, for the 10-ppm level, there was clearly an interrelationship between toxicity of T-2 toxin and nutritional composition of the diet because mice on the low protein diet (L-10) developed anemia and atrophy of lymphoid tissues, whereas those on the other two adequate diets (H-10 and N-10) did not. This observation suggests that under some circumstances the toxicity of T-2 toxin may be potentiated by relatively minor modifications in nutritional composition of the diet.

High levels of dietary T-2 toxin (20 ppm) appeared to depress neutrophilic myelopoiesis. Severely affected, anemic mice on the 20-ppm level of toxin, including those killed on day 28 and, in particular, moribund mice killed between days 21 and 39, had almost total depletion of bone marrow populations. These mice on the 20-ppm level did not develop a high neutrophilia similar to that induced either by the 10-ppm level of toxin, or by the 20-ppm level both in mice in group N-20-A and

in the previous experiment (Section 8.4.2.2). This supports the previous suggestion (Section 8.4.3) that myeloid populations in mice are sensitive to dietary T-2 toxin, but are less susceptible than erythroid and lymphocytic populations.

During the longer period of feeding employed in the present experiment, impairment of erythropoiesis by dietary T-2 toxin was completely overcome. Similarly, regeneration of lymphoid tissues was observed after 8 weeks in mice in group N-20-A, and after 4 weeks in mice in groups H-10 and N-10, indicating that these effects were also transient. These observations further support the hypothesis derived from the previous experiment (Section 8.5.1) that during continuous exposure mice can develop resistance to the suppressive effects of T-2 toxin on the hematopoietic cells. However, mice did not overcome the effects of T-2 toxin on the gastric mucosa because gastric lesions were similar at all stages in all groups receiving T-2 toxin in the present experiment.

Severely affected mice also developed hemorrhagic enteritis and bloody diarrhea and, as a result, became anemic more rapidly than others with aplastic anemia alone. One of these mice had villus and crypt atrophy (Fig. 60C), but most did not. In fact, most mice on the high level of T-2 toxin exhibited hyperplasia of the small intestinal epithelium, as previously observed. Thus, although repeated doses of T-2 toxin cause necrosis and atrophy of intestinal crypts in cats (Lutsky et al., 1978), mice apparently resemble most other species because aplasia of intestinal crypts is not a feature of alimentary toxic aleukia of man (Mayer, 1952a), nor does it occur in experimental trichothecene toxicosis of chickens (Chi et al., 1977b) or swine (Weaver et al., 1978c) when T-2

toxin is fed in the diet. However, the mechanism by which T-2 toxin causes hemorrhagic enteritis in mice should be further examined because T-2 toxin may play a role in hemorrhagic intestinal diseases such as mouldy-corn poisoning of cattle and swine (Smalley, 1973).

An incidental observation in the present experiment was that one mouse in group L-20 had an infection with Eperythrozoon coccoides. The affected mouse had an extremely atrophic spleen and, as this organism is known to sometimes proliferate after splenectomy (Baker et al., 1971), this suggests that exposure to T-2 toxin may have led to a deficiency in splenic histiocyte activity.

The mechanism by which the toxicity of trichothecenes is overcome during continuous exposure remains unknown. The suggestion from the previous experiment that it may be due to anacquired hepatic metabolic competence to detoxify T-2 toxin into a metabolite that does not impair proliferation or maturation of precursors of erythrocytes and lymphocytes remains possible. Mice on softwood bedding in group N-20-A, and in the previous experiment, were able to overcome suppression of hematopoiesis more rapidly than were mice housed in suspension cages in the present experiment. Mice in group N-20-A had relatively larger livers (greater than 7% of body weight) than did mice fed the same diet but housed in suspension cages (see Table 50). This difference could be due to an enhancement of the activity of hepatic microsomal enzymes by volatile components of softwood bedding (Wade et al., 1968; Schulte-Hermann, 1974). However, T-2 toxin itself may have induced such activity because relative enlargement of the liver occurred in all mice fed T-2 toxin and housed in suspension cages (see Fig. 64D, p. 306). The observed direct

relationship between relative hepatic weight and the dietary level of T-2 toxin supports this suggestion but, because control mice were fed ad lib and were much heavier than treated mice, the apparent dose-response relationship does not necessarily indicate that treated mice had larger livers than they would have had if they had been fed toxin-free diet at the same rate.

The suggestion in the previous experiment (Section 8) that the nutritional composition of the diet influenced the rate of regeneration of suppressed hematopoietic cells by modifying the rate of induction of hepatic microsomal enzyme activity remains equivocal. Although the diet does influence the activity and synthesis of microsomes (Campbell and Hayes, 1976), mice fed T-2 toxin at 10 ppm had similar hepatic weights irrespective of the diet, even after 2 and 4 weeks when mice on diet L-10 were still exhibiting signs of suppression of hematopoiesis. Further investigations, incorporating microsomal enzyme function studies, are needed to determine the mechanism by which the diet modifies the rate at which mice become resistant to T-2 toxin.

The mechanism by which the 10-ppm level of T-2 toxin caused anemia in mice fed the low protein diet was apparently more complicated than a simple arrest of cell production. Two of five mice with erythroid aplasia and absolute reticulocytopenia on day 14 had early signs of erythroid regeneration. By day 28, bone marrow and splenic red pulp had many immature erythroid cells, and the blood contained elevated numbers of reticulocytes. For the rest of the trial, erythropoietic tissues remained hyperplastic, but the erythrocyte count became elevated only slightly. Such a lag in restoration of erythrocyte count suggests a

problem with survival of erythrocytes in circulation, or, alternatively, continuous loss of blood. Many poikilocytes, hypochromic or euchromic macrocytes, and some microcytes were evident in circulation during this period, indicating that the newly produced erythrocytes probably had abnormal cell membranes, and deficient amounts of hemoglobin. The spectrum of morphological changes in mice on diet L-10 was similar to that reported in mice with iron-deficiency types of anemia, such as inherited sex-linked anemia (Loeb et al., 1978) or hereditary microcytic anemia (Edwards and Hoke, 1972). Each of these anemias is due to an inherited disorder of iron uptake or transport. Anemic mice in the present study had elevated rather than decreased MCV's suggesting that the anemia was not due to impaired supply or uptake of iron. However, the high MCV might have been due to the release of a disproportionately large number of reticulocytes when mice entered the regenerative phase of erythropoiesis, after having overcome the aplastic stage between 2 and 4 weeks. The semipurified diet contained 75 mg/kg of iron as  $\text{FeSO}_4 \cdot \text{H}_2\text{O}$ ; this provided more iron than the 50 mg/kg reported to be adequate in semipurified diets for mice (Sorbie and Valberg, 1974). Possibly, the reduced rate of consumption of diets containing T-2 toxin, and the high requirement for iron during extremely active erythropoiesis could have resulted in a conditioned marginal iron deficiency, but this seems unlikely because anemic mice had normal stores of iron in macrophages of spleen and bone marrow.

The range of hematologic abnormalities in mice after 4 weeks on diet L-10 may have been due to impairment of hemoglobin synthesis at any locus in the synthetic pathways of heme or globin. Trichothecene mycotoxins

are potent inhibitors of eukaryotic ribosomes in vitro (Vazquez, 1979); therefore, T-2 toxin might have inhibited globin synthesis in erythroblasts. An inherited disorder of mice, termed a "hemoglobin deficit", is characterized by hypochromia, anisocytosis, leptocytosis, poikilocytosis, basophilic stippling, but not microcytosis (Bannerman et al., 1974; Loeb et al., 1978). Such a spectrum of changes is virtually identical to that observed in mice in the present experiment. Although the pathogenesis of these hematological abnormalities in mice with the "hemoglobin deficit" has not been demonstrated, the anemia has been considered to closely resemble thalassemia of man, an inherited disorder in the synthesis of globin chains (Bannerman et al., 1974).

Regardless of the mechanism by which the erythrocytic abnormalities arose in these mice, the abnormal cells probably had a reduced lifespan. Erythrocytes with membrane abnormalities such as may occur during iron deficiency are removed from circulation at a greater rate than normal erythrocytes (Card and Weintraub, 1971). A shortened lifespan of erythrocytes would explain the lag period observed between the appearance of circulating reticulocytes and the elevation of erythrocyte counts in anemic mice in the present study.

## 10.0 SUBACUTE TOXICITY OF DIETARY T-2 TOXIN IN ADULT MICE AND YOUNG RATS

### 10.1 Abstract

Adult male Swiss mice and young male Wistar rats were fed with a natural-ingredient diet containing levels of 0, 10 or 20 ppm of T-2 toxin. Mice were examined hematologically and by necropsy after 14 and 28 days on the diets. Rats were bled and subjected to cytological examination of bone marrow on day 14, and again on day 28, at which stage they were necropsied. In both species, T-2 toxin caused dose-dependent reductions in food consumption and body weights and also caused hyperkeratosis of the squamous gastric mucosa, especially at the 20-ppm level. In adult mice, T-2 toxin at 20 ppm caused splenic atrophy, thymic atrophy, leukopenia, lymphopenia, eosinopenia and slight anemia, but not aplastic anemia. For comparison, juvenile mice were fed the same toxic diet and these mice became lymphopenic and anemic. However, by day 28, some exhibited marked regenerative activity in erythropoietic populations of spleen and bone marrow. In rats, T-2 toxin caused dose-related hypoproteinemia, hepatomegaly, splenic atrophy, neutrophilia, lymphopenia and eosinopenia. The 20-ppm level of T-2 toxin caused reticulocytopenia in rats at day 14, but otherwise, erythropoiesis was not affected. These observations indicated that young growing mice were more susceptible to the inhibition of erythropoiesis by dietary T-2 toxin than were either adult mice or rats. However, T-2 toxin consistently affected the lymphoid tissues and the stomach in all animals, although more severely in young mice.

## 10.2 Introduction

The effects of dietary T-2 toxin observed in young growing male Swiss mice in Sections 8 and 9 depended on the composition of the diet, the duration of exposure, the level of toxin in the diet, and the method of housing of the experimental mice. T-2 toxin was fed to young and to adult mice in the following experiment to determine the influence of age on susceptibility. In addition, because susceptibility to toxins can vary widely between different species (Parke and Williams, 1969), and because T-2 toxin was previously reported to cause minimal effects in rats (Marasas et al., 1969), the effect of dietary T-2 toxin in young Wistar rats was examined, with particular attention to effects on hematopoietic and lymphopoietic tissues.

## 10.3 Materials and Methods

All animals were housed in stainless-steel suspension cages and supplied with diets and tap water ad lib. Rats were caged individually and mice were kept in groups of four. Otherwise, all animal care procedures were as described in Section 9.3.

### 10.3.1 Experiment using adult mice

Twenty-four young adult male white Swiss mice (Animal Resources Centre, University of Saskatchewan) weighing  $32.2 \pm 0.4$  g were fed a natural-ingredient rat and mouse chow (Table 64, Appendix B) containing T-2 toxin at levels of 0, 10 or 20 ppm, used as previously described in Section 9.3.1. In addition, four young mice weighing  $20.5 \pm 0.5$  g were fed the diet containing the 20-ppm level. Mice were subjected to necropsy and hematological examination as described in Section 9.3.2,

according to the experimental design in Table 51. In addition to the observations listed in Section 9.3.2, the testes were examined histologically in this group.

TABLE 51: Effect of dietary T-2 toxin in mice and rats. Experimental designs. Sections 10.3.1 and 10.3.2.

Type of animals	Level of T-2 toxin (ppm)	Number of animals examined at	
		14 days	28 days
Adult mice	0	4	4
Adult mice	10	4	4
Adult mice	20	4	4
Juvenile mice	20	-	4
Young rats	0	5*	5
Young rats	10	5*	5
Young rats	20	5*	5

\* These rats were bled for hematological examination and bone marrow samples were collected. They were then re-examined on day 28.

### 10.3.2 Experiment using young rats

Fifteen young adult male Wistar rats (Animal Resources Centre, University of Saskatchewan) weighing  $188 \pm 13$ g were divided into three groups of five and were fed on the same three diets used in Section 10.3.1 above. On day 14, all rats were anesthetized with a combination of fentanyl (0.04 mg/kg) and droperidol (2 mg/kg) (Innovar-Vet, Pitman-Moore, Scarborough, Ontario) given by intramuscular injection. Samples of bone marrow were aspirated from the left tibial crest, smeared and stained with Wright's-Giemsa. Blood was collected from the tail and examined as previously described for mice (Section 9.3.2). On day 28, all rats were

anesthetized by ether inhalation and decapitated. Blood was collected into EDTA for hematological examination, and serum was obtained from clotted blood for biochemical determinations of total protein, blood urea nitrogen (BUN), creatinine, cholesterol, glucose, and activities of glutamate-pyruvate transaminase (GPT), glutamate oxaloacetate transaminase (GOT), alkaline phosphatase (AP) and creatine phosphokinase (CPK) (ABA-100, Abbott Laboratories, Dallas, Texas). Impression smears from the right-tibial marrow were stained with Wright's-Giemsa. The liver, spleen, thymus, and both testes were weighed, and rats were subjected to gross necropsy examination. Samples of stomach, duodenum, jejunum, Peyer's patches, mesenteric lymph node, spleen, thymus, liver, kidney, adrenal gland, testis, and bone marrow were fixed in buffered neutral 10% formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin.

### 10.3.3 Analysis of data

At each time, means of variables from groups exposed to the three levels of toxin were compared using a one-way analysis of variance and SNK multiple range tests. All calculations were conducted on an IBM 370 computer using a commercially-prepared statistical program described by Nie et al. (1975).

## 10.4 Results

### 10.4.1 Effect of dietary T-2 toxin in adult mice

The young adult mice exhibited few physical changes during exposure to dietary T-2 toxin. The 20-ppm level caused mice to initially have a mild degree of dryness of the fur, but towards the latter part of the 4 week trial, all adult mice appeared physically normal. Mice did not develop perioral dermatitis. Mice on the toxic diets spilled large quantities of food throughout the experiment, whereas those on the control did not.

T-2 toxin caused dose-dependent reductions in body weight by day 14, but by day 28, no significant differences occurred between the groups on the three levels of toxin (Table 52). Mice on the 20-ppm level had significantly smaller spleens and thymuses, but hepatic weights were not different (Table 52). However, the reductions in splenic weight were small and on days 14 and 28, relative splenic weights were not significantly different ( $p > 0.05$ ) among the three treatment groups (Table 52).

By day 28, treated mice had slightly, but significantly, lower hemoglobin, RBC, and PCV values (Table 53). With the exception of the 20-ppm group on day 28, all other treated mice had significant elevations in circulating numbers of reticulocytes (Table 53). This was associated with corresponding increases in MCV and MCH.

Total leukocyte counts were low in the 20-ppm group on days 14 and 28, due mainly to reductions in numbers of lymphocytes (Table 54). T-2 toxin caused a dose-related reduction in numbers of circulating eosinophils on day 14, but not on day 28 (Table 54).

Macroscopic findings in young adult mice exposed to dietary T-2 toxin

for 2 weeks were not as marked as had been observed previously in juvenile mice (Section 9.4.3). The 10-ppm level did not cause any abnormalities in the hematopoietic or lymphoid tissues. Of mice on the 20-ppm level, some had moderate thymic atrophy and atrophy of splenic red pulp with focal regeneration of erythropoietic colonies. Complete atrophy of these organs, similar to that previously observed in juvenile mice fed the 20-ppm level, was not observed in these young adult mice. However, two had megaloblastic and dysplastic erythropoiesis on day 14.

On day 28, one mouse had erythroid hyperplasia in the splenic red pulp and also had megaloblastic erythropoiesis with a predominance of immature stages. The three others had normal splenic and marrow hematopoiesis. All had thymic atrophy; in the mouse with splenomegaly, the thymus was completely atrophic.

All adult mice fed the 10-ppm or 20-ppm levels of T-2 toxin developed hyperkeratosis and ulceration of the squamous gastric mucosa, similar to that previously described (Section 9.4.3). Some on the 20-ppm level had increased numbers of phagosomes in intestinal crypt epithelial cells. Intestinal lymphoid populations appeared microscopically normal. No abnormalities were observed in the seminiferous tubules of any of the treated mice.

By comparison, young juvenile mice fed the 20-ppm level of toxin for 4 weeks displayed the same range of physical and hematological changes previously observed in mice of this age fed dietary T-2 toxin. Mice grew slowly for the first three weeks on the toxic diet and appeared pale with dry ruffled fur. During the fourth week, some began to improve and developed normal skin coloration, but one remained pale until it was

killed on day 28.

Hematological examination of the four juvenile mice on day 28 revealed that three had marked elevations of reticulocyte counts and MCV, but had erythrocyte counts in the low normal range (Table 55). The pale mouse on the other hand was anemic and had a low reticulocyte count, consistent with non-regenerative anemia (Table 55). Two of these four mice were leukopenic due to lymphopenia, and the other two had neutrophilia, but leukocyte counts were normal. All four had absolute eosinopenia.

The two juvenile mice with pronounced regeneration of erythrocytes had extremely large spleens and hyperplastic erythropoietic activity in red pulp and bone marrow. The one anemic mouse had splenic atrophy, but there were small pale colonies of regenerating hematopoietic cells visible in the spleen. Three of the four had marked thymic atrophy, and all four had hyperkeratosis of the squamous gastric mucosa. All four had relative hepatomegaly (Table 55).

Histologically and cytologically, the changes in these juvenile mice were similarly varied. The anemic mouse had generalized atrophy of all lymphoid and hematopoietic tissue, but had early regenerative erythroid hyperplasia in splenic and marrow populations. The mice in active regenerative stages had splenomegaly due to infiltration of erythropoietic tissue throughout the red pulp.

#### 10.4.2 Effects of dietary T-2 toxin in rats

T-2 toxin caused dose-related depression of food consumption and growth rate (Table 56) throughout the 4-week trial. Rats on the 20-ppm level were smaller than the others and developed coarse, dry fur. None

TABLE 52: Body and organ weights of young adult mice fed for 28 days on a balanced natural diet containing T-2 toxin.

Variable	Day	Level of dietary T-2 toxin			P
		0 ppm	10 ppm	20 ppm	
Body weight	14	37.8 $\pm$ 0.5 <sup>a</sup>	32.7 $\pm$ 0.5 <sup>b</sup>	29.7 $\pm$ 0.4 <sup>c</sup>	<0.001
(b.w.) g	28	39.7 $\pm$ 1.45	36.4 $\pm$ 1.39	34.6 $\pm$ 0.4	0.09
Hepatic weight	14	2.06 $\pm$ 0.16	1.83 $\pm$ 0.03	1.87 $\pm$ 0.09	0.32
g	28	1.93 $\pm$ 0.16	1.85 $\pm$ 0.10	2.06 $\pm$ 0.17	0.58
Relative hepatic weight (% of b.w.)	14	5.43 $\pm$ 0.36	5.60 $\pm$ 0.17	6.29 $\pm$ 0.30	0.13
	28	4.84 $\pm$ 0.31 <sup>a</sup>	5.07 $\pm$ 0.11 <sup>a</sup>	6.32 $\pm$ 0.54 <sup>b</sup>	0.04
Thymic weight	14	71 $\pm$ 4 <sup>a</sup>	58 $\pm$ 6 <sup>a</sup>	35 $\pm$ 8 <sup>b</sup>	0.01
mg	28	73 $\pm$ 4 <sup>a</sup>	69 $\pm$ 10 <sup>a</sup>	10 $\pm$ 6 <sup>b</sup>	< 0.001
Relative thymic weight (% of b.w.)	14	0.19 $\pm$ 0.01	0.18 $\pm$ 0.02	0.12 $\pm$ 0.03	0.07
	28	0.19 $\pm$ 0.01 <sup>a</sup>	0.19 $\pm$ 0.02 <sup>a</sup>	0.03 $\pm$ 0.02 <sup>b</sup>	< 0.001
Splenic weight	14	199 $\pm$ 22 <sup>a</sup>	134 $\pm$ 15 <sup>b</sup>	141 $\pm$ 12 <sup>b</sup>	0.04
mg	28	180 $\pm$ 5 <sup>a</sup>	193 $\pm$ 16 <sup>a</sup>	132 $\pm$ 12 <sup>b</sup>	0.02
Relative splenic weight (% of b.w.)	14	0.53 $\pm$ 0.06	0.41 $\pm$ 0.05	0.48 $\pm$ 0.04	0.33
	28	0.45 $\pm$ 0.01	0.53 $\pm$ 0.03	0.33 $\pm$ 0.08	0.06

a, b, c Means followed by the same superscript letter or by no letter do not differ at p = 0.05.

TABLE 53: Alterations in erythrocyte values of young adult mice fed for 28 days on a balanced natural diet containing T-2 toxin.

Variable	Day	Level of dietary T-2 toxin			P
		0 ppm	10 ppm	20 ppm	
Hemoglobin g/100 ml	14	16.4±0.3	15.8±0.5	15.2±0.5	0.19
	28	17.0±0.2 <sup>a</sup>	15.6±0.2 <sup>b</sup>	15.3±0.4 <sup>b</sup>	0.01
Erythrocyte count (RBC) x 10 <sup>6</sup> /μl	14	10.1±0.2	9.8±0.4	9.6±0.3	0.57
	28	10.1±0.4 <sup>a</sup>	8.5±0.2 <sup>b</sup>	8.4±0.3 <sup>b</sup>	0.002
Packed cell volume (PCV) percent	14	47.1±1.1	45.0±1.5	43.1±1.8	0.24
	28	45.0±1.1 <sup>a</sup>	39.3±0.9 <sup>b</sup>	39.4±1.2 <sup>b</sup>	0.006
Mean corpuscular volume (MCV) μ <sup>3</sup>	14	46.5±0.6	46.3±0.9	44.8±0.8	0.26
	28	44.5±0.5	46.5±0.9	47.3±0.8	0.06
Mean corpuscular hemoglobin (MCH) pg	14	16.2±0.2	16.2±0.4	15.7±0.1	0.45
	28	16.8±0.6 <sup>a</sup>	18.5±0.4 <sup>b</sup>	18.3±0.2 <sup>b</sup>	0.03
Mean corpuscular hemoglobin concentration (MCHC) %	14	34.8±0.4	35.1±0.3	35.2±0.5	0.71
	28	37.7±0.9	39.8±0.5	38.8±0.5	0.17
Reticulocytes percent	14	2.4±0.4 <sup>a</sup>	4.8±1.1 <sup>ab</sup>	6.2±0.4 <sup>b</sup>	0.01
	28	2.8±0.5 <sup>a</sup>	9.1±1.6 <sup>b</sup>	2.8±0.7 <sup>a</sup>	0.003
Reticulocytes x 10 <sup>4</sup> /μl	14	23.8±4.0 <sup>a</sup>	47.9±12.3 <sup>ab</sup>	59.1±3.5 <sup>b</sup>	0.03
	28	28.4±6.6 <sup>a</sup>	77.3±14.3 <sup>b</sup>	23.1±5.6 <sup>a</sup>	0.005

a,b,c Means followed by the same superscript letter or by no letter do not differ at p = 0.05.

TABLE 54: Leukocyte values of young adult mice fed for 28 days on a balanced natural diet containing T-2 toxin.

Variable	Day	Level of dietary T-2 toxin			P
		0 ppm	10 ppm	20 ppm	
Total white cell count x 10 <sup>3</sup> /μl	14	15.0±1.8 <sup>a</sup>	11.7±0.8 <sup>ab</sup>	9.2±0.8 <sup>b</sup>	0.02
	28	12.1±1.3 <sup>a</sup>	16.2±1.7 <sup>b</sup>	6.0±0.5 <sup>c</sup>	0.001
Neutrophil count x 10 <sup>3</sup> /μl	14	1.69±0.10	1.62±0.21	2.07±0.59	0.06
	28	3.78±0.69 <sup>a</sup>	1.37±1.01 <sup>b</sup>	1.07±0.15 <sup>b</sup>	0.05
Lymphocyte count x 10 <sup>3</sup> /μl	14	12.70±1.79 <sup>a</sup>	9.77±0.92 <sup>ab</sup>	6.93±0.95 <sup>b</sup>	0.06
	28	7.76±1.25 <sup>a</sup>	13.24±1.38 <sup>a</sup>	4.68±0.38 <sup>b</sup>	0.001
Eosinophil count x 10 <sup>3</sup> /μl	14	0.19±0.14	0.09±0.03	0.0±0.0	0.40
	28	0.12±0.12	0.39±0.11	0.11±0.04	0.12
Monocyte count x 10 <sup>3</sup> /μl	14	0.37±0.09	0.21±0.15	0.0±0.0	0.1
	28	0.40±0.08	0.45±0.15	0.14±0.04	0.13

a,b,c Means followed by the same superscript letter or by no letter do not differ at p = 0.05.

TABLE 55: Individual values measured from 4 young mice after 4 weeks on a normal diet containing 20 ppm T-2 toxin (Section 10.4.1).

	Mouse				$\bar{X} \pm \text{SEM}$
	1	2	3	4	
Hemoglobin; g/dl	14.6	7.8	15.8	14.0	13.1 $\pm$ 1.8
RBC; $\times 10^6/\mu\text{l}$	7.9	4.9	8.7	6.9	7.1 $\pm$ 0.8
PCV; %	36.4	20.6	44.6	34.9	34.0 $\pm$ 5.1
MCV; $\mu^3$	47	41	51	50	47.3 $\pm$ 2.2
Reticulocytes; %	22.4	1.2	16.2	38.4	19.6 $\pm$ 7.7
WBC; $\times 10^3/\mu\text{l}$	15.8	4.0	6.0	15.0	10.2 $\pm$ 3.0
Neutrophils; $\times 10^3/\mu\text{l}$	7.1	2.1	2.5	5.3	4.2 $\pm$ 1.2
Lymphocytes; $\times 10^3/\mu\text{l}$	8.4	1.6	3.5	9.0	5.6 $\pm$ 1.8
Eosinophils; $\times 10^3/\mu\text{l}$	0	0	0	0	0.0 $\pm$ 0.0
Monocytes; $\times 10^3/\mu\text{l}$	0.3	< 0.1	0.1	0.5	0.2 $\pm$ 0.1
Body weight; g	22.2	21.2	26.1	26.3	24.0 $\pm$ 1.3
Hepatic weight; % B.W.	7.8	8.2	6.7	8.1	7.7 $\pm$ 0.3
Splenic weight; % B.W.	1.0	0.4	0.6	1.3	0.8 $\pm$ 0.2
Thymic weight; % B.W.	0.02	0.02	0.1	0.03	0.04 $\pm$ 0.02

developed perioral dermatitis, even on the 20-ppm level. However, all rats on the toxic diets developed hyperkeratosis of the squamous gastric mucosa, especially at the margo plicata (Fig. 66A-D). Discrete ulcers, surrounded by papillary proliferations of keratinized epithelium, were observed in some rats (Fig. 67).

No significant abnormalities in the erythron were found in rats exposed to T-2 toxin except that rats on the 20-ppm level were reticulocytopenic on day 14, but not on day 28 (Table 57). Cytological examination of bone marrow revealed no abnormalities on days 14 and 28. The bone marrow was histologically similar in each of the three treatment groups on day 28.

T-2 toxin caused dose-related neutrophilia on days 14 and 28, but this was significant only on day 14 (Table 58). On day 28, there was a significant dose-dependent reduction in lymphocyte and eosinophil counts in treated groups (Table 58).

Serum protein levels decreased in a dose-related fashion (Table 59). Blood glucose and cholesterol values were similarly decreased (Table 59). Serum GOT activity was significantly, but slightly, elevated in treated rats, whereas serum alkaline phosphatase activity decreased in treated rats (Table 59).

Rats on the toxic diets developed relative enlargement of the liver (Table 56), and those on the high level of toxin had both absolute and relative decreases in thymic weight (Table 56). The spleen was smaller in rats on the 20-ppm level of T-2 toxin, but the degree of reduction in size was proportional to the overall reduction in body weight (Table 56).

Few microscopic changes were found in treated rats. Hyperkeratosis

Figure 66

Effects of dietary T-2 toxin on the gastric mucosa of rats fed a natural-ingredient diet for 4 weeks.

- A. Control rat.  
Note the thin margo plicata (M) between the squamous gastric mucosa (S) and the fundus (F).  
Note the esophageal opening (arrow).
  
- B. Treated rat, 10 ppm T-2 toxin, 4 weeks.  
Note the thickened squamous mucosa beside the margo plicata.  
Hyperkeratosis is most severe near the esophageal region (arrow).
  
- C. Treated rat, 20 ppm T-2 toxin, 4 weeks.  
In addition to the hyperkeratosis along the margo plicata, there is also a solitary nodule of keratin (arrow).
  
- D. Treated rat, 20 ppm T-2 toxin, 4 weeks.  
This rat has a large proliferation of keratin around an ulcerated region (arrow).  
There are many small thickened areas over the entire squamous mucosa.

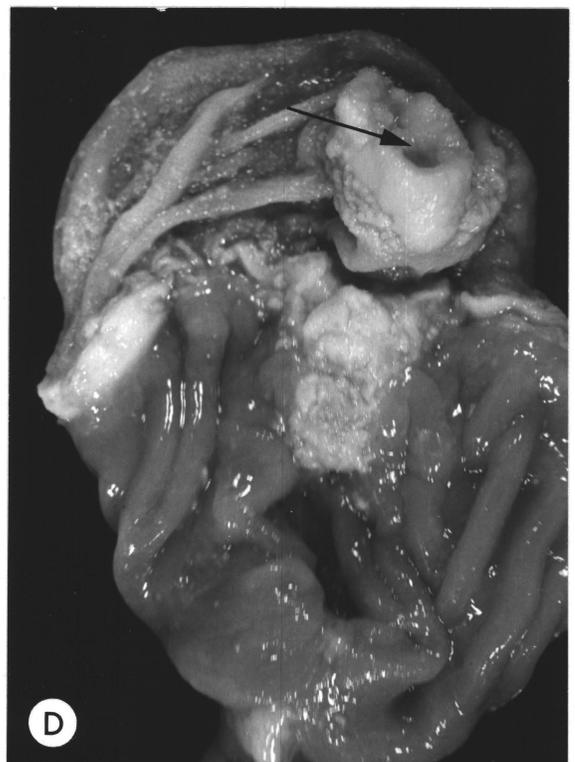
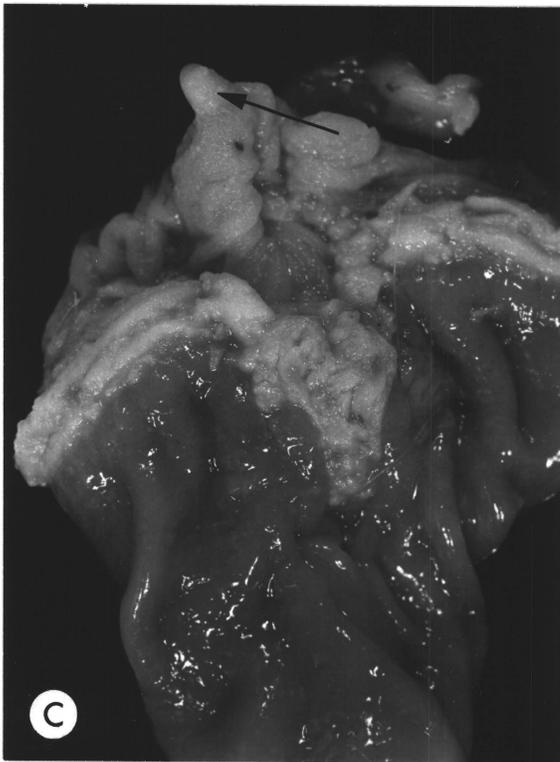
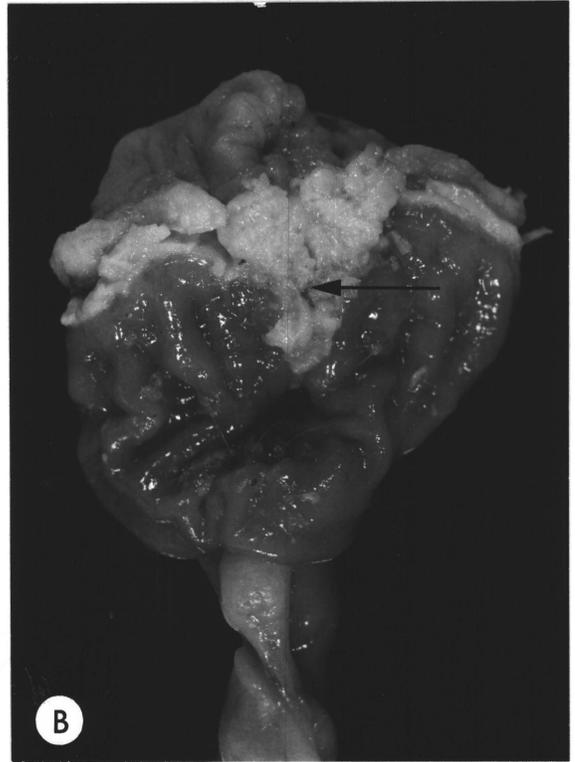


Fig. 66

TABLE 56: Body and organ weights of young rats fed for 28 days on a balanced natural diet containing T-2 toxin

Variable	Day	Level of dietary T-2 toxin			P
		0 ppm	10 ppm	20 ppm	
Body weight (b.w.) g	14	278 $\pm$ 5 <sup>a</sup>	249 $\pm$ 9 <sup>b</sup>	197 $\pm$ 5 <sup>c</sup>	<0.001
	28	336 $\pm$ 8 <sup>a</sup>	304 $\pm$ 9 <sup>b</sup>	222 $\pm$ 5 <sup>c</sup>	<0.001
Hepatic weight g	28	12.42 $\pm$ 0.35 <sup>a</sup>	14.17 $\pm$ 0.42 <sup>b</sup>	10.69 $\pm$ 0.52 <sup>c</sup>	<0.001
Relative hepatic weight (% of b.w.)	28	3.63 $\pm$ 0.04 <sup>a</sup>	4.66 $\pm$ 0.11 <sup>b</sup>	4.82 $\pm$ 0.21 <sup>b</sup>	<0.001
Thymic weight g	28	0.79 $\pm$ 0.05 <sup>a</sup>	0.62 $\pm$ 0.04 <sup>b</sup>	0.29 $\pm$ 0.04 <sup>c</sup>	<0.001
Relative thymic weight (% of b.w.)	28	0.23 $\pm$ 0.02 <sup>a</sup>	0.20 $\pm$ 0.01 <sup>a</sup>	0.13 $\pm$ 0.02 <sup>b</sup>	0.004
Splenic weight g	28	0.72 $\pm$ 0.06 <sup>a</sup>	0.71 $\pm$ 0.05 <sup>a</sup>	0.51 $\pm$ 0.03 <sup>b</sup>	0.013
Relative splenic weight (% of b.w.)	28	0.21 $\pm$ 0.02	0.23 $\pm$ 0.01	0.23 $\pm$ 0.01	0.58
Testicular weight g	28	4.69 $\pm$ 0.11 <sup>a</sup>	4.65 $\pm$ 0.26 <sup>a</sup>	3.96 $\pm$ 0.12 <sup>b</sup>	0.03
Relative testicular weight (% of b.w.)	28	1.37 $\pm$ 0.01 <sup>a</sup>	1.53 $\pm$ 0.06 <sup>b</sup>	1.79 $\pm$ 0.03 <sup>c</sup>	<0.001

a,b,c Means followed by the same superscript letter or by no letter do not differ at p = 0.05.

TABLE 57: Alterations in erythrocyte values of young rats fed for 28 days on a balanced natural diet containing T-2 toxin

Variable	Day	Level of dietary T-2 toxin			P
		0 ppm	10 ppm	20 ppm	
Hemoglobin g/100ml	14	15.5±0.4	14.6±0.5	15.5±0.4	0.35
	28	14.3±0.3 <sup>a</sup>	15.2±0.2 <sup>b</sup>	13.8±0.2 <sup>a</sup>	0.02
Erythrocyte count (RBC) x 10 <sup>6</sup> /μl	14	7.48±0.03	7.38±0.15	7.26±0.13	0.51
	28	7.22±0.26	7.48±0.13	6.88±0.20	0.21
Packed cell volume (PCV) percent	14	38.9±0.3	39.5±0.6	37.8±0.8	0.20
	28	37.1±1.1 <sup>a</sup>	40.2±0.5 <sup>b</sup>	35.4±0.7 <sup>a</sup>	0.01
Mean corpus- cular volume (MCV) μ <sup>3</sup>	14	52.0±0.4	53.6±0.6	52.0±0.4	0.07
	28	51.4±0.8	53.8±0.8	51.5±0.5	0.08
Mean corpus- cular hemo- globin (MCH) pg	14	20.7±0.5	19.8±0.8	21.3±0.5	0.27
	28	19.8±0.5	20.3±0.2	20.1±0.5	0.72
Mean corpus- cular hemo- globin concen- tration (MCHC) %	14	39.8±1.1	37.0±1.4	41.0±1.0	0.08
	28	38.6±0.4	37.8±0.3	39.0±0.6	0.23
Reticulocytes percent	14	4.3±1.3 <sup>a</sup>	4.0±0.5 <sup>a</sup>	1.5±0.3 <sup>b</sup>	0.05
	28	2.8±0.6	3.5±0.9	4.6±1.0	0.34
Reticulocytes x 10 <sup>4</sup> /μl	14	31.9±10.0 <sup>a</sup>	29.2±3.6 <sup>a</sup>	11.1±2.6 <sup>b</sup>	0.04
	28	20.0±3.4	25.8±6.4	31.1±6.4	0.36

a,b,c Means followed by the same superscript letter or by no letter do not differ at p = 0.05.

TABLE 58: Leukocyte values of young rats fed for 28 days on a balanced natural diet containing T-2 toxin

Variable	Day	Level of dietary T-2 toxin			P
		0 ppm	10 ppm	20 ppm	
Total white cell count x 10 <sup>3</sup> /μl	14	11.6±0.7	9.8±0.6	13.3±1.7	0.15
	28	7.6±0.9	5.8±0.4	6.2±0.9	0.25
Neutrophil count x 10 <sup>3</sup> /μl	14	2.46±0.53 <sup>a</sup>	3.33±0.48 <sup>a</sup>	7.31±0.87 <sup>b</sup>	0.001
	28	1.51±0.36	1.62±0.80	3.52±0.79	0.12
Lymphocyte count x 10 <sup>3</sup> /μl	14	8.68±0.69	6.34±0.77	5.77±0.82	0.06
	28	5.88±0.52 <sup>a</sup>	3.91±0.49 <sup>b</sup>	2.48±0.21 <sup>c</sup>	0.001
Eosinophil count x 10 <sup>3</sup> /μl	14	0.18±0.11	0.07±0.04	0.0±0.0	0.16
	28	0.16±0.06 <sup>a</sup>	0.03±0.02 <sup>b</sup>	0.0±0.0 <sup>b</sup>	0.02
Monocyte count x 10 <sup>3</sup> /μl	14	0.31±0.09	0.07±0.05	0.18±0.07	0.07
	28	0.06±0.05	0.19±0.06	0.15±0.04	0.27

a,b,c Means followed by the same superscript letter or by no letter do not differ at p = 0.05.

TABLE 59: Serum biochemical alterations in young rats after 28 days on a balanced, natural diet containing T-2 toxin

Variable	Day	Level of dietary T-2 toxin			P
		0 ppm	10 ppm	20 ppm	
Total protein g/dl	28	6.0 $\pm$ 0.2 <sup>a</sup>	5.8 $\pm$ 0.1 <sup>a</sup>	5.2 $\pm$ 0.0 <sup>b</sup>	<0.001
Blood urea nitrogen (BUN) mg/dl	28	14 $\pm$ 1	13 $\pm$ 1	10 $\pm$ 2	0.17
Creatinine mg/dl	28	0.3 $\pm$ 0.1	0.3 $\pm$ 0.0	0.3 $\pm$ 0.0	0.82
Cholesterol mg/dl	28	63 $\pm$ 3 <sup>a</sup>	50 $\pm$ 4 <sup>b</sup>	45 $\pm$ 3 <sup>b</sup>	0.007
Glucose mg/dl	28	144 $\pm$ 5 <sup>a</sup>	115 $\pm$ 4 <sup>b</sup>	89 $\pm$ 8 <sup>c</sup>	<0.001
Glutamate pyruvate transaminase activity (GPT) IU/1	28	26 $\pm$ 1	24 $\pm$ 1	21 $\pm$ 2	0.09
Glutamate oxaloacetate transaminase activity (GOT) IU/1	28	151 $\pm$ 12 <sup>a</sup>	233 $\pm$ 11 <sup>b</sup>	213 $\pm$ 4 <sup>b</sup>	<0.001
Alkaline phosphatase activity (AP) IU/1	28	304 $\pm$ 21 <sup>a</sup>	212 $\pm$ 17 <sup>b</sup>	160 $\pm$ 11 <sup>c</sup>	<0.001
Creatine phosphokinase activity (CPK) IU/1	28	363 $\pm$ 40	544 $\pm$ 103	399 $\pm$ 48	0.20

a,b,c Means followed by the same superscript letter or by no letter do not differ at p = 0.05.

of the squamous gastric mucosa and terminal esophageal mucosa occurred consistently. Some rats had increased numbers of cytolysosomes in epithelial cells of intestinal crypts. The livers appeared unremarkable except for a consistent observation of nucleolar enlargement in rats on T-2 toxin. Hematopoietic tissues exhibited few abnormalities. The white pulp of the spleen was mildly depleted of mature lymphocytes in the peripheral regions of periarterial sheaths and follicles. Few hematopoietic cells were present in the red pulp of both the controls and the treated rats. No abnormalities were observed in bone marrow except for marked reduction in eosinophil production in all rats on the 20-ppm level and in two of the five on the 10-ppm level. The thymic cortex was moderately depleted in one rat on the 20 ppm level, but otherwise thymic lymphopoiesis appeared normal.

#### 10.5 Discussion

The observations on the toxic effects in young adult mice demonstrated that age is a factor that may influence the toxicity of dietary T-2 toxin. Juvenile mice exhibited transient aplasia of erythroid tissues with regeneration after 3 to 4 weeks, similar to observations in previous experiments (Sec. 8;9). However, in adult mice, erythroid aplasia or hypoplasia was not evident after 2 weeks, although two mice had megaloblastic erythroid development. Thus, anemia did not occur in adult mice fed either level of T-2 toxin; elevated reticulocyte counts on day 14 suggested a regenerative stage had begun before anemia could have developed.

Observations in young rats failed to reveal evidence of impaired hematopoiesis, suggesting that rats are more resistant than mice to the suppressive effects of T-2 toxin.

The direct irritant effects of T-2 toxin were consistently observed in the stomachs of adult mice, juvenile mice, and young rats fed all toxic diets in the present study. This observation supports the hypothesis that the gastric lesions are due to a direct action of T-2 toxin on the gastric mucosa and would therefore not be modified by adaptive capabilities of the animals to biotransform T-2 toxin into an inactive metabolite. Furthermore, these observations in rats are similar to those obtained in other studies, including rats (Marasas et al., 1969; Schoental et al., 1979 ), mice (Ohtsubo and Saito, 1977) and poultry (Wyatt et al., 1972b) fed T-2 toxin in the diet. In the present study, and in those reported, hematopoietic failure was not observed. This indicates that juvenile mice are more sensitive to the effects of dietary T-2 toxin on hematopoietic tissues than are adult mice and rats. In the juvenile mice, erythropoiesis is apparently inhibited for a longer period, sufficiently long enough for aplastic anemia to develop. Thus, juvenile mice should be a more suitable experimental animal in which to examine the effects of dietary trichothecenes on the hematopoietic system.

Effects on the lymphoid tissues of adult mice and young rats were also less severe than were observed in young juvenile mice, both previously and in the present experiment. Nevertheless, some mice and rats exhibited moderate degrees of thymic atrophy. Since paired feeding controls were not conducted, it is not possible to determine the extent to which effects on the lymphoid tissues may have been mediated by stress. Both mice and rats developed eosinopenia and lymphopenia while on the toxic diets, suggesting that stress probably contributed to the cause of the lymphoid atrophy.

GENERAL DISCUSSION

## 11.0 GENERAL DISCUSSION

### 11.1 Introduction

The foregoing series of experiments examined three types of toxicity of T-2 toxin in mammals. These were cutaneous irritation after topical application (Sections 3-5), acute systemic toxicity of a single oral dose (Sections 6, 7), and subacute toxicity from dietary exposure (Sections 8-10). The principal objectives of each of these studies were to characterize the toxic effects in the various target organs and to determine some of the factors that influenced the development of these effects. The overall objective was to obtain information that might mitigate the task of defining and resolving the problems of ingestion of foodborne trichothecenes by man and animals.

### 11.2 Cutaneous irritation by trichothecenes

Sequential histological examination of skin of rats and rabbits exposed to T-2 toxin in Section 3 established that T-2 toxin evokes an acute, non-specific inflammatory reaction in the skin. Topical doses as low as 20 ng were effective (Section 4) indicating that T-2 toxin is a powerful skin irritant, but the mechanism of this toxic activity is unknown. Dermal and epidermal necrosis occurred in intense reactions, but it could not be determined if necrosis was a direct effect of the toxin or a secondary effect of the acute inflammatory reaction in the dermis.

Dose-response studies in Section 4 indicated that the intensity of cutaneous inflammation increased with increasing topical dose of T-2 toxin or diacetoxyscirpenol, but the intensity of inflammation to a given dose varied widely among different test animals. Because such variation in sensitivity of different test animals reduced the quantitative

performance of conventional methods of measuring irritancy, an accurate method described in Section 5 was designed. The advantage of this new method was that reactions to test applications of T-2 toxin were appraised directly in units of equivalent concentration of T-2 toxin and were thus independent of the sensitivity of test rats.

Such a method provides a precise means of measuring irritancy of trichothecenes or of other strong irritants. An immediate application of this procedure could be to determine the efficiency of using cutaneous irritation as a screening bioassay for trichothecenes in feed extracts. Since these bioassays are commonly used to select extracts for chemical analysis (Eppley *et al.*, 1974; Chung *et al.*, 1974), it would be unsatisfactory if harmful levels of relatively non-irritant toxins should escape detection in the bioassays used. Deoxynivalenol (vomitoxin) appears to be one example of a relatively non-irritant trichothecene (Mirocha, 1979), but it is an important cause of the refusal and emesis of corn infested with *F. roseum* (Vesonder *et al.*, 1976) and other feed-stuffs (Mirocha *et al.*, 1976). Because some solvents diminished the cutaneous response to T-2 toxin and diacetoxyscirpenol (Section 4.4.3), the irritancy of deoxynivalenol, which is one of the most polar trichothecenes (Table 3), should be evaluated in various solvents used for extraction of toxins from feed samples.

The assay in Section 5 was designed to quantify dermal irritancy of pure trichothecene toxins, but it could be adapted to measure toxicity of feed extracts believed to contain trichothecenes. Cutaneous reactions to trichothecene toxins in unpurified extracts of feedstuffs are weaker than to the same amount of toxin in a purified extract (Pathre and

Mirocha, 1977). Such interference might be due to suppression of cutaneous reactions by some anti-inflammatory component in crude feed extracts, or to alteration of the characteristics of the vehicle by the impurities. Thus, the sensitivity of detection of trichothecenes in crude extracts would be lower than for purified solutions, but because variation in sensitivity of the skin does not appear to greatly influence the accuracy of measurements obtained, this method could be used quantitatively provided that the extracts were not heavily pigmented, and provided that the standards were made in a similar extract. The range of standard concentrations would need to be adjusted to correspond to the effective range of irritancy of T-2 toxin in the crude extract.

The non-specific nature of the dermal response to trichothecene toxins requires that the presence of trichothecene mycotoxins in irritant test extracts be confirmed by other tests. If analytical methods are not available, the presence of a trichothecene mycotoxin in a feed extract could be tentatively confirmed by administering some test extract to mice. Mitoses cease and phagosomes of cell fragments appear in intestinal crypts and lymphoid follicles within 3 hours of administration of T-2 toxin (Sections 6.4.1.4 and 7.6.2). However, many other cytotoxic agents cause similar effects (Philips et al., 1975) so the observation of these lesions does not prove the presence of a trichothecene.

Identification of trichothecenes can be achieved only by physico-chemical methods, and usually after much difficulty. Thin layer chromatography (TLC) methods are most widely used and will qualitatively identify T-2 toxin and other trichothecenes (Pathre and Mirocha, 1977). Such methods are not quantitative (Eppley, 1975) and require at least partial

purification of test extracts (Pathre and Mirocha, 1977). Interfering substances may also complicate identification and quantitation of trichothecenes by gas-liquid chromatography (GLC) methods (Pathre and Mirocha, 1977). Presently, definitive identification of some trichothecenes in feed extracts can be achieved by gas chromatography-mass spectrometry (GC/MS) using selected ion monitoring (SIM) (Mirocha et al., 1976a).

To interpret the significance of trichothecenes in feedstuffs associated with disease, it is necessary to have a measurement of toxicity of the feed. The complex extraction and purification processes needed for physicochemical detection and measurement of trichothecenes may reduce recovery of toxins resulting in underestimation of their levels (Hsu et al., 1972; Mirocha et al., 1976a). Furthermore, because there are many trichothecenes with different polarity characteristics, it is not possible to simultaneously measure more than a few members of the group (Mirocha et al., 1976a). For these reasons, measurement of the biological toxicity of the feedstuff, using minimal extraction, is a necessary complement to physicochemical analysis.

Cutaneous irritancy might be a useful measurement of biological toxicity, but at present the relationship between dermal irritancy and systemic toxicity is not known. Toxins such as T-2 toxin, HT-2 toxin, and diacetoxyscirpenol have low acute LD<sub>50</sub> values and are also highly irritant, whereas some others, namely nivalenol and fusarenon-X, were found to be much less irritant although they were similarly acutely toxic (Ueno et al., 1970 ; Sato and Ueno, 1977). Cutaneous irritancy of trichothecenes is destroyed when the epoxide group is altered (Bamburg

and Strong, 1971), and this group is also essential for various other acute toxic activities, including inhibition of protein synthesis, cytotoxicity to cells in culture (Bamburg, 1976; Ueno, 1977a) and acute systemic toxicity in mice (Ueno, 1977a). However, there is presently little information to indicate that the acute radiomimetic, cytotoxic activity of trichothecenes plays a part in naturally-occurring mycotoxic disease. In fact, in those mycotoxicoses in which the causative role of trichothecenes is best-documented, namely the food refusal and emesis syndrome (Kotsonis et al., 1975b; Mirocha et al., 1976a) and fusario-toxicosis (Wyatt et al., 1972a; Greenway and Puls, 1976; Puls and Greenway, 1976), topical irritation of the upper alimentary tract appears to be the main toxic effect (Wyatt et al., 1972b; Palyusik and Koplícková, 1975); radiomimetic toxic effects have not been described.

The effects of dietary T-2 toxin in poultry (Wyatt et al., 1972b; Chi et al., 1977b; Chi and Mirocha, 1978) and swine (Weaver et al., 1978c) also appear to be largely due to the food refusal effects and to the irritant effect on the mucosa of the upper alimentary tract. Thus, a measurement of the concentration of irritant toxins in an extract from feed containing T-2 toxin may be a useful estimate of toxicity of the feed. However, other trichothecenes such as deoxynivalenol which are potent inhibitors of food intake, might not be measurable on an irritation bioassay (Mirocha, 1979).

### 11.3 Acute toxicity of T-2 toxin

T-2 toxin and many other trichothecenes are undoubtedly potent toxins that are capable of causing severe injury to germinal populations of cells in the intestinal crypts, bone marrow, and lymphoid tissues. However, there is some question as to the role of these acute radiomimetic effects in animals and people consuming trichothecenes in naturally contaminated food.

After single, relatively large, but sublethal doses of T-2 toxin were administered to mice (Section 6.4.1.4) severe necrotizing lesions appeared in the intestinal crypts, bone marrow and lymphoid tissues, but such effects were temporary and damaged tissues regenerated shortly after exposure. The subacute toxic effects of T-2 toxin in mice (Sections 8.4 and 9.4), and in rats (Section 10.4) did not appear to be solely due to prolongation of the acute "radiomimetic" effects because some tissues such as the gastric and intestinal mucosae were actually hyperplastic after repeated dietary exposure to T-2 toxin. Only in a minority of severely-affected mice fed T-2 toxin did atrophy of intestinal mucosa occur (see Fig. 60) during subacute dietary T-2 toxicosis.

Abnormalities of intestinal villi and crypts have not been observed in various animal species continuously exposed to trichothecenes in the diet (Pier et al., 1976; Witlock et al., 1977; Chi et al., 1977b, 1977c; Richard et al., 1978; Weaver et al., 1978c; 1978d). Furthermore, the intestinal mucosa is apparently normal in fusariotoxicosis (Greenway and Puls, 1976) and mouldy-corn poisoning of cattle (Smalley, 1971), and atrophy of crypts and villi is not a feature of the pancytopenic mycotoxicoses (stachybotryotoxicosis and alimentary toxic aleukia) which

are believed to be caused by trichothecenes (Forgacs and Carll, 1962; Mayer, 1952; Joffe, 1978). Superficial focal necrotizing lesions have been described in the alimentary tract in these diseases, but they are possibly due to direct irritation (Palyusik and Koplic-Kovács, 1975), or to secondary bacterial or mycotic infection subsequent to leukopenia (Forgacs and Carll, 1962; Danko, 1972). Repeated forced administration of T-2 toxin has been shown to cause atrophy of intestinal crypts and villi in cats (Lutsky et al., 1978), but such effects might be a manifestation of repeated acute toxic effects of the intragastric doses.

The lymphoid and erythropoietic germinal cells of mice were susceptible to T-2 toxin during both acute and subacute toxicity, suggesting that the atrophy of these populations during subacute exposure may have been due to the radiomimetic activity of T-2 toxin. However, this interpretation is questionable because granulocytic precursors were hyperplastic in some mice with both lymphoid and erythroid hypoplasia during subacute T-2 toxicosis.

The mechanisms both of acute radiomimetic toxicity and of subacute toxicity to lymphoid and erythroid cells need to be determined before the relationship between the two can be determined. The observed differences between the acute and the subacute effects in mice suggest that toxicological information from acute, high dosage studies is probably of little value in estimating hazards due to levels of trichothecenes likely to be formed in foodstuffs. Concerns that trichothecenes in food may be hazardous to human health have been reinforced by recognition of the potency of the acute radiomimetic toxicity (Bamburg and Strong, 1971; Wilson, 1973). Such acute toxicity information could be misleading and

lead to an exaggeration of the cause for concern if the subacute, subclinical effects of trichothecenes are caused by mechanisms different from those involved in the acute radiomimetic effects.

#### 11.4 Subacute toxicity of dietary T-2 toxin

Mice appear to be suitable animals in which to examine the potential for trichothecenes to cause subclinical and overt impairment of hematopoiesis and impairment of immune function. By using highly-palatable, moist diets containing T-2 toxin, it was possible to cause lethal subacute toxicosis in mice. Thus, the mouse was a useful experimental animal in which to study these toxic effects of T-2 toxin because influences of various factors such as diet, strain of animal, age, environment, or other toxins can be more readily evaluated in animals in which positive effects can be predictably reproduced by the higher levels of exposure. Other species used in feeding studies with trichothecenes, including rats (Marasas et al., 1969; and in Section 10), chickens (Chi et al., 1977b), cattle (Matthews et al., 1978) and swine (Weaver et al., 1978c) have not developed the range of effects observed in mice. These species might have been resistant or might have refused to consume dietary levels high enough to cause effects on hematopoiesis or lymphopoiesis.

The development of aplastic anemia, atrophy of bone marrow, and generalized lymphoid depletion in mice fed T-2 toxin in the diet (Sections 8, 9 and 10) provided the first evidence that dietary T-2 toxin can cause potentially fatal suppression of hematopoiesis, thereby supporting suggestions that diseases such as alimentary toxic aleukia and stachybotrytoxicosis could be trichothecene mycotoxicoses (Ueno et al., 1972c;

Eppley and Bailey, 1975; Szathmary et al., 1976). However, mice did not develop granulocytopenia nor thrombocytopenia, each of which is reported to be a major effect of the naturally occurring mycotoxins. This difference might merely be a difference between susceptibility of hematopoietic populations of different species. Another possible explanation is that different trichothecenes or other toxins might be involved in the natural mycotoxicoses.

Several observations made in mice with experimental subacute dietary T-2 toxicosis may have implications that are relevant to the prediction of hazards of dietary trichothecenes, both to humans and livestock. Mice were able to overcome the effects of T-2 toxin on hematopoietic and lymphoid tissues after several weeks. In adequate diets, low levels of T-2 toxin (10 ppm) had no visible effects on bone marrow and lymphoid tissues after 4 weeks, whereas for higher levels of toxin (20 ppm), regeneration of these tissues was delayed and incomplete after 6 or 8 weeks (Sections 8 and 9). In adult mice fed the 20-ppm level of toxin (Section 10), hematopoietic tissues had regenerated after 4 weeks. If other species respond in a similar fashion, the implication that the effects of low dietary levels of trichothecenes on hematopoietic and lymphoid tissues are transient means that the harmful effects of chronic exposure to dietary trichothecenes would be less than if the effects were constant during exposure.

The findings that recovery from the suppressive effect of dietary T-2 toxin is delayed under various conditions including lowered dietary protein (Fig. 51), increased levels of exposure (Table 36), altered environment (i.e., removal of bedding; Section 9, Table 50), and in

juvenile animals (Section 10) of some species (i.e., mice compared with rats; Section 10) have important implications in eventual definition of acceptably safe levels of trichothecenes in food. Many such potentiating factors could exist and influence toxicity of trichothecenes to populations naturally at risk. For example, if other mycotoxins or dietary components were to interfere with the mechanism by which individuals develop a resistance to the potentially harmful effects on lymphoid and hematopoietic tissues, the toxicity of any given level of trichothecene would be increased.

A limitation to the use of mice for toxicologic studies on T-2 toxin is that dietary T-2 toxin apparently causes different effects in different species. It is not yet known if other species are susceptible to similar effects on erythropoiesis and lymphopoiesis as were observed in mice fed T-2 toxin in the diet. Rats fed T-2 toxin (Section 10) did not exhibit suppression of bone marrow nor did they have the same degree of depletion of lymphoid tissues as did mice. However, mice that overcame the effects of T-2 toxin on hematopoiesis exhibited similar effects as did rats, namely reduced food consumption, reduced weight gain, irritation of the squamous gastric mucosa, and atrophy of lymphoid tissues. This spectrum of effects is similar to that reported in chickens fed T-2 toxin in the diet (Wyatt et al., 1972b; Doerr et al., 1974; Chi et al., 1977b; Richard et al., 1978).

Mice are not the most ideal experimental animals in which to produce severe pathologic effects of trichothecenes for the purpose of comparison with naturally occurring pancytopenic diseases of livestock. Mice have

an extremely short erythrocytic lifespan, in comparison with larger species of animals (Schalm et al., 1975) and they appeared to be less susceptible to depletion of neutrophilic granulocytes and thrombocytes. Nevertheless, it was notable that the generalized hemorrhagic diathesis comparable to the hemorrhagic syndrome of mouldy-corn poisoning of swine and poultry (Forgacs and Carll, 1962), and cattle (Albright et al., 1963; Smalley et al., 1970), was not observed in mice, although some mice in Sections 8 and 9 had localized intestinal hemorrhage with bloody diarrhea. In one of these mice, atrophy of the intestinal mucosa was observed, but in others, no origin of hemorrhage was found. Localized intestinal hemorrhage has been reported in calves given repeated intraruminal doses of T-2 toxin (Pier et al., 1976), and in rats given extracts of Fusarium tricinctum (Kosuri et al., 1970). The difference between patterns of hemorrhage observed in experimental T-2 toxicosis of rodents and calves, and in the naturally occurring hemorrhagic syndromes of swine, cattle and poultry, supports the view of Smalley and Strong (1974) that the hemorrhagic syndromes may be caused at least in part by other mycotoxins. Unfortunately, the roles of coagulopathy, thrombocytopenia, or vascular damage in the pathogenesis of hemorrhage in the naturally occurring diseases have not been clearly demonstrated (see Section 2.2), so various hemorrhagic syndromes reported to affect livestock could include many different diseases, only some of which may be mycotoxicoses.

### 11.5 Effects of T-2 toxin on lymphoid tissues

The morphological changes in lymphoid tissues of mice during acute (Section 6.4) and subacute toxicosis (Section 8.4) indicated that T-2 toxin is toxic to lymphocytes. Juvenile mice were more severely affected than were adult mice and young rats (Section 10.4).

The effects of T-2 toxin on immune function were not examined. However, all lymphoid tissues became severely atrophic in mice exposed to high dietary levels of T-2 toxin, and it is likely that this would be associated with impairment in immune function. Impaired T- and B-cell functions, and thymic atrophy, have recently been reported in mice given single or repeated doses of T-2 toxin, diacetoxyscirpenol, or crude extracts of Fusarium poae (Rosenstein et al., 1979; LaFarge-Frayssinet et al., 1979). There might have been a more pronounced effect on lymphoid populations in mice exposed to repeated bolus doses, rather than to a continuous intake of toxin in the diet, so the impairment observed in the former situation does not necessarily indicate that immune function would be impaired under dietary exposure.

Subacute dietary T-2 toxicosis in poultry has minimally impaired B-cell dependent functions, but T-cell function has not been examined. Turkey poults, but not chicks, developed thymic atrophy after having consumed a diet containing 10 ppm of T-2 toxin for 4 weeks (Richard et al., 1978). However, these authors found no impairment in the ability of turkey poults to form agglutinating antibody against Pasteurella multocida. A previous study by Boonchuvit et al., (1975) showed that dietary T-2 toxin (16 ppm) caused reduction in the relative size of the bursa of Fabricius and the spleen in day-old chickens fed for 3 weeks. Affected

chickens were less resistant to oral inoculation with various isolates of Salmonella, but serum titers of anti-Salmonella agglutinins were not affected by T-2 toxin.

In juvenile and adult mice, the thymus and thymic dependent lymphoid populations (splenic periarteriolar sheaths, paracortical zones of lymph nodes, and intestinal theliolymphocytes) appeared to be more susceptible to T-2 toxin than were B-cell populations. Often, germinal follicles were observed in the spleen and lymph nodes (Fig. 45E,F) in which depletion of T-cells was marked, indicating that the effects of dietary T-2 toxin on T-cell dependent immune function should be further examined. Depletion does not necessarily imply that there is impairment in function.

The mechanism of depletion of lymphoid tissues may not be a simple direct cytotoxic ("radiomimetic") effect of T-2 toxin on germinal cells of thymus and other tissues, although such an effect could be a major component. Contributions to the degree of atrophy have likely been made by chronic stress. Mice fed control diets at the restricted rate developed thymic atrophy and lymphopenia (Section 8, Fig. 39), although these effects were much less severe than in mice consuming toxic diets at the same rate. Reduced levels of dietary protein (Gebhardt and Newberne, 1974; Bell et al., 1976) and protein-calorie malnutrition (Smythe et al., 1971) depress T-cell populations, probably by cortisone-induced lympholysis and nutritional deprivation of precursor cells (Bell et al., 1976).

T-2 toxin may have depleted lymphoid populations by stress-induced lympholysis. Mice on diets containing high or low levels of T-2 toxin consistently exhibited lymphopenia and absolute eosinopenia, the degree

of which was greater than observed in control mice fed at the restricted rate (Appendix C, Table 72). Even mice consuming optimal diets containing 10 ppm of T-2 toxin were eosinopenic and lymphopenic, but had only minor depressions in rates of growth and food consumption. Absolute eosinopenia may result from elevated levels of endogenous corticosteroids due to stress (Schalm et al., 1975). Regardless of the mechanism by which it occurs, the lymphoid depletion observed in mice can be considered to be an effect of diets contaminated with T-2 toxin. Thus, the immune function should be examined, both in mice and in other species fed T-2 toxin in the diet.

#### 11.6 Nutritional influences on toxicity of T-2 toxin

For high levels of dietary T-2 toxin (20 ppm), voluntary consumption of diets by mice was greatly reduced and all mice were undoubtedly poorly nourished. The extent to which such malnutrition contributed to the development of the various toxic effects was not determined. However, mice fed control diets at a similarly restricted rate did not develop aplasia of bone marrow nor gastric hyperkeratosis indicating that such effects were due to T-2 toxin.

Minor variations in protein content of semipurified diets had minimal influence on the severity of toxic effects of T-2 toxin at the 20-ppm level in the diet (Section 8). This may have been because the change in composition was minor in relation to the degree of malnutrition. However, for the lower dietary level of 10 ppm used in Section 9, mice fed a low protein diet (8%) developed severe effects on bone marrow and lymphoid organs, whereas these tissues in mice on optimal diets were only

mildly affected, if at all. Thus, low dietary protein potentiated the toxicity of dietary T-2 toxin, probably in part by decreasing the ability of mice to overcome the suppressive effects of T-2 toxin, as discussed in detail in Section 8.5.2. There are no other reports of dietary modification of toxicity of trichothecenes.

Nutritional deficiency diseases in livestock could be precipitated or potentiated by exposure to trichothecenes. By causing general reduction in food intake, diets that are marginally deficient in one or more nutrients might no longer provide enough of the particular nutrient to fulfill the animal's requirements. Thus, animals could be subjected to general malnourishment, but have effects due to lack of the more deficient nutrient, be it a vitamin, a mineral, or a macronutrient. Exposure to trichothecenes could also become manifested as a nutritional deficiency disease if the toxin acts as an antimetabolite. At present, these possibilities are largely speculative because the interrelationships between nutrition and exposure to trichothecenes have not been studied previously. There are several reports on strong interactions between dietary components and other mycotoxins such as aflatoxin and ochratoxin (Hamilton, 1977).

Observations in mice and rats exposed to T-2 toxin in different diets support the speculation that trichothecenes could cause secondary nutritional problems in livestock. Firstly, due to their ability to inhibit voluntary food intake in the absence of other signs, as was observed in rats fed the 10-ppm level (Section 10), exposure to diets containing T-2 toxin or other trichothecenes could result in a state of malnutrition, manifest as poor growth or production. Such effects have

been demonstrated in poultry fed low levels of T-2 toxin (less than 4 ppm) (Speers et al., 1977; Chi et al., 1977b). Secondly, mice fed dietary T-2 toxin at 10 ppm in a semipurified diet developed an abnormality of erythropoiesis during the stage of regeneration of aplastic bone marrow.

Many erythrocytes had abnormalities of shape, size, and degree of hemoglobination, the reason for which was not determined. However, the major findings of leptocytosis, poikilocytosis, hypochromia, and reticulocytosis are most consistent with an iron deficiency type of anemia (Bannerman et al., 1972) or with an anemia due to impaired synthesis of hemoglobin (Bannerman et al., 1974). Mice had adequate levels of iron in their diets (Sorbie and Valberg, 1974) and in their tissues, but T-2 toxin might have inhibited uptake of iron by young red cells. Alternatively, T-2 toxin might have inhibited the synthesis of globin. Trichothecenes are potent inhibitors of eukaryotic synthesis of protein (Cundliffe et al., 1974; Ueno, 1977a) but such effects have not yet been demonstrated in intact animals. If T-2 toxin depressed the rate of globin synthesis, abnormal erythrocytes would more likely be formed in an animal that was protein deficient but with a high demand for hemoglobin synthesis such as would have occurred during rapid regeneration of erythropoietic cells in anemic mice that had become resistant to the antiproliferative effects of T-2 toxin. Under such circumstances, the putative effect of T-2 toxin on globin synthesis could be considered to be an example of potentiation of a state of protein deficiency. If T-2 toxin, instead, inhibits iron metabolism, the potentiation could likewise be considered to be an enhanced iron deficiency. Iron deficiency anemia induced by dietary ochratoxin has recently been recognized in chickens (Huff et al., 1979), and ochratoxin has been found

to depress absorption of carotenoids in chickens (Huff and Hamilton, 1975), further supporting the concept that mycotoxins might induce nutritional deficiency diseases.

#### 11.7 Harmful effects in humans due to foodborne trichothecenes

The implications of trichothecenes for human health are virtually unmeasured. Efficient analytical methods needed to survey suspect food products for trichothecenes are not generally available at present (Stoloff, 1979), although recent developments in gas chromatographic-mass spectrometric (GC-MS) (Pathre and Mirocha, 1977) and radioimmunoassay techniques (Chu et al., 1979) are promising. If such methods can be implemented in surveillance programs, the necessary information on the natural occurrence of at least some of the trichothecenes could be collected. Not only must such data be gathered, but the significance of the prevalent levels must also be determined by empirical comparative toxicological studies in different species of experimental animals. Then, by integrating knowledge of occurrence with knowledge of effects, the hazards of trichothecenes to the human population could be estimated, and maximum safe levels specified.

Because of the differences among various species in their response to dietary T-2 toxin, the estimation of the possible effects of trichothecenes in humans may be difficult. Some progress might be made if the basis for the differences between various species could be identified. If humans were shown to possess the mechanism by which rodents, swine and poultry resist hematopoietic suppression by T-2 toxin, the estimation of hazards of trichothecenes to humans could more readily be conducted by comparative studies in rodents and other species.

### 11.8 Harmful effects of trichothecenes to livestock

The trichothecene mycotoxicoses presently include several overt diseases of livestock, some of which still have not been proven to be due to dietary trichothecenes (see Section 2.2). Some diseases such as fusariotoxicosis (Wyatt et al., 1972a; Greenway and Puls, 1976) of poultry and swine, or the syndrome of vomition and refusal of feed contaminated by F. roseum (Vesonder et al., 1973; Mirocha et al., 1976a), are clearly due to dietary trichothecenes because identical diseases have been reproduced experimentally with pure trichothecene toxins such as T-2 toxin and deoxynivalenol (Wyatt et al., 1972b; Palyusik and Koplik-Kovács, 1975; Chi et al., 1977b; Forsyth et al., 1977; Weaver et al., 1978c). However, there are relatively few reports of outbreaks of fusariotoxicosis or feed refusal (Funnell, 1979; Mirocha, 1979), so it seems that such problems are sporadic, except after occasional wet years (Curtin and Tuite, 1966; Prentice and Dickson, 1968; Vesonder et al., 1973). To what extent unnoticed food refusal of diets containing trichothecenes reduces growth or production in livestock is unknown, but it might be significant.

There have been suggestions that trichothecenes might increase losses of livestock by predisposing to infectious disease (Richard et al., 1978). As discussed in Section 11.5, T-cell populations of lymphocytes are depleted in mice during consumption of T-2 toxin in the diet. However, no studies have been conducted to evaluate T-cell function of livestock exposed to trichothecenes in the diet.

The observations that various factors can influence the duration of the aplastic state in mice during exposure to dietary T-2 toxin may have

implications in explaining how trichothecenes might sporadically cause hematopoietic failure in livestock. If mice resemble other species in their ability to spontaneously develop resistance to suppression of hematopoiesis by T-2 toxin under ideal nutritional and environmental conditions, livestock might rarely develop lethal hematopoietic failure because resistance might occur before critical depletion of circulating populations of blood cells. However, under non-ideal conditions, such as malnutrition, or in the presence of other mycotoxins that interfere with the development of resistance, dietary T-2 toxin could impair hematopoiesis for a sufficiently long period to cause thrombocytopenic purpura, or granulocytopenia and sepsis. From a diagnostic viewpoint, the apparent complexity of conditions needed for T-2 toxin to cause hematopoietic failure would make it difficult to assign toxicological significance to levels of T-2 toxin or other trichothecenes found in a natural outbreak of mycotoxic disease. Conversely, when a level of T-2 toxin is found in a diet of optimal nutritional composition, it seems unlikely that lethal effects would occur unless other mycotoxins are present because animals would probably refuse to consume enough food containing high levels of trichothecenes to develop hematopoietic failure.

#### 11.9 Economic loss due to wastage of foodstuffs contaminated with trichothecenes

Feeds contaminated with T-2 toxin or deoxynivalenol may be refused by swine, resulting in financial losses to farmers because feed stocks may be wasted due to an inability to counteract the refusal factors. At present, it is not advisable to feed livestock rations containing trichothecenes because it has not been established whether or not this would lead to potentially harmful residues of toxin in eggs, meat or milk products. However, recent studies with isotope-labelled T-2 toxin in chickens (Chi et al., 1978b; 1978c) and rodents (Matsumoto et al., 1978) have shown that doses are eliminated rapidly in the feces and urine, although T-2 toxin has been detected in milk (Robison et al., 1979) and eggs (Chi et al., 1978b). If it can be established that residues of trichothecenes are quickly excreted from the body, it would be possible to safely feed rations containing trichothecenes to other animals such as cattle if they would consume them, and provided that losses due to possible reductions in production were acceptable. The studies with experimental subacute T-2 toxicosis in mice and rats have shown that different species have different susceptibilities to T-2 toxin. Some of the various conditions found to minimize toxicity of T-2 toxin in mice in Sections 8-10 could be adapted into a procedure for safe utilization of feeds containing trichothecenes. For example, it may be advisable to avoid feeding trichothecenes to young animals, and to ensure that the ration is balanced and adequate.

A potential situation resulting in large financial loss could occur if consignments of grain intended for human consumption are found to

contain trichothecenes. Until now, such problems have not arisen because of the lack of suitable, sensitive tests for trichothecenes in feed. As more sensitive methods are adapted for use in surveillance programs, some lots of grain may be found to contain low levels of trichothecenes. The lack of toxicological data suitable for prediction of possible harmful effects of such low levels would lead to the conservative approach of diverting grain into animal feedstuffs, or even outright disposal. The attendant financial losses would be large. Such a possibility further emphasizes the need to evaluate the toxicological significance of various dietary levels of trichothecenes.

## 12.0 SUMMARY AND CONCLUSIONS

The foregoing experiments attempted to define various toxic effects of T-2 toxin in mammalian tissues. Conclusions were derived from the results of pathological and toxicological examinations of: 1) dermal irritation by topical application of T-2 toxin to rats and rabbits; 2) acute toxicity of intragastric T-2 toxin in mice; and 3) subacute toxicity of dietary T-2 toxin in mice and rats.

### A. Summary of findings

#### 1. Dermal irritation by topical T-2 toxin

- a. T-2 toxin evoked a non-specific, acute dermal inflammatory reaction in rats and rabbits. At all stages of development, lesions were histologically similar to those produced by croton oil on rats.
- b. Dermal and epidermal necrosis occurred in all intense reactions, but was inconsistently present in mild reactions.
- c. Reactions were grossly visible within 24 hours as hyperemic, edematous plaques. Maximum intensity of mild reactions occurred at 24 hours, whereas strong reactions intensified until 48 hours.
- d. Sensitivity of the skin to T-2 toxin was greater, but more variable, in rabbits than in rats.
- e. The intensity of inflammation was dose-dependent in rats and rabbits.
- f. The frequency of rats responding to T-2 toxin depended on the topical dose.
- g. Concentrations of T-2 toxin in the range of 5 to 60  $\mu\text{g}/\text{ml}$  could be assayed precisely and accurately when reactions to test applications of T-2 toxin on rats were rated in units of equivalent concentration of T-2 toxin, rather than by scores of intensity or by frequency of response.

#### 2. Acute toxicity of oral T-2 toxin in mice

- a. T-2 toxin (2.0 or 2.5 mg/kg) selectively injured germinal cells of thymic cortex, lymphoid follicles, splenic red pulp, bone marrow, and

crypts of the small intestine, and thereby caused a "radiomimetic" distribution of necrosis.

b. T-2 toxin arrested mitotic activity in lymphoid follicles, thymic cortex, and crypts of the small intestine within two hours after administration.

c. T-2 toxin was selectively toxic to lymphocytes and plasma cells in the lamina propria of the small intestine.

d. Within 90 minutes after administration, T-2 toxin caused nuclear pyknosis, karyorrhexis, and fragmentation of cytoplasm of columnar epithelial cells of intestinal crypts; of lymphoblasts in germinal centres of splenic follicles; of hematopoietic cells in the spleen; and of mature lymphocytes and plasma cells in the lamina propria of the small intestine.

e. Fragments of cells destroyed by T-2 toxin were enclosed in heterophagosomes within neighbouring, surviving macrophages or epithelial cells, and were rapidly degraded by lysosomal activity.

f. The severity of necrosis in the intestinal and lymphoid follicles was dose-dependent, but varied widely among different mice given the same dose.

g. All damaged lymphoid, hematopoietic, and intestinal epithelial tissues regenerated rapidly after acute injury by T-2 toxin. Mitotic activity had resumed by 12 hours, and most heterophagosomes had disappeared by 24 hours after administration. By 96 hours, all target tissues had begun to repopulate.

### 3. Subacute toxicity of dietary T-2 toxin

a. T-2 toxin at dietary levels of 10 or 20 ppm caused dose-dependent depressions of growth and of food consumption by mice and rats.

b. Dietary T-2 toxin caused hyperkeratosis and ulceration of the squamous gastric mucosa in mice and rats. Some mice developed perioral dermatitis.

c. Swiss mice were more susceptible to the effects of dietary T-2 toxin than were Wistar rats.

d. Juvenile mice were more susceptible than young adult mice.

e. In mice, dietary T-2 toxin (20 ppm) caused hypoplasia of bone marrow, splenic red pulp, and all lymphoid tissues within 1 week. Dietary T-2 toxin at 20 ppm did not suppress hematopoiesis in rats, but caused thymic atrophy and lymphopenia.

f. The suppressive effects of dietary T-2 toxin on hematopoiesis of mice were transient. After 2 to 4 weeks, neutrophilic myelopoiesis, megakaryocytopoiesis, erythropoiesis, and lymphopoiesis, in that order, resumed during continuous exposure to dietary T-2 toxin.

g. Neutrophilic myelopoiesis of mice on dietary T-2 toxin resumed within 2 weeks and subsequently became hyperplastic. Affected mice developed neutrophilia that was probably a response to inflammation of the upper alimentary tract caused by the irritant activity of T-2 toxin.

h. Eosinophilic myelopoiesis was suppressed in mice and rats fed dietary T-2 toxin (10 or 20 ppm).

i. In mice, erythropoietic cells were more susceptible to dietary T-2 toxin than were myeloid cells. Suppression of erythropoiesis by T-2 toxin for periods longer than 3 weeks caused aplastic anemia in juvenile mice.

j. The duration of suppression of erythropoiesis by dietary T-2 toxin depended on the diet. In juvenile mice fed a semipurified diet of low protein content (8%) containing 20 ppm of T-2 toxin, regeneration of erythropoietic populations was delayed. Juvenile mice fed this low protein diet containing T-2 toxin at 10 ppm developed aplastic anemia over an 8 week period, whereas similar mice fed nutritionally optimal diets containing 10 ppm T-2 toxin did not become anemic.

k. Suppression of erythropoiesis of mice by T-2 toxin was overcome gradually. Erythroid maturation initially resumed at a low rate and in a megaloblastic and dysplastic fashion, but after 6 to 8 weeks erythropoiesis returned to normal.

l. The thymus and T-cell-dependent populations of lymphocytes were more severely depleted than were B-cell-dependent populations in mice and rats fed dietary T-2 toxin. The degree of lymphoid depletion was dose-dependent, and was more severe in mice than in rats.

m. Lymphopoiesis was more susceptible to inhibition by dietary T-2 toxin than was erythropoiesis. Juvenile mice fed T-2 toxin at 10 ppm in nutritionally adequate diets developed lymphopenia and thymic atrophy but not anemia. Depleted lymphoid tissues eventually began to repopulate after 6 to 8 weeks in these mice. The mechanism of lymphoid depletion and its functional significance are undetermined.

n. Recovery of mice from hematopoietic suppression by dietary T-2 toxin might be mediated by acquisition of hepatic metabolic competence to biotransform T-2 toxin. Recovery of erythropoiesis was associated with hepatomegaly, the degree of which depended on the dietary level of T-2 toxin and the duration of exposure. Mice housed on softwood shavings, which may contain components that stimulate hepatic microsomal activity, overcame suppression of erythropoiesis more rapidly than mice housed in suspension cages.

o. The suppressive effects of dietary T-2 toxin on hematopoietic and lymphoid tissues of mice were readily reversible. Active regeneration occurred in all atrophic tissues within 7 days after mice were transferred from diets containing 20 ppm of T-2 toxin to toxin-free control diets.

p. The effects of dietary T-2 toxin (10 or 20 ppm) on the mucosa of the upper alimentary tract of mice and rats did not diminish during continuous exposure. These effects, including perioral dermatitis, gastritis and hyperplasia of the gastric and duodenal mucosa, may have been caused by a direct irritant effect of T-2 toxin.

q. Dietary T-2 toxin at 20 ppm occasionally caused hemorrhage into the intestine of juvenile mice after 1 to 2 weeks. Crypt atrophy rarely occurred, but increased numbers of heterophagosomes were present in response to T-2 toxin at the 20-ppm level.

## B. Conclusions

The lesions in mice with subacute toxicosis from dietary T-2 toxin were attributed both to the irritant toxicity of T-2 toxin, and to its inhibitory effects on rapidly dividing cells. The irritant toxicity to the mucosa of the upper alimentary tract caused inflammation and epithelial

hyperplasia both of which persisted throughout exposure periods up to 8 weeks. Suppressive effects on rapidly dividing hematopoietic and lymphoid cells were transient because depleted populations gradually regenerated during continued exposure. Rats were not susceptible to hematopoietic suppression by levels of T-2 toxin sufficient to cause aplastic anemia in mice. When the suppressive effects of T-2 toxin had been overcome, mice exhibited lesions due to irritant toxicity similar to those observed in rats and reported in poultry and swine fed T-2 toxin. The mechanism by which mice overcame the toxic effects of T-2 toxin to hematopoietic cells was not established, but the possibility that it was an acquired metabolic ability to detoxify T-2 toxin was supported by several observations. Suboptimal levels of protein increased the toxicity of T-2 toxin to hematopoietic and lymphoid tissues of mice by prolonging the period of suppression. Thus, other species including rats, poultry, and swine might also be more susceptible to hematopoietic suppression by T-2 toxin if the unidentified mechanism by which they are normally resistant is impaired by suboptimal nutrition or by other toxins. Because the lymphopoietic system was highly sensitive to dietary T-2 toxin in mice, immunosuppression could occur in animals ingesting T-2 toxin in the diet.

The task of defining the significance of food-borne trichothecenes to human and animal health should be directed towards: 1) determination of the prevalence of trichothecenes in food and feed; 2) identification of the mechanisms by which trichothecenes are metabolised; 3) identification of factors that modify toxicity of trichothecenes; 4) determination of the functional significance of lymphoid depletion by trichothecenes; and 5) examination on the chronic toxicity and potential carcinogenicity of trichothecenes.

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APPENDIX A

Individual values from Section 4.

TABLE 60: Draize scores of New Zealand rabbits exposed to topical doses of T-2 toxin in ethyl acetate. Section 4.4.1.

Level of T-2 toxin (µg/ml)	Stage of appraisal (hrs.)	Rabbit Number						Mean ± SD
		1	2	3	4	5	6	
0	24	0,0 <sup>a</sup>	0,0	0,0	0,0	0,0	0,0	0
	48	0,0	0,0	0,0	0,0	0,0	0,0	0
	72	0,0	0,0	0,0	0,0	0,0	0,0	0
20	24	3,2	3,2	2,1	2,2	2,2	2,0	3.8±1.2
	48	2,1	2,1	1,1	2,1	1,1	1,0	2.3±0.8
	72	3,0	1,0	1,0	2,0	1,0	0,0	1.3±1.0
40	24	3,1	2,0	3,1	4,2	2,2	2,2	4.0±1.3
	48	4,1	3,1	4,2	3,2	2,1	2,2	4.5±1.0
	72	1,0	2,1	1,0	2,2	1,0	2,1	2.2±1.3
80	24	2,1	2,0	3,2	2,2	3,3	2,2	4.0±1.4
	48	2,2	4,2	2,1	4,3	3,2	4,3	5.3±1.6
	72	1,0	3,1	2,0	1,0	2,1	3,2	2.7±1.6
160	24	2,0	2,1	2,2	3,2	2,2	3,2	3.8±1.2
	48	3,4	3,3	3,1	2,3	4,2	2,1	5.2±1.5
	72	3,0	4,3	3,2	2,1	2,1	2,1	4.0±1.7
320	24	2,0	2,0	3,2	2,1	3,3	2,2	3.6±1.6
	48	3,3	4,3	4,4	4,3	4,4	3,2	6.8±1.2
	72	2,0	4,3	3,3	3,1	4,4	3,2	5.3±2.2

<sup>a</sup> Values are scores (0-4) for erythema and edema respectively.

TABLE 61: Draize scores of Wistar rats exposed to topical doses of T-2 toxin in ethyl acetate. Section 4.4.1.

Level of T-2 toxin ( $\mu\text{g/ml}$ )	Stage of appraisal (hrs)	Rat Number										Mean $\pm$ SD	
		1	2	3	4	5	6	7	8	9	10		
0	24	0,0 <sup>a</sup>	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0
	48	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0
20	24	1,0	0,0	1,1	2,0	2,1	0,0	0,0	1,1	1,0	1,0	1,0	1.2 $\pm$ 1.0
	48	1,0	0,0	1,0	1,0	1,1	0,0	0,0	1,0	0,0	0,0	0,0	0.6 $\pm$ 0.7
40	24	2,1	2,1	1,1	2,1	2,1	1,1	2,1	2,2	1,0	2,1	2,1	2.7 $\pm$ 0.8
	48	2,0	2,1	1,0	1,1	1,1	1,1	2,1	2,1	1,1	1,1	1,1	2.2 $\pm$ 0.6
80	24	1,0	1,1	1,0	2,2	1,1	3,2	2,2	2,2	2,1	2,2	2,2	3.0 $\pm$ 1.4
	48	2,1	1,1	1,1	2,1	2,1	2,2	2,1	2,2	2,1	1,2	1,2	3.0 $\pm$ 0.6
160	24	3,2	2,2	2,1	1,1	2,2	2,1	2,3	2,3	2,2	3,3	3,3	4.1 $\pm$ 1.2
	48	3,1	2,2	3,1	3,2	3,2	2,2	2,3	2,2	3,3	3,2	3,2	4.6 $\pm$ 0.7
320	24	1,0	3,2	2,2	2,3	2,2	1,1	3,2	2,3	1,2	1,2	1,2	3.7 $\pm$ 1.4
	48	1,0	3,3	2,2	4,3	3,3	2,3	3,2	3,2	3,2	2,2	2,2	4.8 $\pm$ 1.6

<sup>a</sup> Values are scores (0-4) for erythema and edema respectively.

TABLE 62: Frequency of visible reactions of rats exposed to graded doses of T-2 toxin and DAS.

Concentrations of T-2 toxin ( $\mu\text{g/ml}$ )	2.5	5	10	15	20	40	80
At 24 hours	1 <sup>a</sup>	1	3	6	9	10	10
At 48 hours	0	0	1	7	9	9	10

Concentrations of T-2 toxin ( $\mu\text{g/ml}$ )	5.3	8	12	18	26.7	40	60	90
At 24 hours	3	4	6	8	9	10	10	10
At 48 hours	4	3	6	9	10	10	9	10
At 72 hours	1	1	3	5	9	9	9	9

Concentrations of DAS ( $\mu\text{g/ml}$ )	5.3	8	12	18	26.7	40	60	90
At 24 hours	1	2	2	8	9	10	10	10
At 48 hours	1	1	4	5	5	9	10	10
At 72 hours	1	0	1	1	5	2	8	9

<sup>a</sup> Each number is the number of rats reacting out of a group of 10.

TABLE 63: Draize scores for erythema of rats exposed to seven replicate applications of each of three solutions of DAS.

Rat	At 24 hours	At 48 hours	Concentration of DAS (µg/ml)
1	0 0 0 0 0 0 0	0 0 0 0 0 0 0	20
	0 0 0 0 0 0 0	0 0 0 0 0 0 0	30
	0 0 0 0 0 0 0	0 0 0 0 0 0 0	60
2	2 2 2 2 2 2 2	1 1 1 1 1 1 1	60
	0 0 0 0 0 0 0	0 0 0 0 0 0 0	20
	0 0 1 1 1 1 1	0 0 0 0 0 0 0	30
3	0 0 0 0 0 0 0	0 0 0 0 0 0 0	30
	1 1 1 1 1 1 1	0 0 0 0 0 0 1	60
	0 0 0 0 0 0 0	0 0 0 0 0 0 0	20
4	0 0 0 0 0 0 0	0 0 0 0 0 0 0	20
	2 1 3 3 3 3 3	2 1 2 2 2 2 2	60
	1 1 1 1 1 1 1	0 0 1 0 1 1 0	30
5	1 1 1 1 1 1 1	1 1 1 1 1 1 1	30
	0 0 0 0 1 0 0	0 0 0 0 0 0 0	20
	2 1 2 2 2 2 2	1 1 1 1 1 1 1	60
6	3 3 3 1 1 3 3	1 1 1 1 1 1 1	60
	1 1 0 2 1 1 1	1 0 0 1 1 1 1	30
	0 0 0 0 0 0 0	0 0 0 0 0 0 0	20
7	0 0 0 0 0 0 0	0 0 0 0 0 0 0	20
	1 0 0 1 0 0 0	0 0 0 0 0 0 0	30
	1 1 0 1 1 1 1	1 1 0 1 1 1 1	60
8	0 0 0 0 0 0 0	0 0 0 0 0 0 0	30
	1 1 1 1 1 1 1	0 0 0 0 0 1 0	60
	0 0 0 0 0 0 0	0 0 0 0 0 0 0	20
9	2 2 2 2 2 2 2	1 1 1 1 1 1 1	60
	0 0 1 0 0 0 0	0 0 0 0 0 0 0	30
	0 0 0 0 0 0 0	0 0 0 0 0 0 0	20
10	1 2 2 2 2 2 2	0 0 1 1 0 0 0	30
	1 1 1 2 2 2 1	0 1 1 1 1 1 1	20
	3 3 3 3 3 3 3	1 1 2 2 2 2 2	60

APPENDIX B

Formulations of Experimental Diets

TABLE 64:

Formulation of the natural-ingredient based laboratory rat and mouse diet\*used in Sections 6-10.

<u>Basal diet</u>	<u>Per cent</u>
Ground wheat grain	39.4
Ground barley grain	33.2
Soybean meal	7.6
Fish meal	5.0
Stabilized lard	2.0
Molasses	3.0
Alfalfa meal	5.0
Dicalcium phosphate	1.6
Brewer's yeast	2.0
Ground limestone	0.7
Iodized salt	0.5
 <u>Supplements</u>	
Zinc oxide	2.2 g/100 kg
Vitamin A	3.66 g/100 kg
Vitamin D (200,000 i.u/g)	0.55 g/100 kg
Vitamin B <sub>12</sub> (132 mg/kg)	36.7 g/100 kg
 <u>Analysis</u>	
Protein	18.6
Fat	3.9
Crude fibre	5.3
Calcium	1.1
Phosphorus	0.9

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\* Animal Resources Centre and Department of Animal Science,  
University of Saskatchewan.  
Slightly modified from Bell (1972)

TABLE 65

Composition of semipurified mouse diet<sup>a</sup>

<u>Basic Diet Ingredients</u>	<u>Grams per kilogram</u>
α-cellulose	50.0
gelatin	20.0
l-methionine-supplemented casein <sup>b</sup>	140.0 <sup>c</sup>
carbohydrate mixture <sup>d</sup>	590.0 <sup>c</sup>
fat mixture <sup>e</sup>	100.0
vitamin mixture <sup>f</sup>	50.0
mineral mixture <sup>g</sup>	50.0

<u>Composition of prepared diet</u>	<u>Proportions</u>
basic diet	100.0 g
water	70.0 ml
ethanol (+ T-2 toxin 10 mg/ml)	0.2 ml

<sup>a</sup> Modified from Bell (1972) and John and Bell (1976).

<sup>b</sup> Vitamin-free casein, 96.9%; l-methionine, 3.1%.

<sup>c</sup> Proportions shown are for a protein level of 16% (approx).

<sup>d</sup> Cornstarch, 50%; dextrose, 30%; sucrose, 20%.

<sup>e</sup> Lard, 60%; sunflower oil, 40%.

<sup>f</sup> 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<sub>12</sub>, 0.0005; meso-inositol, 0.04; choline chloride, 80; retinyl palmitate (Vitamin A, 500,000 IU/g) 0.2; cholecalciferol (Vitamin D<sub>3</sub>, 4x10<sup>7</sup> IU/g) 0.0001; dl-α-tocopherol acetate (Vitamin E, 1000 IU/g) 2.8; menadione, 8.0; sunflower oil, 110.0, cornstarch, 794.3.

<sup>g</sup> Mineral mixture components (g/kg): NaCl (iodized), 40.0; CaHPO<sub>4</sub>·2H<sub>2</sub>O; 687.0; KHCO<sub>3</sub>, 136.0; MgO, 18.0; MnSO<sub>4</sub>·H<sub>2</sub>O, 3.0; FeSO<sub>4</sub>·H<sub>2</sub>O, 4.5; CuSO<sub>4</sub>·5H<sub>2</sub>O, 1.0; ZnO, 1.0; cornstarch, 109.5.

Preparation and administration of gelled diets:

A. Semipurified diet

1. Premix all dry ingredients (carbohydrate mix, cellulose, methionine-supplemented casein, and mineral mixture).
2. For each 100 g of complete diet, dissolve 2 g of powdered gelatin in 70 ml of water (65°C). Add 10 g of fat mixture and 5 g of vitamin mixture and mix thoroughly.
3. Add T-2 toxin in ethanol to the wet mixture and mix thoroughly.
4. Blend in the mixed dry ingredients (83 g per 100 g of diet).
5. Place in sealed containers and store at 4°C until used.

B. Natural ingredient diet

1. Grind the prepared ration.
2. Use 2 g of gelatin in 70 ml of water for each 100 g of prepared, ground diet.
3. Add T-2 toxin to the water-gelatin solution.
4. Mix in ground diet.
5. Store in sealed containers at 4°C until used.

Note: If the natural ingredient diet has been pelleted before being ground, gelatin is not needed.

APPENDIX C

Group means, standard errors  
and analyses of variance from Section 8.

Table 66: Sequential changes in body weights ( $\bar{X} \pm \text{SEM}$ ) of mice consuming semipurified diets containing T-2 toxin (20 ppm) and different levels of protein.

Diet	Group	Weeks on diet					
		0	1	2	3	4	6
8%- protein semi- purified	A (T-2 toxin)	16.8 $\pm$ 0.3	12.9 $\pm$ 0.3	12.3 $\pm$ 0.4	12.2 $\pm$ 0.5	12.0 $\pm$ 0.8	--
	B (restricted control)	15.1 $\pm$ 0.3	15.5 $\pm$ 0.3	15.5 $\pm$ 0.4	16.3 $\pm$ 0.5	17.6 $\pm$ 0.8	--
	C (ad lib control)	17.0 $\pm$ 0.5	18.8 $\pm$ 0.7	24.8 $\pm$ 1.2	29.8 $\pm$ 1.2	30.4 $\pm$ 1.4	--
12%- protein semi- purified	A (T-2 toxin)	15.6 $\pm$ 0.3	12.9 $\pm$ 0.3	13.0 $\pm$ 0.5	12.0 $\pm$ 0.3	12.0 $\pm$ 0.6	--
	B (restricted control)	15.8 $\pm$ 0.3	17.2 $\pm$ 0.3	16.7 $\pm$ 0.3	16.7 $\pm$ 0.6	17.7 $\pm$ 0.9	--
	C (ad lib control)	15.3 $\pm$ 0.4	22.4 $\pm$ 0.5	26.7 $\pm$ 0.6	32.7 $\pm$ 0.9	32.8 $\pm$ 1.4	--
16%- protein semi- purified	A (T-2 toxin)	16.0 $\pm$ 0.2	13.9 $\pm$ 0.3	14.3 $\pm$ 0.3	14.6 $\pm$ 0.3	15.6 $\pm$ 0.6	14.8 $\pm$ 1.6
	B (restricted control)	16.0 $\pm$ 0.4	18.9 $\pm$ 0.4	18.2 $\pm$ 0.4	18.3 $\pm$ 0.7	18.9 $\pm$ 0.8	21.2 $\pm$ 2.0
	C (ad lib control)	16.1 $\pm$ 0.5	24.2 $\pm$ 0.5	29.3 $\pm$ 0.7	33.6 $\pm$ 1.0	34.4 $\pm$ 1.2	--
Natural- ingredient diet	D (ad lib)	17.2 $\pm$ 0.5	25.8 $\pm$ 0.5	30.0 $\pm$ 0.6	32.0 $\pm$ 0.7	34.5 $\pm$ 0.8	--

Table 67 : Influence of dietary protein level on body weights of mice<sup>a</sup> consuming T-2 toxin over a 4-week period.

Source of variation	Analysis of Variance				
	Sum of squares	Degrees of freedom	Mean square	F ratio	P
Main effects:					
dietary protein	42.178	2	21.089	6.598	0.004**
time on diet	2.793	3	0.931	0.291	0.831
2-way interactions					
diet x time	20.620	6	3.437	1.075	0.395
Explained	65.591	11	5.963	1.866	0.078
Residual	115.065	36	3.196	--	--
Total	180.656	47	3.844	--	--

<sup>a</sup> Measured in grams.

\*\* Significant,  $p < 0.001$ .

Table 68: Dietary consumption rates for mice consuming T-2 toxin (20 ppm) in diets of different protein levels.

Diet	Group	Weeks on diet					
		1	2	3	4	5 and 6	
8% protein	A (20ppm T-2)	11.3 $\pm$ 0.1 <sup>a</sup>	8.3 $\pm$ 1.8	9.1 $\pm$ 0.9	13.1 $\pm$ 3.2	--	
		1.4 $\pm$ 0.1 <sup>b</sup>	1.0 $\pm$ 0.3	1.2 $\pm$ 0.2	1.5 $\pm$ 0.3		
	B (restricted control)	15.2 $\pm$ 0.5	11.5 $\pm$ 0.6	13.6 $\pm$ 0.8	11.9 $\pm$ 0.7	--	
		2.4 $\pm$ 0.0	1.9 $\pm$ 0.0	2.0 $\pm$ 0.0	1.9 $\pm$ 0.0		
	C ( <u>ad lib</u> control)	22.7 $\pm$ 1.7	18.5 $\pm$ 0.3	14.3 $\pm$ 0.1	13.7 $\pm$ 0.4	--	
		4.2 $\pm$ 0.1	4.5 $\pm$ 0.0	4.2 $\pm$ 0.1	4.1 $\pm$ 0.2		
	12% protein	A (20ppm T-2)	10.8 $\pm$ 1.4	10.4 $\pm$ 1.1	12.9 $\pm$ 0.6	11.5 $\pm$ 3.7	--
			1.4 $\pm$ 0.2	1.5 $\pm$ 0.2	1.5 $\pm$ 0.2	1.3 $\pm$ 0.4	
		B (restricted control)	12.3 $\pm$ 0.3	12.2 $\pm$ 0.6	11.9 $\pm$ 0.6	11.7 $\pm$ 0.5	--
2.4 $\pm$ 0.1			1.9 $\pm$ 0.0	2.0 $\pm$ 0.0	2.0 $\pm$ 0.0		
C ( <u>ad lib</u> control)		18.0 $\pm$ 0.4	15.7 $\pm$ 1.1	12.0 $\pm$ 0.1	12.2 $\pm$ 0.5	--	
		4.0 $\pm$ 0.0	4.4 $\pm$ 0.1	3.9 $\pm$ 0.0	4.0 $\pm$ 0.1		
16% protein		A (20ppm T-2)	13.4 $\pm$ 0.8	10.1 $\pm$ 2.0	13.9 $\pm$ 1.3	12.4 $\pm$ 1.2	12.8 $\pm$ 1.2
			1.9 $\pm$ 0.1	1.4 $\pm$ 0.3	2.0 $\pm$ 0.2	1.9 $\pm$ 0.3	1.8 $\pm$ 0.1
		B (restricted control)	13.3 $\pm$ 0.3	10.8 $\pm$ 0.2	12.0 $\pm$ 1.1	11.1 $\pm$ 0.1	10.1 $\pm$ 1.1
	2.5 $\pm$ 0.1		2.0 $\pm$ 0.1	2.0 $\pm$ 0.0	2.0 $\pm$ 0.0	2.0 $\pm$ 0.0	
	C ( <u>ad lib</u> control)	17.0 $\pm$ 0.1	15.3 $\pm$ 0.8	12.6 $\pm$ 1.0	10.1 $\pm$ 0.6	--	
		4.1 $\pm$ 0.1	4.4 $\pm$ 0.2	4.2 $\pm$ 0.4	3.5 $\pm$ 0.3		

<sup>a</sup>Mean consumption ( $\pm$ SEM) expressed as g/100g bw/day.

<sup>b</sup>Mean consumption ( $\pm$ SEM) expressed as g/mouse/day.

Quantities relate to basic diet ingredients.

Table 69 : Comparison of diet consumption rates<sup>a</sup> of mice consuming T-2 toxin and mice consuming the corresponding control diets at a restricted rate.

Source of variation	Analysis of Variance				
	Sum of squares	Degrees of freedom	Mean square	F ratio	P
Main effects					
Diet	3.551	2	1.776	0.236	0.790
Time	62.981	3	20.994	2.793	0.046*
Toxin	18.305	1	18.305	2.435	0.123
2-way interactions					
Diet x time	34.203	6	5.701	0.758	0.605
Diet x toxin	43.636	2	21.818	2.903	0.061
Time x toxin	27.673	3	9.224	1.227	0.306
3-way interactions					
	31.377	6	5.230	0.696	0.654
Explained	221.726	23	9.640	1.283	0.211
Residual	541.173	72	7.516	--	--
Total	762.899	95	8.031	--	--

<sup>a</sup> Measured in g/100g/day.

\* Significant,  $p < 0.05$ .

Table 70: Erythrocyte indices\* of young Swiss mice after 28 days on semipurified diets containing T-2 toxin (20 ppm) and different levels of protein.

Variable	Dietary protein level	Group				
		A 20 ppm T-2 toxin	B Restricted control	C Toxin withdrawn	D Ad lib control	E Pellet control
Mean corpuscular volume (MCV) u <sup>3</sup>	8%	46.2±0.5 a	46.7±0.8 a	48.5±0.9 ab	50.8±0.6 b	49.8 b
	12%	44.5±1.7 a	50.0±1.3	48.3±1.2	50.2±0.5	49.8
	16%	46.5±0.6 a	48.7±0.3 b	48.8±0.8 b	50.0±0.4 b	49.8 b
Diet groups combined		45.8±0.6 a	48.5±0.7 b	48.5±0.5 b	50.3±0.3 b	49.8±0.3 b
Mean corpuscular hemoglobin (MCH) pg	8%	17.6±0.6	18.2±0.6	18.5±0.4	18.1±0.4	18.3
	12%	17.1±0.3 a	18.8±0.6 b	19.1±0.8 b	19.3±0.3 b	18.3 ab
	16%	17.9±0.4	18.9±0.5	17.7±0.3	19.3±0.7	18.3
Diet groups combined		17.7±0.3 a	18.4±0.4 ab	18.4±0.3 ab	19.1±0.3 b	18.3±0.3 ab
Mean corpuscular hemoglobin concentration (MCHC) percent	8%	39.8±1.0	38.4±0.8	39.0±0.5 <sup>e</sup>	37.6±0.4	37.5
	12%	37.2±0.7	38.4±0.2	39.7±1.1 <sup>ef</sup>	39.3±0.5	37.5
	16%	39.1±0.6	39.9±1.1	37.1±0.3 <sup>f</sup>	39.7±1.2	37.5
Diet groups combined		38.7±0.5	38.8±0.5	38.5±0.5	38.9±0.5	37.5±0.6

\* Values are means (+SEM) of separate groups of 4 mice.

a,b

In any horizontal row, means followed by the same letter, or by no letter, do not differ at p = 0.05, by SNK multiple range test.

e, f

In any vertical column of 3 means, those followed by the same letter, or by no letter in this position, do not differ at p = 0.05 by SNK multiple range test.

Table 71: Comparison of hemoglobin concentrations of mice during a 4-week period of consumption of T-2 toxin (20 ppm) in diets containing 8%, 12% and 16% protein.

Source of variation	Analysis of Variance				
	Sum of squares	Degrees of freedom	Mean squares	F ratio	P
Main effects					
Dietary protein	38.052	2	19.026	3.218	0.052
Weeks on diet	137.022	3	45.674	7.726	0.000**
2-way interactions					
Explained	195.442	11	17.767	3.006	0.006
Residual	212.814	36	5.911	--	--
Total	408.256	47	8.686	--	--

\*\* Significant,  $p < 0.001$ .

Table 72 : Leukocyte values\* of young Swiss mice after 28 days on semipurified diets containing T-2 toxin (20 ppm) and different levels of protein.

Variable	Dietary protein level	Group				
		A 20 ppm T-2 toxin	B Restricted control	C Toxin withdrawn	D <u>Ad lib</u> control	E Pellet control
Total leukocyte count, (WBC) x10 <sup>3</sup> /ul	8%	8.0±1.9	7.9±1.7	10.9±3.3	13.9±1.5	11.1
	12%	4.6±0.8 <sup>a</sup>	9.5±0.6 <sup>b</sup>	7.0±1.7 <sup>ab</sup>	13.6±0.6 <sup>c</sup>	11.1 <sup>c</sup>
	16%	5.9±1.0 <sup>a</sup>	6.3±1.5 <sup>a</sup>	4.7±0.7 <sup>a</sup>	11.2±0.8 <sup>b</sup>	11.1 <sup>b</sup>
Diet groups combined		6.2±0.8 <sup>a</sup>	8.0±0.8 <sup>ab</sup>	7.6±1.4 <sup>ab</sup>	12.9±0.7 <sup>c</sup>	11.1±0.7 <sup>bc</sup>
Neutrophils, x10 <sup>3</sup> /ul	8%	6.9±1.5 <sup>a</sup>	1.0±0.2 <sup>b</sup>	8.2±2.5 <sup>a</sup>	1.9±0.4 <sup>b</sup>	1.4 <sup>b</sup>
	12%	4.6±0.7 <sup>a</sup>	2.2±0.6 <sup>ab</sup>	4.5±1.2 <sup>a</sup>	1.9±0.5 <sup>ab</sup>	1.4 <sup>b</sup>
	16%	4.9±0.7 <sup>a</sup>	2.0±0.3 <sup>bc</sup>	3.0±0.5 <sup>b</sup>	0.9±0.2 <sup>c</sup>	1.4 <sup>c</sup>
Diet groups combined		5.5±0.7 <sup>a</sup>	1.7±0.3 <sup>b</sup>	5.3±1.1 <sup>a</sup>	1.6±0.2 <sup>b</sup>	1.4±0.2 <sup>b</sup>
Lymphocytes, x10 <sup>3</sup> /ul	8%	1.0±0.0 <sup>a</sup>	6.8±1.8 <sup>b</sup>	2.5±0.8 <sup>a</sup>	11.8±1.3 <sup>c</sup>	9.5 <sup>bc</sup>
	12%	0.3±0.1 <sup>a</sup>	7.1±0.1 <sup>b</sup>	2.3±0.6 <sup>c</sup>	11.5±0.2 <sup>d</sup>	9.5 <sup>bd</sup>
	16%	0.9±0.4 <sup>a</sup>	4.2±1.3 <sup>b</sup>	1.5±0.2 <sup>a</sup>	10.1±0.7 <sup>c</sup>	9.5 <sup>c</sup>
Diet groups combined		0.8±0.2 <sup>a</sup>	6.2±0.8 <sup>b</sup>	2.1±0.3 <sup>a</sup>	11.1±0.5 <sup>c</sup>	9.5±0.8 <sup>c</sup>

\* Values are means (±SEM) of separate groups of 4 mice.

a,b,c,d In any horizontal row, means followed by the same letter, or by no letter, do not differ at p = 0.05, by SNK multiple range test.

In all vertical columns of 3 means for groups A, B, C and D, no differences were detected at p = 0.05 by SNK multiple range test.

Table 73 : Hepatic, gastric and testicular weights\* of young Swiss mice after 28 days on semipurified diets containing T-2 toxin and different levels of protein.

Variable	Dietary protein level	Group				
		A 20 ppm T-2 toxin	B Restricted control	C Toxin withdrawn	D <u>Ad lib</u> control	E Pellet control
Hepatic weight; mg	8%	616 $\pm$ 58	768 $\pm$ 41	612 $\pm$ 24	1110 $\pm$ 75	1750
	12%	821 $\pm$ 112 <sup>a</sup>	807 $\pm$ 40 <sup>a</sup>	504 $\pm$ 100 <sup>a</sup>	1470 $\pm$ 166 <sup>b</sup>	1750 <sup>b</sup>
	16%	943 $\pm$ 123 <sup>a</sup>	714 $\pm$ 13 <sup>a</sup>	637 $\pm$ 95 <sup>a</sup>	1300 $\pm$ 131 <sup>b</sup>	1750 <sup>c</sup>
Diet groups combined		809 $\pm$ 70 <sup>a</sup>	763 $\pm$ 21 <sup>a</sup>	592 $\pm$ 44 <sup>b</sup>	1300 $\pm$ 81 <sup>c</sup>	1750 $\pm$ 95 <sup>d</sup>
Gastric weight; mg	8%	273 $\pm$ 66	207 $\pm$ 8	221 $\pm$ 19	259 $\pm$ 25	240
	12%	236 $\pm$ 22	225 $\pm$ 14	194 $\pm$ 26	232 $\pm$ 17	240
	16%	256 $\pm$ 30	222 $\pm$ 16	253 $\pm$ 13	220 $\pm$ 15	240
Diet groups combined		253 $\pm$ 20	218 $\pm$ 7	226 $\pm$ 12	237 $\pm$ 11	240 $\pm$ 17
Testicular weight; mg	8%	89 $\pm$ 22 <sup>a e</sup>	200 $\pm$ 26 <sup>b</sup>	101 $\pm$ 10 <sup>a</sup>	240 $\pm$ 6 <sup>b</sup>	282 <sup>c</sup>
	12%	123 $\pm$ 12 <sup>a ef</sup>	213 $\pm$ 15 <sup>b</sup>	136 $\pm$ 4 <sup>a</sup>	226 $\pm$ 39 <sup>b</sup>	282 <sup>b</sup>
	16%	158 $\pm$ 13 <sup>a f</sup>	220 $\pm$ 9 <sup>b</sup>	171 $\pm$ 18 <sup>a</sup>	256 $\pm$ 14 <sup>bc</sup>	282 <sup>c</sup>
Diet groups combined		126 $\pm$ 12 <sup>a</sup>	211 $\pm$ 10 <sup>b</sup>	136 $\pm$ 12 <sup>a</sup>	241 $\pm$ 13 <sup>bc</sup>	282 $\pm$ 16 <sup>c</sup>

\* Values are means ( $\pm$ SEM) of separate groups of 4 mice.

a,b,c,d In any horizontal row, means followed by the same letter, or by no letter, do not differ at  $p = 0.05$ , by SNK multiple range test.

e,f In any vertical column of 3 means, those followed by the same letter, or by no letter in this position, do not differ at  $p = 0.05$  by SNK multiple range test.

Table 74: Relative hepatic, gastric and testicular weights\* of young Swiss mice after 28 days on semipurified diets containing T-2 toxin and different protein levels.

Variable	Dietary protein level	Group				
		A 20 ppm T-2 toxin	B Restricted control	C Toxin withdrawn	D <u>Ad lib</u> control	E Pellet control
Relative hepatic weight; % of body weight	8%	5.7 $\pm$ 0.4	4.7 $\pm$ 0.2	4.9 $\pm$ 0.1	3.4 $\pm$ 0.1	5.0
	12%	6.7 $\pm$ 0.5 <sup>a</sup>	4.8 $\pm$ 0.4 <sup>b</sup>	4.2 $\pm$ 0.7	4.2 $\pm$ 0.3	5.0 <sup>b</sup>
	16%	6.3 $\pm$ 0.6 <sup>a</sup>	4.1 $\pm$ 0.1 <sup>b</sup>	4.3 $\pm$ 0.4 <sup>b</sup>	3.8 $\pm$ 0.3	5.0 <sup>b</sup>
Diet groups combined		6.3 $\pm$ 0.3 <sup>a</sup>	4.5 $\pm$ 0.2 <sup>bc</sup>	4.5 $\pm$ 0.2 <sup>bc</sup>	3.8 $\pm$ 0.2 <sup>b</sup>	5.0 $\pm$ 0.2 <sup>c</sup>
Relative gastric weight; % of body weight	8%	2.5 $\pm$ 0.6	1.3 $\pm$ 0.1 <sup>ab</sup>	1.8 $\pm$ 0.1 <sup>b</sup>	0.8 $\pm$ 0.1 <sup>a</sup>	0.7 <sup>a</sup>
	12%	2.0 $\pm$ 0.2 <sup>a</sup>	1.3 $\pm$ 0.0 <sup>b</sup>	1.7 $\pm$ 0.4 <sup>ab</sup>	0.7 $\pm$ 0.0 <sup>c</sup>	0.7 <sup>c</sup>
	16%	1.7 $\pm$ 0.2 <sup>a</sup>	1.3 $\pm$ 0.1 <sup>b</sup>	1.8 $\pm$ 0.1 <sup>a</sup>	0.7 $\pm$ 0.0 <sup>c</sup>	0.7 <sup>c</sup>
Diet groups combined		2.0 $\pm$ 0.2 <sup>a</sup>	1.3 $\pm$ 0.0 <sup>b</sup>	1.8 $\pm$ 0.1 <sup>a</sup>	0.7 $\pm$ 0.0 <sup>c</sup>	0.7 $\pm$ 0.0 <sup>c</sup>
Relative testicular weight; % of body weight	8%	1.0 $\pm$ 0.1 <sup>a</sup>	1.2 $\pm$ 0.1 <sup>a</sup>	0.8 $\pm$ 0.1 <sup>ab</sup>	0.7 $\pm$ 0.1 <sup>b</sup>	0.8 <sup>ab</sup>
	12%	1.0 $\pm$ 0.1 <sup>a</sup>	1.2 $\pm$ 0.1 <sup>ab</sup>	1.2 $\pm$ 0.2 <sup>a</sup>	0.7 $\pm$ 0.1 <sup>c</sup>	0.8 <sup>bc</sup>
	16%	1.1 $\pm$ 0.1 <sup>a</sup>	1.3 $\pm$ 0.0 <sup>a</sup>	1.2 $\pm$ 0.1 <sup>a</sup>	0.8 $\pm$ 0.0 <sup>b</sup>	0.8 <sup>b</sup>
Diet groups combined		1.0 $\pm$ 0.1 <sup>a</sup>	1.2 $\pm$ 0.0 <sup>b</sup>	1.0 $\pm$ 0.1 <sup>a</sup>	0.7 $\pm$ 0.0 <sup>c</sup>	0.8 $\pm$ 0.0 <sup>ac</sup>

\* Values are means ( $\pm$ SEM) of separate groups of 4 mice.

a,b,c In any horizontal row, means followed by the same letter, or by no letter, do not differ at  $p = 0.05$ , by SNK multiple range test.

In each vertical column of 3 means, for each of groups A, B, C and D, no differences were detected at  $p = 0.05$  by SNK multiple range tests.

Table 75 : Relative and absolute splenic weights of young Swiss mice after 28 days on semipurified diets containing T-2 toxin (20 ppm) and different levels of protein.

Variable	Dietary protein level	Group				
		A 20 ppm T-2 toxin	B Restricted control	C Toxin withdrawn	D <u>Ad lib</u> control	E Pellet control
Relative splenic weight; % of body weight	8%	0.12±0.02 <sup>*a</sup>	0.25±0.02 <sup>ab</sup>	0.35±0.07 <sup>b</sup>	0.39±0.05 <sup>b</sup>	0.31 <sup>b</sup>
	12%	0.13±0.02 <sup>a</sup>	0.23±0.02 <sup>ab</sup>	0.31±0.13 <sup>ab</sup>	0.41±0.04 <sup>b</sup>	0.31 <sup>ab</sup>
	16%	0.44±0.14	0.18±0.01	0.35±0.05	0.29±0.02	0.31
Diet groups combined		0.24±0.07	0.22±0.01	0.34±0.04	0.36±0.02	0.31±0.03
Absolute splenic weight; mg.	8%	14±3 <sup>a</sup>	41±4 <sup>a</sup>	45±10 <sup>a</sup>	126±9 <sup>b</sup>	110 <sup>b</sup>
	12%	16±2 <sup>a</sup>	40±4 <sup>a</sup>	37±17 <sup>a</sup>	142±12 <sup>b</sup>	110 <sup>c</sup>
	16%	70±26 <sup>abc</sup>	32±2 <sup>a</sup>	51±7 <sup>ab</sup>	99±11 <sup>bc</sup>	110 <sup>c</sup>
Diet groups combined		35±12 <sup>a</sup>	38±2 <sup>a</sup>	45±6 <sup>a</sup>	120±8 <sup>b</sup>	110±20 <sup>b</sup>

\* Values are means (±SEM) of separate groups of 4 mice.

a,b,c In any horizontal row, means followed by the same letter, or by no letter, do not differ at p = 0.05, by SNK multiple range test.

In each vertical column of 3 means, for each of groups A, B, C and D, no differences were detected at p = 0.05 by SNK multiple range tests.

## APPENDIX D

A sample of crystalline T-2 toxin was generously supplied by Dr. H.R. Burmeister, Northern Regional Research Laboratory, Peoria, Illinois. This was used as an authentic reference sample.

Crystalline T-2 toxin (Batch Nos. 7581, 7873) was purchased from Makor Chemicals, Jerusalem, Israel. This product was chromatographically pure by TLC in ethyl acetate-hexane (3:1). The Rf was identical to that of the reference sample and with purified T-2 toxin isolated from Fusarium poae. These analyses were performed by Dr. N. Westcott, Research Station, Agriculture Canada, Saskatoon. The biological potency of the purchased T-2 toxin was similar to the potency of the reference sample and to the purified sample of T-2 toxin isolated by Dr. Westcott. Comparison of potency was performed by the quantitative bioassay described in Section 5 (p. 118).